PRECLINICAL STUDY OF SIDDHA DRUG VATHA SILETPANA SURA KUDINEER FOR ITS ANTI-INFLAMMATORY, ANALGESIC AND ANTIOXIDANT ACTIVITIES

Dissertation submitted by

Dr. VIDHYA MILANO PRASAD

Under the guidance of

Dr. R. ANTONY DURAICHI, M.D(s).,

Lecturer Grade II,

Department of Gunapadam,

Government Siddha Medical College, Palayamkottai.

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PALAYAMKOTTAI

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Preclinical study of siddha drug *VATHA SILETPANA SURA KUDINEER* for Anti-inflammatory, Analgesic and Antioxidantactivities"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. R. Antony Duraichi M.D(s)., Lecturer Grade II,** 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, Associateship, Fellowship or other similar title.

Date: **Signature of the Candidate**

Place: Palayamkottai **Dr.VIDHYA MILANO PRASAD**

GOVT. SIDDHA MEDICAL COLLEGE
PALAYAMKOTTAI

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"Preclinical study of siddha drug *VATHA SILETPANA SURA KUDINEER* for Anti-inflammatory, Analgesic and Antioxidant activities"** is submitted to The Tamilnadu Dr.M.G.R.Medical University, Chennai-32 is a partial fulfilment of the requirements for the award of degree of M.D (siddha) is the bonafide and genuine research work done by **Dr. Vidhya Milano Prasad** under my guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

Date: **Signature of the Guide**

Place: Palayamkottai **Dr. R. Antony Duraichi, M.D. (s).,**
Lecturer, Grade II,
Department of PG Gunapadam,
Govt. Siddha Medical College,
Palayamkottai.

GOVT. SIDDHA MEDICAL COLLEGE
PALAYAMKOTTAI

BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **"Preclinical study of siddha drug *VATHA SILETPANA SURA KUDINEER* for Anti-inflammatory, Analgesic and Antioxidant activities"** is a bonafide work done by **Dr. Vidhya Milano Prasad,** a candidate of Government siddha medical college, Palayamkottai in partial fulfilment of the University rules and regulations for award of M.D(siddha) - Gunapadam under our supervisionduring the academic year of 2022.

Dr. A. Kingsly, M.D(s).,
Professor & HOD,
Department of PG Gunapadam,
Govt.Siddha Medical College,
Palayamkottai.

Principal,
Govt.Siddha Medical College,
Palayamkottai.

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ABBREVIATIONS

%	-	percentage
CCD	-	Catalytic Combustion Detector
CCD_S	-	Charge Coupled Devices.
DC	-	Differential Count
ED_{50}	-	Effective Dose $_{50}$
EDTA	-	Ethylene Diamine Tetra Acetic Acid
EMB	-	Eosin Methylene Blue agar medium.
ESR	-	Erythrocyte Sedimentation Rate
F	-	Female
FID	-	Flame Ionization Detector
FTIR	-	Fourier Transform Infra Red spectroscopy
g	-	Gram
g%	-	Gram percentage
Hb	-	Haemoglobin
HDL	-	High Density Lipoprotein
IAEC	-	Institutional Animal Ethical Committee.
ICP-OES	-	Inductively Coupled Plasma Optical Emission Spectrometry
Ig E	-	Immunoglobulin E
Kg	-	Kilogram
LD	-	Low Dose
LD_{50}	-	Lethal Dose $_{50}$
LDH	-	Lactate Dehydrogenase
LDL	-	Low Density Lipoprotein
M	-	Male
MCV	-	Mean Corpuscular Volume
Mg	-	Milligram
ML	-	Milliliter
MLD	-	Minimum Lethal Dose
MTD	-	Maximum Tolerated Dose
NOAEL	-	No-Observed-Adverse-Effect-Level
OECD	-	Organisation for Economic Co-operation and Development
PCV	-	Packed Cell Volume.

PDF	-	Powder Diffraction File
PGE	-	Prostaglandin E
R&D	-	Research and Development
RBC	-	Red Blood Corpuscles
SEM	-	Scanning Electron Microscope
SGOT	-	Serum Glutamic Oxaloacetic Transaminase
SGPT	-	Serum Glutamic Pyruvic Transaminase
SPME	-	Solid Phase Micro Extraction
TCD	-	Thermal Conductivity Detector
VLDL	-	Very Low Density Lipoprotein
VSSK	-	Vatha Siletpana Sura Kudineer

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1. INTRODUCTION

For the primary health care needs, most people in this world rely on plant drugs. Siddha Medicine is one of the ancient Tamil medical system. Siddha is the mother medicine of ancient Tamils / Dravidians of peninsular South India. The word *Siddha* means established truth. The persons who were associated with establishing such a Siddha school of thought were known as Siddhars. Siddhars, mainly hailing from Tamil Nadu laid the foundation for Siddha system of medicine. Hence, it is called Siddha medicine. Siddhars were spiritual masters who attained the *ashta* (eight) *siddhis* or unique powers. **Agathiyar** is believed to be the founding father of Siddha Medicine. **Siddha** (**siddha**; "perfected one") is a term that is used widely in Indian religions and culture. It means "one who is accomplished". It refers to perfected masters who have achieved a high degree of physical as well as spiritual perfection or enlightenment.

Disease caused in the human body shows different symptoms. The treatment method of Siddha Medicine is based on three vital humours (Vatha,Pitha and Kapha) which in turn based on panchabootham. Siddha diagnosis of disease is based on eight types of diagnostic tools(en vagai thervu) in which naadi plays an important role. Siddhars said about various diseases and the treatment methods with herbs, minerals and animal products. Accordingly thirteen Siddha text books were reviewed, in which the term suram, kaichal and jevram were the names taken in to consideration. There are 182 types of suram along with their sign and symptoms mentioned in text books. Siddha Medicine says that fever is not a symptom, it consider fever as a disease and among these 182 types of suram(fever), the sign and symptoms of covid-19 infection can be compared to kapha suram, pitha kapha suram and vatha kapha suram. But it is highly related to vatha kapha(siletpanam) suram mentioned in Siddha text books.

According to Siddha Medicine, "*Andathil ullathe pindam*" that means whatever in universe is present in our body. Our body and this universe is made up of basic five elements called panchabootham, they are earth, water, fire, air and space. The three important unit – uyir thathukal of life is vatha, pitha and kapha and the seven structural unit –udal thathukal (*Saram, senner, oon, kolupu, enbu, moolai, sukkilam*) are made up of panchabootham. Three vital humors(functional units)are in the ratio 1:1/2:1/4;. Any imbalance in this, cause disease. The imbalance may cause due to improper behavior,

wrong food habits and climate change. In order to boost the immune system, some taste of foods should be taken, again the taste is based on panchabootham combination. In Siddha, taste plays an important role in the treatment of disease. Siddha medicine also says about some kayakatpam herbs. kaayam means body and katpham means to prevent body from disease. Such herbs will help to improve the immune system.

In Siddha System, the Diseases are raised based on the derangement of Mukkutram. Usually, Thottru Noigal (communicable diseases) associated with Aiya kutram (Respiratory related Illness), are gets affected due to its Sthiram gunam (stability factor). In Guru Naadiquoted, Thottru Noigal generally caused by Kirumi(Pathogens). The symptoms are due to Noiyinan vanmai (Immunity of individual), if it is good, he/she will not be affected. So, the Siddha medicines are used to neutralize the Aiya kutram.

This corono virus infection can be compared to vathakapha suram in Siddha Medicine because, Vatha affected due to two reasons. When udal thathukal affected,(plasma and blood) *saram* and *senner* is affected, as a result they are unable to protect the body against organisms. Secondly, when *senne*r decreases, it blocks the vessels known as thathu imbalance, and due to this the vatha get imbalance. There are ten types of Vatham and Vatha plays a major role in doing five main functions of the body. They are breathing, heart and brain function (Pranan), blood flow(viyanan), digestion (samanan), expulsion of urine and feces(abanan), vocal sounds and others(uthanan).when the vatha get imbalance, the above functions get affected. At the same time, along with samanan vayu, pashaka pitham, kilethaka kapham and jadarakini get affected and cause digestive problem which results in the formation of aamam. This aamam get absorbed in to the blood and by viyanan it reaches the pores of skin and blocks sweating. This affects pitham (pirajaka pitham) and increases temperature in body. Due to vatha (Kirukaran) imbalance, sore throat appears. As the pashaka pitham get imbalance it affects pothaka kapam and kilethaka kapham and so kapha increases. when this is not treated, all the three vital humours get imbalance and leads to disease called muppini suram in which severe complications arises and lead to death.

The corono virus(COVID 19) infection is a pandemic disease and it needs special attention. It becomes a challenge to all medical fields as the spread can only be controlled by isolation and self prevention. No medicines were found yet. The major

symptoms of COVID 19 infection are dry cough, fever and breathing difficulty. The other symptoms include aches and pains, tiredness, sore throat, running nose, diarrhea, nausea, tingling sensation, loss of smell and taste, ,bluish lips or face, fever mild to moderate and high, new confusion or inability to arouse, headache, general feeling of unwell, chills, sweating, malaise, dizziness. The authors decided to find out the symptoms related to types of suram mentioned in Siddha Medicine text books. All together nineteen major and minor symptoms were found to be related with vathakapha suram and also more then this some of the other minor symptoms are also mentioned in Siddha texts for vathakapha suram.

EPIDEMIOLOGY

Corona virus is a pandemic which affects the whole world from 2020 march till up to date. The virus get mutated and affecting many people. Most people infected with the virus will experience mild to moderate respiratory illness and recover without requiring special treatment. However, some will become seriously ill and require medical attention. Older people and those with underlying medical conditions like cardiovascular disease, diabetes, chronic respiratory disease, or cancer are more likely to develop serious illness. Anyone can get sick with COVID-19 and become seriously ill or die at any age. Globally, as of 5:19pm CEST, 31 March 2022, there have been 485,243,022 confirmed cases of COVID-19, including 6,137,553 deaths, reported to WHO. As of 26 March 2022, a total of 11,054,362,790 vaccine doses have been administered. COVID-19; SARS-CoV-2 is the major public health burden in the world, the morbidity and mortality of global community is dramatically increasing from time to time from 2020 onward with different variants like delta and omicron.

In Siddha text books, this disease has been compared with *Vatha siletpana suram* and the treatment was already given. There are various effective Siddha drugs are mentioned in Siddha classical literatures and manuscripts by Siddhars for "*Vatha Siletpana Suram*". Therefore identification and scientific validation of the effective Siddha drug to prevent this pandemic is very essential in this current world. Therefore, I have selected *VATHA SILETPANA SURA KUDINEER* from the literature of ***Pararajasekaram- suram, sanni, vali, vikkal, sathi roga nithanankal part- III , Author ponniayah.I. page no. 24-25.***

2. AIM & OBJECTIVES OF THE STUDY

AIM:

To validate the Siddha formulation *Vatha Siletpana Sura Kudineer* scientifically for Vatha Siletpana Suram through preclinical studies of its Anti inflammatory, Analgesic and Antioxidant activities.

OBJECTIVES:

The following methodologies were done for standardize and evaluate the safety and efficacy of the trial drug

1. To collect the complete details of trial medicine and disease from classical Siddha literature and modern scientific resources
2. To prepare the trial medicine as per classical text literature mentioned
3. To perform the Physico - chemical analysis of the test drug
4. To carry out the Biochemical analysis of the trial drug
5. To conduct the Phytochemical analysis of the test drug
6. To evaluate the Anti-microbial activity of the trial drug
7. To carry out the microbial limit test of test drug
8. To perform the instrumental analysis of the trial drug
    i. Scanning Electron Microscope (SEM)
    ii. Fourier Transform Infrared Spectroscopy (FTIR)
    iii. Inductively Coupled Plasma Optical Emission Spectrometry (ICPOES)
    iv. X-RAY powder diffraction (XRD)
9. To evaluate the acute and sub-acute toxicity of the test drug according to the OECD guidelines 423 & 407 respectively
10. To assess the following pharmacological activities of the test drug in animal experimental study
    i. Anti inflammatory activity
    ii. Analgesic activity
    iii. Anti oxidant activity

3.REVIEW OF THE LITERATURE

3.1. கண்டங்கத்தரி (*Solanum xanthocarpum / Solanum surattense*, Burm.f)

3.1.1 GUNAPADAM ASPECT:-

Fig 1*: Solanum xanthocarpum*

Organoleptic characters:

Taste - Pungent

Potency - Heat

Post Absorptive change- Pungent

Parts used : leaf, flower, pod, fruit, seeds, root, whole plant

Therapeutic action:

Expectorant

Diuretic

Carminative

General Properties of whole plant:

“வேரிலைபூ காய் பழமவ் வித்துமதன் பட்டையுமிவ்
வூரி லிருக்க உடற்கனப்பும் - நீராய்
வரும்பீந சங்கயஞ்சு வாசமுந்தங் காதே
அருங்கண்டங் கத்தரியு ளாய்”

It is useful in the treatment of running nose, breathing difficulty, cold and accumulation of body fluids.

CLASSICAL PREPARATION:

1. ***Kandarasa mezhugu***

   Dose: ½ -1kundri

   Adjuvant: Palm jaggery, butter, jiggery

   Indication: Fever, Sanni, abdominal pain, chest pain, vaayukal,

   (Anuboga vaithya navaneetham, part V, pg-146)

2. ***Kabasura kiyalam:***

   Dose: 30-60 ml (twice a day)

   Indications : kaba suram

   (Siddha Vaithya pathartha Guna vilakam,pg-220)

3. ***Kandankathari veer kudineer***

   Dose : 30-60 ml (Twice a day)

   Indication : vatha suram

   (Gunapadam porutpanbu nool, part I, pg-334)

4. ***Kandankathari rasayanam***

   Dose : external application in foot for children, ½ g (twice a day)

   Indication : External application for kakkuvan. Internally for cough, breathing difficulty.

   (Siddha Vaithya pathartha Guna vilakam,pg-220)

5. Ayavathi chooranam

Dose: 1½ -1 varagan

Adjuvant : jaggery, honey

Indication: All types of vatha disorders, Vatha, pitha and kapha diseases

(Anupoga vaithya navaneetham, part VI, pg-57)

3.1.2 BOTANICAL ASPECT

Taxonomical classification

Kingdom - Plantae
Subkingdom - Tracheobionta
Division - Magnoliophyta
Class - Magnoliopsida
Subclass - Asteridae
Order - Solanales
Family - Solanaceae
Genus - Solanum
Species- xanthocarpum

Synonym : S. surattense Burm.f. S. virginianum Linn. S. maccanni Sant.

Habitat Throughout India.

English : Wild Eggplant, YellowBerried Nightshade.

Action : Stimulant, expectorant, diuretic, laxative, febrifuge.

Description:

A very prickly diffuse bright green perennial herb, somewhat woody at the base; stem is somewhat zigzag; branches are numerous, the younger ones clothed with dense stellate tomentum; prickles are compressed, straight, yellow, glabrous and shining, often exceeding 1.3 cm. Leaves are usually 5-10

in numbers and 2.5-5.7 cm in length, ovate or elliptic, sinuate or sub pinnatified, obtuse or sub acute, stellately hairy on both sides, sometimes becoming nearly glabrous in age, armed on the midrib and often on the nerves with long yellow sharp prickles, base usually rounded and unequal-sided; petiole 1.3-2.5 cm long, stellately hairy. The berries are green and white strips when young but yellow when mature. They are 1.3-2 cm in diameter, yellow, or white with green veins, surrounded by the enlarged calyx. Seeds are 2.5 mm in diameter and glabrous. Calyx is nearly 1.3 cm long, densely hairy and prickly; tube short, globules. Lobes are 11 mm long, linear-lanceolate, acute and hairy outside. Filaments are 1.5 mm long, glabrous; anthers 8 mm long, oblong lanceolate, opening by small pores. Ovary is ovoid, glabrous; style glabrous.

Used in the treatment of cough, bronchitis, asthma, for dislodging tenacious phlegm; also used against rheumatism, enlargement of liver and spleen, vomiting, difficult urination, bladder stones, skin diseases. Fruit—used as an adjuvant for promoting conception. Fruits gave solasonine, solamargine, beta-solamargine and solasodine; petals yielded apigenin; stamens gave quercetin diglycoside and sitosterol. The glycoalkaloid content of fruits collected from Jammu and Kashmir is reported to be 3.5% (total alkaloids, 1.1%). The presence of diosgenin in the plant has been reported. Both glycoalkaloid and fatty acid fractions of the plants extracts cause liberation of histamine from chopped lung tissue. The beneficial effect of the drug on bronchial asthma may be attributed to the depletion of histamine from bronchial and lung tissue. Dosage Whole plant—20-30 g for decoction. (API, Vol. I.)

3.1.3 LATERAL RESEARCH

Table 1: Lateral research on *Solanum xanthocarpum.*

study	Dosage	extraction	pharmacological activity	reference
invivo	15 days both topically (Gel at 2.5%, 5% and 10%) as well as orally (at 100, 200 and 400mg/kg p.o.)	ethanolic extract	anti psoratic activity antioxidant, antimicrobial and cellular proliferative activities	Parmar KM, Itankar PR, Joshi A, Prasad SK. Anti-psoriatic potential of Solanum xanthocarpum stem in Imiquimod-induced psoriatic mice model. J Ethnopharmacol. . 2017;198:158-166.
in vivo	10, 30 and 100 mg/kg p.o. in rats for 7 days	ethanolic extract	anti inflammatory	More SK, Lande AA, Jagdale PG, Adkar PP, Ambavade SD. Evaluation of anti-inflammatory activity of Solanum xanthocarpum Schrad and Wendl (Kaṇṭakāri) extract in laboratory animals. Anc Sci Life. . 2013;32(4):222-226.
in vivo	100-200 mg/kg bw	methanolic extract	antihyperglycemic antioxidant	Poongothai K, Ponmurugan P, Ahmed KS, Kumar BS, Sheriff SA.

				Antihyperglycemic and antioxidant effects of Solanum xanthocarpum leaves (field grown & in vitro raised) extracts on alloxan induced diabetic rats. Asian Pac J Trop Med. 2011;4(10):778-785.
in vivo	100 and 200 mg/kg b.w. of SXAF for 14 d	methanolic extract	antioxidant hepatoprotective	Jalali Ghassam B, Ghaffari H, Prakash HS, Kini KR. Antioxidant and hepatoprotective effects of Solanum xanthocarpum leaf extracts against CCl4-induced liver injury in rats. Pharm Biol. 2014;52(8):1060-1068

3.2 சிறுதேக்கு (*Clerodendrum serratum)*

3.2.1 GUNAPADAM ASPECT

Fig 2: ***Clerodendrum serratum***

Organoleptic characters:

Taste - Bitter, Astringent

Potency -Heat

Post Absorptive change -Pungent

Parts used :root, leaf

Therapeutic actions

Antipyretic

Anti malarial

Stimulant

Sedative

General Properties of root:

;கண்டுபா ராங்கியெனுஞ் சிறுதேக்கு ண்டால்

காலெங்கே பித்தமெங்கே கபந்தானெ ங்கே

தொண்டுதொட்டுத் தொடர்சுவாச காச மெங்கே

சுரமெங்கே வெற்யெங்கே தொனிநோ யெங்கே

மிண்டுபுரி பீநசநீர்க் கோவை யெங்கே

வெளிநீருண் ணீரெங்கே விறற்கா லெங்கே

அண்டுபடாச் சீதசுரங் கடுப்பு மெங்கே

யழலையக நோயெங்கே யறைகு வீரே!"

It is useful in the treatment of vitiated tridosha, delirium, fever, sinusitis, chills, vali disease, body heat.

CLASSICAL PREPARATION

1. Siruthekku kudineer

Dose: 30-60 ml (twice a day)

Indication : kapha suram, soma disease.

(Gunapadam porutpanbu nool, part I, pg-336)

2. Thalisabathri chooranam

Dose: 1½ - 2 varagan

Adjuvant : honey, ghee, milk, ilagam

Indication: cough, bone fever, running eyes, cold, breathing difficulty.

(Anubogaa vaithya navaneetham, part VI, pg-40)

3. Sarabarasa mathirai

Dose: ½ -1 varagan

Adjuvant : ginger extract, *Smilax chinensis* powder, extract of *classampelos Pereira*.

Indication: vatha disease, pain, fever, megam, kaasam

(Anuboga vaithya navaneetham, part VII, pg-135)

4. Seeragathi ilagam

Dose: 1½ - 2 varagan

Indication : Agnimantham, suvaiinmai, diarrhea, fever, cough, sinusitis.

(Anuboga vaithya navaneetham, part VIII, pg-34)

5. Parankichakkai chooranam

Dose: ¼ - ½ Thoola, twice a day, 20-40 days

Adjuvant: sugar

Indications: soothaga vayu, padarkiranthi, kaikal pidipu, vetttai, padai, vandukadi, karumegam, sori,sirangu, karappan

(Cikitcha rathna theepam ennum vaithya nool, pg-116)

3.2.2 BOTANICAL ASPECT

Taxonomical classification

Kingdom: Plantae
Subkingdom: Tracheophytes
Division: Angiosperms
Class: Eudicots
SubClass: Asterids
Order: Lamiales
Family: Lamiaceae
Subfamily: Ajugoideae
Genus: Clerodendrum
Species : serratum

Habitate:A shrub distributed throughout the country, especially common in Assam and Bengal.

English : Blue-flowered Glory tree, Beetle Killer.

Description :

It is a small shrub, 2-4meter tall bearing opposite leaves and having woody rootstock. The plant has quadrangular, glabrous branches. Leaves are large and ovate or oblong, usually ternate whorled, coarsely and sharply serrate, glabrous and pale beneath with six pairs of lateral nerves.

Flowers are large pinkish-white in colour and numerous appearing in May to August month. It has a stout deflexed compressed pedicel in lax, dichotomous, long terminal panicles. It has leafy bracts and the calyx is cup shaped 5 mm long. Corolla is pale to pinkish blue with tube about 6-7 mm long; the lower larger lip like lobe is sky blue in colour. Stamens are long, exerted, curved and bluish.The drupes are 1-4 lobed, bluish-black and glossy.

Action:Root—Antiasthmatic, antihistaminic, antispasmodic, antitussive carminative, febrifuge. Leaf—febrifuge. The Ayurvedic Pharmacopoeia of India indicated the use of the dried roots in cough, bronchitis, dyspnoea, chest diseases and sinusitis. The bark contains triterpenoids— serratagenic, oleanolic and queretaric acids; leaves contain alpha-spinasterol and flavonoids, including luteolin, apigenin, baicalein, scutellarein, phenolic acids—caffeic and ferulic acids. EtOH (50%) extract of the plant exhibited hypotensive and spasmolytic activity. Polyhydric property on isolated guinea pig ileum. Antiasthmatic effect was also observed pharmacologically. Dosage Root—3-6 g powder; 10-20 g for decoction

3.2.3 LATERAL RESEARCH

Table 2: Lateral research on *Clerodendrum serratum.*

Study	**dosage**	**extraction**	**pharmacological activity**	**reference**
in vivo	50, 100 and 200 mg/kg	Methanol extract	antinociceptive, anti-inflammatory and antipyretic activities	Narayanan N, Thirugnanasambantham P, Viswanathan S, Vijayasekaran V, Sukumar E. Antinociceptive, anti-inflammatory and antipyretic effects of ethanol extract of Clerodendron serratum roots in experimental animals. J Ethnopharmacol. 1999;65(3):237-241.
in vivo	100 and 200 mg/kg I.p	ethanol extract	anti asthmatic. Bronchodialator, anti inflammatory	Sreenu Thalla , Jyothibasu Tammu, Bhavani Pentela and Subba Reddy Thalla. Antiasthmatic Activity of Alcoholic Extract of Clerodendrum serratum Induced by Ovalbumin . International Journal of Chemical and Pharmaceutical Sciences. , 2012, Mar., Vol.3 (1)

3.3 கடுக்காய் *(Terminalia chebula)*

3.3.1 GUNAPADAM ASPECT

Fig.3 : ***Terminalia chebula***

Organoleptic characters

Taste - mostly Astringent, it also has sweet, sour, bitter and pungent. (Except salt)

Potency -Heat

Post absorptive change- Sweet

Part used – fruit without seed

Therapeutic Action:

Astringent

Anti microbial

Anti diabetic

Hepatoprotective

Immune modulatory

Cardio protective

General Properties of fruit

“கடுக்காயுந் தாயுங் கருதிலொன்றென் றாலும்

கடுக்காய்த் தாய்க்கதிகங் காண்நீ – கடுக்காய்நோய்

ஓட்டி உடற்தேற்றும் உற்றவன்னை யோசுவைகள்

ஊட்டியுடற் தேற்று முவந்து

This is considered more than a mother, thus it will boost the body.

CLASSICAL PREPARATION

1. Kaddukai nei

Dose : 2-4 vaaragan

Indication : agni mantham, soolai, palavagai vayu, piles, rectal and urinary retention

(Anuboga vaithya navaneetham, part VI, pg-26)

2.Kadukkai lekiyam

Dose : punnai kaai azhavu

Indicaiton : Agni mantham, soolai,vayu, piles

(Gunapadam porut panbu nool, part I, pg-336)

3.Bhavana kadukkai

Dose: pakkalazhavu , twice a day

Indication : Soolai, ulcers, piles, arosagam, sinusitis, vomiting, paandu, cough, throat pain

(Siddha vaithya pathartha guna vilakkam, 1500 mooligaigal, pg-212)

4.Thiripalathi chooranam

Dose : ½ - 1 varagan

Adjuvant : warm water

Indication: Athi moothiram, mathumoothiram, katti

(kannusamy parambarai vaithiyam, pg-125)

5.Saamuthra chooranam

Dose : 3 varagan

Adjuvant : ghee

Indications : Gunmam-8, vatham-80, piles-3, kiragani -11, paandu-5, magotharam-8

(Anuboga vaithya brahma ragasiyam, Part VI, Pg-354)

3.3.2. BOTANICAL ASPECT

Taxonomical classification:

kingdom: Plantae

Subkingdom: Tracheophytes

Division: Angiosperms

Class Eudicots

Subclass: Rosids

Order: Myrtales

Family: Combretaceae

Genus: Terminalia

Species: chebula

Habitat :Abundant in Northern India. Also occurs in the forests of Assam, West Bengal, Bihar, Assam, especially in Konkan.

English : Chebulic Myrobalan, Black Myrobalan.

Description

Terminalia chebula is a medium to large deciduous tree growing to 30 m (98 ft) tall, with a trunk up to 1 m (3 ft 3 in) in diameter. The leaves are alternate to subopposite in arrangement, oval, 7–8 cm (2.8–3.1 in) long and 4.5–10 cm (1.8–3.9 in) broad with a 1–3 cm (0.39–1.18 in) petiole. They have an acute tip, cordate at the base, margins entire, glabrous above with a yellowish pubescence below. The fruit is drupe-like, 2–4.5 cm (0.79–1.77 in) long and 1.2–2.5 cm (0.47–0.98 in) broad, blackish, with five longitudinal ridgesThe dull white to yellow flowers are monoeious, and have a strong,

unpleasant odour. They are borne in terminal spikes or short panicles. The fruits are smooth ellipsoid to ovoid drupes, yellow to orange-brown in colour, with a single angled stone.

Action Gentle purgative, astringent (unripe fruits are more purgative, ripe ones are more astringent; sennoside A and anthraquinone glycoside is laxative, tannins are astringent), stomachic, antibilious, alterative. Used in prescriptions for treating flatulence, constipation, diarrhoea, dysentery, cyst, digestive disorders, vomiting, enlarged liver and spleen, cough and bronchial asthma, and for metabolic harmony. Bark—diuretic. The Ayurvedic Pharmacopoeia of India, along with other therapeutic applications, indicated the use of powder of mature fruits in intermittent fevers, chronic fevers, anaemia and polyuria. The fruits of T. chebula are used in combination with Emblica officinalis and T. bellirica (under the name Triphalaa) in the treatment of liver and kidney dysfunctions. The main purgative ingredient of Triphalaa is T. chebula (the purgative principle is in the pericarp of the fruit). Shikimic, gallic, triacontanoic and palmitic acids, beta-sitosterol, daucosterol, triethyl ester of chebulic acid and ethyl ester of gallic acid; a new ellagitannin, terchebulin, along with punicalagin and teaflavin A have been isolated from the fruits. A new triterpene, chebupentol, and arjungenin, terminoic acid and arjunolic acid were also isolated from the fruit. Antioxidant constituents of the plant, phloroglucinol and pyrogallol have been isolated along with ferulic, vanillic, p-coumaric and caffeic acids. Ether extract showed higher antioxidant activity than BHA and BHT, Acid esters present in phenolic fraction of extract, were found most effective. Dosage Pericarp of mature fruit— 3-6 g powder.

3.3.3 LATERAL RESEARCH

Table 3: Lateral research on *Terminalia chebula.*

Study	dosage	extraction	pharmacological activity	reference
in vitro	54-69% and 33-37%	methanolic extract	antiinflammatory property	Yang MH, Ali Z, Khan IA, Khan SI. Anti-inflammatory activity of constituents isolated from Terminalia chebula. Nat Prod Commun. 2014;9(7):965-968.
in vivo	50 to 500 mg/kg, (antioxidant-10 to 100 µg/ml)	hydroalcoholic extract	antiinflammatory property ,antioxidant, anti-lipid peroxidative and membrane-stabilizing effects	Bag A, Kumar Bhattacharyya S, Kumar Pal N, Ranjan Chattopadhyay R. Anti-inflammatory, anti-lipid peroxidative, antioxidant and membrane stabilizing activities of hydroalcoholic extract of Terminalia chebula fruits. Pharm Biol. 2013;51(12):1515-1520.
in vivo		aquoeus extract	antioxidant, radioprotector	Naik GH, Priyadarsini KI, Naik DB, Gangabhagirathi R, Mohan H. Studies on the aqueous extract of Terminalia chebula as a potent antioxidant and a probable radioprotector. Phytomedicine. 2004;11(6):530-538.

3.4. பற்படாகம் *(Mollugo cerviana / Hedyotis corymbosa(Linn)Lam.*)

3.4.1. GUNAPADAM ASPECT

Fig 4: ***Mollugo cerviana***

Organoleptic characters

Taste - Bitter

Potency -Heat

Post Absorptive change- Pungent

Part used: Whole plant

Therapeutic Action

Laxative

Stomachic

Antiseptic

Febrifuge

Diaphoretic

General Properties of whole plant:

“சீதவா தச்சுரமுந் தீராத தாகமும்போம்

போதவிரு கண்குளிரும் பொய்யலவே – பூதலத்துள்

வுற்பார் பயித்தியமு மாபித்த முந்தொலையும்

புற்பாட கத்தையுன்னிப் பார்”

It is useful in the treatment of fever, thirst, pitha disease, very noigal, it is coolant to eyes.

CLASSICAL PREPARATION

1. Seeraga lekiyam

Dose : pakkalavu

Indication : Puli eppam, delirium, thirst, abdominal discomfort, pain, vaineerural, arosagam

(kannusamy parambrai vaithyam, pg-175)

2.Sarvasura kiyalam

Dose : 2 ounce (Twice a day)

Indication : Vatha pitha silethuma thontha suram

(kannusamy parambrai vaithyam, pg-57)

3.Sakala surankalai alikum kasayam

Dose : 30- 60 ml (Twice a day)

Indication : all types of fever

(Anuboga vaithya brahma ragasiyam, pg-192)

4.asthisura nkiyalam

Dose : 30 ml (once for every 4 hours for 3 days)

Indications : (Asthi suram,evening fever)

(kannusamy parambrai vaithyam, pg-59)

5.Panchapathra kashayam

Dose: 30-60 ml (twice a day)

Indication : vathapitha suram

(Anuboga vaithya brahma ragasiyam, pg-193)

3.4.2 BOTANICAL ASPECT

Taxonomical classification

Kingdom: Plantae

Subkingdom: Tracheophytes

Class: Angiosperms

Subclass: Eudicots

Order: Caryophyllales

Family: Molluginaceae

Genus: Mollugo

Species: cerviana

Habitat : Upper Gangetic Plains, Punjab, Delhi, Rajasthan, Gujarat,Maharashtra, Madhya Pradesh,Orissa, Tamil Nadu, Karnataka.

