STANDARDIZATION AND PHARMACOLOGICAL SCREENING OF *SIDDHAR KULIGAI*

The dissertation Submitted by

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Standardization and Pharmacological screening of *Siddhar kuligai*"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.S.Visweswaran M.D(S)** Head of the Department i/c, Department of *Gunapadam*, National Institute of Siddha, Chennai – 47 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date: **Signature of the Candidate**

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CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"Standardization and Pharmacological screening of *siddhar kuligai*"**is submitted to The Tamilnadu Dr. M.G.R. Medical University in partial fulfillment of the requirements for the award of degree of M.D (Siddha) is the bonafide and genuine research work done by **Dr.R.Keerthana** under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date: **Seal &Signature of the Guide**

Place: Chennai **Dr.S.Visweswaran M.D(S)**

ENDORSEMENT BY THE HOD, PRINCIPAL / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"Standardization and Pharmacological screening of *Siddhar kuligai*"**is a bonafide work carried out by **Dr.R.Keerthana** under the guidance of **Dr.S.Visweswaran M.D(S).,** Head of the Department i/c, Department of *Gunapadam*, National Institute of Siddha, Chennai – 47

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1. INTRODUCTION

Siddha system is one of the oldest system of medicine in India. The term Siddha means achievements and Siddhars were saintly persons who achieved results in medicine. Siddha literature is in Tamil and it is practiced largely in Tamil speaking part of India and abroad. The Siddha System is largely therapeutic in nature.

All the systems of medicine mainly focus on the prevention and cure. Whereas Siddha system of medicine not only focus on prevention and cure but also emphasis in kaya kalpa i.e. making one's body immortal [1]. This form of medicine was professed and practiced by siddhars who were aware of the constitute of the body and mysteries of the mind their thought and teaching were crystalized in the form of a great system of medicine.

According to thirumoolar

மறுப்ப துடல் நோய் மருந்தென லாகும்
மறுப்ப துளநோய் மருந்தெனச்சாலும்
மறுப்ப தினிநோய் வாரா திருக்க
மறுப்பது சாவையு மருந்தென லாமெ''

- திருமூலர்

Siddha medicine has miraculous remedy for incurable, chronic and non communicable diseases, specific treatment for Gyneacological diseases mentioned in Siddha text. In our ancient literature Tholkaapiyam, Poruliyal version[2]. women are mentioned as follows,

'செறிவும் நிறைவும் செம்மையும் செப்பும் அறிவும் அருமையும் பெண்பாலான''

- தொல்காப்பியம்

Women suffered many chronic diseases from ancient times. Some of them are Maladu (infertility), Yoni Thabitha Noikal (pelvic inflammatory diseases), Marbusilanthi (breast cancer,) Vippurithi (tumour), Yoniputru (cervical cancer) etc. In the above mentioned diseases one of the life threatening diseases which reduces the quality of life of a woman Yoni putru may be correlates with cervical cancer in modern world which is considered to be the second most cause of death in women in India.

Cervical cancer is initially affects in the cervix, the lower narrow part of the uterus. These uncontrolled growth of cancer cells form a tumour. Most of the cervical cancers are caused by the Human Papilloma Virus (HPV) [3].

According to National Cancer Registry Program recent report of 2008, the load of breast and cervical cancer together was 23.6 to 38.7% of the total cancers [4]. In 2009 the number of cervical cancer cases were 1, 01,938 which has increased to 1, 07,690 in 2012. 55,000 new cases were reported per year in Tamilnadu during the period of year 2012 to 2016 [4a]. Day by day the morbidity is increasing. During the year 2007-2016 New cases registered – 123000 /year, Deaths – 67500 /year Median age – 38 years (age 21-67 years) [5].

Rural women are at higher risk of developing cervical cancer as compared to their urban counter parts. Cervical cancer is the second largest cause of cancer mortality in India accounting for nearly 10% of all cancer related deaths in the country. It is estimated that by the year 2020 there will be almost 20 million new cases[6]. For this life threatening disease radiation is combined with low dose chemotherapy however this modality often leads to severe toxicity.

The well known practice of chemotherapy to reduce the risk factor of cancer also may leads to many adverse effects such as nausea, vomiting, alopecia (loss of hair), bone marrow depression, amenorrhoea in women like major problems [7]. So the failure of conventional chemotherapy to reduce mortality invites attention towards new alternative approaches that would reduce morbidity as well as side effects conferred by conventional chemotherapy.

Recently, a greater emphasis has been given towards the involving traditional Siddha medicine that includes herbal, Herbo -mineral and metallic preparations has been used from the immemorial to treat chronic ailments such as cancer. Siddhar are well known masters in preparation of a Herbo-mineral formulation by proper purification, using herbal juices to reduce the toxicity of the metals A Siddha text clearly specifies use of Mercury, Sulphur, Copper, Arsenic and Gold as therapeutic agents [8]. Siddha system of medicine believes that Herbo-mineral formulation to be more effective for chronic diseases [9]Up to date, lesser studies have been conducted on standardization of such

preparations [10]. In view of above the trial drug *siddhar kuligai* may reach the next higher level in treating cervical cancer by the upcoming activity on pharmacological and analytical studies. An integrated approach is the need of the day to manage cancer using the growing knowledge gained through scientific development

The Author is interested in proving the trial drug *siddhar kuligai* which is literally evident as told by great scientists Siddhar, but there is no scientific data. The need of the hour is to develop a for scientific validation which may explore the wealth of siddha medicine to global level. So the author chosen this formulation for the standardization and pharmacological screening (anti inflammatory, analgesic activity & Anti-cancer activity in in-vitro HeLa cell line models .

2. AIM AND OBJECTIVES

Aim

To ***Standardize and evaluate the Pharmacological screening of the test drug "Siddhar Kuligai"*** in an animal model and anti-cancer activity in in-vitro cell line model.

OBJECTIVES

- Collection of various information relevant to the study from various *Siddha* and modern literature
- Identification of the ingredients
- Preparation of the test drug as per classical Siddha literature.
- Standardization of the prepared test drug Siddhar kuligai as per AYUSH Guidelines.
- Physicochemical and phytochemical analysis
- chemical analysis for determining acidic and basic radicals.
- HPTLC fingerprinting of test drug.
- Estimation of elements through instrumental analysis.

Pharmacological activities

Facts to be proved by pharmacological activities include

- Anti-inflammatory activity - Carrageenan-induced rat paw edema in animal model
- Analgesic activity - Hot plate method in animal model
- Anti-cancer activity - In-vitro HeLa cell line models by XTT assay

3. MATERIALS AND METHODS

SOP for preparation of *"Siddhar kuligai"*

The test drug *siddhar kuligai*, mentioned in siddha text Veeramamunivar Vagata Thiratu part 1 has been used for Parpala Viranangal, Kiranthi, Rongangal, Yoniputru, Soolaigal, Kallipukiranthi Vippurathi,8 Vagaigunmam, MaegaRanangal[11]

Ingredients

Chithiramoola Verpatai Chooranam (Plumbaco zylanica)	-1.25 varagan(5.25 gram)
Purified Rasakarpooram (Mercurous chloride)	- 1.25 varagan(5.25 gram)
Ooma Chooranam (Carum copticum)	- 1.25 varagan(5.25 gram)
Panaivellam (Jaggery)	-3.4varagan(14 grams)

Procurement of Raw Drugs:

The raw drugs were procured from a well reputed country shop in Parrys corner, Chennai. All the ingredients were purified and the medicine was prepared in the *Gunapadam* laboratory of National Institute of Siddha

Identification and Authentication of the drug:

- The plant materials were identified and authenticated by the Botanist, Department of Medicinal Botany, National Institute of Siddha.
- The raw drug pooram was was authenticated by Department of Geology, University of Madras, Guindy campus ,chennai

Purification of the drugs

All the drugs mentioned were purified as per the Siddha literature

METHOD OF PURIFICATION

Purification of Pooram (Calomel)

A paste was made of black pepper and betel leaves weighing about 8.5 gm mixed this paste mixed with 1.3 litter of water and taken in an earthen pot. pooram was enclosed in a cotton piece cloth placed in the pot filled with the above mixer by using Thula enthiram and boiled till the liquid was fully evaporated finally pooram was taken out washed and dried[12].

Purification Of Cithiramoolam (Plumbago Zeylanica)

The inner root of Plumbago Zeylanica root was removed and the root bark was powdered well. The powdered drug was placed in a cloth and tied in the mouth of a pot filled with milk. Then, the pot was closed with suitable lid and boiled for 3 hours. Then it is allowed to dry completely and ground well[13].

Purification Of Omam (Bishops Weed)

The seeds are soaked in supernatant of limestone solution for the samam (3 hours)[14]

Purification Of Panaivellam (Jaggery)[15]

Dust, stones and dander are removed.

METHOD OF PREPARATION

Purified Mercurous chloride, purified powder of plumbago Zeylanica,purified power of carum copticum are ground well in motar for 1 hour.To this purified jiggery added these are again ground for 6 hours and rolled into a pepper sized(56 mg)pills

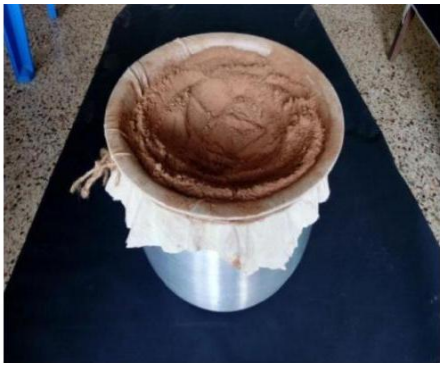
Labling

Name of the preparation	- Siddhar kuligai
Dose	- one to one and half pills 56 to 84 mg, two times a day
Adjuvant/Vehicle	- Sugar/ paste of dried ginger /butter
Route of administration	- Oral
Duration	- 10 days
Indications	- Parpalaviranangal, Kiranthi, Rongangal, Yoniputru, Soolaigal, Kallipukiranthi Vippurathi, 8 Vagaigunmam, Maegaranangal

Kodiveli

Before purification

After purification



Purification of *Kodiveli* in *Pittaviyal* Method

Preparing *Kodiveli* for *Pittaviyal*

Process of *Pittaviyal*

Kodiveli Chooranam

Before purification **After purification**

Omum (Carum copticum)

Carum copticum powder

Panaivellam (palm jaggery)

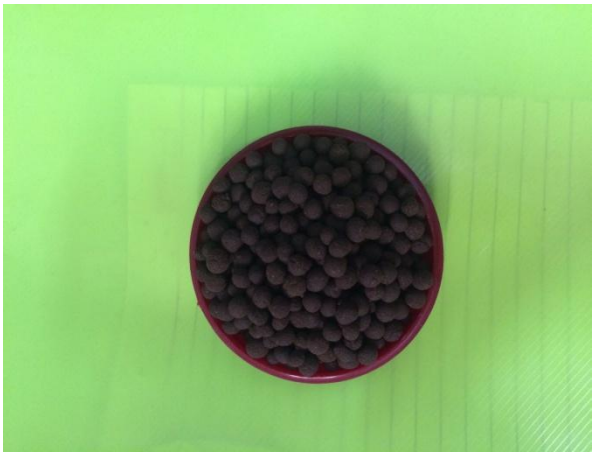
POORAM

Before purification

After purification

Purification process

Siddhar Kuligai



4. LITERATURE REVIEW

4.1. GUNAPADAM REVIEW

POORAM (Rasa Karpooram)

It is prepared by the combination of Rasam and salt

Method of preparation:

Ingredients

Mercury – 336gm

Sulphur – 67.2gm

Sodium Chloride – 650gm

Procedure:

Sulphur is melted in a mud pot and Mercury is added to it and kindled well and there forms a black coloured pot. Brick stone powder is placed up to half of the level of a pot. Sodium Chloride is placed over it. Mercury Sulphur mixture is placed over the salt and sealed with mud pasted cloth. It is burnt for 12 hours with kadakkini. After it is cooled, the Mercurous chloride is found deposited on the upper pot and the same are collected.