Folk : Jeem Shaak.

Description:

Mollugo cerviana is a species of flowering plant known by the common name **threadstem carpetweed**. It can be found on most continents growing as a weed in many types of dry, sandy habitat types. It is an annual herb producing a thin, erect stem up to about 20 centimeters tall. The narrow, waxy leaves are up to 1.5 centimeters long, linear in shape, and arranged in whorls around the stem. The inflorescence is a loose umbel of tiny flowers each made up of whitish, petal-like sepals less than 2 millimeters long, and no true petals.

Action : Plant—stomachic, aperient, febrifuge, antiseptic, blood purifier(used for venereal diseases),emmenagogue. Root—used in rheumatism and gout. Flowers and shoots—diaphoretic, given in fevers. An infusion of the plant is given to promote lochial discharge. The plant contains orientin (leteolin- 8-C-glucoside), vitexin (apigenin- 8-C-glucoside) and their 2”O-glucosides. The plant is cardiostimulant, also antibacterial.

3.4.3 LATERAL RESEARCH

Table 4 : Lateral Research on *Mollugo cerviana*

Study	dosage	extraction	pharmacological activity	reference
in vivo	100 mg/100 g body wt	Alcohol extract	Antiinflammatory	Sadique J, Chandra T, Thenmozhi V, Elango V. The anti-inflammatory activity of Enicostemma littorale and Mollugo cerviana. Biochem Med Metab Biol. 1987;37(2):167-176.
systematic review			antimicrobial, anti-inflammatory, antioxidant activity. Heptoprotective. Phytoprotective	A.A. Aglin. Medicinal Effects of Mollugo cerviana - A Review . International Journal of Scientific Research in Multidisciplinary Studies. , Issue.9, pp.34-37, September (2018)
in vitro	84.12 ± 1.06%		Antioxidant	Valarmathi R, Senthamarai R, Akilandeswari S, Sivagamy M and Saratha R. Phytochemical investigation and in-vitro antioxidant screening of the entire plant of Mollugo cerviana Linn. World journal of pharmacy and pharmaceutical sciences. 2015; 4(5): 1183-1188.
in vitro		methanol extract	antipyretic, analgesic, spasmolytic	Padmapriya, s. Maneemegalai. Qualitative and quantitative analysis of the phytochemical

				constituents of mollugo cerviana (l.). International journal of pharmaceutics and drug analysis. Vol: 2; Issue: 9

3.5 சீந்தில் (*Tinospora cordifolia)*

3.5.1 GUNAPADAM ASPECT

Fig 5 : ***Tinospora cordifolia***

Organoleptic characters

Taste -Bitter

Potency -Heat

Post absorptive change -Pungent

Part used -stem

Therapeutic actions:

Alterative

Antiperiodic

Aphrodisiac

Demulcent

Stimulant

Stomachic

Tonic

Mild diuretic

General Properties:

“ஈங்குப் பெருந்தாகம் என்புருக்கி ரத்தபித்தம்

ஓங்கும் மதுமேக முட்டிணமம் போம் - ஓங்கிவளர்

கூற்தல் முடிமாதே கூறஞ் சஞ்சீவி யெனுஞ்

சீந்தில் சருக்கரையின் சீர்”

The salt taken from the stem is called seenthil sarkarai and it is used in the treatment of thirst, diabetic, enbu uruki, blood vomiting, kanachchoodu.

CLASSICAL PREPARATION

1. Kukkil nei

Dose : 1 varagan

Adjuvant : honey, jiggery, butter

Indication : 8 types of Gunmam, pavuthram, pilavai, kai kal mudakku, wound in tongue

(Anuboga vaithya navaneetham, part VI, Pg-61)

2***. Santhanathi chooranam***

Dose: 1½ - 2 varagan

Adjuvant : ghee, butter, honey, milk, tender coconut

Indication : cough, breathing difficult, fever, body heat

(Anuboga vaithya navaneetham, part VI, Pg-83)

3.Ilagu sutharsana chooranam

Dose: ¼ thoola (twice a day for 20 days)

Adjuvant : water

Indication : fever

(Chikitcha rathna theebam enum vaithya nool, pg-120)

4.Mahara sutharsana chooranam

Dose : ¼ thoola

Adjuvant : warm water

Indications : all types of fever, chest pain, vatha suram, pitha suram, kapha suram, breathing difficulty

(Chikitcha rathna theebam enum vaithya nool, pg-121)

5.Rathakaasam nei

Dose: 1-2 varagan

Indication : Ratha kaasam, eelai, nenju pun.

(Anuboga vaithya navaneetham, part X, Pg-106)

3.5.2. BOTANICAL ASPECT

Taxonomical classification

Kingdom: Plantae

Subkingdom: Tracheophytes

Class: Angiosperms

Subclass: Eudicots

Order: Ranunculales

Family: Menispermaceae

Genus: Tinospora

Species: cordifolia

Habitat : Tropical India and the Andamans.

Folk : Giloya.

Description:

It is a large, deciduous, extensively-spreading, climbing shrub with several elongated twining branches. Leaves are simple, alternate, and exstipulate with long petioles up to 15 cm (6 in) long which are roundish and pulvinate, both at the base and apex with the basal one longer and twisted partially and half way around. It gets its name **heart-leaved moonseed** by its heart-shaped leaves and its reddish fruit. Lamina are broadly ovate or ovate cordate, 10–20 cm (4–8 in) long or 8–15 cm (3–6 in) broad, seven nerved and deeply cordate at base, membranous, pubescent above, whitish tomentose with a prominent reticulum beneath. Flowers are unisexual, small on separate plants and appearing when the plant is leafless, greenish-yellow on axillary and terminal racemes. Male flowers are clustered, but female flowers are usually solitary. It has six sepals in two series of three each. The outer ones are smaller than the inner. It has six petals which are smaller than sepals, obovate, and membranous. Fruits aggregate in clusters of one to three. They are ovoid smooth drupelets on thick stalks with sub terminal style scars, scarlet or orange colored.

Action : Herb−antipyretic, antiperiodic, anti-inflammatory, antirheumatic, spasmolytic, hypoglycaemic, hepatoprotective. Water extract increases urine output. Stem juice−prescribed in high fever; decoction in rheumatic and bilious fevers. Aqueous extract of the plant−fabrifuge. Starch−antacid,antidiarrhoeal and antidysenteric. *The Ayurvedic Pharmacopoeia of India,* alongwith other therapeutic applications, recommends the dried stems in jaundice, anaemia, polyuria and skin diseases. The stem contains alkaloidal constituents, including berberine; bitter principles, including columbin, chasmanthin, palmarin and tinosporon, tinosporic acid and tinosporol. The drug is reported to possess onefifth of the analgesic effect of sodium salicylate. Its aqueous extract has a high phagocytic index. Alcoholic extract of the stem shows activity against *E. coli.* Active principles were found to inhibit *in vitro* the growth of *Mycobacterium tuberculosis.* Oral administration of alcoholic extract of the root resulted in a significant reduction in blood and urine glucose and in lipids in serum and tissues of alloxan diabetic rats.A significant reduction in levels of SGOT, SGPT, ALP and bilirubin were observed following *T. cordifolia* treatment during CCl4 intoxication in mature rats. The plant extract showed *in vitro* inactivating activity in Hepatitis-B surface antigen A new hypoglycaemic agent was isolated from the plant; it was found

to be 1,2-substituted pyrrolidine. The starch from roots and stem, used in chronic diarrhoea and dysentery, contains a polysaccharide having1,4 glucan with occasional branching points.

Dosage _ Stem−3-6 g powder; 20-30 g for decoction. (*API,* Vol. I.)

3.5.3 LATERAL RESEARCH

Table 5: Lateral Research on *Tinospora cordifolia*

Study	**dosage**	**extraction**	**pharmacological activity**	**reference**
in vitro		alcoholic and water extracts	antioxidant and anti-inflammatory properties	Reddi KK, Tetali SD. Dry leaf extracts of Tinospora cordifolia (Willd.) Miers attenuate oxidative stress and inflammatory condition in human monocytic (THP-1) cells. Phytomedicine. 2019;61:152831.
in vivo	300 mg/kg orally	commercially available extract	analgesic activity	Goel B, Pathak N, Nim DK, Singh SK, Dixit RK, Chaurasia R. Clinical evaluation of analgesic activity of guduchi (tinospora cordifolia) using animal model. J Clin Diagn Res. 2014;8(8):HC01-HC4.
in vitro	The LD50 of CETC lies above 2000	chloroform extract	antiinflammatory	Philip S, Tom G, Vasumathi AV. Evaluation of the anti-inflammatory activity of Tinospora cordifolia (Willd.) Miers chloroform extract - a preclinical study.

	mg/Kg body weight. HPLC techniqu e			J Pharm Pharmacol. 2018;70(8):1113-1125
in vivo		Extract	analgesic, anti-inflammatory and anti-pyretic	Hussain L, Akash MS, Ain NU, Rehman K, Ibrahim M. The Analgesic, Anti-Inflammatory and Anti-Pyretic Activities of Tinospora cordifolia. Adv Clin Exp Med. 2015;24(6):957-964.

3.6 கோட்டம் (Sausurea lappa)

3.6.1 GUNAPADAM ASPECT

Fig 6 : ***Sausurea lappa***

Organoleptic characters

Taste - Bitter

Potency -Heat

Post absorptive change - Pungent

Part used - root

Therapeutic action:

Stomachic

Expectorant

Tonic

Stimulant

Diaphoretic

General Property of root:

“நாட்டிலுறு வெட்டை நடுக்கம் எனுநோய்கள்

கோட்டமெனச் சொன்னால் குலையுங்காண் - கூட்டிற்

சுரதோடந் தொண்டைநோய் தோலாத பித்தம்

பரதேசம் போமே பறந்து”

It is used in the treatment of disease caused in eyes, ears,neck, mouth, head and abdomen, along with fever,wounds, rat and snake bites and delirium.

CLASSICAL PREPARATION

1. Surahara mathirai

Dose- 1 tablet

Adjuvant- ingi surasam, honey

Indication- Vatha suram, Pitha suram, Silethma suram, Thontha suram

(Anuboga vaithya brahma ragasiyam, pg-95)

2.Suganantha sanjeevi mathirai

Dose- 1 tablet

Adjuvant- honey, ginger, breast milk.

Indication – Sarva suram

(Anuboga vaithya brahma ragasiyam, pg-92)

3.Emathanda kuligai

Dose- 1 to 2 tablet

Adjuvant- honey, jaggery, certain decoction

Indication- All types of fever, Sanni, koolai kattu, moorchai

(Anuboga vaithya navaneetham , part 9, pg-53)

4.Neithal nei

Dose- 1/2-2 varagan

Adjuvant- jaggery, poriarisi maa

Indication- Vatha pitha siletma suram

(Anuboga vaithya navaneetham, part 9, pg-81)

5.Venthaya ilagam

Dose- 1- ½ varagan

Indication- diarrhoea, abdominal pain

(Anuboga vaithya navaneetham, part 6, pg-36)

3.6.2 BOTANICAL ASPECT

Taxonomical classification

Kingdom: Plantae

Subkingdom: Tracheophytes

Class: Angiosperms

Subclass: Eudicots

Clade: Asterids

Order: Asterales

Family: Asteraceae

Genus: Saussurea

Species: lappa

Synonym : *S. costus* (Falc.) Lipsch.

Habitat :Kashmir, Himachal Pradesh and Garhwal at 2500-3000 m; cultivated in Kashmir and neighbouring regions.

English : Kuth, Costus.

Folk : Sugandha-Kuutth.

Description:

Saussurea lappa is a perennial with a typical growth of 1–2 m (3.3–6.6 ft) tall by 1 m (3.3 ft) wide. It has long lyrate leaves and heads of purple florets. The leaves take the shape of being auricled at base, with jagged, toothed patterns running down the sides of the leaves and are an average of 0.50–1.25 m (1.6–4.1 ft) long. The roots of the plant are stout and can travel up to 40 cm (16 in) in length

Action : Root−antispasmodic, expectorant, carminative, astringent, antiseptic. An ingredient of prescriptions for dyspepsia, asthma, cough, chronic rheumatism, skin diseases. Applied locally to wounds and ulcerations. Powdered root, mixed with mustard oil, is applied to scalp in prurigo. *The Ayurvedic Pharmacopoeia of India* recommends the root in cough, bronchitis, dyspnoea; erysipelas and gout. Theroot (containing both the essential oil and alkaloid, saussurine) is used for asthma, particularly of vagotonic type. It produces a definite relaxtion of the bronchioles. The relief obtained is comparable to that of conventional bronchodilators without side effects, like a rise in blood pressure, sweating or headache even on repeated administration. Saussurine depresses parasympathetic nervous system. Theaminoacidsesquiterpene adducts, saussureamines A, B and C show antiulcer effect. The aqueous extract of the root exhibits antianginal activity. Essentialoil inhibitsperistalicmovement of the gut. It is absorbed from the gastro-intestinal tract and partly excreted by lungs producing an expectorant action and partly by the kidneys producing diuretic effect. (In Western herbal, Kuth essential oil is not prescribed internally.) Kuth roots contain resinoids (6%), and essential oil (1.5%), alkaloid

(0.05%) inulin (18%), saussurea lactone (20-25%), a fixed oil and minor constituents like tannin and sugars. Roots obtained fromKashmir are, in general, richer in essential oil content than roots obtained from Garhwal and Nepal. The roots of Punjab variety gave costunolide, dehydrocostuslactone, costic acid, palmitic and linoleic acids, betasitosterol and alpha-cyclocostunolide. The Kashmir variety, in addition, gave alantolactone, beta-cyclocostunolide and *iso*-alantolactone. The essential oil of the roots exhibit strong antiseptic and disinfectant activity against *Streptococcus* and *Staphylococcus. Costus speciosus* Sm. Synonym *Banksea speciosa,* also known as Kushtha, is a different herb of *Zingiberaceae* family. Rhizomes and stems yield diosgenin.

Dosage : Root—0.2-1.0 g powder.

3.6.3. LATERAL RESEARCH

Table 5 : Lateral Research on *Saussurea lappa*

Study	**dosage**	**extraction**	**pharmacological activity**	**Reference**
Clinical	450 (Polyglceryl-3-Methyl Glucose Distearate) emulsifier and final emulsion was loaded with 4 % extract of	botanical extract	antioxidant and anti-ageing	Adnan Q, Akhtar N, Khan BA. Phytoformulation of Sassurea lappa plant extract: A Single blind, noninvasive and split face study of cream on various skin parameters. Pak J Pharm Sci. 2017;30(5(Supplementary)):1981-1986.

	SL in aqueous phase.			
in vivo	200 mg/kg	ethanolic extract	analgesic, anti inflammatory	Tejaswi JKD, Rajan R, Sara P. Biological evaluation of saussurea lappa root extract for analgesic and anti-inflammatory activity. Asian Journal of Pharmaceutical Research and Development [Internet]. 23Aug.2018 6(4):35-8
in vitro		aqueous	antioxidant and anti-scavenging effect. Anti bacterial, anti fungal	Hanan Saeed Alnahdi, Enas Nabil Danial, Manal Elsayed Abd Elgaffar Elhalwagy and Najla Othman Ayaz, 2017. Phytochemical Studies, Antioxidant Properties and Antimicrobial Activities of Herbal Medicinal Plants Costus and Cidir Used in Saudi Arabia. International Journal of Pharmacology, 13: 481-487.

3.7திப்பிலி (*Piper longum)*

3.7.1.GUNAPADAM ASPECT

Fig 7 : *Piper longum*

Organoleptic characters

Taste -Pungent

Potency - Heat

Post absorptive change- Sweet

Part used : Dried fruit

Therapeutic actions:

Stimulant

Carminative

General Property of dried fruit:

“ஈளை யிரும லிரைப்புப் புசப்பிணிகள்

முாள வொழியாமல் வாட்டுமே – யாளுமுறை

பாங்கா யறிந்து செய்வீர் பண்டிதத்தை பண்டிதரே

வேங்கைவாய்ப் பான்கணை மெய்”

It is used in the treatment of cough, breathing difficulty, abdominal discomfort.

CLASSICAL PREPARATION

1.Agathiar kooda suli

Dose: 1-2 tablet

Adjuvant : Jaggery, banana, butter

Indication : All types of fever, cough, gunmam, paandu, sayam, neerkattu, neeradaipu

(Anuboga vaithya navaneetham, part 9, pg-19)

2.Koorosanai mathirai

Dose : 1 tablet

Adjuvant : jaggery, honey, cumin seeds decoction.

Indication : All types of fever

(Anuboga vaithya navaneetham, part 9, pg-58)

3.Sura sangara mathirai

Dose : 1 tablet

Adjuvant : jaggery, inji surasam

Indication: Kapha suram

(Anuboga vaitha navaneetham, part 9, pg-59)

4.Kalingathi ilagam

Dose : Kottai pakku alavu

Indications : Vau, soolai vayu, thiratchi kendai

(Uyir kakkum siddha maruthuvam , pg-449)

5.Kasthuri mathirai

Dose: 1 tablet

Adjuvant : Honey, breast milk, Thulasi saaru

Indication : All types of fever for children

(Chikitcha rathna theebam enum vaithya nool, pg.132)

3.7.2 BOTANICAL ASPECT

Taxonomical classification

Kingdom: Plantae

Subkingdom: Tracheophytes

Class: Angiosperms

Subclass: Magnoliids

Order: Piperales

Family: Piperaceae

Genus: Piper

Species: longum

Habitat : Warmer parts of India, from Central Himalayas to Assam, lower hills of West Bengal; Uttar Pradesh, Andhra Pradesh, Western Ghats from Konkan southwards to Trivandrum. Often cultivated.

English : Indian Long Pepper, Joborandi.

Description:

Dioecious, low creeping under shrub. Leaves membranous, pale green, hairs microscopic; in sterile branches broadly ovate-cordate, 6-11x5-10 cm; petiole up to 8 cm long; in erect fertile branch ovate to lanceolate, 8-10x4-6 cm, acuminate, base round or slightly oblique ; petiole short, 0.5-1.0 cm . Stem terete, green, weak, rooting at the node in the sterile branch. Spikes erect, flowers densely arranged in thickly cylindrical

pubescent rachis. Male spikes 5-8 cm long, peduncle 2-3 cm long. Female spikes 1.5-3 cm long.

Action: Fruits−used for diseases of the respiratory tract (cough, bronchitis, asthma); as sedative (in insomnia and epilepsy); as cholagogue (in obstruction of bile duct and bladder), as emmenagogue, as digestive, appetizer and carminative (in indigestion); as general tonic and haematinic (in anaemia, chronic fevers and for improving intellect). Applied locally on muscular pains and inflammations. Several aristolactams and dioxoaporphines have been isolated from Indian long pepper. It also contains the long chain isobutyl amide, longamide, besides guineensine and the lignans, pluviatilol, methyl pluviatilol (fargesin), sesamin and asarinine. Piperine is themajor alkaloid of peppers. Piperine is antipyretic, hypotensive, analeptic, CNS stimulant. It has been reported to exert significant protection against CCl・-induced hepatotoxicity in mice. It improves drug availability in experimental animals, and iS. used for enhancing the efficacy of coadministered medicaments. Piperine enhanced bioavailability of hexobarbital, phenytoin, propranolol and theophylline. (Sharon M. Herr.) (Piperine is also a component of Piper nigrum.) N-isobutyl-deca-trans-・-trans-・- dienamide, isolated from the fruit, exhibited antitubercular property. Milk extract of the fruit effectively reduced passive cutaneous anaphylaxis in rats. It protected guinea-pigs against antigen-induced bronchospasm. In China, Piper longum oil constituents were reported to inhibit the increase in serum total cholesterol induced by triton in mice. The root powder exhibited antifertility activity.

A related species, *P. peepuloides* Roxb., is known as Saamvali Peepal. It is used specifically against obstinate skin diseases and as a sialagogue.

Dosage : Fruit−1-3 ; root−1-3 g powder.

3.7.3 LATERAL RESEARCH

Table 7 : Lateral Research on *Piper longum*

study	dosage	extraction	pharmacological activity	reference
in vitro		reflux, ultrasonic and supercritical fluid extraction	anti-inflammatory and antitumour	Guo Z, Xu J, Xia J, Wu Z, Lei J, Yu J. Anti-inflammatory and antitumour activity of various extracts and compounds from the fruits of Piper longum L. J Pharm Pharmacol. 2019;71(7):1162-1171.
in vivo	external	oil	anti inflammatory	Kumar A, Panghal S, Mallapur SS, Kumar M, Ram V, Singh BK. Antiinflammatory Activity of Piper longum Fruit Oil. Indian J Pharm Sci. 2009;71(4):454-456.
in vivo	(1-256 mg/kg/day)	methanolic	stress response suppressing, analgesic, and anti-inflammatory	Yadav V, Chatterjee SS, Majeed M, Kumar V. Preventive potentials of piperlongumine and a Piper longum extract against stress responses and pain. J Tradit Complement Med. 2015;6(4):413-423. Published 2015 Dec 11.
in vitro		active compound	anti-neuroinflammatory	Kim N, Do J, Bae JS, et al. Piperlongumine inhibits neuroinflammation via regulating NF-κB signaling pathways in lipopolysaccharide-stimulated BV2 microglia cells. J

				Pharmacol Sci. 2018;137(2):195-201
in vitro	67µg/ml /24h by the MTT assay	aqueous extract	antioxidant, antimicrobial and cytotoxic activities	Reddy NJ, Nagoor Vali D, Rani M, Rani SS. Evaluation of antioxidant, antibacterial and cytotoxic effects of green synthesized silver nanoparticles by Piper longum fruit. Mater Sci Eng C Mater Biol Appl. 2014;34:115-122.

3.8. கச்சோலம் (*Kaempferia galanga)*

3.8.1.GUNAPADAM ASPECT

Fig 8 : ***Kaempferia galanga***

Organoleptic characters

Taste -Pungent

Potency -Heat

Post absorptive change- pungent

Part used : rhizome

Therapeutic action :

Expectorant

Carminative

It is used in the treatment of cough, perunoikal.

CLASSICAL PREPARATION

1.Mudithaila chooranam

Dose: 1 varagan

Adjuvant: gingilly oil for head bath

Indication : Head disease, back pain, neck pain

(Chikitcha rathna theebam enum vaithya nool, pg-119)

2.Sikamani chooranam

Dose: thirikadi for 20 days

Indication : vatha disease

(Kannusamay parambarai vaithyam, pg-119)

3.Kumari thailam

Dose : Head bath for once a week

Indication : Eye disease, body heat, eye pain

(Kannusamay parambarai vaithyam, pg-283)

4.Siroroga sinthamani thailam

Dose: Head bath once a week

Indication : Headache, running nose, kapha disease

(Kannusamay parambarai vaithyam, pg-288)

5.Sanniku satharana *thailam*

Dose : Head bath for once a week

Indication : Sanni

(Anuboga vaithya brahma ragasiyam, pg-86)

3.8.2 BOTANICAL ASPECT

Taxonomical classification

Kingdom: Plantae

Subkingdom: Tracheophytes

Class: Angiosperms

Subclass: Monocots

Order: Zingiberales

Family: Zingiberaceae

Genus: Kaempferia

Species: galangal

Habitat : Throughout the plains of India, cultivated in gardens.

English : Galanga, Maraba.

Description:

Small, low-growing herb. The plant typically consists of 2-3 (occasionally up to 5) broadly elliptical to suborbicular leaves which occur in a rosette. The leaves are held horizontally, close to the ground and are hairless on top, but hairy below the rhizome, or underground horizontal stem, is white or yellowish and smells like camphor. The inflorescence is composed of 4-12, white tubular flowers. It occurs naturally in forest margins, open forest and bamboo forests at up to 1000 m above sea level.

Action : Tuber–stimulant, carminative, expectorant, diuretic used for respiratory ailments like cough, bronchitis and asthma. Theessential oil fromrhizomes contain *n*-

pentadecane, ethyl-*p*-methoxy cinnamate, ethyl cinnamate, carene, camphene, borneol, *p*-methoxystyrene, *p*-methoxy cinnamate, *p*-methoxy- *trans*-cinnamic acid and cinnamaldehyde. Insecticidal activity of the oil is attributed to ethyl cinnamates. Ethyl-*p*-methoxy-cinnamate shows monoamine oxidase inhibitor activity and a cytotoxic principle (the rhizomes exhibit cytotoxic activity). Leaves and flowers exhibit antiphlogistic and vitamin P activity. Ethyl-*p*methoxy- *trans* cinnamate is the main compound in the root.

3.8.3 LATERAL RESEARCH

Table 8 : Lateral Research on *Kaempferia galanga*

study	**dosage**	**extraction**	**pharmacological activity**	**reference**
in vivo	4, 90, and 180 mg/Kg,	Ethanol	anti inflammatory	Dina fabrina. Anti-Inflammatory Effects of Kaempferia galanga L. Rhizome Extract in Carrageenan-Induced Female Rats. Advances in Health Sciences Research, volume 20
in vitro	4.78 µg/mL and 0.11 µg/mL.		antioxidant and cytotoxicity	Imon Rahman, Md. Tanvir Kabir, Md. Nasiful Islam, Mushfiqa Muqaddim, Shahana Sharmin, Mohammed Sami Ullah, Md. Sahab Uddin. Investigation of Antioxidant and Cytotoxic Activities of Kaempferia galanga L. doi: 10.5958/0974-360X.2019.00365.2

IN VIVO	45 mg/Kg	Ethanol	anti inflammatory	Riasari H, Rachmaniar R and Febriani Y: Effectiveness of Anti-Inflammatory Plaster from Kencur (Kaempferia Galanga L.) Rhizome Ethanol Extract. Int J Pharm Sci Res 2016; 7(4): 1746-49
in vivo and in vitro		chloroform	anti-inflammatory, analgesic and anti-angiogenic	Umar Muhammad Ihtisham, Asmawi Mohd Zaini, Sadikun Amirin, Majid Amin Malik Shah Abdul, Al-Suede Fouad Saleih R., Hassan Loiy Elsir Ahmed et al . Ethyl-p-methoxycinnamate isolated from kaempferia galanga inhibits inflammation by suppressing interleukin-1, tumor necrosis factor-α, and angiogenesis by blocking endothelial functions. https://doi.org/10.6061/clinics/2014(02)10.
clinical	160 mg/day		analgesic and anti inflammatory	Syahruddin AN, Dahlan CK, Taslim NA. The Effects of Kaempferia galanga L. Extract on Pain, Stiffness and Functional Physic in Patient with Knee Osteoarthritis: Double Blind Randomized Clinical Trial. International Journal of Science and Healthcare Research, 2017; 2(4).

3.9 சிற்றரத்தை (*Alpinia officinarum)*

3.9.1. GUNAPADAM ASPECT


Fig 9 : *Alpinia officinarum*

Organoleptic characters

Taste - Pungent

Potency -Heat

Post absorptive change-Pungent

Part used : rhizome

Therapeutic action:

Expectorant

Febrifuge

Stomachic

General property of rhizome:

“வாதபித் தங்கரப்பான் வாதஞ் சிரோரோகஞ்

சேர்ந்தகப முத்தோடஞ் சீதமோடு – நேர்ந்தசுரம்

மற்றரத்தைக் காட்டி வருமிரும லுந்தீரும்

சிற்றரத்தை வன்மருந்நால் தேர்”

It is used in the treatment of kapha disease, vomiting, cough, throat pain, fever, vatha pitha skin disorders.

CLASSICAL PREPARATION

1.Nagarathi urundai

Dose : ½ -1 ½ tablet

Adjuvant : butter milk, curd, honey, ghee

Indication: indigestion, kirani, lung disease, abdominal discomfort

(Anuboga vaithya navaneetham, part-6, pg-2)

2.Thiratchathi mathirai

Dose : 1 – 1 ½ tablet

Indication : cough, kapha disease, all types of vayu

(Anuboga vaithya navaneetham, part-6, pg-81)

3.Elathi chooranam

Dose : 1 ½ - 2 ½ varagan

Adjuvant : Ghee, honey, butter

Indication : Diarrhoea, fever, thirst, cough, apthus ulcer

(Anuboga vaithya navaneetham, part-6, pg-39)

4.Theebakini rasaanam

Dose : ½ - 1 varagan

Indication : 8 types of gunmam, abdominal discomfort, 360 agnimantha disease

(Anuboga vaithya navaneetham, part-8, pg-7)

5.Kasakudori lekiyam

Dose : Puiyam kottai alavu

Indication : Cough, breathing difficulty, suvasa kaasam

(Kannusamy parambarai vaithyam, pg -179)

3.9.2 BOTANICAL ASPECT

Taxonomical classification

Kingdom: Plantae

Subkingdom: Tracheophytes

Class: Angiosperms

Subclass: Monocots

Order: Zingiberales

Family: Zingiberaceae

Genus: Alpinia

Species: officinarum

Habitat : Native to China; cultivated in northern India.

English: Lesser Galangal, Alpinia,Catarrh Root, Chinese Ginger

Description:

It is a herbaceous plant which can grow up to 2 metres in height. The leaves are lanceolate (long and thin), and the flowers are white with streaks of red, growing from a spike at the top. The plant's rhizomes, the part known as galangal, are thin and tough, and they are the principal reason the plant is cultivated. They have orange flesh with a brown coating, and have an aromatic odor and a sweet flavor. These are smaller than greater galangal which have a stronger peppery pine-like bite that is lacking in the sweeter rhizomes of lesser galangal

Action : Rhizome−a circulatory stimulant and carminative. **Key application** _ As a carminative. (*The British Herbal Pharmacopoeia*.) Aqueous and methanolic extracts of the rhizome, on oral administration, exhibited significant decrease in gastric secretion in rabbits and showed anticholinergic effect in pylorus-ligated rats. Flavones from rhizomes are strongly antifungal against a wide variety of pathogenic fungi, responsible for major skin diseases in eastern India. Flavones were also found to be active against a number of Gram-positive and Gram-negative bacteria. The gingerols and diaryheptanoids constituents of the rhizome are potent inhibitors of PG synthetase

(prostaglandin biosynthesizing enzyme); they can also be active against 5-lipoxygenase, an enzyme involved in leukotriene biosynthesis

3.9.3. LATERAL RESEARCH

Table 9 : Lateral Research on *Alpinia officinarum*

Study	extraction	Chemical	pharmacological activity	Reference
in vitro	Hydro alcoholic extract and methanol extract	tannins, alkaloids, flavonoids and saponins. phenol and flavonol	antibacterial antifungal antioxidant	Srividya, ammayappan rajam & Dhanabal, S.P. & Misra, V & Suja, G. (2010). Antioxidant and Antimicrobial Activity of Alpinia officinarum. Indian journal of pharmaceutical sciences. 72. 145-8. 10.4103/0250-474X.62233
in vitro	methanol, ethanol, ethyl acetate, hexane, dichloromethane, aqueous, chloroform, and petroleum ether,	Flavonoids, DAHs, and terpenes	antioxidant, anti-inflammatory, anticancer, and antimicrobial activities	Aida Maryam Basri, Hussein Taha, Norhayati Ahmad. A Review on the Pharmacological Activities and Phytochemicals of Alpinia officinarum (Galangal) Extracts Derived from Bioassay-guided Fractionation and Isolation. Pharmacognosy reviews.,2017,11,21,43-56.
in vitro	rhizome extraction	flavonoids, alkaloids,	antimicrobial, anti-oxidant,	Balamurugan, Vishnu & Velurajan, Sreenithi &

		tannins, steroid and phenols	anti-arthritics, anti-inflammatory	Palanisamy, Arun. (2019). Phytochemical analysis of Alpinia officinarum and to test its Anti -oxidant, Anti-microbial, Anti-inflammatory and Anti -arthritic activity. International Journal Of Advance Research And Innovative Ideas In Education. 5. 1125-1140.
in vitro	95%EtOH	Diarylheptanoid	antiplatelet, antioxidant,antiproliferative, anti-emetic, antihepatotoxic and anti-inflammatory	An, N., Xu, L. -Z., Zou, Z. -M. and Yang, S. -L.(2006)'Diarylheptanoids from Alpinia officinarum',Journal of AsianNatural Products Research,8:7,637 — 641

3.10. DISEASE REVIEW

3.10.1. SIDDHA ASPECT

3.10.1.1. FEVER (SURAM)

In Siddha system of medicine Fever is defined as a disease, and many types of fever had been mentioned in siddha text books with causes, clinical features and treatment.

Causes for fever is mentioned as,

பண்டுள மலத்தி னாலும் பழகிய சீததி தாலும்
உண்டியிற் பொல்லாங் காலு மொண்டொடி வருத்தத் தாலும்
கண்டுயி லாமை யாலுங் கடுகிய நடையி னாலும்
மிண்டிய சுவையி னாலும் வெதுப்புவந் தணுகு மன்றே.
கடுவெயின் மழைக ளாலுங் கதறிய குரலினாலும்
விடமது படித லாலும் வெருவிய வேகத் தாலும்
முடிமிசை பெண்ணெய் தேய்த்து முழுகியும் போகா தாலும்
தடியடி படுகை யாலும் வெதுப்பது சாருங் காணே.
ஈங்கெழு கோபத்தாலு மிளவெயிற் காய்த லாலும்
தாங்களுஞ் சுமையி ணலுஞ் சருகிலை யூற லாலும்
ஓங்கிய பசியிணது முண்டிமே லுண்டி யாலும்
தேங்கிய மலக்கட் டாலுந் தீயவெப் பணுகு மன்றே
(புரராஜசேகரம் சுர ரோக நிதானம்)

According to pararajasekaram, Fever is caused due to abdominal discomfort, worries, walking long distance, variation of taste, travelling in heat, over exposure to sun and rain, shouting too much, poisonous infestation, accidental beatings, too much of anger, lifting heavy weights, too much of hunger and constipation. It is also said that in fever if vatha increases, it cause body pain, if pitha increases it cause vomiting, if kapha increases it cause cough.Manthakini cause fever and if all the doshas get affected, it will cause sanni.

In agathiyar 2000 book , it is mentioned aamam is the main cause for fever

In noinadal noi muthal nadal and siddha maruthuvam- pothu, it is mentioned as body's nature heat increases and spread all over the body and exhibit symptoms.

In noinadal it is mentioned as "குடல்தனில் சீதமல்லாது சுரம் வராது"

In sarabendra vaithya muraigal it is mentioned as vatha reaches aamasayam, and mix with rasa thathu, it decreases agni which causes increase in temperature.

In agathiar vaithyakaviyam 1500, it is mentioned that increase of thee and vayu panchabootha causes fever.

3.10.1.2. TYPES OF FEVER:

- Many types of fever has been mentioned in Siddha books. From a review of 13 siddha books, it is found to have 182 types of fever. Each and every fever mentioned in Siddha Books before, can be compared to modern fevers.

1 வாத(வளி) சுரம்
2 வாத பித்த (வளி அழல) சுரம்
3 வாதகப(வளி ஐய) சுரம்
4 பித்த(அழல்) சுரம்
5 பித்த வாத(அழல் வளி) சுரம்
6 பித்த கப (அழல் ஐய) சுரம்
7 கப (ஐய) சுரம்
8 கப வாத (ஐய வளி) சுரம்
9 கப பித்த (ஐய அழல்) சுரம்
10 முப்பிணி சுரம்
11 மந்த சுரம்
12 நளிர் சுரம்
13 வெஞ் சுரம்
14 மேனி சுரம்
15 உள்ளச் சுரம்
16 உட் சுரம்
17 வெளிச் சுரம்
18 விட்டு வரும் சுரம்
19 விடா சுரம்
20 சாரற சுரம்
21 செங்கரை சுரம்
22 ஊன் சுரம்
23 கொழுப்பு சுரம்
24 என்பு சுரம்
25 முளை சுரம்
26 விந்து சுரம்
27 துன்ப சுரம்
28 முறைச் சுரம்
29 மயக்க சுரம்
30 பிதற்றல் சுரம்
31 புழு சுரம்
32 மஞ்சற் சுரம்
33 காலைச் சுரம்
67 ஆம சுரம்
68 இரத்த சுரம
69 அன்னபனாதி சுரம்
70 மாங்கிச சுரம்
71 அட்சர மாந்த சுரம்
72 சீதவிச சுரம்
73 நளிர் சுரம்
74 தாப சுரம்
75 அஜீரண சுரம்
76 அத்தி சுரம்
77 பிரேத சுரம்
78 சோக சுரம்
79 அபிகாத சுரம்
80 ஆகிக வாத சுரம்
81 சுரபாத சுரம்
82 சாதுர்திக சுரம்
83 வாதவத்தீ சுரம்
84 சங்கிரக அஸ்தி சுரம்
85 முழுத்த சுரம்
86 தீய சுரம்
87 கப சன்னிபாத சுரம்
88 சய சுரம்
89 முழுத்த சுரம்
90 பித்த பிரதாபசுரம்
91ஆபன்யாச சன்னிபாதசுரம்
92 சுக்கில தாது கத சுரம்
93 சாப சுரம்
94 அபிசார சுரம்
95 அவுசதகந்த சுரம்
96 கோப சுரம்
124 வாத அதிசார சுரம்
125 வாத அசீரண சுரம்
126 வாத உதிரச் சுரம்
127 மாங்கிச வைசூரிச் சுரம்
128 பித்தவிட சுரம்
129 பித்த அத்தி சுரம்
130 பித்த அதிசார சுரம்
131 பித்த மாறற் சுரம்
132 பித்த அசீரண சுரம்
133 பித்த சன்னி சுரம்
134 பித்த சீத சுரம்
135 பித்த இரத்த சுரம்
136 பித்த இரத்தப் பிரதாப சுரம்
137 பித்த விரண சுரம்
138 ஆம சிலேற்பன சுரம்
139 சிலேற்பன அதிசார சுரம்
140 சிலேற்பன சன்னி சுரம்
141 சிலேற்பன சுரம்
142 சிலேற்பன கண சுரம்
143 சிலேற்பன சீத சுரம்
144 பித்த சோக சுரம்
145 ஆகிக சிலேற்பன சுரம்
146 விச சிலேற்பன சுரம்
147 கப பஞ்சாகீக சுரம்
148 சீலேத்ம மாகீக சுரம்
149 பித்த பிரதாப சுரம்
150 சிலேற்பன மோக சுரம்
151 ஆம சிலேற்பன சுரம்
152 கப பஞ்சவாகி சுரம்
153 சிலேற்பன சோக சுரம்
154 சிலேற்பன நீர் தோச சுரம்
155 சிலேற்பன பிரமேக சுரம்
156 சிலேற்பன நாலா மாறற் சுரம்
157 மாங்கிச அதிசார சுரம்
158 சிலேற்பன தொந்த அதிசார சுரம்

34 இராச் சுரம்
35 அதி காலை சுரம்
36 நடுக்கற் சுரம்
37 பகற் சுரம்
38 மாலை சுரம்
39 தீவரற் சுரம்
40 வேனற் சுரம்
41 கொட்டாவி சுரம்
42 விக்கற் சுரம்
43 ஏப்பச் சுரம்
44 வியர்வை சுரம்
45 தூங்காச் சுரம்
46 இருமல் சுரம்
47கட்டி சுரம்
48 சீழ்கொள்ளும் சுரம்
49 பழஞ் சுரம்
50 வீகீ க் சுரம்
51 கழிச்சற் சுரம்
52 வாந்தி சுரம்
53 மோது சுரம்
54 நாவேறு சுரம்
55 ஏவற் சுரம்
56 பூத சுரம்
57 மருந்து வேகச் சுரம்
58 நஞ்சு சுரம்
59 சினச் சுரம்
60 பயச் சுரம்
61 வருத்தச் சுரம்
62 காம சுரம்
64 சேர்க்கை சுரம்
65 கணக்காய்ச்சல் சுரம்
66 உடம்பு நோய் சுரம்
97 அவிசங்க சுரம்
98 சீதீ சுரம்
99 சங்கம தோச சுரம்
100 கிருமி சுரம்
101 சுராதி சார சுரம்
102 இரத்தாதி சார சுரம்
103 அம்மை சுரம்
104 சன்னிபாத சுரம்
105 சப்த சுரம்
106 அதிசார சன்னிபாத சுரம்
107 அல்ப சுரம்
108 புனரா வர்த்தி சுரம்
109 விஸ்போடகசுரம்
110 விசச்சிலேத்மச் சுரம்
111 பித்த தாது.மேதை தாது கத சுரம்
112 மச்சை கத சுரம்
113 ஆகாந்துவ சுரம்
114 மாமக சுரம்
115 குழைப்பு சுரம்
116 ஆதிகமாறல் சுரம்
117 ஆகீக சுரம்
118 சர்மதாது கத சுரம்
119 வாத அத்தி சுரம்
120 வாதமாசீத சுரம
121 வாத கன சுரம்
122 அக்னி சுரம்
123 வாத மாந்த கண சுரம்
159 நீருதிரி சுரம்
160 சிலேற்பன பிரதாப சுரம்
161 சிலேற்பன சோக சுரம்
162 சிலேற்பன தொந்த சுரம்
163 சிலேற்பன சன்னி பாத சுரம்
164 தோலை பற்றிய சுரம்
165 தலையை பற்றிய சுரம்
166 தசையில் விட சுரம்
167 சீத வாத சுரம்
168 வாதத்திற் சன்னி பாத சுரம்
169 பித்த இரத்த பிரமேக சுரம்
170 சிலேற்பன அத்தி சுரம்
171 மாங்கிசபிரதாப சுரம்
172 விரண வாத சுரம்
173 பித்த தாக சுரம்
174 பித்த விச சிலேற்பன அத்தி சுரம்
175 பித்த சய சுரம்
176 இரத்த பித்த உதிர் சுரம்
177 கர பாத பித்த சுரம்
178 பித்த பிரமேக சுரம்
179 பித்த இரத்த அத்சார சுரம்
180 பித்த மேக சுரம்
181 சிலேற்பன சய சுரம்
182 சிலேற்பன மாங்கிச உதிர் சுரம்

3.10.1.3. CLINICAL FEATURES OF VATHA SILETPANA SURAM

“பேராதிருமும் நாவூறும் பெருகுஞ்சத்தி யுறக்கமுண்டாம்

சீராருடல் முற்குழிருஞ் சிறுந்த மலமோடு கிர் வெளிறு

நீராமூக்கில் வாயதனில் நீருண்டாம் னாட்செல்லும்

விரவெற்றீரா வெதுப்பபுங்கண் வெளிறும் தீயும் வெயிலும் வேண்டிடுமே

வேண்டும் பலகால் கொட்டாவி மெய்யில் ரோமஞ் சீலிர்க்கும்

ஈண்டுங் குடைச்சல் வாதசிலேத்ம ஜ்வரமென்றதை யறிந்து

மீண்டுமிருண்டு பட்டினிதான் விட்டுச் செய்யும்படி கேளே”

(அகத்தியர் இரண்டாயிரம்)

The sign and symptoms of vatha siletpana suram is mentioned as, continuous cough,vomiting, constipation,chills, running nose,dry eyes, body heat,yawning, wish to go in heat,pain in body,fainting and sleepy always. When these smptoms found, one should not eat any food for two days.

“ஊதையும் மங்கமும் பொங்கி

வுாந்தியுடன் மூச்சு முண்டாய

பாதடுஞ் சுரமும் வீங்கி

யிருமலோ டிளைப்பும் பற்றி

நாதமு மடைத்துக் காய்ந்து

நடுக்கலுஞ் சுரமு மாறா

வாதைசெய் வாத சேற்ப

சுரமென வகுக்க லாமே”

(சித்த மருத்துவம் பொது)

Vomiting, breathing difficulty, edema in limbs, cough, continuous fever, chills are the symptoms

“தொண்டையு முடம்பு நொந்தே

துலங்கிய முகம்வெ ளுத்து

கண்டதுப் பிசமும் விக்கல்

விண்டுவா யுலர்த்துஞ் சோபாய்

வெருண்டுடல் நலிந்தி ருக்கும்

மண்டுகால் கையும் சந்து

வாதமும் சேரென் றோதே”

(சித்த மருத்துவம் பொது)

Throat pain, indigestion, flatulence, hiccups, tiredness, dryness of mouth, limbs pain are the sign and symptoms of vatha siletpana suram

“திண்ணமாம் வாதசிலேட்டுமசு ரந்தான்

சேடமெல்லா நொந்திடுதல் சுவாசங் காணல்

ஏண்ணமாமிருமலுநா வெழுந்தி ருத்தல்

இசைந்துமயிர்க் குச்செறிதன் மிடறு நோதல்

தண்ணமாந் தாபமெ மிகவுண் டாதல்

தயங்தியே சஞசலித்தல் வாய்தான் பேத்தல்

விண்ணமா மிரளியொடு வியர்வை காணல்

மிக்கவழுத் திடலிதன்றன் விபர மாமே”

(யூகி வைத்திய சிந்தாமணி)

Body pain, breathing difficult, cough, dryness of tongue, goose bumps in hairs, bod heat, sweating are the sign and symptoms of vatha siletpana suram

According to ஆத்மரட்சாமிர்தம் எனும் வைத்திய சார சங்கிரகம்”- cough, fever,blabbering, biting teeth, eyes become dull, throat painconstipation,indigestion tiredness are the symptoms.

“திண்மமாம வாதசிலேத் துமச்சுரந்தான்

சடமெல்லாம் நொந்திடுதல் சுவாசங்காணல்

எண்ணமா மிருமலுநா வெழுத்திருத்தல்

இசைந்துமளிர்க் குச்செறித் மிடறுநோதல்

கண்ணமாந் தாபமே மிகவுண்டாதல்

தியங்கியே சஞ்சலித்தல் வாய்பிதற்றல்

விண்ணமா மிரளவொடு வியர்வைகாணில்

மிப்பவழுத் திடலிதன்றன் விவரமாமே”

(சரபேந்திர வைத்திய முறைகள்)

Body pain, joint pain, head ache, breathing difficult, cough, constipation, fainting, tiredness , neck pain, edema, sweating are the symptoms.

3.10.1.2 MODERN ASPECT

COVID 19 INFECTION

COVID-19 is a serious global infectious disease outbreak. It is part of a family of viruses called coronaviruses that infect both animals and people. This particular one originated in China at the end of 2019, in the city of Wuhan, which has 11 million residents. In the past two decades coronavirus outbreaks have caused global concern, including one in 2003 with the Severe Acute Respiratory Syndrome (SARS) and more recently in 2012 with the Middle East Respiratory Syndrome (MERS).

COVID-19 can cause symptoms very similar to the flu – fever and a dry cough (the two most common symptoms), fatigue, aches and pains, and nasal congestion. As the pandemic spread around the world, other symptoms such as a loss of sense of smell or taste have emerged – these are not yet conclusive evidence of infection with the new coronavirus, and the World Health Organization is investigating this.

EPIDEMIOLOGY

Severe cases can lead to serious respiratory disease, and even pneumonia. Those most at risk are the elderly, or people with underlying medical issues, such as heart problems or diabetes. According to the most recent global numbers (27 March 2020), 14.8% of people over 80 years old, infected with the virus, have died from it, compared with 0.4% in people aged 40-49% and none in children under 9 years. The situation across countries is rapidly changing and these numbers will continue to change as the pandemic shifts.

Despite most deaths still being in older people, it is clear that many young people with the virus can still develop serious infection that requires hospitalisation.

Based on available evidence, COVID-19 appears to have a fatality rate of 4.4%, much lower than 10% for SARS and around 30% for MERS-CoV. Yet this is not a reason to relax containment and control measures.

According to Hindustan times dated may 20, 2022 stated that The seven-day average of new infections of Covid-19 across the world in the past week has touched 562,014, according to data collated by Our World In Data.(AP). WHO reported that, as of 15

May 2022, over 518 million confirmed cases and over six million deaths have been reported globally.

SPREADING

The evidence so far indicates that the virus is spread from person to person through small respiratory droplets. When a person coughs or sneezes, these droplets can also land on nearby surfaces. There is also evidence that the COVID-19 virus can last on surface – especially plastic or metal – for up to 3 days. This is why advice to avoid catching COVID-19 has focused on handwashing with soap, the use of alcohol-based hand sanitising gels and keeping a distance from people who are symptomatic.

COVID-19 is a new coronavirus, which means that it is likely no-one has natural immunity to it. Coronaviruses such as MERS-CoV and SARS are on watchlists of infections with pandemic potential, along with Ebola and influenza. Since it began, COVID-19 has spread worldwide, leading the WHO to label it a pandemic and a "public health emergency of international concern."

COVID-19 is more contagious than either SARS or MERS-CoV, and crucially, can be spread undetected. This is because many people with COVID-19 are either asymptomatic or have very mild symptoms, so they may not be adequately isolating themselves, and spreading the infection. Most countries around the world was on lockdown during the year 2020 and 2021, to avoid spreading the virus any further, and allowing "a flattening of the curve" meaning avoiding cases from spiking and overwhelming health systems. Later the virus spread with mutation such as delta and omicron in 2021. In 2022, new variant spread in shanghai, china.

The most propable confirmatory diagnostic method used worldwide for COVID-19 is done through real-time reverse transcription-polymerase chain reaction assay (RT-PCR)

SYMPTOMS

Most common symptoms are,

- fever
- cough
- tiredness
- loss of taste or smell
- Less common symptoms are,
- sore throat
- headache
- aches and pains
- diarrhoea
- a rash on skin, or discolouration of fingers or toes
- red or irritated eyes

Serious symptoms are,

- difficulty breathing or shortness of breath
- loss of speech or mobility, or confusion
- chest pain

ADVICE GIVEN TO PUBLIC DURING COVID

Protect yourself and those around you:

- Get vaccinated as soon as it's your turn and follow local guidance on vaccination.
- Keep physical distance of at least 1 metre from others, even if they don't appear to be sick. Avoid crowds and close contact.
- Wear a properly fitted mask when physical distancing is not possible and in poorly ventilated settings.
- Clean your hands frequently with alcohol-based hand rub or soap and water.
- Cover your mouth and nose with a bent elbow or tissue when you cough or sneeze. Dispose of used tissues immediately and clean hands regularly.

- If you develop symptoms or test positive for COVID-19, self-isolate until you recover.

VACCINES

As this is a viral infection and had no treatment, vaccines are preferred by WHO and the whole world is vaccinated by any one of the following vaccine along with booster dose and certificates of vaccination has been distributed by the government for the individuals and one can travel only with those certificates.

As of 12 January 2022, the following vaccines have obtained EUL:

- The Pfizer/BioNTech Comirnaty vaccine, 31 December 2020.
- The SII/COVISHIELD and AstraZeneca/AZD1222 vaccines, 16 February 2021.
- The Janssen/Ad26.COV 2.S vaccine developed by Johnson & Johnson, 12 March 2021.
- The Moderna COVID-19 vaccine (mRNA 1273), 30 April 2021.
- The Sinopharm COVID-19 vaccine, 7 May 2021.
- The Sinovac-CoronaVac vaccine, 1 June 2021.
- The Bharat Biotech BBV152 COVAXIN vaccine, 3 November 2021.
- The Covovax (NVX-CoV2373) vaccine, 17 December 2021.
- The Nuvaxovid (NVX-CoV2373) vaccine, 20 December 2021

Thus the whole world is fighting against COVID 19 during these days.

3.11. PHARMACEUTICAL REVIEW

3.11.1. SIDDHA ASPECT OF THE FORMULATION

KUDINEER CHOORANAM

Definition:

Kudineer Chooranam are coarse powders of drugs. The term *Kudineer Chooranam* may be applied to the powder of a single drug or a mixture of two or more drugs, which are powdered separately and later they are mixed together.

Equipment Required:

1. A mortar and pestle

Process of preparation:

The drugs enumerated in the recipe are purified, cleaned and were dried.. The drugswhich are to be used in the preparation should be taken from recently collected material.

They should be checked whether they are not infested with pests, deteriorated or spoiled or developed rancidity.

In general, the aromatic drugs are slightly fried, in order to enhance theiraroma and milling properties. Any extraneous material, organic or inorganic shouldbe removed from the drugs by close inspection.

Kudineer chooranam should be grinded as coarse powder so that the extract can be get during doing decoction process.

Storage:

The prepared dry powder should be allowed to cool by spreading and mixing prior to packing. They should be stored in rightly stoppered glass, polythene or tin containers, or in polythene or cellophane bags and sealed. These bags should in turn be enclosed in card board boxes.

The powder (*Kudineer Chooranam*) is said to retain its potency for three months and then gradually deteriorate. However if properly packed, preserved they kept good for a year.

Note:

In large scale manufacture, in factories comminutors, pulverisers and ball mills are employed for powdering.

3.11.2. ANALYTICAL SPECIFICATION OF KUDINEER CHOORANAM – MODERN ASPECT

Table No: 10. Analytical specifications of Kudineer *Chooranam*

Sl. No	TESTS
1.	Description
	Macroscopic, Microscopic
2.	Loss on drying at 105 °C
3.	Total ash
4.	Acid – insoluble ash
5.	Water - soluble extractive
6.	Alcohol – soluble extractive
7.	Particle size (80 – 100 mesh for churna; 40 – 60 for Kvatha churna
8.	Identifications, TLC/HPTLC – with marker (wherever possible)
9.	Test for heavy / toxic metals
	Lead
	Cadmium
	Mercury
	Arsenic
10.	Microbial contamination
	Total bacterial count
	Total fungal count

11.	E-coli Salmonella spp. S.aureus Pseudomonas aeruginosa
12	Pesticide residue Organochlorine peticides Organophosphorus pesticides Pyrethroids
13	Test for Aflatoxins (B1, B2, G1, G2)

4.MATERIALS AND METHODS

4.1 PREPARATION OF THE TRIAL DRUG (VSSK):

Selection of Drug:

The trial drug "*Vatha siletpana sura kudineer*" has been selected for Anti-inflammatory, analgesic and antioxident activities from the Classical Siddha literature of *Pararajasekaram- suram, sanni, vali, vikkal, sathi roga nithanankal part- III , Author ponniayah.I. page no. 24-25.*

Ingredients of the Drug:

Kandankathari (Solanum xanthocarpum)	*- 1 Palam(35 g)*
Siruthekku (Clerodendrum serratum)	*-1 Palam(35 g)*
Kaddukkai (Terminlia chebula)	*-1 Palam(35 g)*
Seenthil (Tinospora cordifolia)	*-1 Palam(35 g)*
Patpadakkam (Mollugo cerviana)	*-1 Palam(35 g)*
Kottam (Saussurea lappa)	*-1 Palam (35 g)*
Thippili (Piper longum)	*- 1 Palam(35 g)*
Kachcholam (Kaemeferia galanga)	*-1 Palam(35 g)*
Sittarathaai (Alpinia officinarum)	*-1 Palam(35 g)*

Collection of the Drugs:

Herbal drugs were purchased from Herbal drug shop, Nagarcoil.

Identification and Authentication:

All raw drugs were identified and authenticated by the experts of *Gunapadam* (Pharmacology) department in Government Siddha Medical College Palayamkottai, Tirunelveli.

The specimen samples of the identified raw drugs were presented to the laboratory of PG *Gunapadam* for future references.

PURIFICATION OF RAW DRUGS:

1. ***Thippili*** **(Long pepper)**

Soaked in lemon juice and dry it.

2. Kaddukkai (myrobalan)

Seeds are removed and dried

3. All other ingredients are cleaned well and dried.

FRESH INGREDIENTS **AFTER DRYING, CLEANING AND PURIFICATION**

1. KANDANKATHARI

2. SIRUTHEKKU

3. KADDUKKAI

4. PATPADDAKAM

5. SEENTHIL

6. KOTTAM

7. KACHCHOLAM

8. THIPPILI

9. SITTARATHAI

Fig 10 : Fresh ingredients and cleaned, purified ingredients

Method of preparation:

Raw drugs are collected and cleaned properly. They are coarsely powdered using large mortor and pestle and stored in air tight container.

Fig 11 : Mortor and pestle

Fig 12 : grinding in motor and pestle

Fig 13: Prepared VSS kudineer chooranam

For 5 g of kudineer chooranam, nalzhi water(1.3 l) is added and boiled and reduced to 1 ullakku (336 ml) of its volume. The decoction is prepared and filtered.

Shelf life:

3 months.

Dosage:

30 – 60ml – Twice a day.

Indication:

Perumuchu (Breathing difficulty)

Irumal (Cough)

Suram (Fever)

Vekkam (Swelling)

Vanthi (Vomiting)

Kathadaippu (unable to hear)

Kulir nadukkam (Chills)

Mugam vaattal (Dryness in face)

nithraiyinmai (insomnia)

4.2. STANDARDIZATION OF THE DRUG

4.2.1. PHYSICAL STANDARDIZATION AS PER SIDDHA CLASSICAL LITERATURE

Organoleptic character

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

Colour:

Trial drug was taken into watch glasses and positioned against white background in white tube light. Its colour was observed by naked eye and note in results.

Odour:

Trial drug was smelled individually. The time interval among two smelling was kept two minutes to overturn the effect of previous smelling. Odour of *Mahalavangathi Chooranam* was noted in results table.

Taste:

The taste of the trial drug should be noted.

4.2.2. STANDARDIZATION OF TEST DRUG BY USING MODERNTECHNIQUES:

Standardization of drug helps to authenticate and determine its quality andefficiency. Thus, the process involves qualitative and quantitative analysis.

1. The Physico-chemical analysis of *VSS kudineer* hasbeen done in IITM Laboratory, Chennai.
2. The Biochemical analysis of *VSS kudineer* has been done in Biochemical laboratory, Government Siddha Medical College, Palayamkottai.
3. The Phytochemical analysis of *VSS kudineer* has been done in IITM Laboratory, Chennai.
4. Microbial limit test of *VSS kudineer* has been done in Vivek Institute ofLaboratory Medicine, Nagercoil.
5. Instrumental analysis.

5.1 Scanning Electron Microscope (SEM) in Kalasalingam Academy of Research and Education, International research center, Srivelliputhur, also assesses the particle size and qualitative analysis of chemical elements of *VSS kudineer*.

5.2 The chemical fingerprints are engaged by using modern analytical technique Fourier Transform Infra-Red Spectroscopy (FT-IR) in Kalasalingam Academy of Research and Education, International research center, Srivelliputhur.

5.3 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES) in IITM Laboratory, Chennai.

5.4 The chemical fingerprints are engaged by using modern analytical technique Powder X-ray (EDAX) (Energy Dispersive X-ray Analysis) diffraction methods in Kalasalingam Academy of Research and Education, International Srivelliputhur. research center,

6. Evaluation of antimicrobial activity of *VSS kudineer* has been done in Vivek Institute of Laboratory Medicine, Nagercoil.

4.2.2.1 PHYSICO CHEMICAL ANALYSIS

The Therapeutic effect of a drug is depended to the influence of various physico chemical properties of the drug. Physicochemical studies of the trial drug have been done according to the WHO guidelines.

DETERMINATION OF LOSS ON DRYING (Indian Pharmacopeia 1996):

10gm of VSS kudineer was accurately weighed in an evaporating dish and was air dried at105^{o}C for 5 hours and then weighed.

DETERMINATION OF TOTAL ASH

3 g of test drug VSS kudineer was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ^{o}C until it turns white in colour which indicates the absenceof carbon. Total ash will be calculated with reference to the weight of the air-dried drug.

Total Ash = Weight of Ash / Weight of the Crude drug taken

DETERMINATION OF ACID INSOLUBLE ASH

The ash obtained by total ash test was be boiled with 25 ml of dilute Hydrochloric acid for 6mins. Then the insoluble matter is collected in a crucible and will be washed with hot water and ignited to constant weight.

Acid insoluble ash will be calculated with reference to the weight of air-dried ash.

Acid-insoluble Ash = Weight of Ash / Weight of the Crude drug taken

DETERMINATION OF WATER SOLUBLE ASH

The ash obtained by total ash test will be boiled with 25 ml of water for 5 mins.The insoluble matter is collected in a crucible and will be washed with hot water, and ignite for 15mins at a temperature not exceeding 450^{o}C. The weight of the insoluble matter will be subtracted from the weight of the ash; the difference in weight represents the water-soluble ash.

Calculate water-soluble ash with reference to the air-dried drug.

Water Soluble Ash = Weight of Ash / Weight of the Crude drug taken

DETERMINATION OF PH

About 5 g of test sample VSS kudineer will be dissolved in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 mins and then subjected to pH evaluation.

4.2.2.2 BIO CHEMICAL ANALYSIS

PRELIMINARY BASIC AND ACIDIC RADICAL STUDIES:

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

A) 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 thepresence of Ca.

Test for Iron (Ferric):

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

Test for Iron (Ferrous):

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

Test for Zinc:

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

B) QUALITATIVE ANALYSIS FOR ACIDIC RADICALS:Test for Sulphate:

2ml of the 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, it gives brisk effervescence. This indicates the presence of carbonate.

Test for starch:

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

Test for albumin:

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

Test for tannic acid:

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

Test for unsaturation:

The extract is treated with potassium permanganate solution. The discolourization of potassium permanganate indicates the presence of unsaturatedcompounds.

Test for the reducing sugar:

5ml of Benedict‟s qualitative solution is taken in a test tube and allowed to boilfor 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. Afterdrying, 1% Ninhydrin is sprayed over the same and dried it well. Formation of violet colour indicates the presence of amino acid.

4.2.2.3 PHYTOCHEMICAL ANALYSIS

GAS CHROMATOGRAPHY- MASS SPECTROSCOPY ANALYSIS FOR THEDRUG VSS KUDINEER FOR PHYTOCHEMICAL ANALYSIS

Derivatization procedure

For the crude ethanol extracts, a small amount of concentrated sample was takenin a separating funnel and shaken by adding water and ethyl acetate in the ratio of 1:4.The upper layer was collected and concentrated in rotary evaporator to about 1.5 ml. Added 100µl N, O-Bis(trimethylsilyl)trifluoroacetamide and trimethyl chlorosilane (BSTFA+TMCS) and 20µl pyridine and heated at 60°c for 30 minutes.

For the layers which are separated from the crude extracts, a small amount of extract was taken and evaporated out totally. To this added acetonitrile and filtered into a conical flask. To the filtrate added 50µl BSTFA+TMCS and heated at 60°c in a water bath for 30 minutes. Filtered using 0.45µ membrane filter to a vial.

GC-MS Procedure

Gas chromatography (GC) analysis was carried out using Agilent 6890N gas chromatography equipped with photon multiplier tube as detector coupled to front injector type 1079. The chromatograph was fitted with HP 5 MS capillary column (30 m ×0.25 mm i.d., film thickness 0.25 _m). The injector temperature was set at 250°C, and the oven temperature was initially at 70 °C hold for 4 mins then programmed to 200°C at the rate of 10°C/min and finally held at 200 °C for 13 min. Helium was used as a carrier gas with the flow rate of 1.5 ml/min. 0.2 microlitre of the sample-PNP

(diluted with methanol 1:10) were injected in the split less mode. GC–mass spectrometry (GC–MS) analysis of sample was performed using Agilent gas chromatography equipped with JEOL GC MATE-II HR Mass Spectrometer. C conditions were the same as reported for GC analysis and the same column was used. The mass spectrometer was operated in the electron impact mode at 70 eV. Ion source and transfer line temperature was kept at 250°C. The mass spectra were obtained by centroid scan of the mass range from 50 to 600 amu. The compounds were identified based on the comparison of their retention indices (RI), retention time (RT), mass spectra of WILEY, NIST library data of the GC-MS system and literature data (Adams, 2009).

4.2.2.4 INSTRUMENTAL ANALYSIS

1. SCANNING ELECTRON MICROSCOPE (SEM)

Fig 14 : Scanning Electron microscope (SEM)

Introduction:

Scanning Electron Microscopy (SEM), also known as SEM analysis or SEM microscopy, is used very effectively in microanalysis. It is used for observation of specimen surfaces. When the specimen is irradiated with a fine electron beam (called Aan electron probe), secondary electrons are emitted from the specimen surface. Topography of the surface can be observed by two-dimensional scanning of the electron probe over the surface and acquisition of an image from the detected secondary electrons.