Potency - Hot

Taste - Salt

Properties - Laxative, tonic, antiseptic and diuretic

General Properties

It cures various types of throbbing pains. Hepatomegaly, pyrexia, Jaundice, Bacillary dysentery, dropsy, chronic ulcers, venereal diseases, indigestion, vomiting, diarrhoea, worm infestation, rheumatism, itching, constipation, scabies etc.

‘இடைவாத சூலைளரிசூலைகுந்மந்

தோடைவாழைவாதமாஞ்சோணி–யிடையாதோ

வொக்குரசகற்பூரமொன்றேயளவொடுநல்

இக்குவெல்லத்தேழுநாளீ’’

-தாது குணப்பாடம்

Medicinal uses:

Poorakattu

Delirium associated with fever and delirium associated with constipation.

It is given along with lingakattu for curing the vatha disease

Rasa Karpoora Kuligai

Scabies, Syphilis, Vulva Cancer, Penis Cancer, Chronic ulcer and pit wounds .

Poora kalimbu

The paste is useful in the treatment of syphilitic ulcer.

Poora ennai

Constipation, Venereal Disease, Ulcers.

Poora podi

The powder is useful in the treatment of venereal ulcer (external)

Sign and symptoms of calomel poisoning

- Multiple red boils may appear on the face,
- acne formation,
- ulcers in the chest, mouth and tongue,
- diarrhoea and dysentery,
- scrotal swelling and ulcer in the uvula.

Antidote for poisoning(calomel)

- Black musali tubers(curculigoorchider)
- Indian penny wort root(centella asiatica root)
- Root of sessile plant(alternatheriasessiles)
- Beetle killer(clerodendrumserratum- kanduparangi)[16]

VENKODIVELI

Synonyms	**:**	Venchithramoolam, Venkodimoolam
Botanical Name	**:**	Plumbago zeylanica
Family name	**:**	Plumba Ginaceae
English name	**:**	Ceylon lead- wort
Parts Used	**:**	Root and stem bark
Taste	**:**	Pungent
Nature	**:**	Heat
Division	**:**	Pungent
Activity	**:**	Anti-periodic Diaphoretic
Dose	**:**	¼ - ½ Varagan

General Characters :

கட்டிவிரணங் கிரந்திகால்கள் அரையாப்புக்

கட்டிச்சூ லைவீக்கங் கால்மூலம் - முட்டிரத்தக்

கட்டுநீரேற்றங் கனத்தபெருவயிறும்

அட்டுங் கொடிவேலியாம்

- அகத்தியர் குணவாகடம்

It cures abscess, ulcers, tumours, arthritis, anaemia, piles and ascites.

Uses

Take 40 grams of the root of *kodiveli*, 1.5 liters of sesame oil. Grind the root with cow's milk and make a paste. Put the paste into the oil and boil till dehydration. This is called "*Chiththira Moola Enney".* It cures all types of headache including migraine.

Take 50 grams of the root bark of *P. zeylanica*, 30 grams each of pepper, coriander seeds, long pepper, cumin seeds, asafoetida, rock salt, Carum Capticum. Grind them separately and mix them. 2 pinch of powder with honey and sugar cures peptic ulcer, indigestion etc[17].

OMAM

Synonyms	**:**	Asamootha,Theepiyam
Botanical Name	**:**	Carumcopticum
Family name	**:**	Apiaceae
English name	**:**	Bishop weed
Parts Used	**:**	Seed
Taste	**:**	Pungent
Nature	**:**	Heat
Division	**:**	Pungent
Activity	**:**	❖ Stomachic ❖ Anti spasmodic ❖ Carminative ❖ Antiseptic ❖ Stimulant ❖ Tonic ❖ Sialogogue
Dose	**:**	¼ - ½ Varagan

General Characters :

கட்டிவிரணங் கிரந்திகால்கள் அரையாப்புக்

கட்டிச்சூ லைவீக்கங் கால்மூலம் - முட்டிரத்தக்

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அட்டுங் கொடிவேலியாம்

- அகத்தியர் குணவாகடம்

It curesfever,cough,indigestion,diarrhoea,palnoi,kudaleraisal,kaalara,asthma.

Uses

Take 34 grams of carum copticum and pepper, slightly heat and Grind them separately and mix with jiggery 34 grams. Take morning and night 5oo mg cures Diarrheoa, indigestion etc.

Oma Theener

Dose 30-60 ml

Kalara, abdominal pain, indigestion, etc[17]

Oma Thylam

Dose 1-3 drops.

PANAI VELLAM

Synonyms	**:**	Thaalam, Karumpuram Aedagam, Kamam Tharuviragan, Thaali
Botanical Name	**:**	Borassusflabellifer
Family name	**:**	Arecaceae
English Name	**:**	Palmyra Palm Brab Tree
Parts Used	**:**	Whole plant
Taste	**:**	Sweet
Nature	**:**	Coolant
Division	**:**	Sweet

General Characters:

---------- தங்குபனை

வெல்லத்தால் வாதபித்தம்வீறுகபஞ் சன்னிநோய்

வல்லருசிகுன்மமறுமால்

- அகத்தியர் குணவாகடம்

It cures gastric ulcerdelirium, tridosha, and ageusia

4.2. BOTANICAL REVIEW

VENKODIVELI

Botanical Name		:	Plumbagozeylanica
Family		:	Plumbaginaceae
Vernacular names			
	Tamil	:	Kodiveli, Akkini, Angodiveli, Kanilam, sittramulam.
	English	:	Ceylon leadwort, White flowered leadwort
	Telugu	:	Agnimata, Chitrmulamu, Tellachitramulamu
	Malayalam	:	Tumpu kotuveli, Vella koduveli
	Hindi & Beng	:	Chita, Chitrak, Chitra
	Sanskrit	:	Agni, Agnimata, Agnisikha, Anala
Scientific classification			
	Kingdom	:	Plantae
	Clade	:	Angiosperms
	Clade	:	Eudicots
	Order	:	Caryophyllales
	Family	:	Plumbaginaceae
	Genus	:	Plumbago
	Species	:	P.zeylanica

Distribution

It is indigenous to South-East Asia. Found wild in peninsular India and West Bengal and cultivated in gardens throughout India[18].

Description

A perennial, sub- scandent shrub;

Leaves

Thin, 3.8-7.5 by 2.2-3.8 cm ovate, glabrous, reticulately veined shortly and abruptly attenuated into a short petiole;

Petiole- narrow, amplexicaul at the base

Flowers

- White, in elongated spikes; Rachis glandular striate; bracteoles ovate, shorter than the calyx
- Calyx 1-1.3 cm long, narrowly tubular, densely covered with stalked glands
- Corolla white, slender; tube 2-2.5 cm long; lobes 8 mm long obovate-oblong, acute, apiculate
- Filaments as long as corolla tube; anthers exserted just beyond the throat

Fruit

Capsules oblong, pointed contained in viscid glandular persistent calyx.

Phytochemical constituent[19]

Leaf

Leaf of the plant contain mainly plumbagin that is the prime chemical component of the plant, along with plumbagin it contains

- Plumbagic acid
- Beta-sitosterol4-hydroxy-benzaldehyde
- Trans-cinnamic acid • 2,5-dimethyl1-7-hydroxy chromone
- Isoshinanolone
- Indole-3-carboxaldehyde
- Vanillic acid

Roots

Roots contain mainly Napthoquinone it includes

- 5,7-dihydroxy-8-methoxy-2-methyl-1,4-napthoquinone (plumbagin)
- Biplumbagin
- Chloroplumbagin
- Maritinone
- Elliptinone
- 2-(1-hydroxy-1-methyl-ethyl)-9methoxy-1,8-dioxadicyclopenta(b.g)
- Naphthalene-4,10-dionic,9-hydroxy-2-isopropeny1-1,8-dioxa-dicyclopenta(b.g)
- Isoshinanolone
- 2-isopropeny,1-9-methoxy-1,8-di-oxadicyclopenta(b.g)

Coumarins

- 5-methoxy seselin
- Seselin
- Suberosin
- Xanthylctin
- Xanthoxylctin Plumbic Acid
- 3-o-β-glucopyranosylplumbagic acid
- 3-o-β-glucopyranosylplumabagic acid methyl ester

Enzymes

- Invertase
- Protease

Other Compounds

- Zeylinone, glucose, fructose, isozeylinone, droscrone, plumbaginol

Flower

- Plumbagin
- Glucose
- Zeylanone

Stem

- Plumbagin
- Dihydroflavinolplumbaginol
- Sitosterol
- Isozeylanone

Fruit

- Plumbagin
- Glucopyranoside
- Sitosterol

Seed

- Plumbagin

Medicinal Uses[20]

Root Bark

- ❖ Stomachic
- ❖ Carminative
- ❖ Astringent
- ❖ Anthelmintic
- ❖ Alterative
- ❖ Cures intestinal troubles, dysentery, leucoderma, inflammation, piles, bronchitis, *Vatha* and *Kapha* itching, liver diseases, ascites, *tridosha*
- ❖ Good in anaemia
- ❖ Tincture of root bark is used as an anti periodic

Root

- ❖ Laxative
- ❖ Expectorant
- ❖ Stomachic
- ❖ Tonic
- ❖ Abortifacient
- ❖ Alexipharmic

- Useful in laryngitis, rheumatism, disease of spleen, leucoderma, ring worm, scabies
- Increase the digestive power to promote appetite, to be useful in dyspepsia, piles, anasarca, diarrhoea, skin diseases
- Root mixed with milk, vinegar or salt and water applied externally for leprosy and other skin diseases of obstinate character.
- Infusion of root used in influenza and black-water fever.