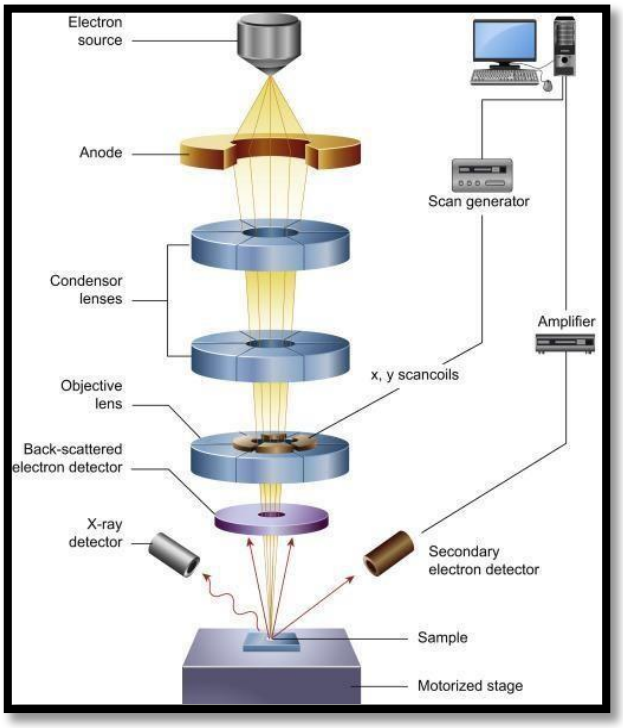


Fig 15: Mechanism of scanning Electron Microscope (SEM)

Principle

The beam is then transferred over the specimen in synchronism with the beam of a cathode ray tube display screen. The elastically 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.

Procedure

Electromagnetic lenses onto the specimen surface focus an electron beam passing through an evacuated column. 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. In addition, 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 specimen (exciting X- rays or other quanta in the process) absorbs the remainder. If the specimen insufficiently 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* herbal preparation of *VSSK*. For this analysis the coarse powder of kudineer chooranam was grinded in to powder form using mortor and pestle. SEM results of *VSSK* were represented in results section.

2. FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR)

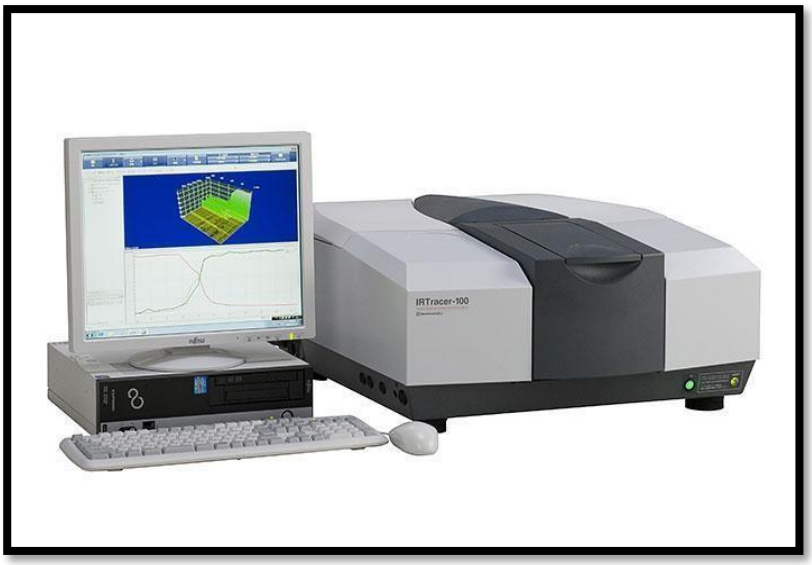


Fig 16 : FTIR instrument

The analysis was carried out using IRTracer-100. The IRTracer-100 offers high sensitivity with a 60,000:1 S/N ratio. This s ensitivity combined with the Lab Solutions IR Contaminant Analysis Macro enables easier, quicker and more accurate analysis ofsmall samples. It can be customized by the user, with a range of accessories and user- friendly software options to meet the needs of a specific application.

Introduction:

FTIR (Fourier Transform Infra - Red Spectroscopy) is a sensitive technique particularly for identifying organic chemicals in a whole range of applications althoughit can also characterize some inorganics. Examples include paints, adhesives, resins, polymers, coatings and drugs. FTIR is an effective analytical instrument for detecting functional groups. 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 branchof spectroscopy. 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.

Principle:

Spectrophotometric tests are commonly used in the identification of chemical substances and quantification of polymorphic forms. The test procedures are applicableto substances that absorb IR radiation. The IR absorption spectrum of a substance compared with that obtained concomitantly for the corresponding reference standard / reference substance provide conclusive evidence of the identity of the substance being tested.

The Main Features of the IRTracer-100 FTIR Spectrophotometer:

Resolution	:	0.25 cm^{-1}
Sensitivity	:	Highest SN ratio in its class at 60,000:1
Speed	:	20 spectra/second
Source	:	Nernst Glower
Beam splitter :		It is made up of a transparent material. Thin films of Silicon deposited on Potassium bromide (KBr) Bromide (KBr)
Detectors	:	Deuterated Triglycine Sulphate (DTGS).
Scan Range	:	MIR 400 to 4000 cm^{-1}
Software	:	LabSolutions IR Series

FTIR Mechanism

A common FTIR spectrometer consists of a source, interferometer,sample compartment, detector, amplifier, A/D convertor, and a computer.

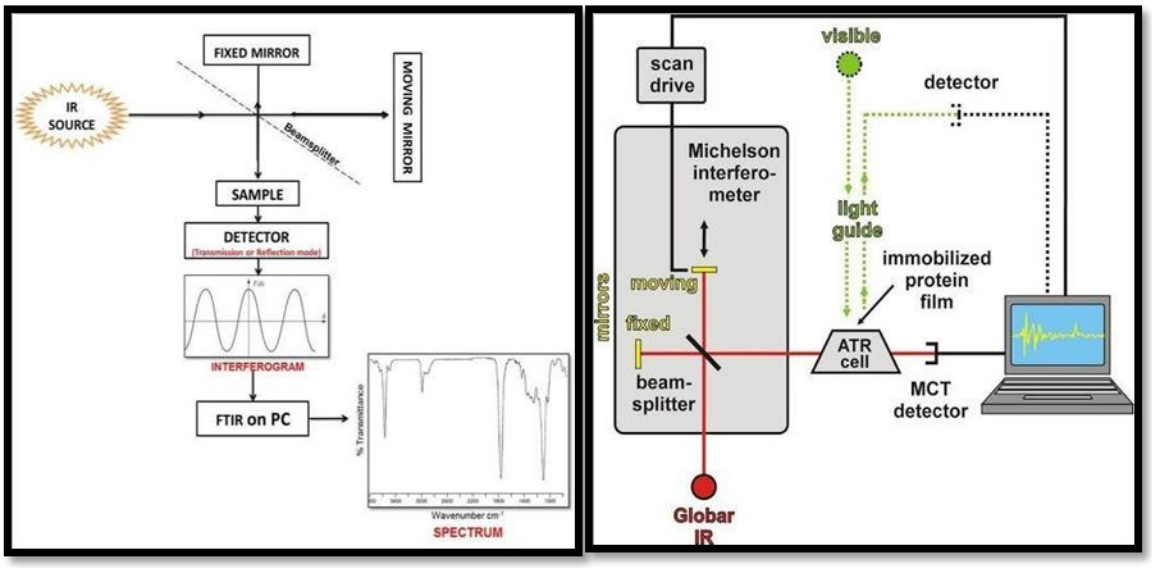


Fig 17 : Mechanism of FTIR instrument analyser

The source generates radiation which passes the sample through theinterferometer and reaches the detector. Then the signal is amplified and converted to digital signal by the amplifier analog-to-digital convertor, respectively. Eventually, thesignal is transferred to a computer in which Fourier transform is carried out.

Sampling Techniques

There are a variety of techniques for sample preparation depending on the physical form of the sample to be analysed.

1. Solid : Solution, Nujol mulls, KBr pellets.
2. Liquid : Liquids placed on KBr plates.
3. Gas : Gas cells

1. Liquids:

Place a small drop of the compound on one of the KBr plates. Place the secondplate on top and make a quarter turn to obtain a nice even film. Place the plates into the sample holder and run a spectrum. If the sample is too concentrated, separate the plates and wipe one side clean before putting them back together.

2. Solids (in solution):

Prepare a concentrated solution of your compound in a suitable solvent (e.g. CH2Cl2). Either place a small amount (2-5 mg) of compound directly on the plates andadd one drop of solvent, or dissolve it in a small test tube first and transfer this solutionwith a pipet onto the IR plates. 3. Solids (as Nujol mulls): Alternative methods to obtain

IR spectra of solids are Nujol (mineral oil) mulls between KBr plates. Good results areobtained by this method only if the average particle size of the solid is somewhat less than the wavelength of light the particles are to transmit. Samples should therefore be ground in a mortar to reduce the average particle size to 1 to 2 microns. About 5 to 10 mg of finely ground sample are then placed onto the face of a KBr plate, a small drop of mineral oil is added and the second window is placed on top. With a gentle circularand back-and-forth rubbing motion of the two windows, evenly distribute the mixture between the plates. The mixture should appear slightly translucent, with no bubbles, when properly prepared. Place the sandwiched plates in the spectrometer and obtain a spectrum. Ideally, the strongest band should have a transmission ob. 0 to 10% and should not be totally absorbing for more than 20 cm-1.

3.Powder (KBr pellets/disks):

In order to prepare a KBr pellet, follow the procedure given below:

Sample/KBr Ratio:

The concentration of the sample in KBr should be in the range of 0.2% to 1%. The pellet is much thicker than a liquid film, hence a lower concentration in the sampleis required (Beer's Law). Too high a concentration usually causes difficulties obtaining clear pellets. The IR beam is absorbed completely, or scattered from the sample which results in very noisy spectra. KBr allows transmission of IR radiation in the range 370

– 10000cm-1.

Sample Preparation:

Although a homogeneous mixture will give the best results, excessive grindingof the potassium bromide is not required. The finely powdered potassium bromide will absorb more humidity (it is hygroscopic) from the air and therefore lead to an increased background in certain ranges. Make sure to work fast. Transfer some KBr out of the oven into a mortar. Add about 1 to 2 % of your sample, mix and grind to a fine powder. For very hard samples, add the sample first, grind, add KBr and then grind again. The sample must be very finely ground as in the Nujol mulling technique to reduce scattering losses and absorption band distortions.

Take two stainless steel disks out of the desiccator. Place a piece of the precut cardboard (in the tin can next to the oven) on top of one disk and fill the cutout hole with the finely ground mixture. Put the second stainless steel disk on top and transfer the sandwich onto the pistil in the hydraulic press. With a pumping movement, move the hydraulic pump handle downward. The pistil will start to move upward until it reaches the top of the pump chamber. Then, move the pump handle upwards and pump until the pressure reaches 20,000 prf. Leave for a few seconds and with the small lever on the left side, release the pressure (hold until the sample and pistil are all the way down). Remove the disks measure about 13mm diameter and 0.3mm in thickness and pull apart. Remove the film, which should be homogenous and transparent in appearance. Insert into the IR sample holder and attach with scotch tape. Run the spectrum.

Procedure:

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

Then, 300 Al of 20 mm buffer, prepared in H2O or 2H2O, pH or p2H 7.2, wereadded and the sample concentrated again. The p2H value corresponds to the pH meter reading + 0.4. The concentration and dilution procedure were repeated several times inorder to completely replace the original buffer with the buffer.

The washings took 24 hours, which is the time of contact of the protein with the2H2O medium prior FT-IR analysis. In the last washing, the protein was concentrated to fine a volume of approximately 40 Al and used for the infrared measurements. The concentrated protein sample was placed in CaF2 windows and a 6 Am tin spacer or a 25 Am Teflon spacer for the experiments in H2O or 2H2O, respectively. FT-IR spectra were recorded by means of IRTracer - 100 FT-IR spectrometer using a deuterated triglycinesulfate detector.

At least 24 h before, and during data acquisition, the spectrometer was continuously purged with dry air at a dew point of 40ºC. Spectra of buffers and samples were acquired at 0.25 cm-1 resolution under the same scanning and temperature conditions. In the thermal denaturation experiments, the temperature was raised in 5ºCsteps from 20 to 95ºC.

Before spectrum acquisition, samples were maintained at the desiredtemperature for the time necessary for the stabilization of temperature inside the cell (6min). Spectra were collected and processed using the Spectrum software from LabSolutions IR Series. Correct subtraction of H2O was judged to yield an approximately flat baseline at 1900-1400 cm-1 , and subtraction of 2H2O was adjustedto the removal of the2H2O bending absorption close to 1220cm-1.

Measurements Techniques:

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

- Air is first scanned for the reference and stored. The sample is then recorded and finally the ratio of the sample and reference data is computed to give required %T or %A at various frequencies. (%T - percentage of transmittance of lights through sample without absorbance, %A - percentage of absorbance oflights by sample without transmittance)
- Study of substances with strong absorbance bands and weak absorbance bandsas well as possible.
- Small amount of samples are sufficient
- High resolution is obtained.

Advantages:

FT-IR was the most advanced and the major advantage was its Speed,Sensitivity. Mechanical Simplicity, Internally Calibrated.

Applications:

- Quantitative scans
- Qualitative scan solids, liquids, gases
- Organic samples, inorganic samples
- Unknown identification
- Impurities screening
- Formulation
- Pharmaceuticals

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

3. INDUCTIVELY COUPLED PLASMA OPTIC EMISSIONSPECTROMETRY (ICP-OES)

Fig 18: ICP- OES Perkin Elmer Optima 5300 dv

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.

Principle

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

To generate plasma, first, argon gas is supplied to torch coil, and high frequency electric current is applied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas

is ionized and plasma is generated. This plasma has high electron density and temperature (10000K) and this energy is used in the excitation-emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the center of the torch tub

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. The RF generator, which is effectively, creates this RF signal a high power radio transmitter driving the "work coil" 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, 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 analyses for every element to which the unit is sensitive all at once. Thus, samples can be analyzed very quickly. The intensity of

each line is then compared to previously measured intensities of known 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

1. Trace analysis of environmental soil and water samples
2. Assessment of metal ores for mass balances and process control
3. Trace metal analysis of any material that can be digested into an aqueous matrix
4. Boron and Lithia in glasses
5. Forensic analysis
6. Trace analysis of food and drink samples such as; metals in wine; and elements bound to proteins
7. Metal release testing of tableware

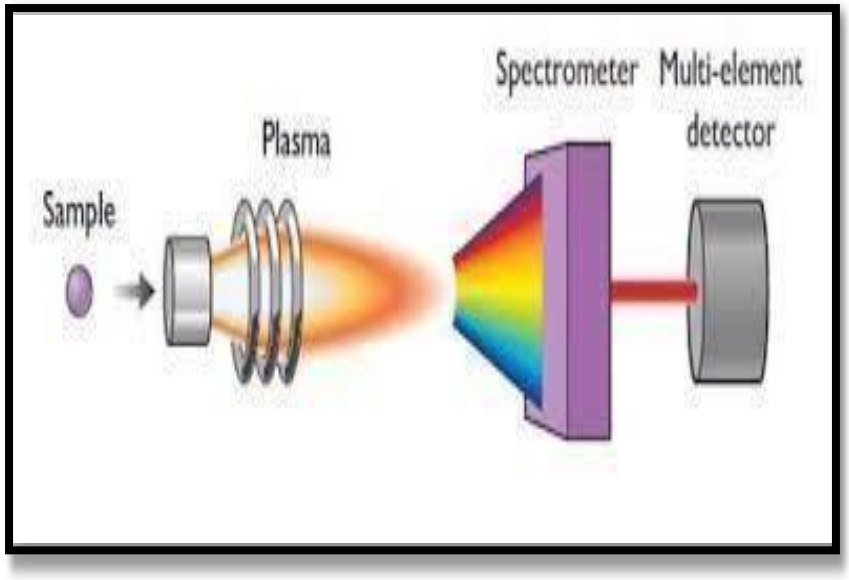


Fig 19 : Schematic view of inductively coupled plasma optical emission spectrometry

4. X-RAY POWDER DIFFRACTION (XRD)

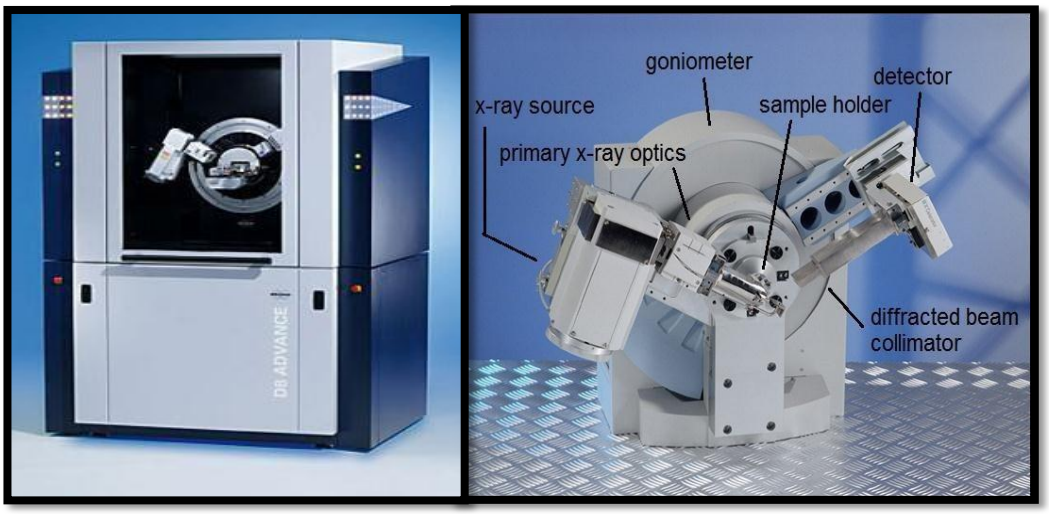


Fig 20 :XRD – Instrumentation (BRUKER ECO DS ADVANCE)

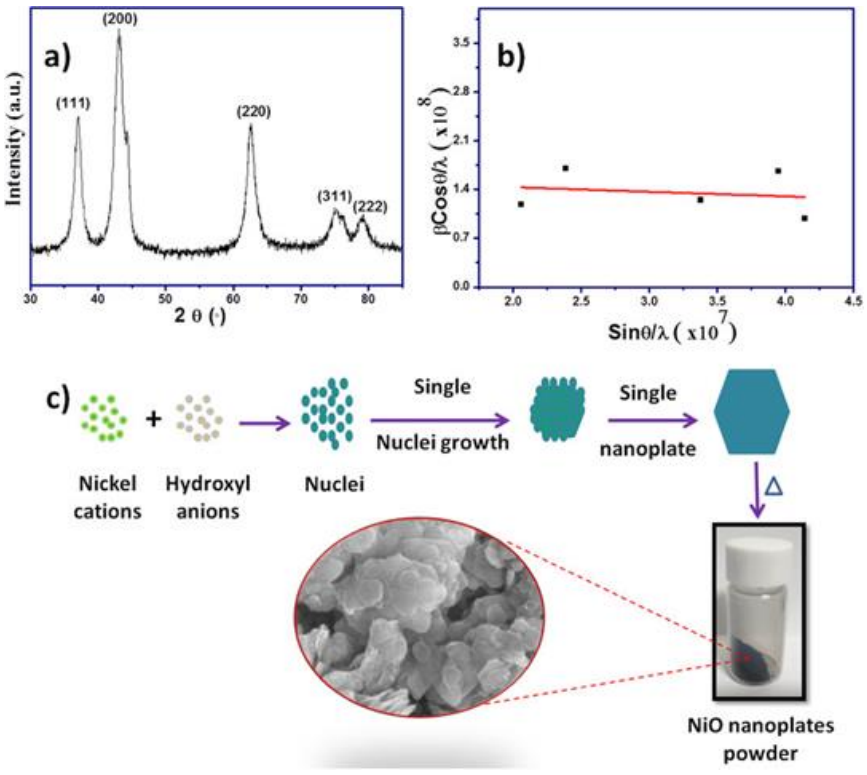


Fig 21: Schematic view of XRD - instrumentation

Introduction

X-ray diffractometers consist of three basic elements: an X-ray tube, a sample holder and an X-ray detector. 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 Kα and Kβ. Kα consists in part of Kα1 and Kα2. Kα1 has a slightly shorter wavelength and twice the intensity of Kα2. The specific wavelengths are characteristic of the target material (Cu, Fe, Mo, and Cr). Filtering, by foils or crystal monochrometers, is required to produce monochromatic X-rays needed for diffraction. Kα1 and Kα2 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 CuKα radiation = 1.5148A0. 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 -50 to 700, 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 straightforward.

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 (dspacings, hkls)
3. Requires tenths of a gram of material, which must be ground into a powder.
4. For 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’.

Benefits of Bruker D8 Advance with EIGER2 detector

1. Switch easily between 1D and 2D geometries, for traditional powder scans and microdiffraction
2. Automatic optimisation for acquiring high quality data easily

3. Flexible, modular system
4. Maintenance-free goniometer
5. Lifetime alignment guarantee

Sample Collection and Preparation:

1. Determination of an unknown requires: The material, an instrument for grinding and a sample holder.
2. Obtain a few tenths of a gram (or more) of the material, as pure as possible.
3. 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. i. Powder less than -10 μm (or 200-mesh) in size is preferred.
4. Place into a sample holder or onto the sample surface. i. Packing of the fine powder into a sample holder. Smear uniformly onto a glass slide, assuring a flat upper surface.
5. Pack into a sample container
6. Sprinkle on double sticky tape i. typically the substance is amorphous to avoid interference 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- spacing appropriate to diffract X-rays at that value of θ. Although each peak consists of two separate reflections (Kα1 and Kα2), at small values of 2θ the peak locations overlap with Kα2 appearing as a hump on the side of Kα1. 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 reported either 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/I1 x 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- spacing 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- spacing, matching these d- spacing provides an identification of the unknown sample. A systematic procedure is used by ordering the d- spacing in terms of their intensity beginning with the most intense peak. Files of d- spacing 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- spacing 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. The author used it for elemental identification and quantitative compositional information of the selected drug sample.

4.2.2.5. MICROBIAL LIMIT TEST FOR VATHA SILETPANA SURA KUDINEER

STERILITY TEST BY POUR PLATE METOD

Objective

The pour plate techniques were adopted to determine the sterility of the product. Contaminated / un sterile sample (formulation) when come in contact with the nutrition rich medium it promotes the growth of the organism and after stipulated period of incubation the growth of the organism was identified by characteristic pattern of colonies. The colonies are referred to as Colony Forming Units (CFUs).

Methodology

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45ºC were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10 minutes). Plates were then inverted and incubated at 37º C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.

Test for Specific Pathogen

Methodology

Test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37ºC for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic color with respect to pattern of colony formation in each differential media.

Table 11: Detail of Specific Medium and their abbreviation

Organism	Abbreviation	Medium
E-coli	***EC***	***EMB Agar***
Salmonella	***SA***	***Deoxycholate agar***
Staphylococcus Aureus	***ST***	***Mannitol salt agar***
Pseudomonas Aeruginosa	***PS***	***Cetrimide Agar***

ANTI BACTERIAL POTENTIAL OF VSSK

Agar Well Diffusion Test

The antibacterial screening of the **Vatha Siletpana Sura Kudineer (VSSK)** was carried out by determining the zone of inhibition using agar well diffusion method (Bauer., 1996). The drug extracts were tested against pathogenic bacteria including 1Gram positive *(Staphylococcus aureus),* 3 Gram negative organism *(E.coli, Pseudomonas aeruginosa, Proteus vulgaris)* and a fungi *Candida* sp.

Bacterial Inoculums Preparation

Inoculum of *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcu aureus and Candida* sp. were prepared individually in a respective broth and kept for incubation at suitable temperature.

Antibacterial Test:

The medium was prepared by dissolving 38 g of Muller Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 121^0C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured petriplates (25 ml/plate) the plates were swabbed with Pathogenic Bacteria culture viz. analysis analysis *E.coli, Proteus vulgaris, Candida albicans, Pseudomonas aeruginosa and Staphylococcus aureus* Finally, About 10 µL of sample (Aqueous extract of KC) was loaded onto the disc then placed on the surface of Mullar-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimetres. The size of the zone of inhibition (including disc) was measured in millimeters. The absence of zone inhibition was interpreted as the absence of activity (Kohner *et al*., 1994; Mathabe *et al*., 2006). The activities are expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm (Assam *et al*., 2010).

4.3. A PRECLINICAL TOXICITY STUDIES OF VSSK ON WISTAR ALBINO RATS

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

OBJECTIVES

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

Guidelines followed:

(a) OECD Guidelines No. 423,

Study Design and Controls:

1) Female Wister Rats in controlled age and body weight were selected.

2) *The test drug VSSK* was administered at 5 mg/kg, 50 mg/kg, 300 mg/kg, 2000 mg/kg body weight of animal as suspension along with water.

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

EXPERIMENTAL PROCEDURE

1. ANIMALS

1.1 Supply

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

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

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

1.2. **Housing**

The Female Wister Rats were housed in metabolic cages (55 x 32.7 x 19 cm), placed on racks. From the week before initiation of the treatment, each cage contained a maximum of 3 rats of the same sex and treatment group.

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

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

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

2.DIET

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

2.1. Water

The water was offered ad libitum in bottles.

3. 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 12 : Numbering and identification

Group No	Animal Marking
1	Head
2	Body
3	Tail

Table 13 : Numbering and identification of animal marking

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

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

3. ADMINISTRATION ROUTE AND PROCEDURE

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

3.1. Doses

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

Table 14 : Animal dose level

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

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

Dose Preparation

VSSK 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.

3.2.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.

3.3.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.

3.4.Mortality and Morbidity

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

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

1. Objective

The objective of this 'sub-acute toxicity study of VSSK on wister rats' was to assess the toxicological profile of the test item when treated as a single dose daily. Animals should be observed for 28 days after the drug administration. This study provides information on the possible health hazards likely to arise from exposure over a relatively limited period of time.

2.Test Guideline Followed

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

3. Test Item Detail

Name: *VSSK*

4.Test System Detail

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

5. Acclimatization

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

6.Randomization& grouping

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

7.Numbering and Identification

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

Table 15 : Numbering and identification

Group No (C0NCENTRATION/DOSE)	**Animal Marking**
CONTROL	H,B,T (MALE) H,B,T(FEMALE)
V. CONTROL	H,B,T (MALE) H,B,T(FEMALE)
LOW DOSE OF VSSK	H,B,T(MALE) H,B,T (FEMALE)
MIDDLEDOSE OF VSSK	H,B,T (MALE) H,B,T (FEMALE)
HIGH DOSE OF VSSK	H,B,T (MALE) H,B,T(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 Above

8. Husbandry

8.1 Housing

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

8.2 Environmental conditions

The animals were kept in a clean environment with 12 hour light and 12 hour dark cycles. The air was conditioned at 22 ± 3^{0}C and the relative humidity was maintained between 30-70% with 100% exhaust facility. The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

8.3 Feed & feeding schedule

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

8.4 Water

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

8.5 Doses

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

Table 16: DOSE LEVEL TO ANIMALS

TEST GROUP	**C0NCENTRATION/DOSE TO ANIMALS (ml/kg body-weight/day)**	**NUMBER OFANIMALS**
Group-1	1. CONTROL	6 (3MALE and3 FEMALE)
Group-II	V. CONTROL	6 (3MALE and3 FEMALE)
Group-III	2. LOW DOSE OF VSSK	6(3MALE and 3 FEMALE)
Group-IV	3. MIDDLE DOSE OF VSSK	6(3MALE and 3 FEMALE)
Group-V	4. HIGH DOSE OF VSSK	6(3MALE and 3 FEMALE)

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

Dose Preparation

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

8.6 Administration

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

9. OBSERVATIONS

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

9.1. Clinical signs of toxicity

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

9.2. Food intake

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

9.3. Water intake

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

9.4 Bodyweight:

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

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

LABORATORY STUDIES

During the 4th week of treatment, samples of blood were withdrawn from the orbital sinus of 6 rats from each group, under light ether anesthesia after fasting for 16 hours. The blood samples are used to evaluate Hematological parameters like RBC, WBC, and PLATELETS etc….. The collected blood samples also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, ALP and BILIRUBIN etc……...

Hematology

The following hematological parameters were analysed using Autoanalyser

Hb	:	Haemoglobin
PCV	:	Packed Cell Volume
WBC	:	White Blood Corpuscles
RBC	:	Red Blood Corpuscles

Blood Platelet count

Differential WBC count:

N	:	Neutrophils
L	:	Lymphocytes
M	:	Monocytes
E	:	Eosinophils

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)

Electrolytes

Sodium

Potassium

Chlorides

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.

Statistical analysis

The statistical analysis was carried by one way ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean ± standard error .A statistical comparison was carried out using the Dunnet's test for the control and treatment group.

4.4 PHARMACOLOGICAL STUDIES

4.4.1.1 ACUTE ANTI-INFLAMMATORY ACTIVITY OF SIDDHA FORMULATION VATHA SILETPANA SURA KUDINEER - IN VITRO STUDY

Carrageenan induced rat paw edema

Evaluation of Acute Anti-inflammatory activity Carrageenan induced rat paw oedema the rats were divided into four groups containing six rats in each group. 0.1ml of 1.0% carrageenan in normal saline (0.9% w/v NaCl) was injected to the sub plantar region of right hind paw. The trial drug VSSK was administered to the rats 1 h before carrageenan injection. Different groups were treated as follows:

Group I: Carrageenan (0.1 ml of 1.0% carrageenan/rat to the sub plantar region).

Group II: Carrageenan + Indomethacin (10 mg/kg b. w., p. o.)

Group II and IV: Carrageenan +VSSK (200 mg/kg and 400 mg/kg b. w., p. o.respectively). The paw volume was measured initially and at 1, 2, 3 and 4 h after carrageenan injection, using Plethysmograph, inflammation was calculated for comparison.

4.4.1.2. CHRONIC ANTI-INFLAMMATORY ACTIVITY OF SIDDHA FORMULATION VATHA SILETPANA SURA KUDINEER - IN VITRO STUDY

Cotton pellet granuloma pouch method

Chronic inflammation was induced by cotton pellet granuloma method. Rats were divided into four groups. First two groups received oral doses of 100 mg/kg and 200 mg/kg of VSSK respectively. The reference drug indomethacin (10mg/kg) was used as a positive control and the other negative control group received saline solution. Sterilized Cotton pellets 50 mg were implanted under light ether anesthesia in the axilla and groin region of each rat by making a small incision Drugs (VSSK100, VSSK200 and Indomethacin) and saline for control group were administered orally to four groups of rats once daily for 7 consecutive days from the day of cotton pellet implantation. The

8th day, the animals were sacrificed and cotton pellets were removed and dried in an oven at 600C for 24 hours.

They were then weighed. The granuloma formation was calculated as a measure of increment in the dry weight of the pellet. The percentage of inhibition of granuloma was calculated using the following formula.

$P = (1 - Wt /Wc) \times 100$, where,
Wt – Dryweight of the cotton in test animals and Wc - Dry weight of the cotton in control animals.

Statistical Data were presented as mean ± S.E.M. Statistical differences between control and treated groups were tested by one way ANOVA followed by dunnett's test

4.4.2 ANALGESIC ACTIVITY OF VATHA SILETPANA SURA KUDINEER – IN VITRO STUDY

Hot plate method

Animals

Young wistar rats of either sex aged 4-5 weeks, average weight 20-25 gm were used for the experiment. The mice were purchased from the animal jipmer. They were kept in standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and fed ICDDRB formulated rodent food and water ad libitum. The set of rules followed for animal experiment were approved by the institutional animal ethical committee (Zimmermann, 1983).