OMAM

Botanical name : Trachyspermum ammi

Synonyms : Ammi coptium, Carum copticum, Trachyspermum, copticum

Family : Apiaceae

Vernacular names

Tamil	:	Ayanodakan
English	:	Bishops seed
Telugu	:	Vaamu
Hindi	:	Ajowan,ajwain
Malayalam	:	Omam
Kannadam	:	oma,omu.
Gujarathi	:	Ajamo
Bengali	:	Yamani,javan
Punjabi	:	Jawin

Scientific Classification

Kingdom	:	plantae
Clade	:	angiosperms
Clade	:	eudicots
Clade	:	asterids
Order	:	apiales
Family	:	Apiacea
Genus	:	trachyspermum
Species	:	T.ammi

Description

Carum copticum grows as a herb whose roots are fusiform. stems are 30 to 90cms long leaves are 2-3 pinnate bracts several and linear the fruit is ovoid in shape and grayish brown in colour[21]

Habitate

This plant (Carum copticum) grows and is largely cultivated in Eastern India, it is particularly abundant in and around indore and in andra Pradesh. Is also grows in iran, Pakistan and Egypt.

Macroscopic

Fruit, occurs mostly as entire cremocarps with pedicel attached or detached at the base and bifid slopedat the apex ,broadly ovoid ,1.5 to 3 mm in length and 1.2 to 2.8 mm in width yellowish green dorsal surface cover with five distinct longitudinal ridges in reach mericarp, commissural surface flat, showing two darker longitudinal bands representing the vittae, odour, aromatic, taste at first slightly bitter becoming strongly pungent producing slight numbness the tongue.

Microscopic[22]

Diagrammatic TS os fruit shows 5 strongly developed primary ridges ,each with a vascular bundle 4 large vittae on the dorsal surface and 2 on the commissural surface where lies raphae in between the endocarp and testa layer. Epicarp consists of highly papilla seepidermis, interrupted at places with warty unicellular trichome like dome shaped bulging extensions, with striated cuticle. Vittae broad ,measuring 150 to 200 m in width lined with dark brown epithelium layer, innermost layer of the mesocarp some what collapsed, endocarp composed of unequal sized tangentially running thin walled parenchymatous cells; testa layer verybroad ;cells of the endosperm, thick walled, parenchymatous and filled oil globules and aluerone grains embedded with micro rosette crystals of calcium oxalate.

Chemical Constituents

Major

Essential oil, thymol

Others

Carvone, limonene, dillapiol, quercetin, kaemferol, fatty acid

TLC Identity Test

A band corresponding to thymol (0.69- bright orange) is visible in both reference and test solution tracks.

Estimation of thymol in the carum copticum

The percentage of thymol ranges from 0.82 to 1.32 (w/w) in carum copticum.

Extraction of Essential Oil[23]

Carry out hydro distillation of 500 g of the carum copticum powdered with 1000 ml of water in clevengers apparatus for 12 h to obtain the essential oil (2.6 per cent (w/w).

Quantitative Standards

Foreign matter

Not more than 2.0 per cent.

Ash

Not more than 8.1 per cent .

Acid insoluble ash

Not more than 0.3 per cent .

Ethanol – soluble extractive

Not less than 20.0 per cent .

Water soluble extractive

Not less tha 29.0 per cent.

Pharmacology

- Thymol isolated from the fruits of the carum copticum produced hypotension, bradycardia and anti molluscicidal effect .
- The extract of fruit showed significant inhibitory effect in hepatitis c virus protease.
- The essentioal oil obtained from the seeds demonstrated antifungal activity.
- The methanolic extract of seeds showed antioxidant property.

Major therapeutic claims

- Antioxidant
- Carminative
- Anthelmintic

Safety aspects

The drug used traditionally in prescribed doses may be considered safe.

Dosage

3-6 gram

Medicinal uses[24]

Seed

- The seed are hot ,bitter ,pungent ;stomachic appetizer, aphrodisiac, anthelmintic, carminative, laxative; cure ascites abdominal tumours enlargement of spleen piles vomiting abdominal pains, good for heart, increase biliousness. (Ayurveda)
- The seed are cure weakness of limbs and paralysis, chest pain improve speech and the eye sight stimulant the intestine good for ear boils liver spleen hiccough vomiting, dyspepsia kidney troubles, inflammation, galactagogue, diuretic, carminative. (unani)
- The seed is prescribed for snake bite and scorpion sting but is not an antidote to either snake venom or scorpion venom.
- Omum seeds are useful in flatulence, indigestion, colic, atonic dyspepsia, diarrhea, cholera, hysteria and spasmodic affections of the bowels and check chronic discharges such as profuse expectoration in bronchitis.
- Volatile oil is also used in cholera, flatulent, colic, atonic dyspepsia or diarrhea, hysteria and indigestion. Externally it is applied to relieve rheumatic and neuralgic pains.
- "The chief importance of ajowan seeds is for production of thymol, which is a very valuable anthelmintic",
- Seeds are also used as spices along with betel-nuts and pan leaves in flatulence, dyspepsia and spasmodic affections.
- A plaster of poultice or the crushed seeds is used to relieve the pain of colic.
- Omum seeds made hot are used as a dry foementation to the chest in asthma and to the hands and feet in cholera, fainting, syncope and rheumatism.

Root

- It is diuretic and carminative it is used in febrile affection and in stomach disoders
- Whole plant
- The plant is used as a stomachic in loralai (Hughes-buller) [25]

BORASSUS FLABELLIFER, LINN

Botanical Name	:	Borassus flabellifer
Family	:	Arecaceae
English Name	:	Palmyra
Colour	:	Off white top pale yellowish white
Preparation	:	It is prepared by boiling the sap of Palmyrapalm
Physical State	:	Amorphous Solid
Place of Availability	:	India (West Bengal) and Bangladesh

Habitat:

- The Palmyra palm is a large tree upto 30m high and the trunk may have a circumference of 1.7m at the base. There may be 25-40 fresh leaves.
- It is commonly cultivated in India, South east Asia, Malaysia and occasionally in other warm regions including Hawaii and southern Florida. In India, it is planted as a windbreak on the plains.
- Grows on dry soils or sandy localities along river banks, throughout tropical India, especially in South India.[27]

Parts used:

Root, Flowering Stalk, Juice, Bark and Fruit.

Constituents:-

- Gum
- Fat
- Albuminoids

Action:-

- Root is cooling and restorative; juice is diuretic, cooling, stimulant and antophlogistic when fresh;
- Pulp from the unripe fruit is diuretic, demulcent and nutritive, terminal buds are nutritive and diuretic.

Preparations:-

- Palm juice and palm-wine, confection.
- Sago from the trunk; poultice; pulp; ashes of the flowering stalk and decction.

Uses:-

- ❖ It is from the juice of this tree that toddy, jiggery and country-sugar are prepared in large quantities in Southern India.
- ❖ Sugar-candy produced in the manufacture of sugar from the palm is used in cough and pulmonary affections.
- ❖ Fresh saccharine juice obtained by excision of the spadix (young terminal buds) early in the morning is cooling and is a stimulant beverage, also acts as a laxative taken regularly folr several mornings.
- ❖ It is useful for inflammatory affections and dropsy; also in gastric catarrh and to check hiccup; as diuretic it is useful in gonnorhea.
- ❖ "Decoction of the root is also used in gastritis and hiccup". Slightly fermented juice called Tari (toddy)", an intoxicating liquor, is a favourite drink among the labouring classes" is given in diabetes.
- ❖ With aromatics it is a good tonic in emaciation or phthisis. Milky fluid from the immature fruits is a sweet and cooling drink, and checks hiccup and sickness.
- ❖ Toddy poultice prepared by adding fresh drawn toddy to rice flour and subjected to a gentle fire till fermentation takes place, then spread on a cloth forms a valuable stimulant application to in-flamed parts, gangrenous and indolent ulcers, carbuncles, etc..,
- ❖ Yellow pulp surrounding the ripe nuts is sweet, but heavy and indigestible. Ashes of the flowering stalk are useful in enlarged spleen.
- ❖ Bark of the tree burnt, reduced to charcoal and pulverized makes a good dentrifice; decoction of the bark with a little salt added to it is a good astringent gargle for strengthening the gums and teeth.
- ❖ The palm yields a fruit which is eaten with much relish.

Toddy:-

The chief product of the Palmyra is the sweet sap (toddy) obtained by tapping the tip of the inflorescence, as is done with the other sugar.

Jaggery preparation:-

Unrefined sugar made from palm sap.

- Americal heritage Dictionary of the English Language, IV edition

It is the first extract of the palm juice.

- The juice is boiled, a little salt is added to it to act as a preservative and so that the jaggery does not taste too sweet.
- It is cooled and poured into a long cone made of palm leaves.
- Jaggery is an amorphous form of unrefined and non-distilled sugar prepared from sap or juice of plants which contains considerable amount of sucrose or sugar in them, like sugar cane and palms like date palm and Palmyra[28]

Constituents:-

Palmyra palm jaggery (gur) is much more nutritious than crude cane sugar, containing

Protein	:	1.04%
Fat	:	0.19%
Sucrose	:	76.86%
Glucose	:	1.66%
Total minerals	:	3.15%
Calcium	:	0.861%
Phosphorous	:	0.052%
Iron	:	11.01 mg per 100g
Copper	:	0.767 mg of per 100g

The fresh sap is reportedly a good source of vitamin B complex.[29]

1. Sugarcane jaggery:-

Colour	:	golden brown to dark brown
Preparation	:	Prepared by boiling sugar cane juice
Physical state	:	amorphous solid to viscous granular liquid.

2. Date palm jiggery :-

Colour	:	Golden brown to dark brown
Physical state	:	prepared by boiling sap of date palm.
Preparation	:	Amorphous solid and viscous viscous granular to clear red liquid.

3.Palmyrajaggery:-

Colour	:	Off white to pale yellowish white.
Preparation	:	prepared by boiling sap of Palmyra palm.
Physical state	:	Amorphous solid.

4.Toddy palm jaggery:-

Colour	:	Golden brown
Preparation	:	prepared by boiling sap of Toddy palm.
Physical state	:	Amorphous solid

5. Other palm jaggery:-

These days, even the sap of Sago plant and coconut palm are also being used to make jaggery, but they are rarely.

Nutritive value of 100g palm jaggery:-

Energy	:	349 Ecals
Moisture	:	9 gm
Protein	:	2 gm
Fat	:	0 gm
Mineral	:	4 gm
Carbohydrate	:	85 gm
Calcium	:	1252 mg
Phosphorous	:	372 mg[30]

4.3. MINERALOGICAL ASPECT OF POORAM (Mercurous chloride)

Introduction:

Mercury (I) Chloride is the chemical compound with the formula $Hgcl_2$, Also known as Calomel (a mineral form, rarely found in nature) or mercurous chloride, this dense white or yellowish-white, odourless solid is the principal example of a mercury(I) compound. It is a component of reference electrodes in electrochemistry.

A relatively rare mineral, associated with other mercury minerals, probably always secondary and late in the mineral swquence. It will be found in small brilliant crystal in cavities, associated with cinnabar and often perched on crystals of that mercury ore.

Two related anhydrous halides are similar in color to calomel even though they contain copper. Rare nanokite (Cucl; copper chloride) and almost as rare marshite (CuI; copper iodide) are the only colorless or white copper minerals. Both are tetrahedral. Marshite forms triangular lustrous tetrahedral crystals at Chuquicamata, Chile and was formerly found at Broken Hil l, New South Wales. In a mine tunnel near Chuquicamata, iron-stained orange incrustations of marshite, catalysed by iron rails and bolts, form from drainage water.

Marshite is colorless to pale yellow when fresh, as a rule, but seems to turn coppery on exposure to light and air. Iodine vapors emanate when a sealed marshite container is opened, and can be smelled; perhaps copper is freed and remainsto give the color noted in older exposed specimens.

History:-

The name calomel is thought to come from the Greek word beautiful and black. This name (somewhat surprising for a white compound) isprobably due to its characteristic disproportionation reaction with ammonia, which gives a spectacular black coloration due to the finely dispersed metallic mercury formed. It is also referred to as the mineral *horn quicksilver* or *horn mercury.* Calomel was taken internally and used as a laxative and disinfectant, as well as in the treatment of syphilis, until the early 20^{th} century.

Mercury became a popular remedy for a variety of physical and mental ailments during the age of “heroic medicine”. It was used by doctors in America throughout the 18^{th} century, and during the revolution, to make patients regurgitate and release their

body from “impurities”. Benjamin Rush, a famed physician in colonial Philadelphia and signatory to the Declaration of Independence, was one particular well-known advocate of mercury in medicine and famously used calomel to treat sufferers of yellow fever during its outbreak in the city in 1973. Calomel was given to patients as a purgative until they began to salivate. However, it was often administered to patients in such great quantities that their hair and teeth fell out.

Preparation and Reaction:-

Mercurous chloride forms by the reaction of elemental mercury and mercuric chloride

$$Hg + Hgcl_2 \longrightarrow Hg_2Cl_2$$

It can be prepared via metathesis reaction involving aqueous mercury (I) nitrate using various chloride sources including NaCl or HCl

$$2HCl+Hg_2(NO_3)_2 \longrightarrow Hg_2Cl_2+2HNO_3$$

Ammonia causes $HgCl_2$ to disproportionate:

$$Hg_2Cl_2+2NH_3\,Hg + Hg \longrightarrow (NH_2)\,Cl+NH_4Cl$$

Photochemistry:-

Mercurous chloride decomposes into mercury (II) chloride and elemental mercury upon exposure to UV light.

$$Hg_2Cl_2 \longrightarrow HgCl_2+Hg$$

The formation of Hg can be used to calculate the number of photons in the light beam, by the technique of actinometry. By utilizing a light reaction in the presence of mercury (II) chloride and ammonium oxalate, mercury (I) chloride, ammonium chloride and carbon dioxide is produced.

$$2HgCl_2 + (NH_4)_2C_2 + \text{Light} \longrightarrow HgCl_{2(s)}+2[NH_4^+][Cl^-]+2CO_2$$

Physical and Chemical Properties:-

Molecular formula	:	Hg_2Cl_2
Molar mass	:	472.09g/mol
Appearance	:	White solid
Density	:	$7.150g/cm^3$
Melting Point	:	525 C (triple point)
Boiling point	:	383 C (sublimes)
Solubility in water	:	0.2 mg/100 mL
Solubility	:	Insoluble in ethanol, ether
Refractive index (nD)	:	1.973

Related Compounds:-

Other anions	Mercury (I) fluoride
	Mercury (I) bromide
	Mercury (I) iodide
Other cations	Mercury (II) chloride

Stability and Reactivity:-

Stability:-

Stable under ordinary conditions of use and storage. Slowly decomposed by sunlight into mercuric chloride and metallic mercury.

Hazardous Decomposition Products:-

Oxides of the contained metal and halogen, possibly also free, or ionic halogen.

Hazardous Polymerization:-

Will not occur.

Incompatabilities:-

Bromides, iodides, ammonia, alkalis, cyanides, chlorides, copper and lead salts, silver salts, carbonates, sulphides, soap, lime water, iodoform and hydrogen peroxide.

Conditions to avoid:-

Light and incompatible.

Handling and Storage:-

Keep it in a tightly closed container, stored in a cool, dry, ventilated area. Protect against physical damage. Isolate from any source of heat or ignition. Protect from light. Follow hygiene practices. Containers of this material may be hazardous when empty since they retain product residues (dust, solids); observe all warnings and precautions listed for the product.

POISONOUS EFFECTS OF POORAM

Potential health effects:-

Inhalation:

Causes irritation to the respiratory tract. Symptoms include sore throat, coughing, pain, tightness in chest, breathing difficulties, shortness of breath and headache. Pneumonitis may develop. Can be absorbed through inhalation with symptoms to parallel ingestion.

Ingestion:

Toxic Average lethal dose for inorganic mercury salts is about 1 gram. May cause burning of the mouth and pharynx, abdominal pain, vomiting, bloody diarhhoea. May be followed by a rapid and weak pulse, shallow breathing, paleness, exhaustion, tremors and collapse. Delayed death may occur from renal failure.

Skin contact:-

Causes irritation. Symptoms include redness and pain. May cause burns. May cause sensitization. Can be absorbed through the skin with symptoms to parallel ingestion.

Eye contact:-

Causes irritation to eyes.May cause burns and eye damage.

Chronic exposure:-

Chronic exposure through any route can produce central nervous system damage. May cause muscle tremors, personality and behaviour changes, memory loss, metallic taste, loosening of the teeth, digestive disorders, skin rashes, brain damages and kidney damages.

Can cause skin allergies and accumulate in the body. Repeated skin contact can cause the skin to turn grey in colour. Not a known reproductive hazard, but related mercury compounds can damage the developing foetus and decrease fertility in males and females.

Aggravation of Pre-existing Conditions:-

Persons with nervous disorders or impaired kidney or respiratory function or a history of allergies or a known sensitisation to mercury may be more susceptible to the effects of the substance.

Toxicological effects of Pooram:-

Oral rat LD50:210mg/kg

Reproductive Toxicity:-

All forms of mercury can cross the placenta to foetus, but most of what is known has been learned from experimental animals.

Calomel therapeutic applications:-

- Calomel is peculiarly called for as a purgative, whenever, in connection with another demand for cathartic medicine, there is an indication for stimulating the secretary function of the liver.
- Full mercurial purgation will generally entirely relieve this affection and probably prevent the occurrence of some more serious attack as bilious colic, cholera morbus, dysentery or jaundice.
- In all cases of constipation with deficiency of bile in the passages, a purgative dose of calomel may be given. This condition often precedes an attack of jaundice, which may thus be prevented.
- In jaundice itself, of the ordinary kind, attended with clay-coloured passages and bilious urine, a purgative dose of calomel, alone or combined, should be given at the commencement and occasionally repeated in the course of the dose.
- Acute hepatitis generally offers the same indication. Where a purgative is required, calomel should almost always be used, either alone or connected with other cathartics. In the chronic variety, active purgation is seldom desirable, and it is rather the alternative than the cathartic action of the medicine that is wanted.
- In acute splenitis, calomel should be given at the onset, with a view to deplete from the portal circle, so intimately connected with that organ.
- In bilious colic, calomel is strongly called for by the congested state of the liver and in conjunction with opium, is the most important remedy in the disease.
- In gastritis, severe enteritis and peritonitis, calomel may often be advantageously used as a cathartic at the commencement of the disease.
- In infantile diseases, calomel is peculiarly efficacious. It is recommended here by its want of unpleasant taste, by its retention upon the stomach when others are rejected and by the general mildness of its operation. It is useful moreover in the complaints of the children.
- In epidemic cholera, dysentery, yellow fever, etc.., it has been recommended in large doses as a sedative agent. It is asserted that, when given very largely in these cases, so far from causing local or general excitement, it produces, on the contrary, a remarkable sedative effect, allaying the local irritation, checking vomiting and purging, lowering the frequency and force of the pulse and heat of the skin and greatly contributing the cure.

4.4 SCIENTIFIC REVIEW

Carum copticum

The analgesic effect of carum copticum extrat and morphine on phasic pain in mice

The present study supports the claims of Iranian traditional medicine showing the carum copticum extract possesses a clear cut analgesic effect[31]

Role of carum copticum seeds in modulating chromium induced toxicity on human bronchial epithelial cells and human peripheral blood lymphocytes.

The present study pertains to investigate modulatory effects of methanolic extract of carum copticum seeds (MCE) Against hexavalent chromium induced cytotoxicity, genotoxicity, apoptisis and oxidative stress on human bronchial epithelial cells and isolated human peripheral blood lymphocyte in vitro[32]

Evaluation of anticancer activity of Plumbaco zeylanical inn Leaf Extract

The present study was indicates that ethonolic extract of plumbago zeylanicain posses significant anticancer activity and also reduce elevated level of lipid peroxidation due to higher content of terpenoids and flavonoids. Thus ethonolic extract of plumbago zeylanica. linn could have vast therapeutic application against cancer[33]

A comparative anticancer activity of plumbaco zeylanica collected from northern and southern parts of india

The present study was indicates Aqueous extract of plumbaco zeylanica inhibits the growth of MCF -7 breast cancer cells at 5070 and 100mg /ml concentrations. comparision of IC50 values and linearity of the activity, it is observed that sample of northen region has promising result as compared to the sample collected from the southern region [34].

Analgesic activity of methanol extract of plumbago Zeylanical (l) by acetic acid induced writhing method

The present study was designed to investigate analgesic activity of methanolic extract of plumbago using acetic acid induced writhing method .Methanol extract of Plumbago Zeylanica showed significant inhibition in painresponse induced by thermal and mechanical stimuli in dose dependent manner. The obtained result provide

promising baseline information for the potential use of these crude extract in the treatment of pain[36].

Anti –inflammatory and analgesic effect of plumbagin through inhibition of nuclear factor –kB activation

The present study characterized the anti inflammatory and analgesic effects of plumbagin orally administrated in a range of dosages from 5 to 20 mg /kg the result showed that plumbagin significantly and dose dependently suppressed the paw edema of rats induced by carrageenan, the study suggests that plumbagin has a potential to be developed into an anti inflammatory agent for treating inflammatory[37]

Isolation characterization and in vitro anti inflammatory activity of plumbago indica Linn.