Experimental animals of either sex were randomly selected and divided into four groups designated as group-I, group-II, group-III and group-IV consisting of five Rats in each group for control, positive control and test sample group respectively. Each group received a particular treatment i.e. control (1% Tween-80 solution in water, 10ml/kg, p.o.), positive control (Diclofenac sodium 10 mg/kg, p.o.) and the test sample (drug of 200 mg/kg, p.o. & 400 mg/kg, p.o. respectively). The animals were positioned on

Eddy's hot plate kept at a temperature of 55±0.5 0C. A cut off period of 15 s (Franzotti *et al.*, 2000) was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90 min after oral administration of the samples (Eddy *et al.*, 1953; Kulkarni, 1999; Toma *et al.*, 2003).

Statistical analysis

The results of statistical analysis for animal experiment were expressed as mean ± SEM and were evaluated by ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group. The $p<0.05$, 0.001 were considered to be statistically significant

4.4.3. ANTIOXIDANT ACTIVITY OF VATHA SILETPANA SURA KUDINEER – IN VITRO METHOD

DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay.

The antioxidant activity of test drug sample VSSK was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay . Sample VSSK was mixed with 95% methanol to prepare the stock solution in required concentration. From the stock solution the serial dilution the concentration of 10,20,40,60,80,100,250,300 was made respectively. Ascorbic acid were used as standard was prepared in same concentration as that of the sample extract by using methanol as solvent. Final reaction mixture containing 1 ml of 0.3 mM DPPH methanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature. Absorbance in the presence of test sample VSSK at different concentration of 10,20,40,60,80,100,250,300 was noted after 15 min incubation period at 370C. Absorbance was read out at 517 nm using double-beam U.V Spectrophotometer by using methanol as blank.

% scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100.

The effective concentration of test sample VSSK required to scavenge DPPH radical by 50% (IC50 value) was obtained by linear regression analysis of dose-response curve plotting between %inhibition and concentrations

4.5. ANTIMICROBIAL ACTIVITY TEST OF VATHA SILETPANA SURA KUDINEER

Principle

The antimicrobials present in the samples were allowed to diffuse out into the medium and interact in a plate freshl seeded with the test organisms. The resulting zone of inhibition will uniformly circular as there will be a con fluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Agar Well Diffusion Test

The antibacterial screening of the **Vatha Siletpana Sura Kudineer (VSSK)** was carried out by determining the zone of inhibition using agar well diffusion method (Bauer., 1996). The drug extracts were tested against pathogenic bacteria including 1Gram positive *(Staphylococcus aureus),* 3 Gram negative organism *(E.coli, Pseudomonas aeruginosa, Proteus vulgaris)* and a fungi *Candida* sp.

Bacterial Inoculums Preparation

Inoculum of *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcu aureus and Candida* sp. were prepared individually in a respective broth and kept for incubation at suitable temperature.

Antibacterial Test:

The medium was prepared by dissolving 38 g of Muller Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 121^0C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured petriplates (25 ml/plate) the plates were swabbed with Pathogenic Bacteria culture viz. analysis analysis *E.coli, Proteus vulgaris, Candida albicans, Pseudomonas aeruginosa and Staphylococcus aureus* Finally, About 10

µL of sample (Aqueous extract of KC) was loaded onto the disc then placed on the surface of Mullar-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimetres. The size of the zone of inhibition (including disc) was measured in millimeters. The absence of zone inhibition was interpreted as the absence of activity (Kohner *et al*., 1994; Mathabe *et al*., 2006). The activities are expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm (Assam *et al*., 2010).

05. RESULTS AND DISCUSSION

The Siddha system of medicine is a holistic system that gives importance to physical, mental as well as the spiritual wellbeing of mankind. It was originated by the Siddhars who were the ancient spiritual saints of South India particularly Tamilnadu. This traditional system of medicine has numerous medicinal formulations comprising of substances from herbal, mineral / metal and animal origins that are purified and processed by traditional methods to have therapeutic effects.

Standardization is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance program for the manufacturing of an herbal drug. The Siddha Pharmacology now undergoes numerous scientific validations and standardization methods for their safety and efficacy which in turn confirms the safety of these time tested formulations.

Herbal, Mineral, Animal product cannot be considered scientifically valid if the drug tested has not been authenticated and characterized in order to ensure reproducibility in the manufacturing of the product. Till date, lesser studies have been conducted on standardization of such preparations.

Therefore, an attempt was made to ensure the formulation of *Vatha Siletpana Sura Kudineer*. It has been mentioned in Siddha texts for the management of *Vatha Siletpana Suram* - *Solanum xanthocarpum* (Kandankathari), *Clerodendrum serratum* (Siruthekku), *Terminalia chebula* (Kaddukkai), *Mollugo cerviana* (patpaddakkam), *Tinospora cordifolia* (Seenthil), *Saussurea lappa* (Kottam), *Piper longum* (Thippili), *Kaempferia galanga* (Kachcholam), *Alpinia officinarum* (Sittarathai) are used as ingredients for the preparation of *Vatha Siletpana Sura Kudineer*. Studies were performed in order to establish the authenticity of standard drug. The drug *VSSK* has been selected for its Anti-inflammatory, Analgesic and Antioxidant activities.

The study includes literary collections, physical standardization based on *Siddha* aspect, physicochemical, biochemical, phytochemical, microbiological, instrumental analysis, toxicological studies, and pharmacological studies. The results of the above studies were analyzed and discussed below.

STANDARDIZATION OF THE TRIAL DRUG

PHYSICAL STANDARDIZATION AS PER THE SIDDHA CLASSICAL LITERATURE:

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

The following characters have been noted in *VSSK*

Table 17: Physical standardization of *Vatha Siletpana Sura Kudineer* as per *Siddha* aspect

S.No	Physical standardization parameters of *VSSK*	Results of Physical standardization
1	Colour	Brown
2	Odour	Pleasant odour
3	Taste	Pungent, Bitter
4	Sense of touch	Rough
5	Appearance	Coarse powder

INTERPRETATION:

Organoleptic character indicates that the test drug *VSSK* has the following characters; Brownish colour indicates the general colour appearance of *Kudineer choornam*. Pleasant odour indicates the odour of herbal ingredients. Pungent and bitter taste indicates the ingredients taste and the appearance is coarse as the nature of kudineer chooranam.

STANDARDIZATION OF TRIAL DRUG BY USING MODERN TECHNIQUES

Table 18: Physico chemical standardization of *VSSK*

S.No	Physico chemical standardization of *KC*	Result
1	Loss on drying at 105^{o}C	1.50±0.120
2	**Ash value**	
	Water soluble ash	9.90±0.130
	Acid insoluble ash	2.75±0.130
3	**Extractive values**	
	Water soluble extractive	9.90±0.110
4	**Organoleptic characters**	
	Colour in day light	Brown
	pH (power of hydrogen)	7.10
	Odour	pleasant
	Taste	Pungent, Bitter
	Appearance	Coarse
	Touch	Rough

[Values are mean of three determinations ±SEM]

INTERPRETATION:

Determination of loss on drying:

According to physico-chemical standardization parameters, the loss of drying at 105^{o}C of VSSK was found to be 1.50± 0.120. Loss on drying is the loss of weight expressed as percentage w/w resulting from water and volatile matter of any kind that can be driven off under specified conditions. Loss in drying does not usually refer to molecularly bound water or water of crystallization. Normally less than 2% is

recommended. Hence the result of trial drug *VSSK* reveals that the stability and its long shelf-life due to low moisture content.

Determination of Ash value:

Ash values means that the residue remaining after incineration is the ash content of the drug. (Inorganic salts of carbonates, phosphates, silicates of sodium, potassium, calcium and magnesium) is known as ash content.Ash value is a criterion to judge the identity or purity of the crude drug. The object of ashing crude drugs is to remove the traces of organic matter which may be interferes in an analytical determination.

The acid insoluble ash, water soluble ash of *VSSK* was found to be 9.90±0.130 and 2.75±0.130 respectively.

Determination of water-soluble extractive value:

Extractive values by different solvents are used to assess quality, purity and to detect adulteration due to exhausted and incorrectly processed drugs. Thus, water soluble extractive value was determined.

Water-soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating.

Water-soluble extractive value of *VSSK* was 9.90±0.110. Higher water-soluble extractive value indicates that water is a better solvent of extraction for the formulation.

Determination of pH:

The pH can control the availability of nutrients, biological functions, microbial activity, and the behavior of chemicals. Because of this, monitoring or controlling the pH is important. The pH is an indication for the acidity of a substance. It is determined by the number of free hydrogen ions (H+) in a substance.

VSSK shows neutral pH (7.10). The pH level plays a role in enzyme activity by maintaining the internal environment thus regulating the homeostasis. It is also an important factor for drug absorption. Because of the neutral nature, the drug is more readily absorbed in small intestine which enhances the bio availability of the drug.

The pH level plays a role in enzyme activity by maintaining the internal environment thus regulating the homeostasis. Very high or very low pH will lead to the complete loss of the activity of most enzymes. The pH value at which the enzyme is most active is called the optimal pH value. The pH value of the trial drug *VSSK* falls near to the neutral pH value. Hence it has optimal enzymatic reaction.

Organoleptic character:

The Organoleptic character of the *VSSK* has shows Brown colour, Pleasant odour, Pungent and Bitter Taste, Coarse appearance, Rough to touch and the pH of the trial drug was 7.10.

BIO-CHEMICAL ANALYSIS OF *VSSK*

Following bio-chemical properties identified on screening the test drug

Table 19: Biochemical analysis results of preliminary basic and acidic radicals studies

S.No	Experiment	Observation	Results
1	Test for calcium	White precipitate	**Present**
2	Test for sulphate	White precipitate	**Present**
3	Test for chloride	No White precipitate formed	Absent
4	Test for carbonate	No Brisk effervescence is formed	Absent
5	Test for starch	Blue colour is formed	**Present**
6	Test for ferric iron	No Blue colour is formed	Absent
7	Test for ferrous iron	Blood red colour is formed	**Present**
8	Test for phosphate	No yellow Precipitate is formed	Absent
9	Test for albumin	No yellow Precipitate is formed	Absent
10	Test for tannic acid	Blue black Precipitate is formed	**Present**
11	Test for unsaturation	It gets decolorized	**Present**

12	Test for the reducing sugar	Colour change occurs	**Present**
13	Test for amino acid	Violet colour is formed	**Present**
14	Test for zinc	No white precipitate is formed	Absent

INTERPRETATION:

From the above result of preliminary biochemical analysis of *VSSK* reveals that, the trial drug consists of Calcium, Sulphate, Starch, Ferrous iron, Tannic acid, Unsaturated compounds, Reducing sugar, and Amino acids.

Calcium: There are calcium-sensing receptors on vascular smooth muscle cells and on platelets, calcium plays a role in smooth muscle contraction and its role in the electrophysiology of the heart and myocardial function. Antioxidant enzyme responses depend on calcium levels. Calcium carbonate, calcium citrate and calcium gluconate have significant anti inflammatory activity.

Sulphate Chondroitin sulfate (CS) prevents joint space narrowing and reduces joint swelling and effusion. To produce these effects, CS elicits an anti-inflammatory effect at the chondral and synovial levels. Sulphate has been considered as an adjunct therapy for severe and life threatening asthma exacerbation. Nutritionally essential element. Sulphate has anti- bacterial activity and it is one of the macronutrient of cells. It inhibits growth of yeast and moulds in low pH and inhibits growth of enterobacteriae and other gram negative bacteria in high pH. Sulphate important role for the anti-microbial activity

Starch It is a odourless tasteless white substance occuring widely in plant tissue. it is a polysaccharide functions as a carbohydrates store and is an important constituent of the human diet**.** Resistant starch is divided into five different types based on the origin and physical properties of starch. It can produce more butyrate in comparison to other prebiotics. Butyrate is the main SCFA that is produced from the fermentation of RS and acts as an anti-inflammatory agent. Starch is needed during fever condition.

Ferric iron and ferrous iron: Iron is an essential element for blood production. About 70 percent of the body's iron is found in the red blood cells of blood called hemoglobin and in muscle cells called myoglobin. Hemoglobin is essential for transferring oxygen in blood from the lungs to the tissues. In the ferrous state (Fe^{2+}), iron acts as an electron donor, while in the ferric state (Fe^{3+}) it acts as an acceptor.

Tannic acid It has been used as an antidote to soak up poisons historically. In the common day, however, Tannic Acid is used to stop bleeding, treat rashes, and alleviate other conditions of soreness. It is used orally to prevent throat infections and other internal alleviations. Tannic acid is a natural polyphenol which has been reported to possess antioxidant, anti-inflammatory, anticarcinogenic, antimutagenic, antitumor, and antimicrobial activities.

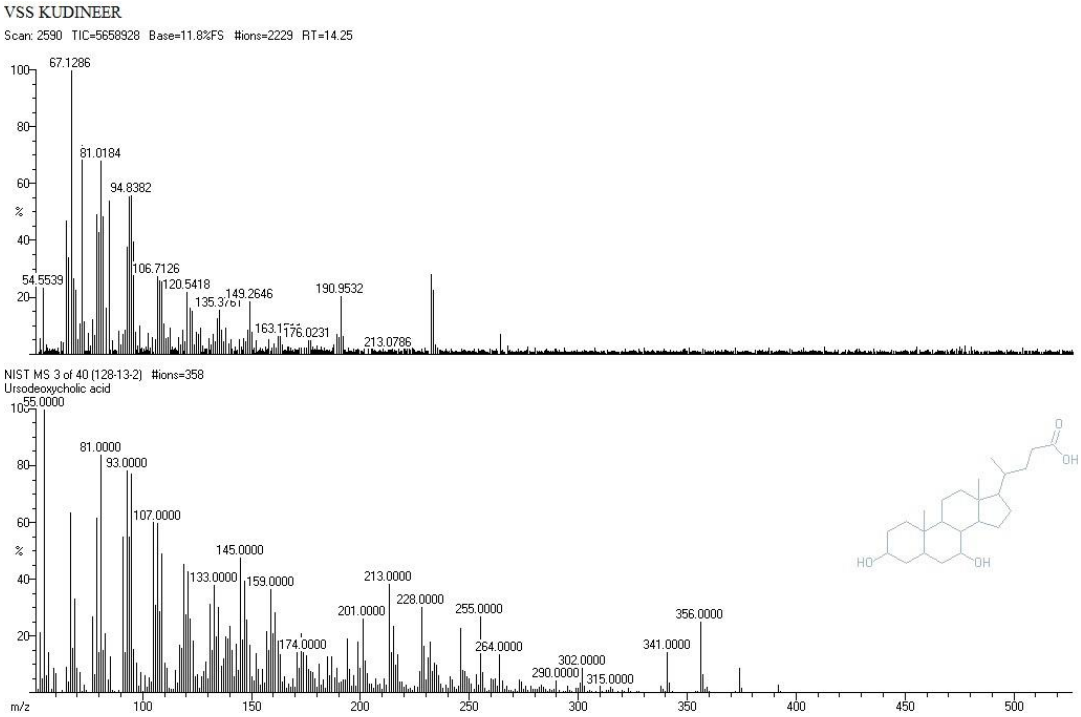
Unsaturated compounds: In other tissues and cell types, unsaturated fatty acids have well known anti-inflammatory effects, which range from the inhibition of the lipoxygenase and cycloxigenase pathways and decrease of neutrophil adhesion to the reduction of inflammatory cytokine expression and inhibition of TLR4 signaling .

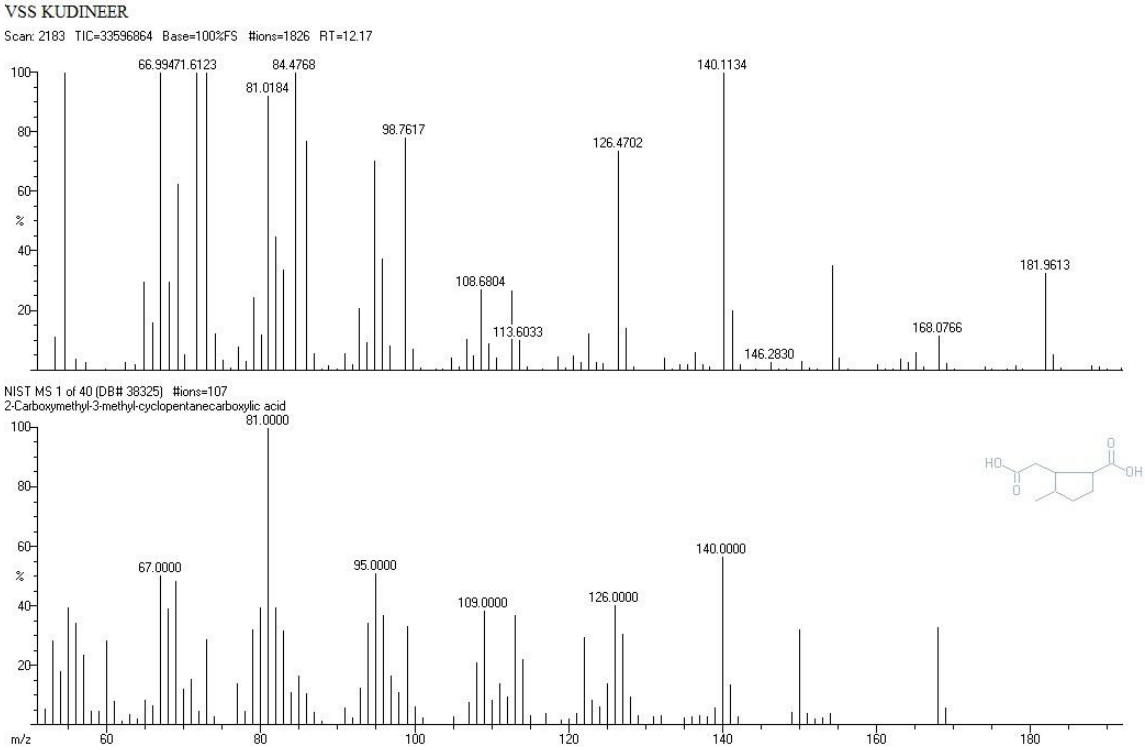
Reducing sugar relaxes mucus, lessens cold and cough symptoms.

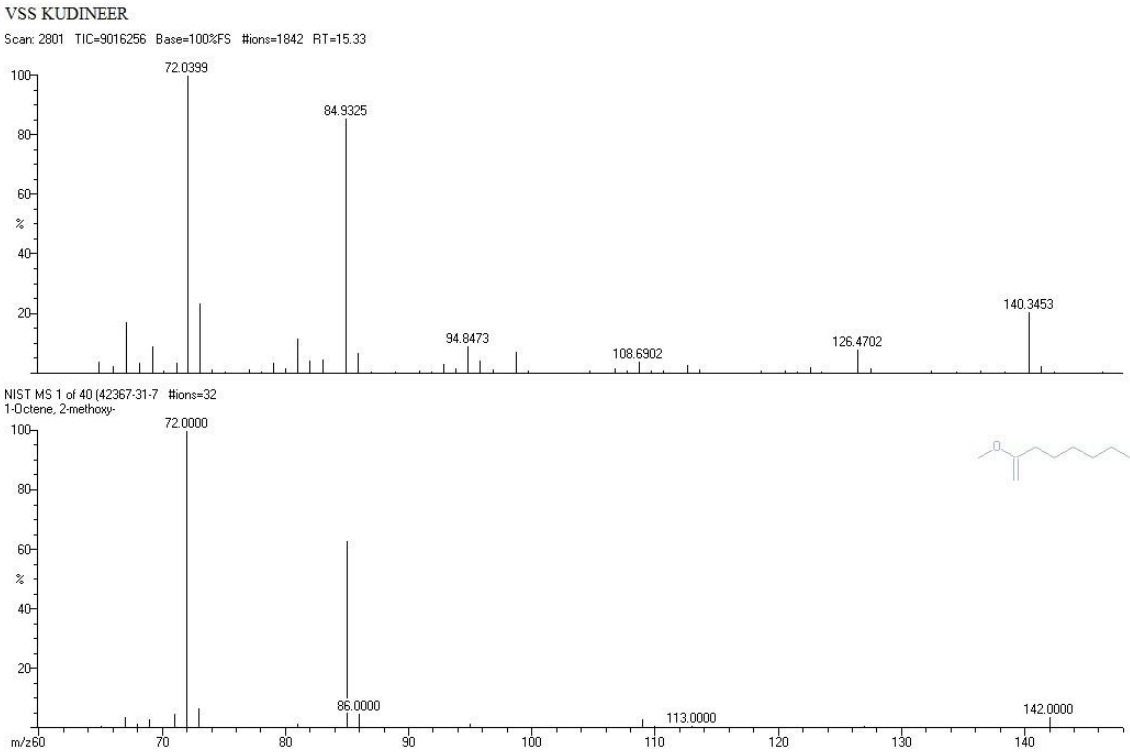
Amino acids: N-acetyl cysteine for cough and other lung conditions. It is also used for flu, dry eye, and many other conditions. NAC is also useful to help fight long-term lung damage in those with chronic obstructive pulmonary disease (COPD). Amino acids contribute to various anti-oxidant and immunological activities relevant to asthma pathogenesis, raising the possibility that differences in amino acids may be involved in asthma aetiology. Cystine reduces the risk of asthma via glutathione metabolism.

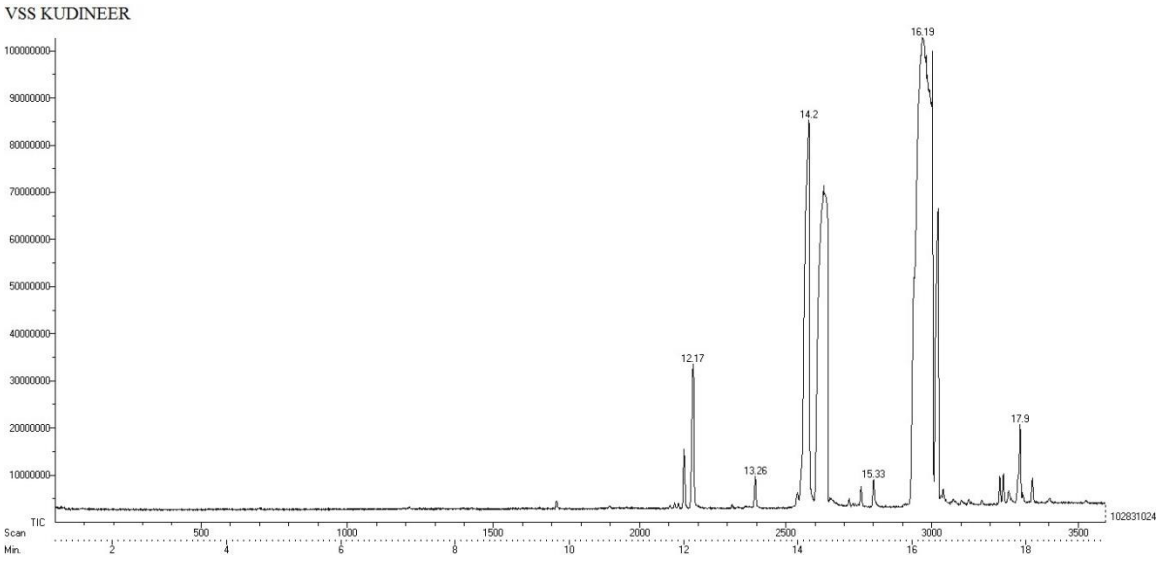
PHYTOCHEMICAL ANALYSIS

GAS CHROMATOGRAPH – MASS SPECTROSCOPY OF VATHA SILETPANA SURA KUDINEER






VSS KUDINEER
Scan: 2801 TIC=9016256 Base=100%FS #ions=1842 RT=15.33
72.0399
84.9325
94.8473
108.6902
126.4702
140.3453
NIST MS 1 of 40 (42367-31-7 #ions=32
1-Octene, 2-methoxy-
72.0000
86.0000
113.0000
142.0000
m/z


VSS KUDINEER
16.19
14.2
12.17
17.9
13.26
15.33
TIC
Scan
Min.

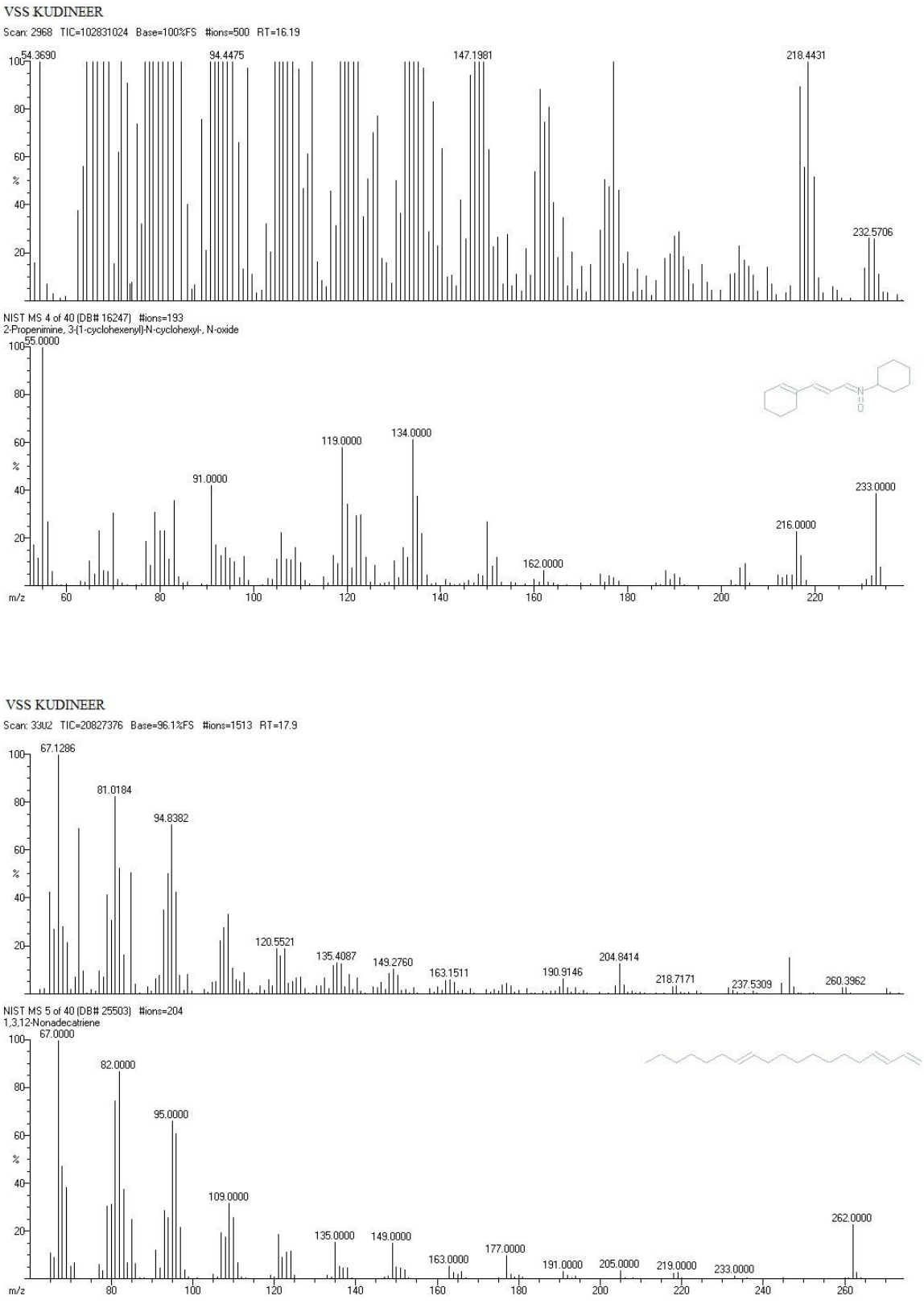


Fig. 22 : mass spectroscopy analysis of VSSK- Gas chromatography

Interpretation

Gas chromatography mass spectroscopy analysis was carried out in crude extracts of the *MLC* such as ethanol extract. The peaks in the chromatogram were integrated and were compared with the database of spectrum of known components stored in the GC-MS library. The detailed of GC-MS analysis of the extracts are given in figures. This study shows the presence of those compounds such as 1,3,12-nonadecarine, 2-propenamine, 3,1 [cyclohexanyl]-N-cyclohexanyl-N-oxide, 1-octane, 2-methoxyl, 2-carboxymethyl, 3-methyl- cyclopentano carboxylic acid, ursodeoxcholic acid.

MICROBIAL LIMIT TEST

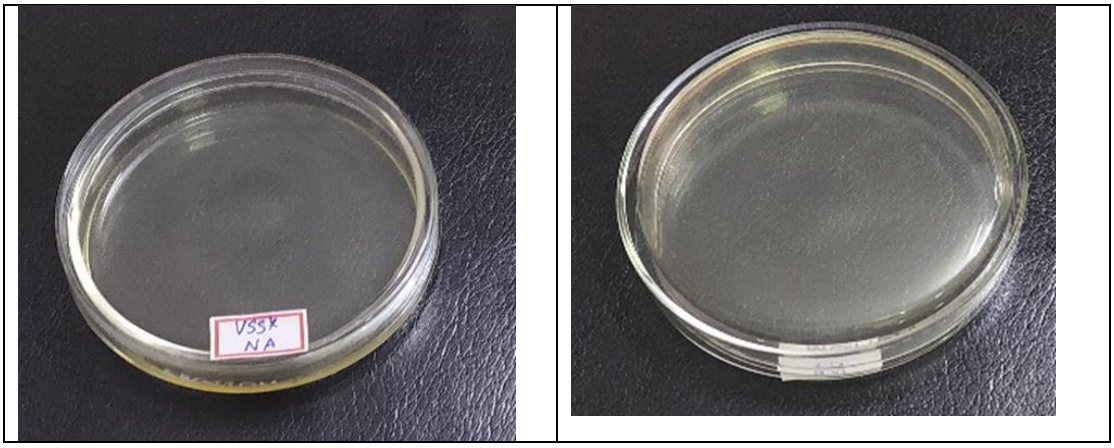


Fig. 23 : Sterility test by pour plate method for VSSK

Observation

No growth was observed after incubation period. Reveals the absence of specific pathogen

Table 20 : Result of Sterility test for VSSK

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10^5CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10^3CFU/g	

Result

No growth / colonies was observed in any of the plates inoculates with the test sample.