The present study plumbico zeylanicalinn evaluate for anti inflammatory activity. The roots of plumbago zeylanica was extracted with methanol and obtained extract was columm chromate graphed using silica gel the result indicates that the compound exhibited significantly anti inflammatory activity at different concentration[38]

Toxicity study

Kumar.M.C.et.al, studied the Oral acute toxicity study of Plumbago Zeylanica that showed no toxicity even at higher dose 3200 mg/kg, indicative of the wide margin of safety of the root extract[21]

Cytoprotective and antioxitant activity studies of jaggery sugar

Jaggery and other sugar namely white, refined and brown sugar were evaluated for cytoprotectivity on NIH 3T3 Fibroblast and erythrocytes ,DPPH radical scavenging activity reducing power and DNA protection[39].

Jaggery :A Revolution in the field of natural sweeteners

Jaggery is know n produce heat and give instant energy to a human body .The micronutrients which are present in jiggery has many nutritional and medicinal aspects like its anti carcinogenic and antitoxic activity[40].

4.5 PHARMACEUTICAL ASPECT OF THE DRUG

Pharmaceutics is a discipline of pharmacy that deals with the process of turning a new chemical entity to be used safely and effectively by the patients. (Formulation of pure drug substance into dosage form)

Siddha pharmaceutics has very minute chemical processes in it. It has several chemical processes like purification of raw substances, grinding them with herbal juices for several days and subjecting the ground material to fire by way of *putam* process. Medicines prepared according to the above methods undergo several chemical changes.

Siddha medicines are classified into internal medicines (32) and external medicines (32). The drug taken for dissertation is in the form of *Kuligai.* Other names of *Kuligai* are *mathirai, Urundai, Vattam. Kuligai* comes under the category of internal medicines[25]. The powder which is required for the preparation of *Kuligai*is *Chooranam*. It also comes under the category of internal medicines.

Purification of the drugs included

Purification of the drugs is mainly done to remove the toxicities, impurities like soil, dust, clay present in the drugs. Also the drugs when subjected to heat like roasting or soaked in liquids undergo certain chemical reactions such as oxidation of toxic substances to non-toxic, reduction of some poisonous chemicals to non-poisonous ones, or undergo enzymatic reactions. In these ways, not only the toxicities and impurities are removed but also enhanced the potency of the drugs.

Concept and Terminology of pills

It is a pill prepared from a finely ground paste of drugs. The term *kuligai* is the most fitting category of medicines as besides indicating the form of medicine that is pills. It also means that the minimal dosage unit is one pill (*kuligai* means 1 unit). Preparation of *kuligai* includes various processes like extraction of juices, making decoction, preparing powders, grinding pastes and rolling into pills or pressing into tablets. The raw drugs are dried in the sun or shade and the drugs which are aromatic are to be roasted separately. The raw drugs are purified and the raw drugs are grounded separately then the compounded drug be grounded in a mortar for the prescribed period with the addition of prescribed juices and decoctions. If green drugs are to be added they should be made into fine paste before being used. Vegetable drugs which require frying are fried and powdered. However scented vegetable drugs like cinnamon leaves and cloves,

cinnamon bark are dried only in shade as otherwise their volatile oil is lost by drying in the sun.

The individual drugs should be separately weighed after being powdered and then taken in the prescribed ratio. After the pill mass has been prepared by following the processes outlined in the recipe, it is convenient to roll it into long uniform pencils and then cut into bits of uniform length to give suitable pill weight and then rolling a pill from each piece. This is a fast process to prepare uniform pills as pinching and rolling every time is invariably a tiresome, tedious and time consuming messy process. The pill mass when rolled between the fingers should not stick. This is the correct consistency for rolling into pills. The pills should be always dried in a warm, dry shady place and never in the sun because volatile matters in the pills are easily lost and photochemical breakdown of active principles are faster in sunlight of the tropics, if the pill mass sticks to the fingers, a speck of ghee may be smeared on the fingers. Pills should be well dried in shade [41]

Storage and Usage[41]

Almost all the *kuligai* contain highly active ingredients. Hence they should be preserved in well stoppered glass vials with relevant labels and instructions. If the *kuligai* lose their natural shape, colours, smell, taste etc, it is not advisable to consume them. If properly stored, we can keep them for a year.

Preparation of *Kuligai* in Manufacturing Units

In the manufacturing unit, *chooranam* is compressed into tablets. Tablets are unit forms of solid medicinal substances with or without suitable diluents prepared by compressing and they are mostly discoid in form. Binders like Gum acacia, lubricants like liquid paraffin and disintegrators like Talcum powder are used. *Chooranam* is first prepared according to the above procedures. Then the ingredients are mixed with in the form of granules before compressing as tablets. Too much fine powder refuse to form satisfactory tablets and so they must be mixed with some adhesive substances or binders such as gum acacia. To prevent the sticking of the tablets to the punches and dyes a lubricant like liquid paraffin is added. If the tablet is to dissolve quickly, a disintegrator like Talcum powder is added. [42]

Initial step of preparation of *Kuligai* (Preparation of *chooranam*) [26]

Before the preparation of *kuligai*, *chooranam* is prepared. Then it is subjected to various procedures as described above before becoming *kuligai*.

Chooranam is the fine dry powder of drugs. The term *chooranam* may be applied to the powder of a single drug or a mixture of two or more drugs, which are powdered separately prior to their being mixed homogeneity. If the constituent drugs are dry ones, they should not be dried. If they are fresh or green drugs with moisture they should be dried. The drugs should be cleaned and foreign matter removed before powdering. The drugs enumerated in the recipe in clean and well dried state are grounded in a mortar and a pestle and sieved through a fine cloth of close mesh. The drugs which are to be used in the preparation should be taken from recently collected material. Drugs which are aged by prolonged storage change in colour, taste and scent and those which are insect infested or attacked by fungi should be positively rejected.

In general, the aromatic drugs are slightly fried in order to enhance their aroma and milling properties. Any extraneous material, organic or inorganic material should be removed from the drugs by close inspection. The *chooranam* should be very fine as to be called amorphous and should never damp. The fineness of the sieve should be 100 mesh or still finer. The prepared chooranam should be allowed to cool by spreading and mixing, prior to packing. They should be stored in tightly 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.

Purification of *Chooranam*:[43]

The prepared *chooranam* was purified by a process called *Pittaviyal*. For this process cow's milk and water were taken in equal ratio and half filled in filled mud pot. A clean dry cloth was tied firmly around the mouth of the mud pot. *Chooranam* was placed over the tied cloth. Another mud pot of similar size was kept over the mouth of the mud pot. The gap between mud pots was tied with a wet cloth to avoid evaporation. The mud pot was kept on fire and boiled until the cow's milk reduced to the lower pot. Then the *chooranam* was taken, dried, powdered finely. Using this fine powder as base for *kuligai* preparation, further steps are continued.

Shelf life of the drugs

The shelf life of the drugs depends on the effectiveness of the preparation. The efficacy, smell, taste and appearance of the drugs gradually change as time goes on

resulting in reduced potency thereby the desired effect is not attained. But some drugs appear to be good externally inspite of reduced efficacy. So they should not be considered for consumption and should be discarded.

The shelf life of *kuligai* is 1 year and for *chooranam* it is 3 months. According to recently published guidelines by Ayush, the shelf life period of *chooranam* is 1 year and that of *kuligai* is 2 years (Drug and cosmetic rule, Nov 2005). Also the following are the analytical parameters of specifications of *kuligai.*

Traditional tests for *Kuligai*

Characters

- Non sticky on rolling
- No cracks over the surface after drying
- Shall be rolled uniformly over the plane surface

Based on these characters the drug is assessed as the appropriate one for medication.

According to siddha system of medicine:

- **Meganadha kuligai:**

  A pill made as per process of soothamuni for dyspepsia, dropsy and consumption.
- **Jayarasa kuligai:**

  Pill prepared according to the method of kallanki siddhar, for chronic diarrhea and indigestion.
- **Sirothari kuligai:**

  Pill contemplated in the works of yugimuni, for all kinds of fever.
- **Sudamani kuligai:**

  Pill was made by the SiddharVedamuni for apoplexy and Delerium.
- **Sironmaniboopathi kuligai:**

  Pill as given out by Pambatti siddhar for apoplexy chest pain, fatigue, etc..
- **Ramabanak kuligai:**

  Pills described in theraiyar works for all kinds of fever.
- **Sagalavida kuligai:**

  It used as an antidote pill prescribed by Nandisar, for all kinds of poisoning and poisonous bites.

- **Karuk kuligai:**

  Pill made from the Siddhar Konganavar for uterine disease.
- **Siva kuligai:**

  The one prescribed by Agastyar for mortify diathesis in children.
- **Koodasuri kuligai:**

  Pill described in Bogar's works for dropsy and chronic diarrhea.
- **Chandhirothaya kuligai:**

  Pill contemplated in the work of Kumbamuni siddhar for fever and apoplexy.

According to Ayurvedha some kinds of kuligai are:

- Egadasaruthira kuligai,
- Koorosanaadhi kuligai,
- Pipiliyaathi kuligai,
- Rasaathi kuligai,
- Vachirakandi kuligai,
- Sindhamani kuligai.

In philosophy- Pills made so as to be useful in alchemy, Yoga, philosophy, rejuvenation etc. They are used for purposes of consolidating volatile substances as mercury, sulphur, arsenic, salt etc for flying in the regions of the sky reviving the dead for rejuvenation and for several of the purposes.

They are different kinds of shown under:

- **Siddhar kuligai:**

  Pills made of different kiknds of arsenic or other minerals
- **Manthira Kuligai:**

  Pills made of herbs possessing occult powers
- **Rasa kuligai:**

  Consolidated mercurial pill
- **Rasavatha kuligai**:

  Pill used in alchemy for transmuting metals
- **Vachira kuligai:**

  Pill used for rejuvenating or invigorating the human system
- **Thambana kuligai**:

  A magical pill by which the body is made a shot proof or impenetrale

- **Boopathi kuligai**:

  A pill for reviving the dead so as to make them live only for a period of one and half hours

- **Mouna kuligai:**

  A pill enabling one to enter into silence

- **Yoga kuligai:**

  A pill that assists one to sit at yoga practice for a long time at a stretch without being disturbed by surrounding environments

- **Gnana kuligai:**

  A form of pill assisting one in the bath of wisdoms[44].

Modern Aspect of the formulation:

Tablet (Pill) - *Kuligai*

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

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

Classification:

As per IP2007 tablets are majorly classified into following categories (Indian pharmacopoeia 2007)

1) **Uncoated Tablets**:

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

2) **Coated Tablets:**

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

3. Dispersible Tablets:

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

4. Effervescent Tablets:

These type of tablets which are uncoated and are planned to be dissolved and produce an dispersion before they are administered the dissolution is achieved by the reaction between an organic acid and bicarbonate which produce CO2, thus produced CO2 will disintegrate the tablet so which dissolves in the solution to produce an suspension which was rapidly absorbed.

5. Modified-release Tablets:

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

A) Enteric-coated Tablets:

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

Ex: erythromycin, NSAIDS

B) Prolonged- release Tablets:

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

C) Delayed-release Tablets:

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

6. Soluble Tablets:

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

7. Tablets for Use in the Mouth:

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

8. Tablets for other routes of administration:

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

Tablet Ingredients:

A tablet consist of active medicament with excipents which are in powder form are compressed or pressed into a solid dosage form. In addition to active ingredients, tablet contains a number of inert materials known as additives or excipients[45].

- ❖ Binder and adhesive
- ❖ Disintegrents
- ❖ Lubricants and glidants
- ❖ Colouring agents
- ❖ Flavoring agents
- ❖ Sweetening agents

1. Diluent:

Diluents are fillers are used to make bulk of the tablet when the drug dosage itself was inadequate to produce the bulk these are used. Secondary reason is to provide better tablet properties such as improve cohesion, to permit use of direct compression manufacturing or to promote flow.

2. Binders and Adhesives:

These materials are added either dry or wet form granules or to form cohesive compacts for directly compressed tablet. Ex: Acacia, tragacanth- Solution for 10-25% Concentration.

3. Disintegrants:

It added to the tablet formulations to facilitate its breaking or disintegration, when it contact in water in the GIT. Example: Starch- 5-20% of tablet weight.

Super disintegrants: Swells up to ten fold within 30 seconds when contact water.

Example: Cross carmellose- cross-linked cellulose.

4. Lubricant and Glidants:

Lubricants are planned to prevent adhesion of the tablet materials to the surface of dies and punches, reduce inter particle friction and may improve the rate of flow of the tablet granulation Example: Lubricants-Stearic acid, Stearic acid salt - Stearic acid.

5. Coloring agent:

The use of colors and dyes in a tablet has three purposes:

- ❖ Masking of off color drugs
- ❖ Product Identification
- ❖ Production of more elegant product

All coloring agents must be approved and certified by FDA Example: FD & C yellow 6-sunset yellow

6. Flavoring agents: For chewable tablet- flavor oil are used

7. Sweetening agents: For chewable tablets: Sugar, mannitol. Saccharine (artificial):500 times sweeter than sucrose.

Advantages:

- ❖ These are very easy and handy to use.
- ❖ As these are unit dosage form fixed dose was administered.
- ❖ Modified drug release rate and duration of tablets be able to increase their therapeutic effect and increase the patient compliance by reducing the frequency of drug administration.
- ❖ These are cost-effective dosage forms when compared to other dosage forms
- ❖ The physical, microbial and chemical stability of tablet are superior to other dosage forms.

Disadvantages:

- ❖ The systemic availability of the drug depends on many physiological factors
- ❖ The onset of action was lesser when compared to Intra Venous route. (except sublingual tablets)
- ❖ Geriatric and children's of small age cannot swallow the tablets easily.

6.THE PRELIMINARY PHYTOCHEMICAL SCREENING TEST OF *SIDDHAR KULIGAI*

The preliminary phytochemical screening test was carried out for each extracts of ***siddhar kuligai*** as per the standard procedure at The Tamil Nadu Dr. MGR Medical University, Anna Salai, Guindy, Chennai-600032.

1. Detection of alkaloids

Extracts were dissolved individually in diluted hydrochloric acid and filtered.

Mayer's test

2 ml of extract was treated with few drops of Mayer's reagent; formation of yellow colored precipitate indicates the presence of alkaloids.

Wagner's test

2 ml of filtrate was treated with Wagner's reagent. Formation of brown /reddish precipitate indicates the presence of alkaloids.

2. Detection of carbohydrate

Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for presence of carbohydrates.

Molisch's test

2 ml of filtrate was treated with few drops of alcoholic Alpha naphthol solution in a test tube. Formation of the violet ring at the junction indicates presence of carbohydrates.

Benedict's test

Filtrate was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

3. Detection of Glycosides Liebermann's test

2ml of extract was treated with 2ml chloroform and 2ml of acetic acid, Violet color change into blue and green indicates presence of Glycosides

4. Detection of saponins

Froth test

Extracts was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 centimeter layer of foam indicates the presence of Saponins.

Foam test

0.5-gram extract was shaken with 2 ml of water. If foam produced persists for 10 minutes, it indicates the presence of saponins.

5. Detection of phytosterols Salkowski's test

Extracts was treated with chloroform and filtered; the filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand for few minutes. Golden yellow color indicates the presence of triterpenes.

6. Detection of phenols

Ferric Chloride test: 2 ml of extracts was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

7.Detection of tannins

Gelatin test

To the extracts, 1% of gelatin solution containing sodium chloride was added; formation of white precipitate indicates the presence of tannins.

8. Detection of flavonoids Alkaline reagent test

Extract was treated with few drops of 10% sodium hydroxide, formation of intense yellow color then on addition of diluted hydrochloric acid it becomes colorless, and it indicates the presents of flavonoids.

Lead acetate test

Extract was treated with few drops of lead acetate solution; yellow color precipitate indicates presence of flavonoids.

9. Detection of proteins and amino acids Xanthoproteic

Test:

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

10. Detection of diterpenes

Copper Acetate test

Extracts were dissolved in water and treated with 3-4 drops of copper Acetate solution; formation of emerald green color indicates the presence of diterpenes.

11. Test for gum and mucilage

The extract was dissolved in 10 ml of distilled water and to this 2ml of absolute alcohol with the constant stirring white cloudy precipitate indicates the presence of gum and mucilage.

12. Test for Quinones

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones. The results were tabulated in table number 2.

7. THE PRELIMINARY PHYSICOCHEMICAL SCREENING TEST OF *SIDHHAR KULIGAI*

Physicochemical Properties of *siddhar kuligai* was carried out for each extracts of *siddhar kuligai* as per the standard procedure at The Tamil Nadu Dr. MGR Medical University, Anna Salai, Guindy, Chennai-600032.

Physico-chemical studies of the plant drugs are necessary for standardization, as it helps in under-standing the significance of physical and chemical properties of the substance being analyzed in terms of their observed activities and especially for the determination of their purity and quality. The analysis includes the determination of ash value, Loss on drying of the sample at 105ºC, pH value and Extractive value. These were carried out as per guidelines.

1. Loss On Drying:

An accurately weighed 2g of siddhar kuligai formulation was taken in a tarred glass bottle. The crude drug was heated at 105^0C for 6 hours in an oven till a constant weight. Percentage moisture content of the sample was calculated with reference to the shade dried material.

2. Determination of total ash:

Weighed accurately 2g of siddhar kuligai formulation was added in crucible at a temperature 600^0C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

3. Determination of acid insoluble ash:

Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and Filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffler furnace. The percentage of acid insoluble as was calculated with reference to the air dried drug.

4. Determination of water soluble ash:

Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15min at a temperature not exceeding 450^0C in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

5. Determination of water soluble Extractive:

5gm of air dried drug, coarsely powered siddhar kuligai was macerated with 100ml of distilled water in a closed flask for twenty-four hours shaking frequently.

Solution was filtered and 25 ml of filtrated was evaporated in a tarred flat bottom shallow dish, further dried at 100^0C and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

6. Determination of alcohol soluble extractive:

2.5gm. of air dried drugs; coarsely powdered siddhar kuligai was macerated with 50 ml. alcohol in closed flask for 24 hrs. With frequent shaking it was filtered rapidly taking precaution against loss of alcohol. 10ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100^0C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

7. Determination of pH:

Five grams of *siddhar kuligai* was weighed accurately and placed in clear 100 ml beaker. Then 50 ml of distilled water was added to it and dissolved well. After 30 minutes it was then applied in to pH meter at standard buffer solution of 4.0, 7.0, and 9.2. Repeated the test four times and average was recorded. The results were tabulated in Table –03

8. CHEMICAL ANALYSIS OFSIDDHAR KULIGAI

The chemical analysis of Siddhar Kuligai was carried out in Bio chemistry lab, National Institute of Siddha.

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

Preparation of Extract:

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

S.No	EXPERIMENT	OBSERVATION	INFERENCE
	I. Test For Acid Radicals		
1.	**Test For Sulphate:** 2ml of the above prepared extract was taken in a test tube to this added 2ml of 4% dil ammonium oxalate solution	No cloudy appearance	Absence of Sulphate
2.	**Test For Chloride:** 2ml of the above prepared extract was added with 2ml of dil-HCl until the effervescence ceases off.	No Cloudy appearance was formed	Absence of Chloride
3.	**Test For Phosphate:** 2ml of the extract was treated with 2ml of dil.ammoniummolybdate solution and 2ml of Con.HNo3	Cloudy yellow appearance present	Presence of Phosphate
4.	**Test For Carbonate:** 2ml of the extract was treated with 2ml dil. magnesium sulphate solution.	No Cloudy appearance was evolved.	Absence of carbonate
5.	**Test For Nitrate:** 1gm of the extract was heated with copper turning and concentrated H2So4 and viewed the test tube vertically down.	No Brown gas was evolved	Absence of nitrate
6.	**Test For Sulphide:** 1gm of the extract was treated with 2ml of Con. HCL	No rotten egg smelling gas was evolved	Absence of Sulphide
7.	**Test For Fluoride & Oxalate:** 2ml of extract was added with 2ml of dil. Acetic acid and 2ml dil.calcium chloride solution and heated**.**	No cloudy appearance.	Absence of fluoride and oxalate

8.	**Test For Nitrite:** 3drops of the extract was placed on a filter paper, on that-2 drops of dil.acetic acid and 2 drops of dil.Benzidine solution were placed.	No characteristic changes were noted.	Absence of nitrite
9.	**Test For Borate:** 2 Pinches (50mg) of the extract was made into paste by using dil.sulphuric acid and alcohol (95%) and introduced into the blue flame.	No Appearance of bluish green color.	Absence of borate
II. Test For Basic Radicals			
1.	**Test For Lead:** 2ml of the extract was added with 2ml of dil.potassium iodine solution.	No Yellow precipitate was obtained	Absence of lead
2.	**Test For Copper:** One pinch (25mg) of extract was made into paste with Con. HCl in a watch glass and introduced into the non-luminuous part of the flame.	No blue colourappeared	Absence of copper
3.	**Test For Aluminium:** To the 2ml of extract dil.sodium hydroxide was added in 5 drops to excess.	No yellow Colourappeared	Absence of Aluminium.
4.	**Test For Iron:** a. To the 2ml of extract, added 2ml of dil.ammonium solution b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNO3 were added	No Red colour appeared	Absence of Iron
5.	**Test For Zinc:** To 2ml of the extract dil. sodium hydroxide solution was added in 5 drops to excess and dil. ammonium chloride was added.	No White precipitate was formed	Absence of Zinc