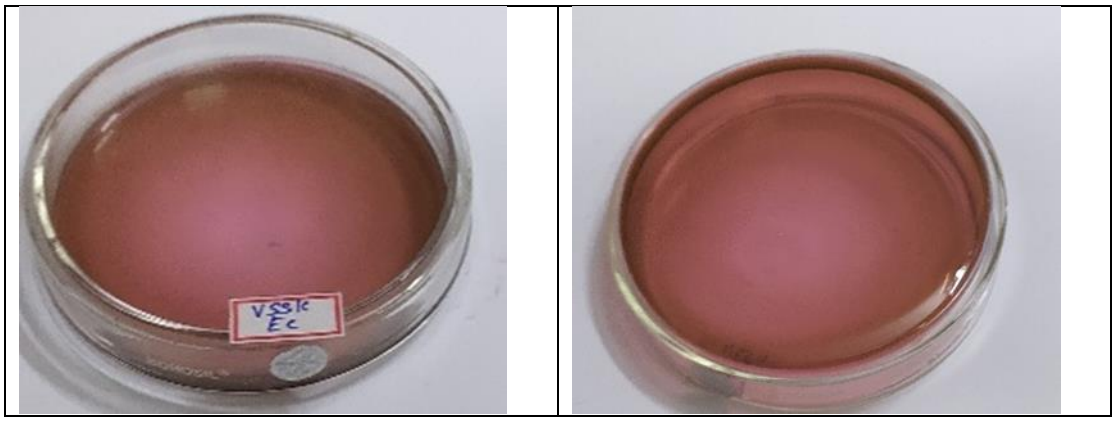


Fig. 24 : ***Culture plate with E-coli (EC) specific medium***

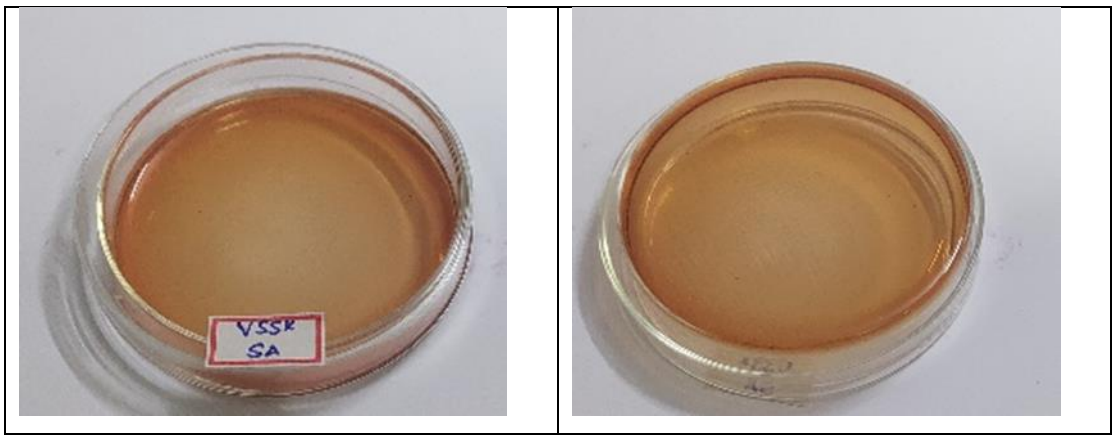


Fig. 25: ***Culture plate with Salmonella (SA) specific medium***

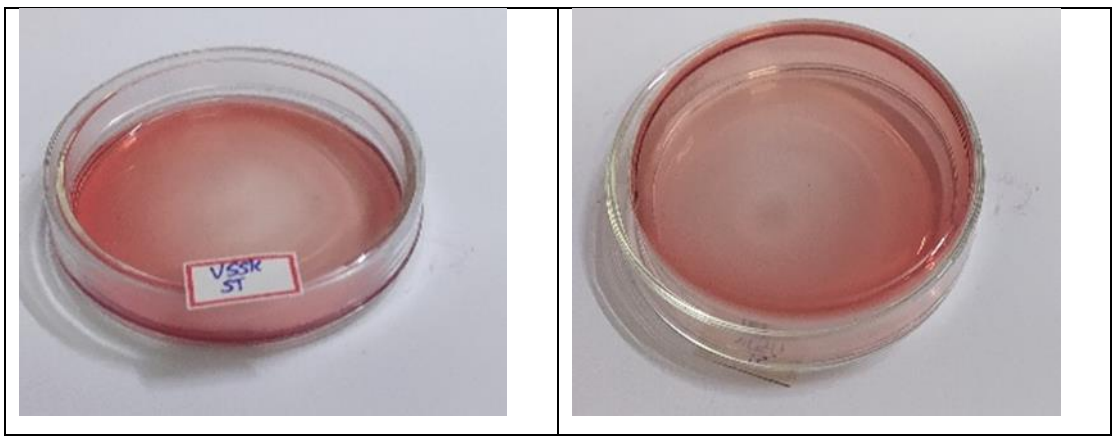


Fig. 26 : ***Culture plate with Staphylococcus Aureus (ST) specific medium***

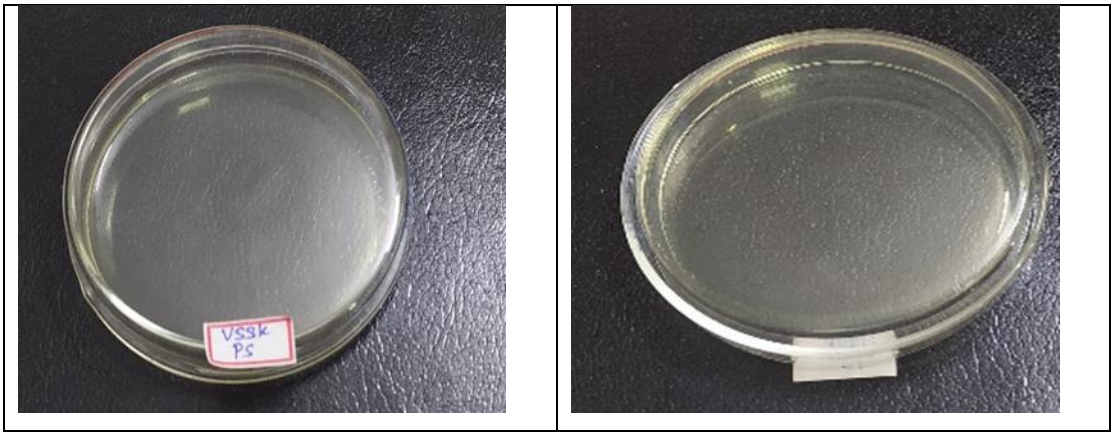


Fig. 27 : ***Culture plate with Pseudomonas Aeruginosa (PS) specific medium***

Observation

No growth was observed after incubation period. Reveals the absence of specific pathogen

Table 21 : Result of Specific pathogen test for VSSK

Organism	**Specification**	**Result**	**Method**
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus Aureus	Absent	Absent	
Pseudomonas Aeruginosa	Absent	Absent	

Result

No growth / colonies were observed in any of the plates inoculated with the test sample.

Interpretation:

The total bacterial count and the total fungal count was nil. This indicates that the drug is free from microbial contamination. The other pathogens like *Escherichia coli, Salmonella sps, Staphylococcus aureus* and *Pseudomonas aeruginosa* were found to be completely absent in the drug

INSTRUMENTAL ANALYSIS

SCANNING ELECTRON MICROSCOPE (SEM)

A scanning electron microscope (SEM) scans a focused electron beam over a surface to create an image. The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition.

Scanning Electron micrographs of the sample is given in both figures. These micrographs revealed that rough surface of the sample had various sized, various shaped irregular particles and these particles were randomly oriented and also aggregated on the surface.

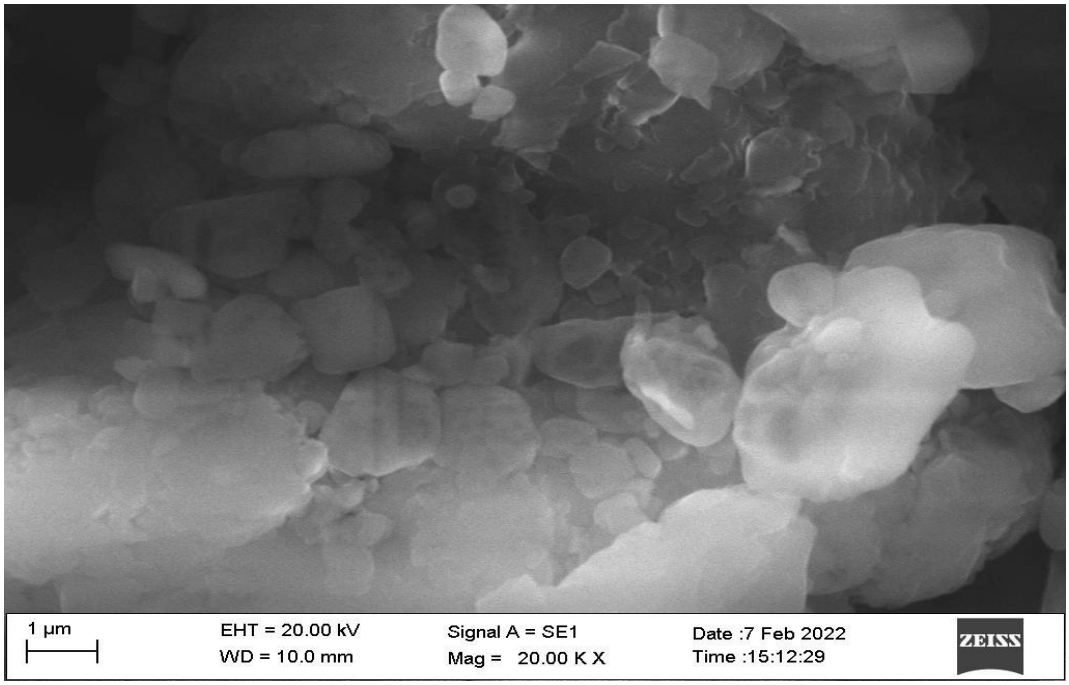


Figure 28: SEM picture 20.00x magnification of VSSK

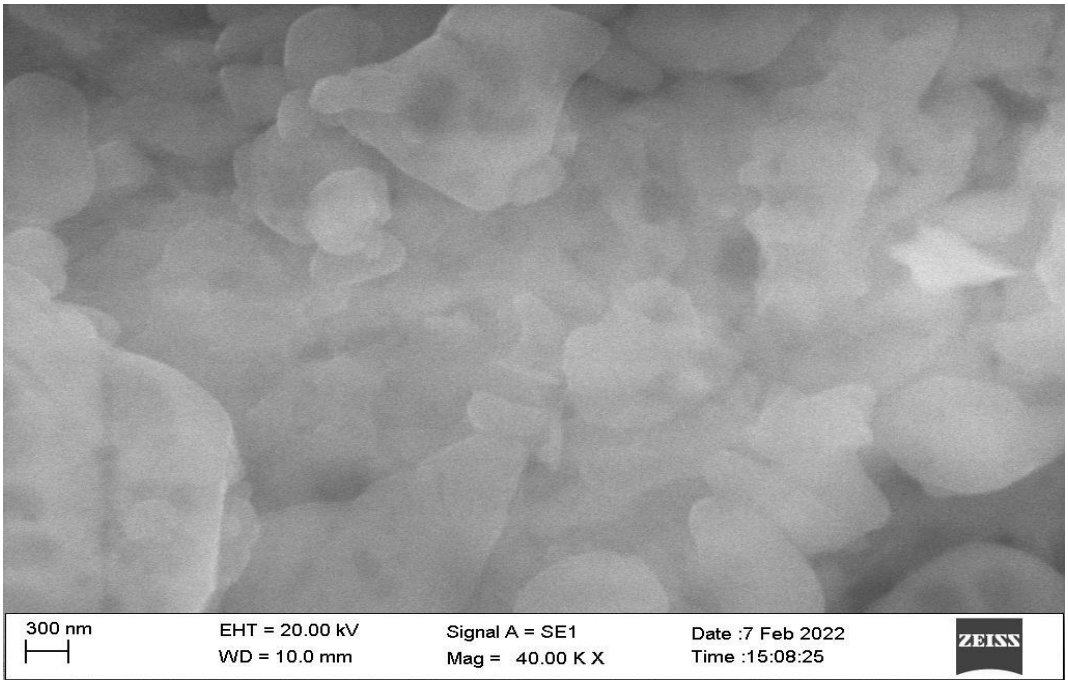


Figure 29 : SEM picture 40.00 kx magnification of VSSK

INTERPRETATION:

The morphology of the *VSSK* 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 300 nm. 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 micro particles. ***VSSK*** exhibited larger sizes and agglomeration of the particles. SEM analysis of the ***VSSK*** shows most of the particles present in the sample are micro size, average particle size is **1µm - 300nm**

FTIR- FOURIER TRANSFORM INFRARED SPECTROSCOPY

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: and Fig no: shows the presence of functional group and inorganic compounds of *Vatha Siletpana Sura Kudineer*..

FTIR results of *VSSK*:

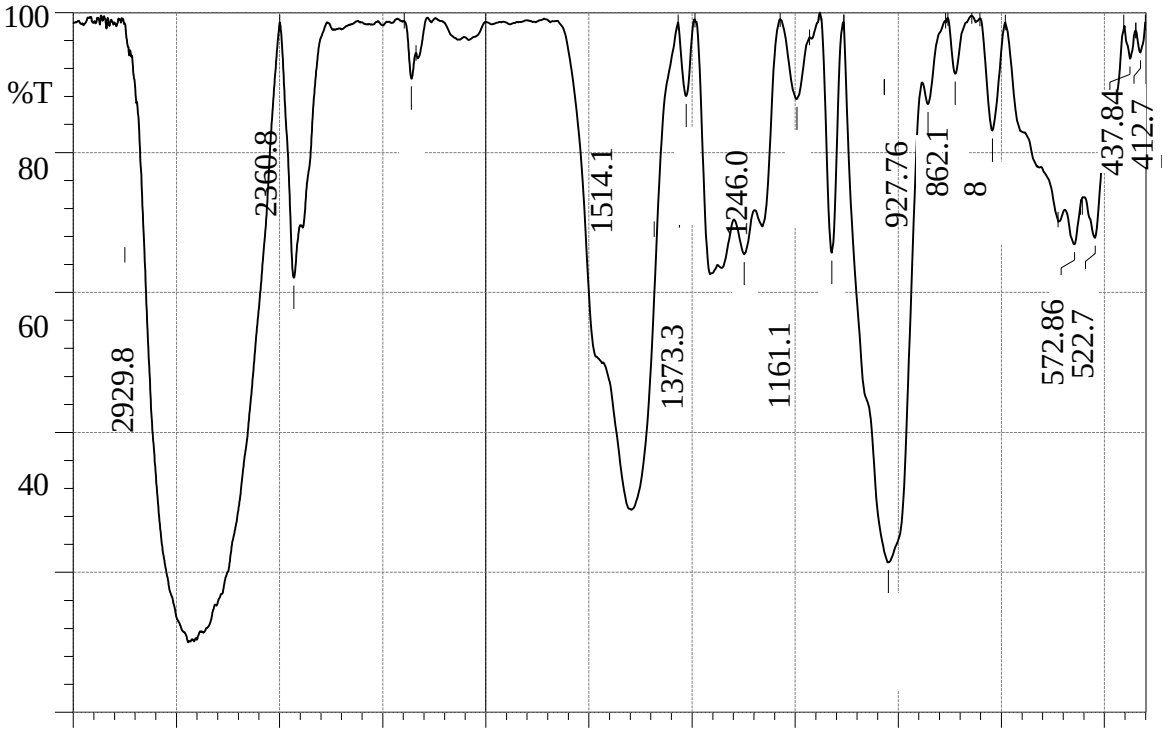


Fig 30: FTIR Spectra of Vatha Siletpana Sura Kudineer

Table 22: FTIR Interpretation of *Vatha Siletpana Sura Kudineer*

S. No	Wave Number (cm^{-1})	Vibrational Modes of SMC in IR Region	Functional groups
1	412.77	C-I Stretching	Alkyl & Aryl Halides
2	437.84	C-I Stretching,	Alkyl & Aryl Halides
3	522.71	C-Br Stretching	Alkyl & Aryl Halides
4	572.86	C-Br Stretching	Alkyl & Aryl Halides
5	771.53	C-Cl Stretching	Alkyl & Aryl Halides
6	862.18	C-H Bending	Aromatics
7	927.76	C =C bending	Alkene
8	1024.20	None	None
9	1161.15	C –O stretching	Tertiary alcohol
10	1246.02	C-N stretching	Amine
11	1373.32	N-O stretching	Nitro compounds
12	1514.12	O-N-O Stretching	Nitro compounds
13	2360.87	None	None
14	2929.87	O-H Stretching	Carboxylic acid

INTERPRETATION:

In FT-IR spectra analysis, this sample *Vatha Siletpana Sura Kudineer* exhibits the peak value at 2929.87, 2360.87, 1514.12, 1373.32, 1246.02,1161.15, 1024.20, 927.76, 862.18, 771.53, 572.86, 522.71, 437.84, 412.77 having O-H stretch, none, O-N-O stretch, N-O stretch, C-N stretch, C-O stretch, None, C=C Bend, C-Cl stretch, C-Br stretch, C-I stretch respectively. This peak indicates the presence of some organic functional groups such as, Carboxylic acid, Isothianate, nitro compounds, amine, tertiary alcohol, alkenes, alkyl halides & aryl halides.

These compounds have some pharmaceutical properties and are briefly discussed below.

Nitro compounds has anti inflammatory , , analgesic, antioxidant ,anti proliferative, . it can act against infectious diseases, it has anti tubular activity, and anti parasitic

activity. Carboxlic acid acts as Anti inflammatory , Analgesic , Anti pyretic and cytotoxic , Anti oxidant ,It depresses cough and its symptoms .

Amines has anti inflammatory, antioxidant,Anti tussive, Bronchodialator activities. Alkl and Aryl halides has anti inflammatory ,Anti microbial , Anti niociceptive activities. Alcohols has analgesic activity .

Inductively coupled plasma optical emission Spectrometry ((ICP-OES):

The drug *VSSK* sample was analyzed by the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to detect the trace elements and other elements quantitatively. Heavy metals were analyzed by ICP-OES, results have been tabulated.

VSS KUDINEER ---(wt:0.3100110g)

Table 23 : Results of ICP-OES of VSSK

S.No	Elements	Wavelength(nm)	Concentration
1.	As	188.979	BDL
2.	C	193.030	195.210 mg/L
3.	Ca	315.807	BDL
4.	Cd	228.802	BDL
5.	Cu	327.393	BDL
6.	Fe	238.204	01.081 mg/L
7.	Hg	253.652	BDL
8.	K	766.491	23.110 mg/L
9.	Mg	285.213	01.131 mg/L
10.	Na	589.592	01.320 mg/L
11.	Pb	220.353	BDL
12.	P	213.617	146.341 mg/L
13.	S	180.731	01.304 mg/L
14.	Zn	206.200	01.200 mg/L

BDL:Below Detectable Limit

1% = 10000ppm,

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

Table 24: The toxic metals and the permissible limits

S.No	Heavy metals	WHO limits for ASU drugs
1	Arsenic (As)	3 ppm
2	Mercury (Hg)	1ppm
3	Lead (Pb)	10ppm
4	Cadmium (Cd)	0.3ppm

INTERPRETATION:

The results indicates that the formulation contains heavy metals are in below detectable level. This results shows Below Detectable Limit (BDL) of Al (Aluminium), As (Arsenic), C (Carbon), Cd (Cadmium), Cu (Copper), Fe (Iron), Hg (Mercury), K (Potassium), Mg (Magnesium), Na (Sodium), S (Sulphur) and Zn (Zinc). So it is considered as safe and free from toxic substances.

1.Ferrous iron

Higher iron stores were inversely associated with asthma and lower body iron and higher tissue iron need were associated with lower lung function.

Iron supplementation resulted in a significant decreases in airway eosinophilia,while systemic iron injections lead to a significant suppression of both allergen- induced airway eosinophilia and hyperactivity compared to placebo

2.Potassium and sodium:

In the presence of Sodium and Potassium regulate the acid-base balance of the body fluids.They regulate the water

balance by maintaining the osmotic pressure of the body fluids.They help to preserve the neuromuscular irritability by maintaining a state of equilibrium on account of their relative proportion in the ECF and ICF.

3.Magnesium :

In the presence of Magnesium and Sulfate (MgSo 4). - Magnesium sulphate has been considered as an adjunct therapy for severe and life threatening asthma exacerbation. Theoretically, Magnesium can induce bronchial smooth muscle relaxation in a dose dependent manner.

4.Phosphorus :

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

5.Zinc:

Zinc is essential for growth.There are conflicting reports about the effect of zinc supplements on asthma.Zinc are required for optimal activity of the immune system and it has been shown that low levels of these trace elements are important factors in acute and chronic inflammatory status such as bronchial asthma

6.Sulphur:

As part of four amino acids, sulphur performs a number of functions in enzyme reactions and protein synthesis. It is necessary for formation of collagen, the protein found in connective tissues in our bodies. Sulphur is important to cellular respiration as it is needed in the oxidation-reduction reactions

that help the cells utilize oxygen, which aids brain function and all cell activity. A physiologic form of sulphur called methylsulfonyl methane (MSM) has recently become available and may be helpful in patients with allergies.

X-RAY DIFFRACTION

Table 25: coupled two theta of VSSK- Measurement conditions and results of XRD

Index	1
Name	Vidhya - VSS.raw #1
Parent	2Theta
Sample Name	Vidhya - VSS
File Name	Vidhya - VSS.raw
Scan Type	Coupled
Scan Status	Completed
Start	10.000
End	80.008
Step Size	0.020
Time per Step	32.0
Temperature	25 °C (Room)
Goniometer radius	255.0
2-theta	10.000
Theta	5.000
Anode	Cu
kα1	1.54060
kα2	1.54439
kα2 Ratio	0.50000
kβ	1.39222
Generator kV	40.0
Generator mA	25.0
Detector Name	LynxEye
Detector opening angle	2.452
Sample rotation speed	0.000
Slit Mode	Fixed
Compute Crystallinity	Yes
Cristallinity - From	10.000
Cristallinity - To	80.008
%-Crystallinity	27.5 %
%-Amorphous	72.5 %
Global Area	219.9
Reduced Area	60.38
Operator Name	Lab Manager
Creation Date/Time	1/21/2

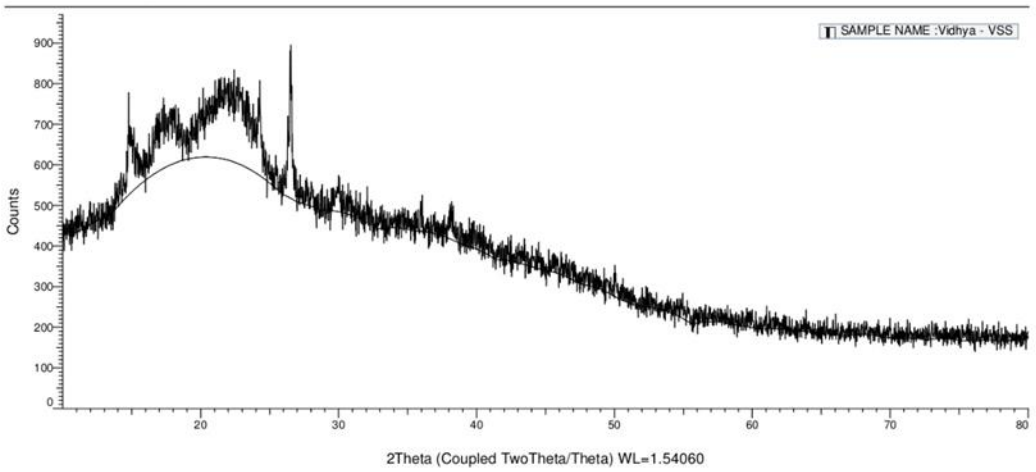


Fig 31 : Peak levels of Elements in XRD spectra

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. The analyzed material is finely ground, homogenized and average bulk composition is determined.

This XRD fingerprint shows both the similarities and differences of the sample successfully and is a valuable primary tool for checking the quality control of minerolo metallic formulations. The different peaks show the presence of minerals in the samples.

Crystallinity refers to the degree of structural order of a solid. The Percentage of crystalinity of the *VSSK* is 27.5 %. Increasing the degree of crystallinity increases hardness and density. It is a Meto – mineral preparation, hence it has the high value of cristalinity. Amorphous means, noncrystalline solid in which the atoms and molecules are not organized in a definite lattice pattern. The Percentage of Amorphous of the *VSSK* is 72.5 %.

TOXICOLOGICAL STUDIES

EFFECT OF ACUTE ORAL TOXICITY STUDY (14 DAYS) OF *VSSK* IN FEMALE WISTAR ALBINO RATS

Table 26: Results of 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	2000mg/kg	Normal	0 of 3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=3); $^{ns}p > 0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group

Data obtained in this study indicated $^{ns}p > 0.05$ no significant changes the physical and behavioral signs of any toxicity due to administration of *VSSK* at the doses of 5mg/kg, 50mg/kg , 300mg/kg and 2000mg/kg to rats.

Table no-27: Home cage activity

Functional and Behavioural observation	**Observation**	**5mg/kg Group (G-I)**	**50mg/kg (G-II)**	**300mg/kg (G-III)**	**2000mg/kg (G-IV)**
		Female n=3	**Female n=3**	**Female n=3**	**Female n=3**
Body position	Normal	3	3	3	3
Respiration	Normal	3	3	3	3
Clonic involuntary Movement	Normal	3	3	3	3
Tonic involuntary Movement	Normal	3	3	3	3

Palpebral closure	Normal	3	3	3	3
ApproVSS Kh response	Normal	3	3	3	3
Touch response	Normal	3	3	3	3
Pinna reflex	Normal	3	3	3	3
Tail pinch response	Normal	3	3	3	3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $^{ns}p > 0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group

Data obtained in this study indicated $^{ns}p > 0.05$ 05 no significance changes in Home cage activity, signs of any toxicity due to administration of *VSSK* at the doses of 5mg/kg, 50mg/kg , 300mg/kg and 2000mg/kg to rats.

Table no-28 Hand held observation

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

Abdominal tone	Normal	3	3	3	3	3
Limb tone	Normal	3	3	3	3	3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $^{ns}p > 0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group

Data obtained in this study indicated $^{ns}p > 0.05$ no significance changes in hand held observation and signs of any toxicity due to administration of *VSSK* at the doses of 5mg/kg, 50mg/kg , 300mg/kg and 2000mg/kg to rats.

Table no-29: 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	2000(mg/kg)	0 of 3

Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $^{ns}p > 0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group

From acute toxicity study it was observed $^{ns}p > 0.05$ that the administration of *VSSK* 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) at *VSSK* is 2000 mg/kg.

SUB-ACUTE TOXICITY STUDY IN WISTAR RATS

Table 30: EFFECT OF SUB- ACUTE DOSE (28 DAYS)OF *VSSK ON* BODY WEIGHT IN GRAM

GROUP	NORMAL CONTROL	VEHICLE CONTROL	LOW	MID	HIGH
1st day	175.34±0.32	176.11±0.21	177.24±0.60	185.06±0.14	190.33±0.76
7th day	180.10±0.22	190.23±0.29	197.33±0.28	188.44±0.34	210.44±0.55
14th day	185.31±0.77	182.05±0.29	187.35±0.32	200.03±0.98	218.96±0.45
21st day	205.05±0.55	217.23±0.66	220.18±0.10	222.33±0.88	225.25±0.09
28th day	200.22±0.34 *	220.55±0.56 *	207.22±0.63 *	190.10±0.88 *	219.15±0.64 *

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

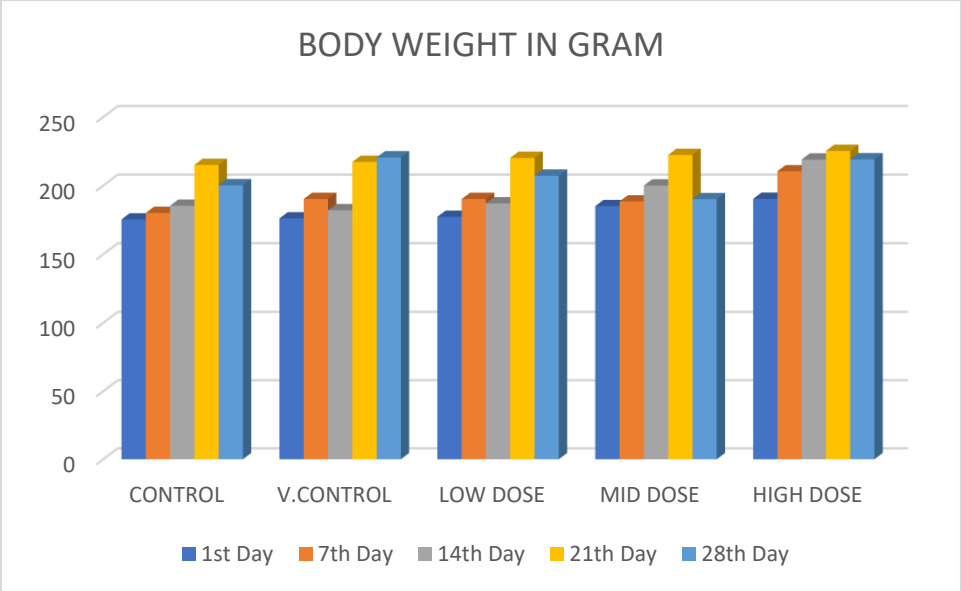


Fig 32 : Effect of sub- acute toxicity study (28 days) of *CPC on* body weight in gram

The effect of VSSK was observed, on the body weight changes, significantly increase (*p<0.05) in body weight in all the treated animals were observed. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV.

Table 31: *VSSK* **ON ORGAN WEIGHT (PHYSICAL PARAMETER) IN GRAM**

GROUP		**CONTROL**	**VEHICLE CONTROL**	**LOW**	**MID**	**HIGH**
HEART		2.75±0.45	2.70±0.61	2.80±**0.14**	2.90±**0.55**	2.99±**0.02**
LIVER		7.02±**0.37**	8.58±**0.62**	7.50.12±**0.42**	8.22±**0.47**	9.30±**0.54**
LUNGS		1.09±**0.32**	1.98±**0.43**	1.24±**0.40**	2.32±**0.32**	2.98±**0.66**
KIDNEY	L	2.84±**0.32**	2.00±**0.89**	3.88±**0.09**	4.02±**0.05**	3.12±**0.44**
	R	3.80±**0.23**	2.78±**0.10**	3.80±**0.43**	4.16±**0.55**	3.89±**0.33**

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

The effects of VSSK on kidney, heart, liver and lungs of the rats were recorded. not significant p>0.05 changes in the weights of various organs of the animals occurred with higher doses of the extract but macroscopic examinations visualized no changes in color of the organs of the treated animals compared with the control group.

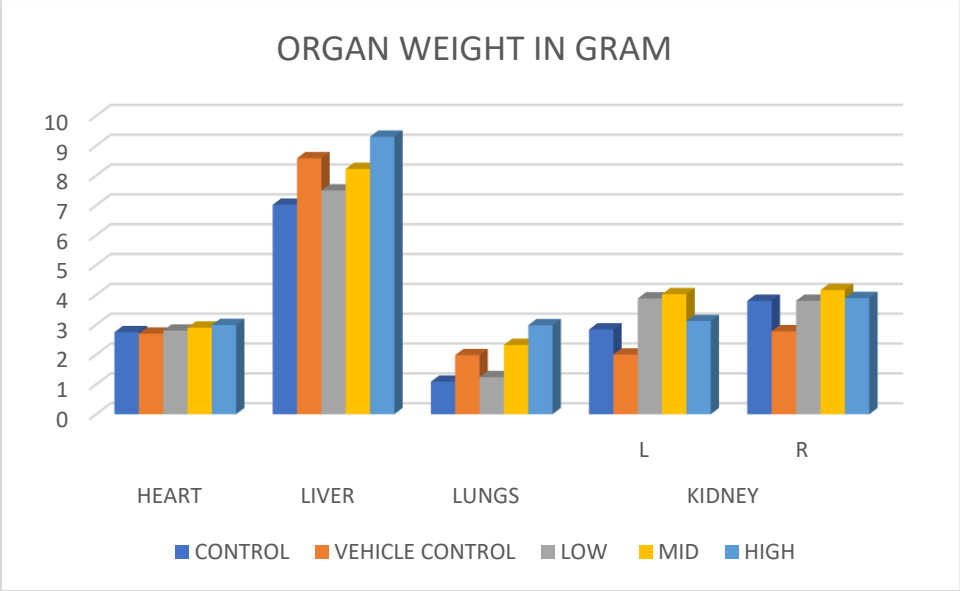


Fig 33: Effect of sub- acute toxicity study (28 days) of *CPC on* organ weight (Physical parameters) in gram

EFFECT OF SUB- ACUTE TOXICITY (28 DAYS) OF *VSSK ON* HAEMATOLOGICAL PARAMETERS

Table no 32: Effect of sub- acute toxicity study (28 days) of *VSSK on* hematological parameters

Drug treatment	RBC 10^{12}/liter	WBC 10^{9}/liter	Haemoglobin gm /liter	Differntial count %			
				Neutrophils	Eosinophils	Monocyte	Lymphocyte
Control	4.02±0.07	5.90±0.66	12.12±0.56	40.77±0.99	5.60±0.88	7.30±0.65	40.65±0.57
Vehicle Control	5.90±0.99	8.10±0.32	18.45±0.12	60.77±0.65	2.00±0.77	5.60±0.55	35.09±0.47
LOW	4.05±0.65	6.45±0.22	15.67±0.09	55.00±0.87	4.40±0.56	8.09±0.08	25.45±0.76
MID	6.02±0.44	7.00±0.55	17.89±0.87	45.55±0.55	3.70±0.98	4.20±0.90	38.99±0.55
HIGH	5.18±0.54	8.85±0.23	20.00±0.43	56.77±0.66	6.00±0.87	6.40±0.34	30.78±0.67

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

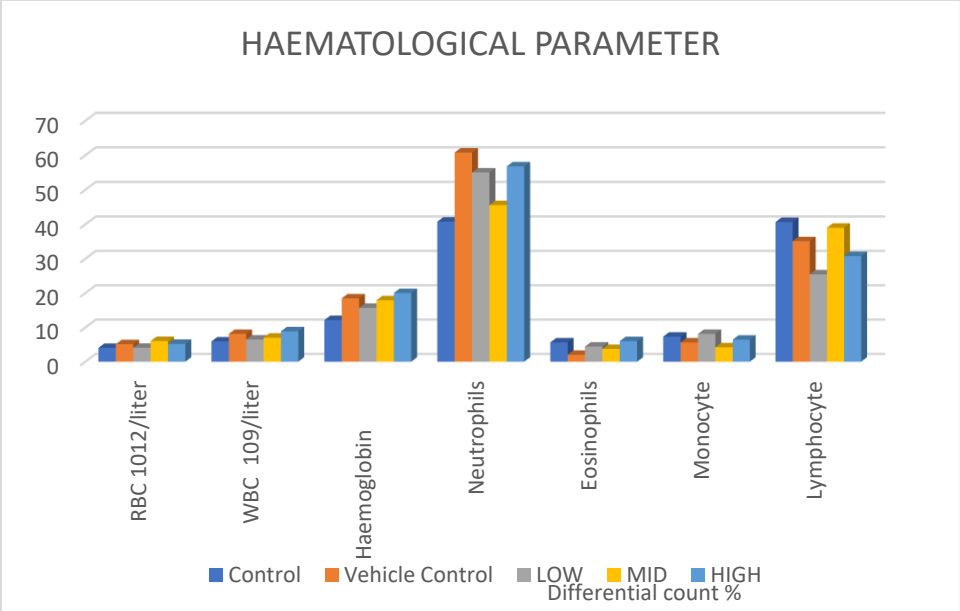


Fig 34 : Effect of sub- acute toxicity study (28 days) of *VSSK on* hematological parameters

The effects of **VSSK** were observed for its effect on hematological parameters in experimental rat. Final study, not significant (p<0.05) in the haemoglobin,RBC values are increased after treated groups. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV.