6.	**Test For Calcium:** 2ml of the extract was added with 2ml of 4% dil.ammonium oxalate solution	No Cloudy appearance and white precipitate was formed	Absence of calcium
7.	**Test For Magnesium:** To 2ml of extract dil. sodium hydroxide solution was added in 5 drops to excess.	No White precipitate was obtained	Absence of magnesium
8.	**Test For Ammonium:** To 2ml of extract 1 ml of Nessler's reagent and excess of dil.sodium hydroxide solution were added.	No Brown colour appeared	Absence of ammonium
9.	**Test For Potassium:** A pinch (25mg) of extract was treated with 2ml of dil. sodium nitrite solution and then treated with 2ml of dil. cobalt nitrate in 30% dil. glacial acetic acid.	No Yellow precipitate was obtained	Absence of potassium
10.	**Test For Sodium**: 2 pinches (50mg) of the extract was made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow colour flame evolved.	Absence of sodium
11.	**Test For Mercury**: 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.	No Yellow precipitate was obtained	Absence of Mercury
12.	**Test For Arsenic:** 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.	No Brownish red precipitate was obtained	Absence of arsenic

III. Miscellaneous			
1.	**Test For Starch:** 2ml of extract was treated with weak dil.Iodine solution	No Blue colour developed	Absence of starch
2.	**Test For Reducing Sugar:** 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes were noted.	No Brick red colour is developed	Absence of reducing sugar
3.	**Test For The Alkaloids**: a) 2ml of the extract was treated with 2ml of dil.potassiumlodide solution. b) 2ml of the extract was treated with 2ml of dil.picric acid. c) 2ml of the extract was treated with 2ml of dil.phosphotungstic acid.	Yellow colour developed	Presence of Alkaloid
4	**Test For Tannic Acid:** 2ml of extract was treated with 2ml of dil. ferric chloride solution	No Blue-black precipitate was obtained	Absence of Tannic acid
5	**Test For Unsaturated Compound:** To the 2ml of extract, 2ml of dil. Potassium permanganate solution was added.	Potassium permanganate was not decolourised	Absence of unsaturated compound
6	**Test For Amino Acid:** 2 drops of the extract was placed on a filter paper and dried well. 20ml of Burette reagent was added**.**	No Violet colour appeared	Absence of amino acid
7	**Test For Type of Compound:** 2ml of the extract was treated with 2 ml of dil. ferric chloride solution.	No green and red colour developed	Absence of quinolepinephrinepyrocatechoantipyrine

			Aliphatic amino acid and meconic acid.
		No Violet colour developed	Apomorphine salicylate and Resorcinol were absent
		No Blue colour developed.	Morphine, Phenol cresol and hydrouinone were Absent.

The results were tabulated in Table –04

9. TLC/ HPTLC FINGER PRINT ANALYSIS

Thin layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound.

TLC/HPTLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation. TLC/HPTLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. The goal of TLC/HPTLC is to obtain well defined, well separated spots.

Retention Factor

After a separation is complete, individual compounds appear as spots separated vertically. Each spot has a retention factor (Rf) which is equal to the distance migrated over the total distance covered by the solvent.

Rf = Distance traveled by sample /Distance traveled by solvent

The Rf value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions.

The compound with the larger Rf value is less polar because it does not stick to the stationary phase as long as the polar compound, which would have a lower Rf value.

The results were tabulated in Table –05

10. MICROBIAL ANALYSIS

Microbial analysis was carried for determination of microbial contamination as per procedures of Indian Pharmacopoeia 2010 and WHO Guidelines. The test included total bacterial count, total fungal count and identification of specified organisms such as Escherichia coli, Salmonella sp., Staphylococcus aureus and Entero bacteriaceae.
The results were tabulated in Table –06

11. AFLATOXIN

The Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2 in ***Siddhar kuligai*** have been analyzed in Regional Research Institute of Unani Medicine (RRIUM), Royapuram, Chennai-600013.The results were tabulated in Table –07

Test for Aflatoxin:

The procedures recommended for the detection of Aflatoxin as per WHO (2007).

Instrument Details:

Name of the Instrument	**:**	CAMAG (CAMAG - Automatic TLC sampler, Scanner and Visualiser)
Spray Gas	**:**	N2
Lamp used	**:**	Mercury Lamp

The samples were processed as per procedures recommended in WHO 2007 and applied for the Thin Layer Chromatography and High Performance Thin Layer Chromatography study with suitable solvent systems. After development the plate was allowed to dry in air and examined under UV 366nm.

INSTRUMENTAL ANALYSIS

12. FT-IR (Fourier Transform Infra-Red)

DEFINITION:

FTIR offers quantitative and qualitative analysis for organic and inorganic samples. Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum. The spectra produce a profile of the sample, a distinctive molecular fingerprint that can be used to screen and scan samples for many different components. FTIR is an effective analytical instrument for detecting functional groups.

APPLICATIONS: Quantitative Scans, Qualitative Scans x Solids, Liquids, Gases

- Organic Samples, Inorganic Samples
- Unknown Identification
- Impurities Screening
- Formulation
- Pharmaceuticals.

Fig 3.FTIR (Fourier Transform Infrared Spectroscopy)

FTIR INSTRUMENT

INSTRUMENT DETAILS

Model	: Spectrum one: FT-IR Spectrometer
Scan Range	: MIR 450-4000 cm-1
Resolution	: 1.0 cm-1
Sample required	: 50 mg, solid or liquid.

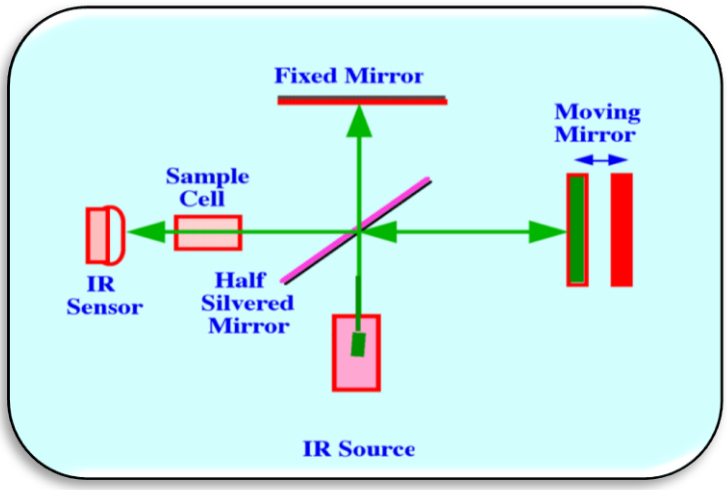
It is the preferred method of infrared spectroscopy. FT-IR is an important and more advanced technique. It is used to identify the functional group, to determine the quality and consistency of the sample material and can determine the amount of compounds present in the sample. It is an excellent tool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

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

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

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated .

Fig 3.FTIR (Fourier Transform Infrared Spectroscopy)



FTIR MECHANISM

13. SEM (SCANNING ELECTRON MICROSCOPE)

DEFINITION

Scanning Electron Microscopy (SEM), also known as SEM analysis or SEM microscopy, is used very effectively in microanalysis and failure analysis of solid inorganic materials. Scanning electron microscopy is performed at high magnifications, generates high-resolution images and precisely measures very small features and objects.

SEM INSTRUMENT

SEM ANALYSIS APPLICATIONS

The signals generated during SEM analysis produce a two-dimensional image and reveal information about the sample including:

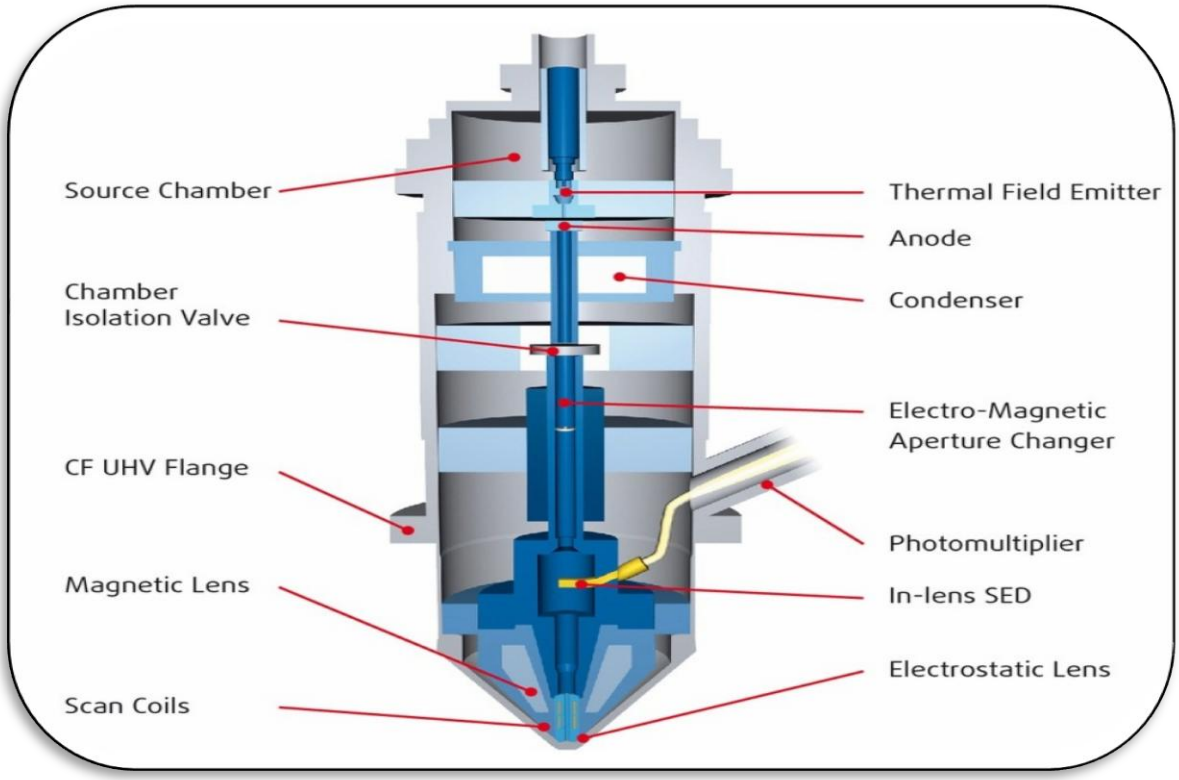
- External morphology (texture)
- Chemical composition (when used with EDS)
- Orientation of materials making up the sample
- The EDS component of the system is applied in conjunction with SEM analysis to:
- Determine elements in or on the surface of the sample for qualitative information
- Measure elemental composition for semi-quantitative results
- Identify foreign substances that are not organic in nature and coatings on metal
- SEM Analysis with EDS – qualitative and semi-quantitative results
- Magnification – from 5x to 300,000x

- Sample Size – up to 200 mm (7.87 in.) in diameter and 80 mm (3.14 in.) in height Materials analysed – solid inorganic materials including metals and minerals.