Table 33: Effect of sub- acute toxiicty (28 days)of *vssk* on biochemical parameter

Drug Treatment	SGPT (U/L)	SGOT(U/L)	ALP(U/L)	Urea (mg/dl)	Creatinine(mg/dl)
Control	65.38±0.37	85.36±0.75	99.97±0.54	12.08±0.75	0.46±0.23
Vehicle Control	77.76±0.39	102.75±0.85	118.37±0.75	17.89±0.36	0.60±0.75

LOW	**63.35±0.95**	**70.35±0.98**	**102.85±0.37**	**14.65±0.35**	**0.56±0.75**
MID	**79.36±0.85**	**83.36±0.46**	**144.36±0.85**	**18.95±0.47**	**0.50±0.25**
HIGH	**72.65±0.75**	**118.65±0.36**	**132.65±0.24**	**15.57±0.86**	**0.72±0.54**

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

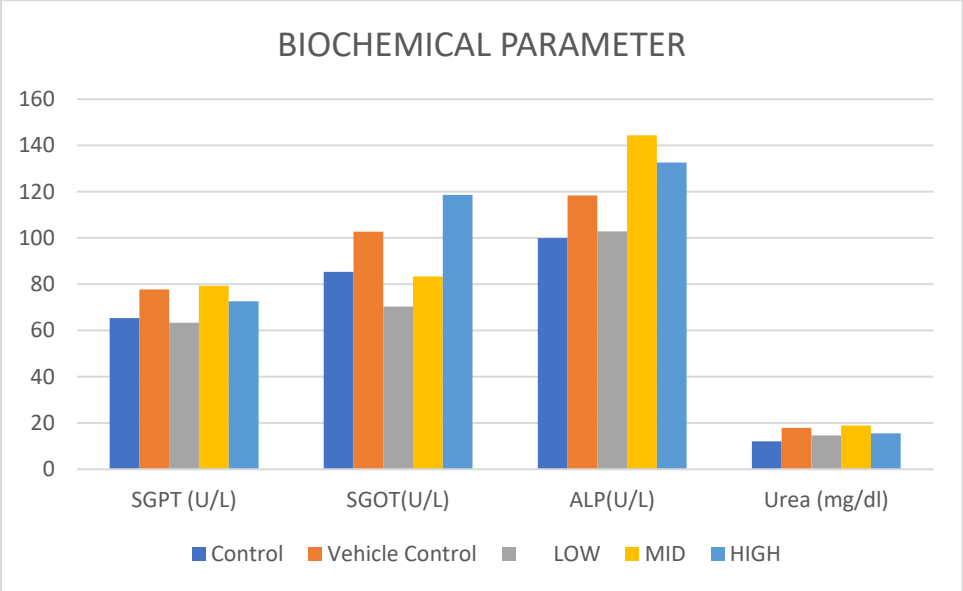


Fig 35: Effect of sub- acute toxicity study (28 days) of *VSSK on* Biochemical parameters

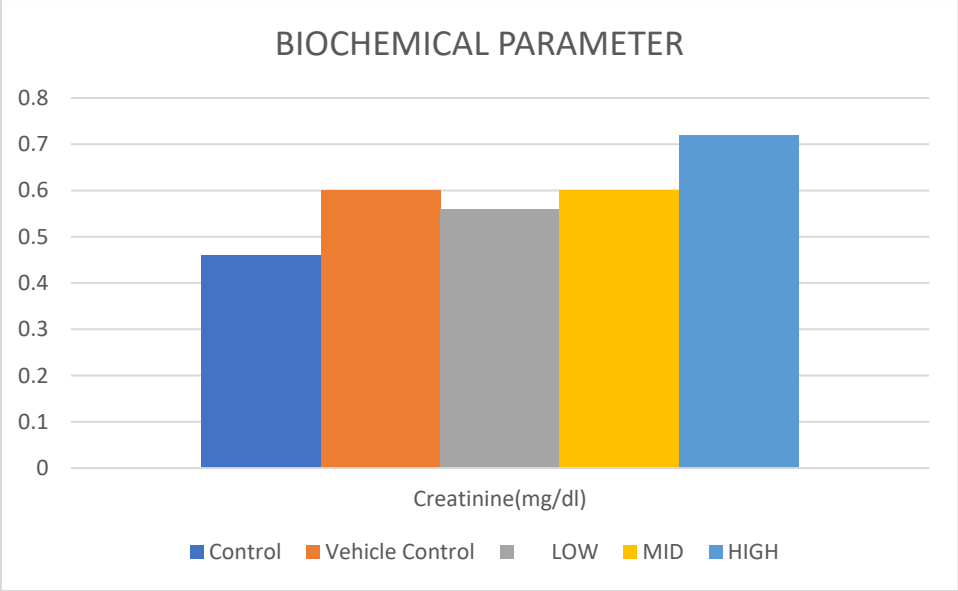


Fig 36: Effect of sub- acute toxicity study (28 days) of *VSSK on* Biochemical parameters (creatinine)

The SGPT, SGOT, ALP, UREA and Creatinine values was compared in Group I and other groups II, III, IV. The biochemical Parameters in experimental rat. not significant ($p<0.05$) in The SGPT, SGOT, ALP,urea and Creatinine values. The values are expressed as mean ± S.E.M. n=6.

EFFECT OF SUB- ACUTE TOXICITY (28 DAYS) OF *VSSK* BIOCHEMICAL PARAMETERS

Table 34: Effect of sub- acute toxicity (28 days) of *vssk* biochemical parameters

GROUP	CONTROL	VEHICLE CONTROL	LOW (300mg/kg)	MEDIUM (1000mg/kg)	HIGH (2000mg/kg)
TOTAL BILIRUBIN (mg/dl)	0.77±0.67	0.87±0.57	0.78±0.20	0.65±0.76	1.00±0.89

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

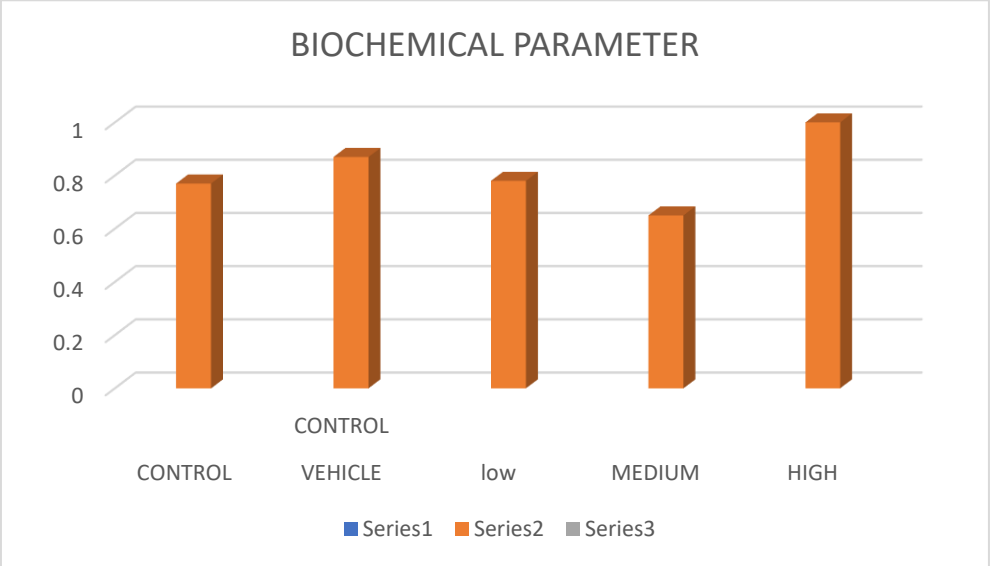


Fig 37: Effect of sub- acute toxicity study (28 days) of *VSSK on* Biochemical parameters (Bilirubin)

The Bilirubin values in experimental rat. Final study, not significant ($p<0.05$) in the bilirubin values are. The values are expressed as mean ± S.E.M. n=6 was compared in Group I and other groups II, III, IV, and V.

Table:35 :EFFECT OF SUB-ACUTETOXICITY (28 DAYS) OF ON FOOD INTAKE IN GRAM

GROUP	CONTROL	VEHICLE CONTROL	LOW	MEDIUM	HIGH
1st DAY	22.32±**0.45**	28.68±**0.68**	31.90±**0.34**	29.46±**0.88**	25.87±**0.76**
7th DAY	35.75±**0.54**	38.67±**0.87**	39.98±**0.57**	32.87±**0.86**	34.90±**0.45**
14th DAY	29.65±**0.65**	24.78±**0.86**	27.98±**0.87**	36.57±**0.36**	22.90±**0.35**
21st DAY	40.56±0.35	39.64±0.46	34.90±0.36	24.60±0.46	37.90±0.35
28th DAY	36.67±0.65*	31.76±0.47*	29.90±0.46*	39.56±0.78*	39.90±0.35*

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

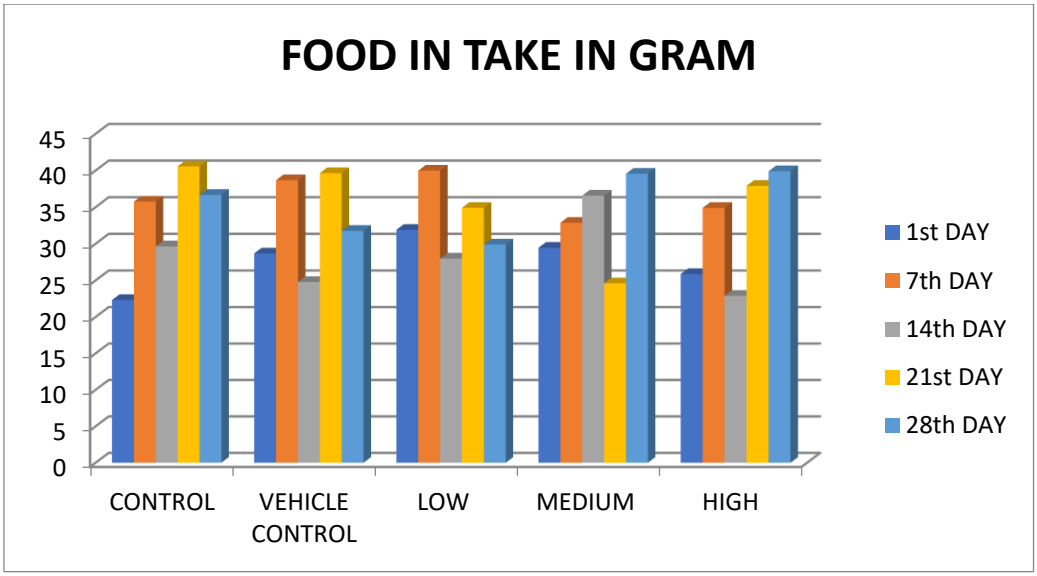


Fig 38: Effect of sub- acute toxicity study (28 days) of *VSSK on* food intake in gram

The Food intake values was compared in Group I to other groups II, III, IV, and V. in experimental rat. Final study, significantly increase in the Food intake values . The values are expressed as mean ± S.E.M. n=6.

Table 36:. Effect of Sub- acute toxicity (28 Days) Of *VSSK* On Water Intake in ml

GROUP	CONTROL	VEHICLE CONTROL	LOW	MIDUM	HIGH 2000
1st DAY	10.32±0.24	11.75±0.78	12.45±0.65	13.25±0.67	13.80±0.56
7th DAY	14.24±0.65	15.65±0.76	16.25±0.66	16.98±0.76	17.25±0.76
14th DAY	18.64±0.46	19.80±0.78	20.30±0.76	20.80±0.87	21.78±0.45

21st DAY	22.65±0.75	23.43±0.54	23.89±0.78	24.64±0.56	25.76±0.75
28t9h DAY	26.55±0.56	27.57±0.66	28.12±0.54	28.87±0.68	29.57±0.76

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

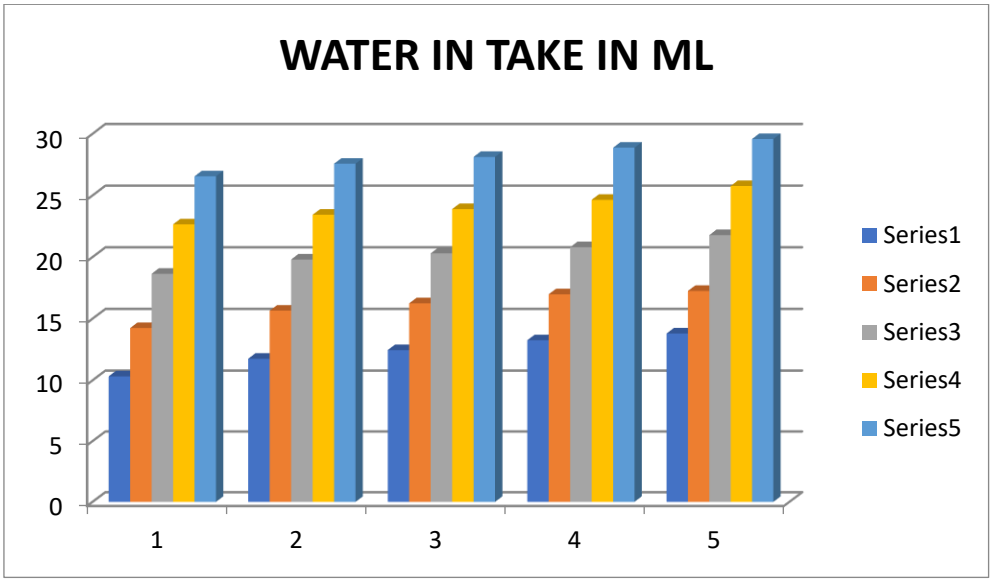


Fig 40: Effect of sub- acute toxicity study (28 days) of *VSSK on* water intake in ml

The water intake values was compared in Group I and other groups II, III, IV, and V. in experimental rat. Final study, significantly increase in the water intake. The values are expressed as mean ± S.E.M. n=6

Table 37 : EFFECT OF SUB -ACUTE TOXICITY (28 DAY) OF *VSSK* ON ELECTROLYTES: -

GROUP	CONTROL	VEHICLE CONTROL	LOW (300mg/kg)	MEDIUM (1000mg/kg)	HIGH (2000mg/kg)
Sodium (mmol/L)	136.56±0.64	145.57±0.57	139.56±0.57	144.56±0.57	140.67±0.75

chloride(mmol/L)	94.34±0.65	91.76±0.67	97.76±0.57	95.86±0.56	99.67±0.67
potassium(mmol/L)	4.90±0.57	3.36±0.46	4.56±0.57	3.50±0.46	5.00±0.02

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

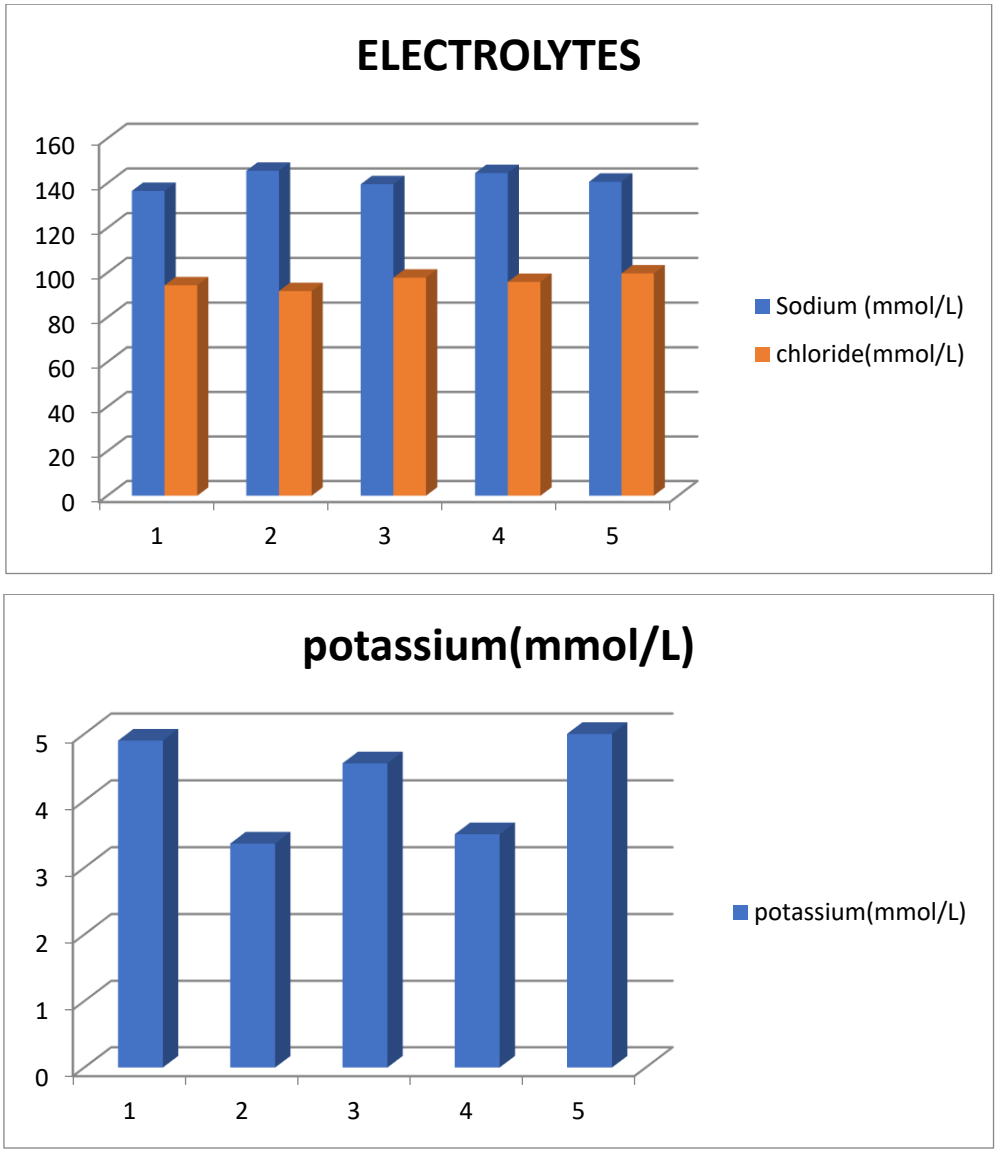


Fig 40: Effect of sub- acute toxicity study (28 days) of *VSSK on* electrolytes

There was no significant changes in the Electrolyte level in all the treated animals compared to the control. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV and V

Discussion

All animals from control and all the treated dose groups survived throughout the dosing period of 28 days. The results for body weight determination of animals from control and different dose groups show comparable body weight gain throughout the dosing period of 28 days. During dosing period, the quantity of food and water consumed by animals also significantly increase. The results of hematological investigations conducted on day 29th day revealed no significant changes in the hematological values when compared with those of respective controls. This gave clear justification that bone marrow and spleen were not influenced by *VSSK*. The clinical biochemistry analysis was done to evaluate the possible alterations in hepatic and renal functions not influenced by the test drug . Results of Biochemical investigations conducted on days 29 and recorded in revealed the no significant changes in the values of different parameters studied when compared with those of respective controls; Urea, SGOT,SGPT, Bilirubin were within the limits. Group Mean Relative Organ Weights are recorded Comparison of organ weights of treated animals with respective control animals on day 29 was found to be normal comparable with respective control group.

CONCLUSION

acute and subacute toxicity were carried out in wister albino 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 observation, haemotological and biochemical investigations were done. There were no significant changes in the biochemical and haematological profile. So the toxicological study of these test drug, VSSK establish the safety of the drug for long time administration.

PHARMACOLOGICAL STUDY RESULTS

ANTI INFLAMMATORY ACTIVITY OF VATHA SILETPANA SURA KUDINEER

Table 38: Acute Anti inflammatory activity of vatha siletpana sura kudineer

	treatment	1hr	2hr	3hr	4hr	% of inhibition
group 1	carragenan(1% w/v)	1.54	3.58	3.85	4.10	-
group 2	carragenan(1% w/v)+indomethacin(10mg/kg)	1.64	2.48	2.48	2.08	48.04
group 3	carragenan(1% w/v)+low dose(200mg/kg)	1.52	2.85	2.74	2.84	30.73
group 4	carragenan(1% w/v)+high dose(400mg/kg)	1.25	2.92	2.10	2.14	47.80

Result and discussion

The carrageenan-induced hind paw oedema model in rats is known to be the acute inflammatory model sensitive to cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of nonsteroidal anti-inflammatory agents (NSAID), which primarily inhibit the cyclooxygenase involved in prostaglandin (PG) synthesis. In case of the time course of oedema development in carrageenan induced paw edema model in rats is generally two phases are found. The first phase, which occurs between 0 to 2.5 h of injection of the phlogistic agent, has been attributed to the release of histamine or serotonin. The edema volume reaches to its maximum approximately 3 h post treatment and then begin to decline. The second phase of inflammatory reaction which is measured at 3h is caused by the release of bradykinin, protease, prostaglandin and lysosome. Therefore, it can be inferred that the inhibitory effect of the extract on the carrageenan induced inflammation could be due to the inhibition of enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. Thus, the results of the present study demonstrate that the *VSSK* exhibited acute anti-inflammatory activity in

the tested models which was found to be the most effective at higher concentrations employed.

CHRONIC ANTI-INFLAMMATORY ACTIVITY OF VSSK

Table 39 : Evaluation of chronic anti-inflammatory activity of VSSK

S NO	TREATMENT	GRANULOMA	% OF INHIBITION
1	CONTROL	78.01±0.21	----
2	INDOMETHACIN	29.02±0.07***	62.80
3	200mg/kg	50.09±0.04***	35.80
4	400mg/kg	33.10±0.10***	57.57

*Values expressed in mean ±SEM (Dunnet test), ***P<0.001 compared to control.*

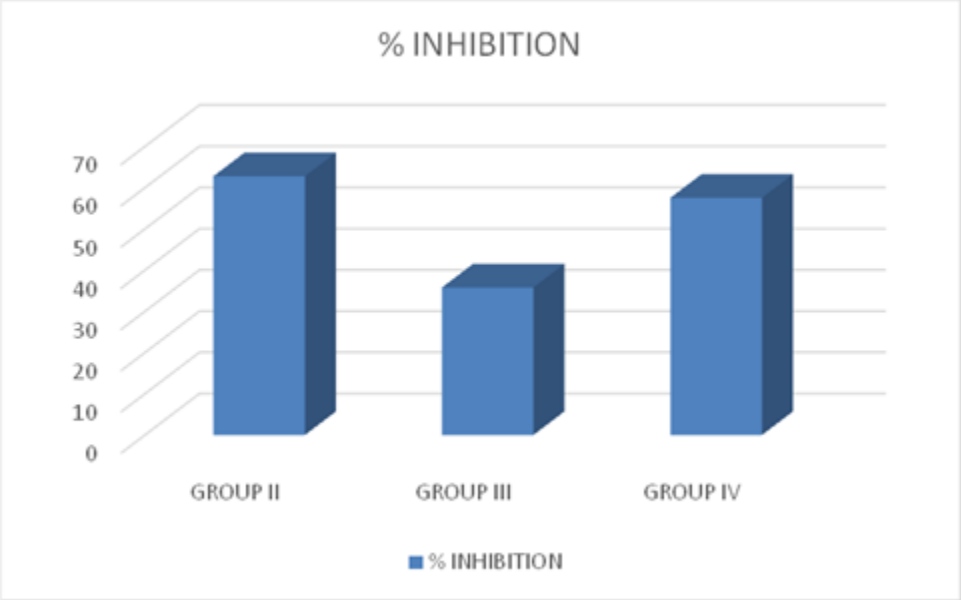


Fig 41: Evaluation of chronic anti-inflammatory activity of VSSK

The percentage of inhibition of granuloma in Cotton pellet granuloma pouch method is shown in table. From this result it was observed that both doses of *VSSK*(VSSK200 mg and VSSK 400 mg)

The percentage of inhibition of VSSK 200 mg and VSSK400 mg were 35.80% and57.57 % respectively which indicated the dose dependent activity of VSSK 200mg exhibited percentage of inhibition more than VSSK *400 mg* and slightly less than the reference drug Indomethacin (10mg/kg) which produced 62.80% of inhibition.

DISCUSSION

The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of chronic inflammation . It has three distinct phases: a first phase which involves transudative phase where the wet weight of the pellet increased during the first 3 hours, Second phase called an exudative phase in which plasma leaking from the blood stream around the granuloma that occurs between 3 hours and 72 hours after the cotton pellet implantation and a final third phase is proliferative phase in which the dry weight of the granuloma increased during the 3 to 6 days after the implantation . The result of present study revealed that the trial drug *VSSK* 200 and *VSSK* 400 decrease the granuloma weight significantly in a dose dependent manner. Presence of various chemical constituents in *VSSK* justifies this anti inflammatory activity. Hence, the drug used for the trial *VSSK* proved to be a better alternative for the commercially available allopathic drugs.

Conclusion

In conclusion, results showed *VSSK* has significant anti inflammatory properties. The *VSSK* showed dose dependant anti inflammatory activity in formalin induced edema and cotton pellet granuloma pouch method. This study results confirmed the validity of traditional indications of *VSSK* in inflammatory disease conditions

ANALGESIC ACTIVITY OF VATHA SILETPANA SURA KUDINEER

Table 40 : Analgesic activity of VSSK

GROUP	DOSE	Mean latency before and after drug administration				% inhibition		
		0 min	30 min	60 min	90 min	30min	60min	90min
Group I	Vehicle	4.63±0.230	5.52±0.237	4.43±0.198	5.42±0.270	·	.	.
Group II	10	3.14±0.098	8.43±0.635	6.24±0.655	17.45±1.008	34.51	29.00	68.93
GROUP III	200	5.13±0.084	7.25±0.285	8.13±0.786	9.52±0.817	23.86	45.51	43.06
Group IV	400	5.17±0.018	6.52±0.872	9.62±0.524	11.51±0.475	15.33	53.95	52.91
GROUP	DOSE	Mean latency before and after drug administration				% inhibition		
		0 min	30 min	60 min	90 min	30min	60min	90min
Group I	Vehicle	4.63±0.230	5.52±0.237	4.43±0.198	5.42±0.270	·	.	.
Group II	10	3.14±0.098	8.43±0.635	6.24±0.655	17.45±1.008	34.51	29.00	68.93
GROUP III	200	5.13±0.084	7.25±0.285	8.13±0.786	9.52±0.817	23.86	45.51	43.06
Group IV	400	5.17±0.018	6.52±0.872	9.62±0.524	11.51±0.475	15.33	53.95	52.91

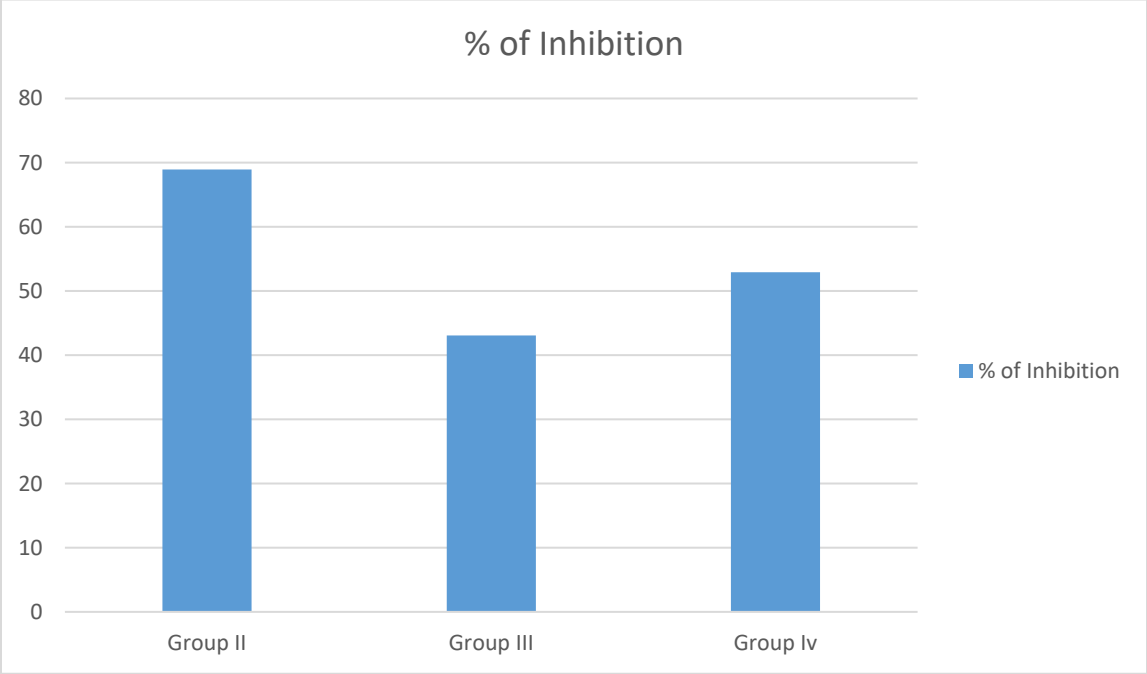


Fig 42: Effet of Analgesic activity of VSSK

Results of hotplate test are presented in Table for drugs respectively. The drug were found to exhibit a dose dependent increase in latency time when compared with control. At 90 minutes, the percent inhibition of two different doses (100 and 200 mg/kg body weight) was 43.06% &52.91% respectively. The results were found to be statistically significant ($p<0.001$)

Discussion

The drug of both the plants doses showed significant analgesic action compared to the reference drug diclofenac sodium but *drug 200 /kg* was found to exhibit higher analgesic activity

ANTIOXIDANT ACTIVITY OF VATHA SILETPANA SURA KUDINEER

Table 41 : Result Analysis of DPPH radical scavenging Assay of VSSK

S.No	Concentration	Ascorbic acid (Standard)		VSSK	
		Absorbance	% inhibition	Absorbance	% inhibition
1	20	1.058 ± 0.0017	88.29 %	0.998 ± 0.023	22.04%
2	40	0.808 ± 0.0015	96.50%	0.892 ± 0.002	27.20%
3	60	0.680 ± 0.0020	109.20%	0.760 ± 0.051	31.39%
4	80	0.468 ± 0.0025	126.40%	0.652 ± 0.020	39.99%
5	100	0.273 ± 0.0026	130.00%	0.569 ± 0.010	46.95%
6	250	0.180 ± 0.0028	150.05 %	0.493 ± 0.019	56.90%
7	300	0.230 ± 0.0031	162.30%	0.389 ± 0.121	65.15%
	Ic 50 values		Ic_{50}= 6.1 µg/ml		Ic_{50} = 26.92µg/ml

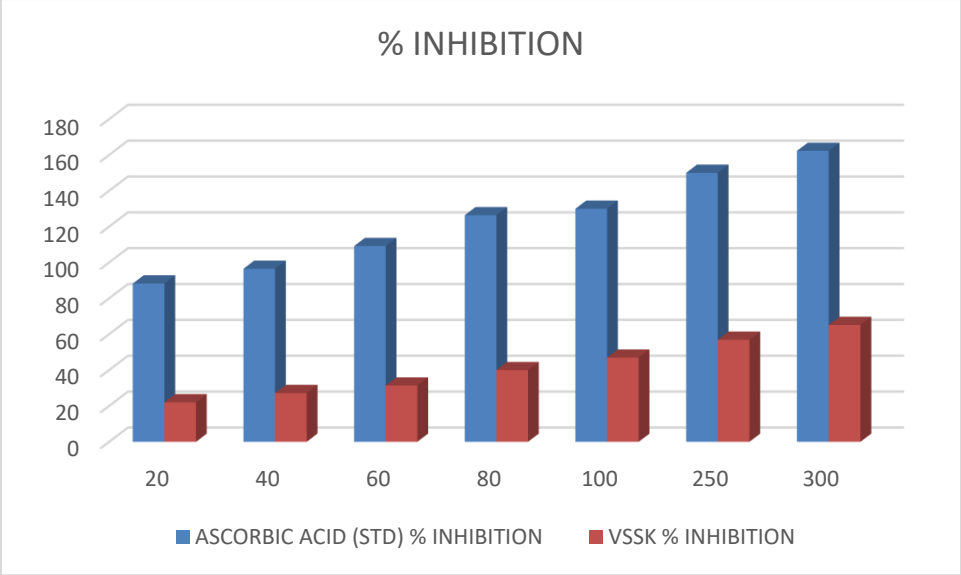


Fig 43 : Effect of Antioxidant activity of VSSK

The results of DPPH radical scavenging assay of the sample VSSK shows that the test drug possesses concentration dependent scavenging activity on DPPH radicals.The value of DPPH free radical scavenging activity of the VSSK was given in(Table 1 and Figure 1). The extract of VSSK showed the highest DPPH scavenging activity 65.15% at conc 300 and the lowest percentage of inhibition(22.04 %) at conc 20 . Ascorbicacid (Standard)showed highest percentage of inhibition(162.30%)at 300 and the lowest percentage of inhibition 88.29 at conc20.

Discussion

Free radicals and other oxidants have gained importance in the field of biology due to their central role in various physiological conditions as well as their implication in a diverse range of diseases. The free radicals, both the reactive oxygen species (ROS) and reactive nitrogen species (RNS), are derived from both endogenous sources (mitochondria, peroxisomes, endoplasmic reticulum, phagocytic cells etc.) and exogenous sources (pollution, alcohol, tobacco smoke,heavy metals, transition metals, industrial solvents, pesticides, certain drugs like halothane, paracetamol, and radiation). Free radicals can adversely affect various important classes of biological molecules such as nucleic acids, lipids, and proteins, thereby altering the normal redox status leading to increased oxidative stress . Large number of medicinal plants has been

investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress.Substantial evidence has accumulated and indicated key roles for reactive oxygen species (ROS) and other oxidants in causing numerous disorders and diseases. The evidence has brought the attention of scientists to an appreciation of antioxidants for prevention and treatment of diseases, and maintenance of human health . Human body has an inherent antioxidative mechanism and many of the biological functions such as the anti-mutagenic, anti- carcinogenic, and anti-aging responses originate from this property . Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells . Recently interest in naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products, because they possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance. The results of DPPH radical scavenging assay of the sample VSSK shows that the test drug possesses concentration dependent scavenging activity on DPPH radicals with the highest percentage inhibition of about 65.15%.

Conclusion

Imbalance between the antioxidants and oxidant leads to increased generation of free radicals which in turn causes vigorous damage to macromolecules such as nucleic acids, proteins and lipids. This leads to tissue damage in various disease conditions such as diabetes mellitus, neurodegenerative diseases, cancer, cardiovascular diseases, cataracts, rheumatoid arthritis, asthma etc. and thus severely hastening the disease progression. From the result obtained from the present investigation it was concluded that the formulation VSSK possess significant antioxidant property and may act therapeutically in treating several oxidative stress related disorder’s. Further present investigation had generated an evidence based data with respect to purity, standards and antioxidant potential of the formulation VSSK.

ANTIMICROBIAL STUDY

Table: 42: Anti-microbial potential of aqueous extract of Vatha Siletpana Sura Kudineer (VSSK)

Sample Code and Conc.		Bacteria Strains Name and Zone of inhibition (mm in diameter)			
VSSK	***Staphylococcus aureus*** **(G+)**	***Pseudomonas aeruginosa*** **(G -)**	***E.coli (G-)***	***Proteus vulgaris (G-)***	***Candid albicans***
25	-	-	-	-	-
50	-	-	-	-	-
75	-	-	-	-	8
100	-	-	-	10	10
Positive Control (Streptomycin 25mg)	18	14	10	12	13
Negative Control	-	-	-	-	-

Keywords: ***PC*** *Positive control (Streptomycin),* ***NC*** *Negative control, "-" No Zone,* ***mm*** *(Millimetre),* ***G+*** *(Gram Positive Organism),****G-*** *(Gram Negative Organism),*

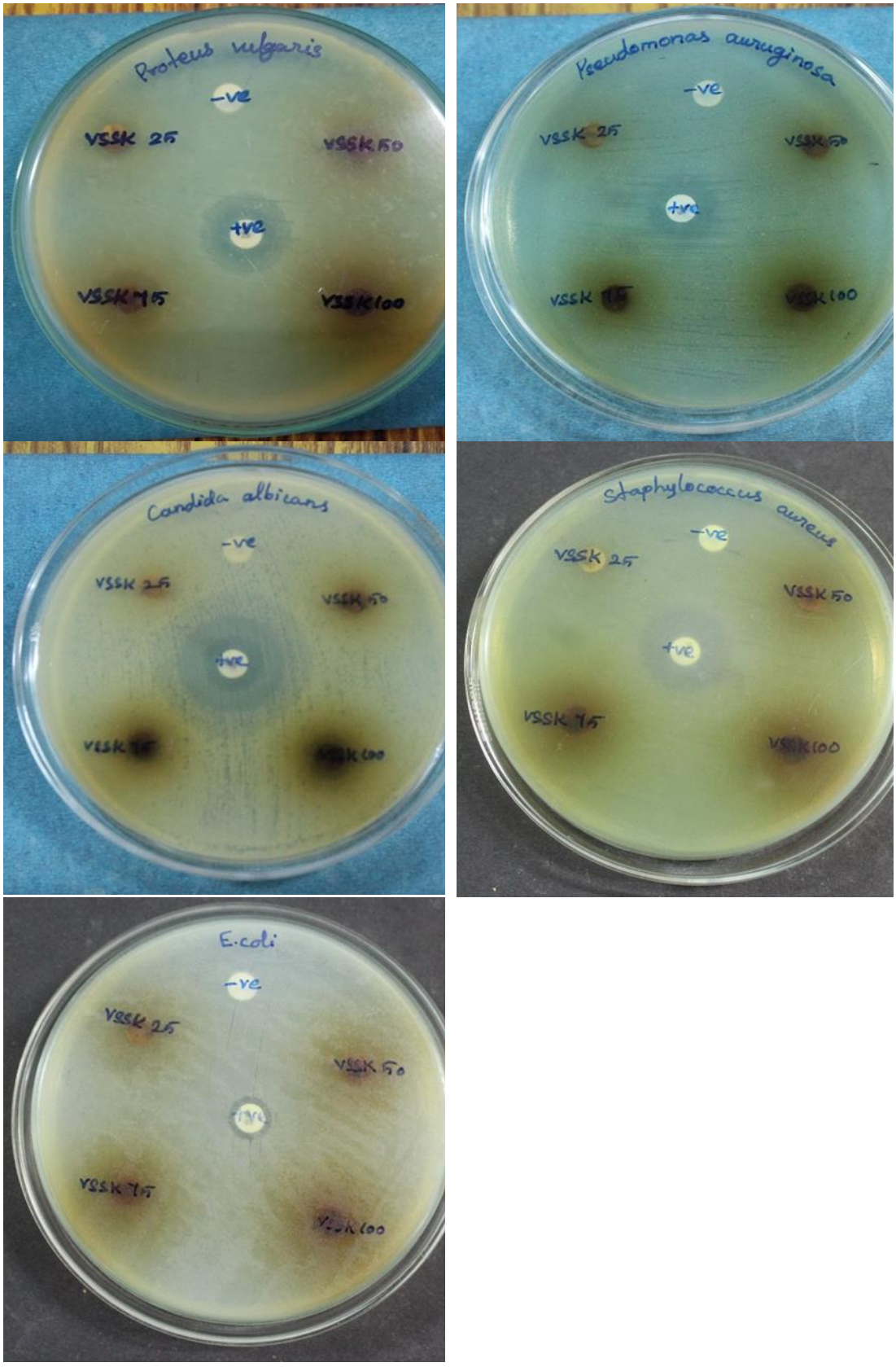


Figure: 44: Anti-bacterial potential of Aqueous extract of VSSK

INTERPRETATION

The sample *VSSK* was screened for their antimicrobial activities against both bacterial (Gram+ve and Gram-ve) and fungal pathogens using agar well diffusion method along with standard broad-spectrum antibiotics for bacterial pathogens. After incubation, the zone production on the plates were read as per the standard methodand correlated with the result of standard antibiotics. The results illustrated that the given samples have antimicrobial activity and anti fungal activity against the tested pathogens in higher concentrations given in the table . VSSK shows anti bacterial potential at 75mg and 100mg dosage and anti fungal potential at 100 mg dose.

06. SUMMARY

In this dissertation work, I have selected the Siddha formulation *Vatha Siletpana Sura Kudineer* is a herbal formulation contains nine ingredients of *Solanum xanthocarpum, Mollugo cerviana, Clerodendrum serratum, Terminalia chebula, Tinospora cordifolia, Saussurea lappa, Piper longum, Kaempferia galangal and Alpinia officinarum* are used as ingredients for the preparation which is mentioned in Siddha Literature of, ***Pararajasekaram- suram, sanni, vali, vikkal, sathi roga nithanankal part- III , Author ponniayah.I. page no. 24-25.*** The drug is useful for the treatment of ***Vatha kapha suram,*** hence it has been selected for its Anti inflammatory, Analgesic and Anti oxidant activities.

Collection of literature reviews regarding the ingredients of trial medicine carried out in *Siddha* and modern literatures to support the fact of Anti inflammatory, Analgesic and Antioxidant activities

All the ingredients of the trial drug *VSSK* were purchased from *M.Gopalan aasan* store, Nagercoil, Kanyakumari District. Each ingredient of the trial drug is verified and authenticated by the *Gunapadam* experts, Department of *Gunapadam*, Government *Siddha* Medical College, Palayamkottai. The trial drug *KC* was prepared as per the procedure given in the above mentioned Literature.

The *Siddha* Standardization of the trial drug *VSSK* indicates the drug is brown in colour, pleasant odour, Pungent and bitter taste, coarse powder in appearance and rough to touch. Based on the *Siddha* aspect, *vatha kapha suram* is caused by the dearrangement of vatham and kapham. The increased *Kapha* humor is normalized by administering the taste which containing fire elements (*theyu boodham*). This trial drug *VSSK* is pungent and bitter taste and has hot potency which can be normalize the deranged *vatha and Kapha* humor. Therefore the derangement of *Vatha and Kapha* humor is gradually normalized by the administration of this trial drug *VSSK*. Hence *VSSK* relieves the basic causes of *Vatha Kapha suram.*

After the preparation of *VSSK*, it was screened for various standardization parameters such as the *Siddha* standardization methods as well as the Modern standardization methods. As per *Siddha* standardization methods, *VSSK* had all the characteristics of properly prepared *kudineer chooranam.*

As per modern standardization methods, following parameters were followed. The Physico-chemical analysis , Bio-chemical analysis , Phyto - chemical analysis , Microbiological Analysis ,Instrumental analysis such as Scanning Electron Microscope (SEM) ,The chemical fingerprints are engaged by using modern analytical technique Fourier Transform Infra–Red Spectroscopy (FTIR), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), The chemical fingerprints are engaged by using modern analytical technique Powder X-ray (EDAX) (Energy Dispersive X-ray Analysis) diffraction methods and the Experimental analysis such as the Toxicological Studies & The Pharmacological studies.

Physicochemical analysis of *VSSK* shows

The percentage of loss on drying at 105ºc is 1.50%. It is within the acceptable range.

The Water soluble ash value of the trial drug *KC* was 9.90% and acid insoluble ash is 2.75%. The Water soluble ash value is higher than the acid insoluble ash. It represents the good quality of the drug *VSSK* and it is easily absorbed in the gut. Acid insoluble ash value is very small amount of the inorganic component is insoluble in acid, lower the acid insoluble value better will be the drug quality.

A water soluble extractive value of *VSSK* is 9.90%. Higher the Water soluble extractive value implies that the water is better solvent of the extraction.

VSSK shows acidic pH 7.10. The pH level plays a role in enzyme activity by maintaining the internal environment thus regulating the homeostasis. Very high or very low pH will lead to the complete loss of the activity of most enzymes. The pH value at which the enzyme is most active is called the optimal pH value. The pH value of the trial drug *VSSK* falls near to the neutral pH value. Hence it has optimal enzymatic reaction.

Biochemical analysis shows,

Biochemical analysis of ***VSSK*** reveals that, the trial drug consists of Calcium, Sulphate, Starch, Ferrous iron, Tannic acid, Unsaturated compounds, Reducing sugar, and Amino acids.

Calcium: Calcium citrate is an effective anti inflammatory agent. There are calcium-sensing receptors on vascular smooth muscle cells and on platelets, calcium plays a role in smooth muscle contraction and its role in the electrophysiology of the heart and myocardial function. Antioxidant enzyme responses depend on calcium levels. Calcium carbonate, calcium citrate and calcium gluconate have significant anti inflammatory activity.

Sulphate Chondroitin sulfate (CS) prevents joint space narrowing and reduces joint swelling and effusion. To produce these effects, CS elicits an anti-inflammatory effect at the chondral and synovial levels.. Sulphate important role for the anti-microbial activity.

Starch It is a odourless tasteless white substance occuring widely in plant tissue. It is a polysaccharide functions as a carbohydrates store and is an important constituent of the human diet**.** Resistant starch is divided into five different types based on the origin and physical properties of starch. It can produce more butyrate in comparison to other prebiotics. Butyrate is the main SCFA that is produced from the fermentation of RS and acts as an anti-inflammatory agent. Starch is needed during fever condition.

Ferric iron and ferrous iron: Iron is an essential element for blood production. About 70 percent of the body's iron is found in the red blood cells of blood called hemoglobin and in muscle cells called myoglobin. Hemoglobin is essential for transferring oxygen in blood from the lungs to the tissues. In the ferrous state (Fe^{2+}), iron acts as an electron donor, while in the ferric state (Fe^{3+}) it acts as an acceptor.

Tannic acid Tannic acid is a natural polyphenol which has been reported to possess antioxidant, anti-inflammatory, anticarcinogenic, antimutagenic, antitumor, and antimicrobial activities.

Unsaturated compounds: In other tissues and cell types, unsaturated fatty acids have well known anti-inflammatory effects, which range from the inhibition of the lipoxygenase and cycloxigenase pathways and decrease of neutrophil adhesion to the reduction of inflammatory cytokine expression and inhibition of TLR4 signaling .

Reducing sugar relaxes mucus, lessens cold and cough symptoms.

Amino acids: N-acetyl cysteine for cough and other lung conditions. It is also used for flu, dry eye, and many other conditions. NAC is also useful to help fight long-term lung damage in those with chronic obstructive pulmonary disease (COPD). Amino acids contribute to various anti-oxidant and immunological activities relevant to asthma pathogenesis, raising the possibility that differences in amino acids may be involved in asthma aetiology. Cystine reduces the risk of asthma via glutathione metabolism.

The phytochemical screening of the alcoholic and aqueous extract of the *VSSK* reveals,

Gas chromatography mass spectroscopy analysis was carried out in crude extracts of the *MLC* such as ethanol extract. The peaks in the chromatogram were integrated and were compared with the database of spectrum of known components stored in the GC-MS library. The detailed of GC-MS analysis of the extracts are given in figures. This study shows the presence of those compounds such as 1,3,12-nonadecarine, 2-propenamine, 3,1 [cyclohexanyl]-N-cyclohexanyl-N-oxide, 1-octane, 2-methoxyl, 2-carboxymethyl, 3-methyl- cyclopentano carboxylic acid, ursodeoxcholic acid.

In instrumental analysis,

The SEM photographs revealed that particles were spherical in shapes and sizes were in the range from 1μm to 300 nm. 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 micro particles. ***VSSK*** exhibited larger sizes and agglomeration of the particles. SEM analysis of the ***VSSK*** shows most of the particles present in the sample are micro size, average particle size is 1μm - 300nm

In FT-IR spectra analysis, VSSK exhibits the peak value at 2929.87, 2360.87, 1514.12, 1373.32, 1246.02,1161.15, 1024.20, 927.76, 862.18, 771.53, 572.86, 522.71, 437.84, 412.77 having O-H stretch, none, O-N-O stretch, N-O stretch, C-N stretch, C-O stretch, None, C=C Bend, C-Cl stretch, C-Br stretch, C-I stretch respectively. This peak indicates the presence of some organic functional groups such as, Carboxylic acid,

Isothianate, nitro compounds, amine, tertiary alcohol, alkenes, alkyl halides & aryl halides.

Nitro compounds has anti inflammatory, analgesic, antioxidant ,anti proliferative, . it can act against infectious diseases, it has anti tubular activity, and anti parasitic activity.Carboxlic acid acts as Anti inflammatory , Analgesic , Anti pyretic and cytotoxic , Anti oxidant ,It depresses cough and its symptoms .Amines has anti inflammatory, antioxidant,Anti tussive, Bronchodialator activities. Alkl and Aryl halides has anti inflammatory ,Anti microbial , Anti niociceptive activities.Alcohols has analgesic activity .

In ICP – OES, the formulation contains heavy metals are in below detectable level. This results shows Below Detectable Limit (BDL) of Al (Aluminium), As (Arsenic), C (Carbon), Cd (Cadmium), Cu (Copper), Fe (Iron), Hg (Mercury), K (Potassium), Mg (Magnesium), Na (Sodium), S (Sulphur) and Zn (Zinc). So it is considered as safe and free from toxic substances.

This XRD fingerprint shows both the similarities and differences of the sample successfully and is a valuable primary tool for checking the quality control of minerolo metallic formulations. The different peaks show the presence of minerals in the samples.

Crystallinity refers to the degree of structural order of a solid. The Percentage of crystalinity of the *VSSK* is 27.5 %. Increasing the degree of crystallinity increases hardness and density. It is a Meto – mineral preparation, hence it has the high value of cristalinity. Amorphous means, noncrystalline solid in which the atoms and molecules are not organized in a definite lattice pattern. The Percentage of Amorphous of the *VSSK* is 72.5 %.

Results of microbiological study shows,

In microbiological limit, Total viable aerobic bacterial counts and total fungal count are within the normal level. Specific pathogens like Salmonella sp, Staphylococcus aureus and E.coli are Nil. Hence, the test drug *VSSK* is free from any

microbial Contamination and it has standard quality. But the trial drug has some range of Pseudomonas sp.

The results of **antimicrobial activity**, illustrated that the given samples had shown antibacterial activity against the tested pathogens including *Proteus vulgaris and candida albicans* at higher concentrations. However, the results showed that the sample had no antimicrobial activity against *Staphylococcus aureus* , *Pseudomonas aeruginosa* and *E-coli.*

In acute oral toxicity study,

The rats were treated with different concentration of *VSSK* from the range of 5mg/kg to 2000mg/kg. This dose level did not produce the signs of toxicity, functional and behavioural changes and mortality in the test groups as compared to the control when observed during 14 days of experimental period. From acute toxicity study it was observed $^{ns}p > 0.05$ that the administration of *VSSK* 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) at 2000 mg/kg.

In Sub - acute oral toxicity study,

Acute and sub-acute toxicity were carried out in Wister albino rats according to OECD guidelines (423 & 407). 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 observation, haemotological and biochemical investigations were done. There were no significant changes in the biochemical and haematological profile. So the toxicological study of this test drug, *VSSK* establish the safety of the drug for long time administration.

In Pharmacological studies,

The Anti inflammatory activity of *VSSK* studied by in-vivo method shows, oedema development in carrageenan induced paw edema model in rats is generally two phases are found. The first phase, which occurs between 0 to 2.5 h of injection of the phlogistic agent, has been attributed to the release of histamine or serotonin. The edema volume reaches to its maximum approximately 3 h post treatment and then begin to decline. The second phase of inflammatory reaction which is measured at 3h is caused by the

release of bradykinin, protease, prostaglandin and lysosome. The inhibitory effect of the extract on the carrageenan induced inflammation could be due to the inhibition of enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. *VSSK* exhibited acute anti-inflammatory activity in the tested models which was found to be the most effective at higher concentrations employed.

Analgesic activity shows that VSSK found to exhibit a dose dependent increase in latency time when compared with control. At 90 minutes, the percent inhibition of two different doses (100 and 200 mg/kg body weight) was 43.06% &52.91% respectively. The results were found to be statistically significant ($p<0.001$) . VSSK of both the plants doses showed significant analgesic action compared to the reference drug diclofenac sodium but *drug 200 /kg* was found to exhibit higher analgesic activity.

Under this study, In - vitro Antioxident activity of Test drug *VSSK* VSSK shows that the test drug possesses concentration dependent scavenging activity on DPPH radicals with the highest percentage inhibition of about 65.15%. So, the present research proposes that, the Test drug *VSSK* has moderate Antioxidant activity. Thus the formulation may be a source of effective herbal drug..

Finally, Toxicological study of Acute, sub-acute toxicity of *VSSK* represent nontoxic and safe drug in rats. As per Siddha literature the primary cause of *Vatha kapha suram* with increased *vatha and kapha dosha* due to certain diets and activities. This *vadha dosha* in association with *kapha dosha* adversely affects the respiratory function such as difficulty in breathing, chest tightness,etc. and causes the disease. The test drug *VSSK* has pungent and bitter taste. Pungent taste of *VSSK* normalized the increased *vatha and Kapha doshas*.

Results and discussion give the necessary and essential justification to prove the potency of test drug with scientific validation. Based on the results presented in this study, it can be concluded that *Vatha Siletpana Sura Kudineer* exerts significant Anti inflammatory, Analgesic and Antioxidant activities.

07. CONCLUSION

It is concluded that the trial drug *VATHA SILETPANA SURA KUDINEER* has significant Anti inflammatory, Analgesic and Antioxidant activities. Anti inflammatory is a good fever reducer and also it relieve pain and reduce inflammation. Analgesic activity helps to relieve all the pains related to fever. Anti oxidant preotect the cells against free radicals, which may play a role in heart disease and other disease. The trial drug is scientifically validated by modern techniques and *Siddha* standard methods. The toxicological study of this trial drug establishes the safety of the drug for long time administration. Hence the trial drug can be safely used to human for *Vatha siletpana (Kapha) Suram.*

08. FUTURE SCOPE

Preclinical evaluation of the test drug *Vatha Siletpana Sura Kudineer* has been done by physio-chemical, bio-chemical, phytochemical, instrumental, toxicological, pharmacological and microbial standard prescribed procedures. In future the drug has to validate by extensive clinical trials as per WHO guidelines to understand the exact molecular mechanisms of action. This *Vatha Siletpana Sura Kudineer* can be used very much in treating vatha siletpana suram.

Thus, the ancient wisdom *Siddhars* will remains as one important source of future medicine and therapeutics.

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de Oliveira, R. B. (Synthesis and evaluation of the anti parasitic activity of aromatic nitro compounds). *European journal of medicinal chemistry*, 2011; *46*(11), 5443–5447

ANNEXURE

GOVERNMENT SIDDHA MEDICAL COLLEGE
PALAYAMKOTTAI
SCREENING COMMITTEE

DEPARTMENT OF GUNAPADAM

Name of the Candidate: Dr. VIDHYA MILANO PRASAD.

Candidate Reg No: 321912009

This is to certify that the dissertation topic **Preclinical Study of Siddha Drug " VATHA SILETPANA SURA KUDINEER" for its ANTI INFLAMMATORY, ANALGESIC AND ANTI OXIDANT** activities has been approved by the screening committee.

Branch	Department	Name	Signature
I	Pothu Maruthuvam	Dr.A. Manoharan MD(s), PhD Professor	A. T. Manoharan 5.11.20
II	Gunapadam	Dr.A. Kingsly MD(s), Associate Professor	5/11/20
IV	Kuzhandhai Maruthuvam	Dr.D.K. Soundararajan MD(s) Professor	05/11/20
V	Noi Nadal	Dr.S. Victoria MD(s) Professor	5/11/2020
VI	Nanju Maruthuvam	Dr.M. Thiruthani MD(s) Professor	5/11/2020
VII	Pura Maruthuvam	Dr.M. Ahamed Mohideen MD(S), Associate Professor	5/11/2020
VIII	Varma Maruthuvam	Dr.A. Muneeswaran MD(S), Associate Professor	05/11/2020
IX	Siddhar Yoga Maruthuvam	Dr.A.S Poongodikanthimathi MD(S) Professor	5/11/2020

Remarks:

5/11/2020

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Palayamkottai

Date: 5.11.2020
Place: Palayamkottai

Arulmigu Kalasalingam College of Pharmacy

(Approved by AICTE, PCI, New Delhi and Affiliated to The Tamil Nadu Dr.M.G.R. Medical University, Chennai)
Anand Nagar, Krishnankoil - 626 126. Srivilliputtur (Via), Virudhunagar Dist., Tamil Nadu
Phone: 04563-289006 Email: akcpprl@yahoo.com Website: www.akcp.ac.in

"Kalvivallal"
T.Kalasalingam, B.Com., Founder

"Ilayavallal"
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Dr.S.Shasi Anand, Ph.D., (USA) Secretary

Er.S.Arjun Kalasalingam, M.S., (USA) Director

Dr.N.Venkateshan, M.Pharm., Ph.D., Principal

CERTIFICATE

INSTITUTIONAL ANIMAL ETHICS COMMITTEE APPROVED BY CPCSEA, NEW DELHI.

Name of the principle investigator	: Dr. Vidhya Milano Prasad
Title of the Project	: Preclinical Study of Siddha Drug Vatha Siletpana Sura Kudineer for its Anti-inflammatory, Analgesic and Antioxidant activities
Proposal Number	: AKCP/IAEC/20/20-21
Date of received after modification (if any)	: Nil
Date of received after second Modification	: Nil
Approval date	: 25.11.2020
Animals	: Rats
Expiry Date	: Nil
Name of IAEC Chairperson	: Dr.N.Venkateshan

Signature of IAEC Chairperson

GOVERNMENT SIDDHA MEDICAL COLLEGE

PALAYAMKOTTAI-627002

CERTIFICATE OF BOTANICAL AUTHENTICITY

Certified that the following plants used in Siddha formulation **VATHA SILETPANA SURA KUDINEER** used as internal medicine for the management of **Vatha Kapha Suram** taken up for the post graduate dissertation study by **Dr. Vidhya Milano Prasad MD(S)** (Reg.No.:321912009) PG, Department of Gunapadam are correctly identified and authenticated through Visual inspection/ Experience/ Education and training/ Organoleptic characters/ Morphology and Taxonomical method

Tamil Name	Botanical Name	Family	Part used (Dried)
Kandankaattai	*Solanum xanthocarpum*	Solanaceae	Whole plant
Siruthekku	*Clerodendrum serratum*	Lamiaceae	Root
Kaddukkai	*Terminalia chebula*	Combretaceae	Fruit without seed
Patpadakam	*Mollugo cerviana*	Molluginaceae	Whole plant
Thippili	*Piper longum*	Piperaceae	Fruit
Kottam	*Saussurea lappa*	Asteraceae	Root
Seenthil	*Tinospora coridifolia*	Menispermaceae	Stem
Kachcholam	*Kaempferia galanga*	Zingiberaceae	Rhizome
Sittarathai	*Alpinia officinarum*	Zingiberaceae	Rhizome

Date : 27.07.2021

Station :Palayamkottai

27/7/21.

Authorized Signature

DR. R. ANTONY DURAICHI, M.D(s)
Lecturer Gr-II
Govt. Siddha Medical College
Palayamkottai, Tirunelveli Dist.

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

69, Anna Salai, Guindy, Chennai - 600 032.

This certificate is awarded to Dr. VIDHYA MILANO PRASAD

for participating as Resource Person / Delegate in the 33rd Workshop on

" How To Do a Good Dissertation & Publish? (Research Methodology and Biostatistics)"

For AYUSH Post - Graduates & Researchers organized by the Department of Siddha,

The Tamil Nadu Dr.M.G.R. Medical University from 24.02.2020 to 28.02.2020.

Dr.N.KABILAN
PROFESSOR & HEAD, DEPT.OF SIDDHA

Dr. M.B.ASWATH NARAYANAN
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Authored

By

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This certificate is presented to Dr. .....VIDHYA..MILANO...PRASAD..... *an acknowledgement of the contribution to the Conference as a* ~~DELEGATE~~ / PRESENTER *of a paper on* .SYSTEMATIC..REVIEW..ON..PLANTS....USED..IN..VATHA..SILEIPANA .....SURA...KUDINEER:.....*in* ORAL / ~~POSTER~~ *session, held on 11th & 12th February 2021 at Govt. Siddha Medical College, Palayamkottai, Tirunelveli - 627002.*

Dr. A. Rajarajeswari, M.D(s).,PGDB., PGDEpi.,
Lecturer, Grade - II
Research Methodology & Biostatistics
GSMC & H, Palayamkottai

Dr. A. Manoharan, M.D(s).,Ph.D.,
Vice Principal
GSMC & H, Palayamkottai

Dr. M. Thiruthani, M.D(s).,PGDYN.,
Principal
GSMC & H, Palayamkottai

Certificate of Appreciation

This certificate is awarded to

Dr.Vidhya Milano Prasad

for securing First place in "Poster Presentation" Competition (PG Category) entitled "Reiew on Vatha Kapha Suram in Siddha Medicine which can be corelated with covid 19 pandemic" conducted on the occasion of Fourth Siddha Day 2021 jointly organized by Central Council for Research in Siddha, National Institute of Siddha in association with Directorate of Indian Medicine and Homoeopathy with the support of Ministry of AYUSH held on 2nd January, 2021 through virtual platform.

Prof. Dr. R. Meenakumari
Director
National Institute of Siddha

Prof. Dr. P. Parthibhan
Joint Director
Directorate of Indian Medicine & Homoeopathy

Prof. Dr. K. Kanakavalli
Director General
Central Council for Research in Siddha

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