THE SEM ANALYSIS PROCESS

Scanning Electron Microscopy uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. In most SEM microscopy applications, data is collected over a selected area of the surface of the sample and a two-dimensional image is generated that displays spatial variations in properties including chemical characterization, texture and orientation of materials. The SEM is also capable of performing analyses of selected point locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations.

The EDS detector separates the characteristic X-rays of different elements into an energy spectrum and EDS system software is used to analyse the energy spectrum in order to determine the abundance of specific elements. A typical EDS spectrum is portrayed as a plot of X-ray counts vs. energy (in keV). Energy peaks correspond to the various elements in the sample. Energy Dispersive X-ray Spectroscopy can be used to find the chemical composition of materials down to a spot size of a few microns and to create element composition maps over a much broader raster area. Together, these capabilities provide fundamental compositional information for a wide variety of materials, including polymers. In scanning electron microscope high energy electron beam is focused through a probe towards the sample material. Variety of signals was produced on interaction with the surface of the sample. This results in the emission of electrons or photons and it is collected by an appropriate detector.



SEM MECHANISM

The types of signal produced by a scanning electron microscope include

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

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

EDAX: (Energy Dispersive X-Ray Analysis)

Energy Dispersive X-Ray Analysis is also known as EDS or EDX. It is an X-Ray technique used to detect the composition of elements present in the given material. It has its attachments to electron microscopy instruments like scanning electron microscopy (SEM) or transmission electron microscopy (TEM) as the imaging competence of the microscope identifies the sample material.

The data produced by the EDX analysis consists of the spectra containing the elements present in the given sample which is being analysed. It is also possible to get the elemental mapping and image analysis of the sample.

EDAX technique is a non-destructive and can be qualitative, quantitative and provide spatial distribution of the elements.

14. x-ray fluorescence (XRF) :-

An x-ray fluorescence (XRF) spectrometer is an x-ray instrument used for routine, relatively non-destructive chemical analyses of rocks, minerals, sediments and fluids. It works on wavelength dispersive spectroscopic principles that are similar to an electron microprobe (EPMA). However, an XRF cannot generally make analyses at the small spot sizes typical of EPMA work (2-5 microns), so it is typically used for bulk analyses of larger fractions of geological materials. The relative ease and low cost of sample preparation, and the stability and ease of use x-ray spectrometers make this one of the most widely used methods for the analyses of major and trace elements in rock, minerals and sediment.

Fundamental principles of X-ray Fluorescence (XRF) :-

The XRF method depends on fundamental principles that are common to several other instrumental methods involving interactions between electron beams and x-rays with samples, including: X-ray spectroscopy (e.g : SEM – EDS), X-ray diffraction (XRD), and wavelength dispersive spectroscopy. Analysis of major and trace elements in geological materials by x-ray fluorescence is made possible by the behavior of atoms when they react with radiation. When materials are excited with high energy, short wavelength radiation (e.g : X-rays), they can become ionized. If the energy of radiation is sufficient to dislodge a tightly held inner electron, the atom becomes unstable and an outer electron replaces the missing inner electron. When this happens, energy is released due to the decreased binding energy of the inner electron orbital compared with an outer one. The emitted radiation is of lower energy than the primary incident X-rays and is termed fluorescent radiation. Because the energy of the emitted photon is characteristic of a transition between specific electron orbital in a particular element, the resulting fluorescent X-rays can be used to detect the abundances of elements that are present in the sample.

Procedure:-

The analysis of major and trace elements in geological materials by XRF is made possible by the behavior of atoms when they interact with X-radiation. An XRF spectrometer works because if a sample is illuminated by an intense X-ray beam, known as the incident beam, some of the energy is scattered, but some is also absorbed within the sample in a manner that depends on its chemistry. The incident X-ray beam is

typically produced from a Rh target, although W, Mo, Cr and others can also be used, depending on the application.

When this primary X-ray beam illuminates the sample, it is said to be excited. The excited sample in turn emits X-rays along a spectrum of wavelength characteristic of the type of atoms present in the sample. How does this happen? The atoms in the sample absorb X-ray energy by ionizing, ejecting electrons from the lower (usually K and L) energy levels. The ejected electrons are replaced by electrons from an outer, higher energy orbital. When this happens, energy is released due to the decreased binding energy of the inner orbital compared with an outer one. This energy release is in the form of emission of characteristic X-rays indicating the type of atom present. If a sample has many elements present, as is typical for most minerals and rocks, the use of a Wavelength Dispersive Spectrometer much like that in an EPMA allows the separation of a complex emitted X-ray spectrum into characteristic wavelengths for each element present. Various type of detectors (gas flow proportional and scintillation) are used to measure the intensity of the emitted beam. The flow counter is commonly utilized for measuring long wavelength (~0.15nm). X-rays that are typical of K spectra form elements lighter than Zn. The scintillation detector is commonly used to analyze shorter wavelengths in X-ray spectrum (K spectra of element from Nb to I; L spectra of Th and U). X-rays of intermediate wavelength (K spectra produced from Zn to Zr and L spectra from Ba and the rare earth elements) are generally measured by using both detectors in tandem. The intensity of the energy measured by these detectors is proportional to the abundance of the element in the sample. The exact value of this proportionality for each element is derived by comparison to mineral or rock standards whose composition is known from prior analyses by other techniques.

X-ray fluorescence is particularly well suited for investigations that involve

- Bulk chemical analyses of major elements (Si, Ti, Al, Fe, Mn, Mg, Ca, Na, K, P) nin rock and sediment.
- Bulk chemical analyses of trace elements (in abundances ~1ppm; Ba, Ce, Co, Cr, Cu, Ga, La, Nb, Ni, Rb, Sc, Sr, Rh, U, V, Zr, Zn) in rock and sediments detection limits for trace elements are typically on the order of a few parts per million.

X-ray fluorescence is limited to analysis of

- Relatively large samples, typically ~1 gm
- Materials that can be prepared in powder form and effectively homogenized
- Materials for which compositionally similar, well-characterized standards are available
- Materials containing high abundances of elements for which

Absorption and fluorescence effects are reasonably well understood.

In most cases of rocks, ores, sediments and minerals, the sample is ground to a fine powder. At this point it may be analysed directly, especially in the case of trace element analyses. However, the widerange in abundances of different elements, especially iron and the wide range of sizes of grains in a powdered sample, makes the proportionality comparison to the standards particularly troublesome. For this reason, it is common practice to mix the powdered sample with a chemical flux and use a furnace or gas burner to melt the powdered sample. Melting created a homogeneous glass that can be analyzed and the abundance of the (now somewhat diluted) elements calculated.

Strengths:-

X-ray fluorescence is particularly well suited for investigations that involve:

- Bulk chemical analyses of major elements (Si, Ti, Al, Fe, Mn, Mg, Ca, Na, K, P) nin rock and sediment.
- Bulk chemical analyses of trace elements (in abundances ~1ppm; Ba, Ce, Co, Cr, Cu, Ga, La, Nb, Ni, Rb, Sc, Sr, Rh, U, V, Zr, Zn) in rock and sediments.

The results were tabulated in Table –07

15. ULTRAVIOLET – VISIBLE SPECTROSCOPY:-

UV spectroscopy is an important tool in analytical chemistry. The other name of UV (Ultra violet) spectroscopy is Electronic spectroscopy as it involves the promotion of the electrons from the ground state to the higher energy or excited state.

Introduction to UV spectroscopy:-

UV spectroscopy is type of absorption spectroscopy in which light of ultra violet region (200-400nm) is absorbed by the molecule. Absorption of the ultra violet radiations results in the excitation of the electrons from the ground state to higher energy state.

Principle of UV spectroscopy:-

UV spectroscopy obeys the Beer-Lambert law, which states that: when a beam of monochromic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution.

Procedure:-

Monochromators generally composed of prisms an slits. The most of the spectrometers are double beam spectrophotometers. The radiation emitted from the primary source is dispersed with the help of rotating prisms. The various wave lengths of the light source which are separated by the prism are then selected by the slits such the rotation of the prism results in a series of continuously increasing wave length to pass through the slits for recording purpose. The beam selected by the slit is monochromatic and further divided into two beams with the help of another prisms.

Uses:

Identification of an unknown compound

An unknown compound can be identified with the help of UV spectroscopy. The spectrum of unknown compound is compared with the spectrum of a reference compound and if both the spectrums coincide then it confirms the identification of unknown substance.

Determination of the purity of a substance:

Purity of a substance can also be determined with the help of UV spectroscopy. The absorption of the sample solution is compared with the absorption of the reference solution. The intensity of the absorption can be used for the relative calculation of the purity of sample solution.

16. PHARMACOLOGICAL ACTIVITY

16.1.ANTICANCER ACTIVITY-IN-VITRO BY XTT ASSAY

XTT ASSAY

Cell proliferation assays are widely used in cell biology for the study of growth factors, cytokines or media components. They are also applied in the screening of cytotoxic agents and lymphocyte activation. In order to determine the number of viable cells *Cell Proliferation Kit XTT* employs 2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide salt (XTT). Only in living cells mitochondria- are capable to reduce XTT to form an orange colored water soluble dye. Therefore, the concentration of the dye is proportional to the number of metabolically- active cells[46].

KEYWORDS

- XTT assay
- cytotoxicity testing
- non radioactive assay
- quantitating and viability testing of cells

Background Principle of the XTT Assay

- The XTT cell proliferation assay was first described in 1988 by Scudiero*et al*. (3) as an effective method to measure cell growth and drug sensitivity in tumor cell lines. XTT is a colorless or slightly yellow compound that when reduced becomes brightly orange (Figure 1). This color change is accomplished by breaking apart the positively-charged quaternary tetrazole ring (2). The formazan product of XTT reduction is soluble and can be used in real-time assays.

- XTT is thought to be excluded from entering cells by its net negative charge (2). Considerable evidence suggests that XTT dye reduction occurs at the cell surface facilitated by trans-plasma membrane electron transport. Mitochondrial oxido reductases are thought to contribute substantially to the XTT response with their reductants being transferred to the plasma membrane (Figure 2). It has been proposed that XTT assays actually measure the pyridine nucleotide redox status of cells (2, 4).

❖ XTT can be used alone as a detection reaction but the results are not optimal. XTT assay results are greatly improved when an intermediate electron acceptor, such as PMS (N-methyl dibenzopyrazine methyl sulfate), is used with XTT (Figure 2). PMS is the Activation Reagent included in the XTT Cell Proliferation Assay Kit. Findings suggest that PMS mediates XTT reduction by picking up electrons at the cell surface, or at a site in the plasma membrane that is readily accessible, and forms a reactive intermediate that then reduces XTT to its highly pigmented formazan product.

Figure 1.The Reduction of XTT to form the Colored Formazan Derivative

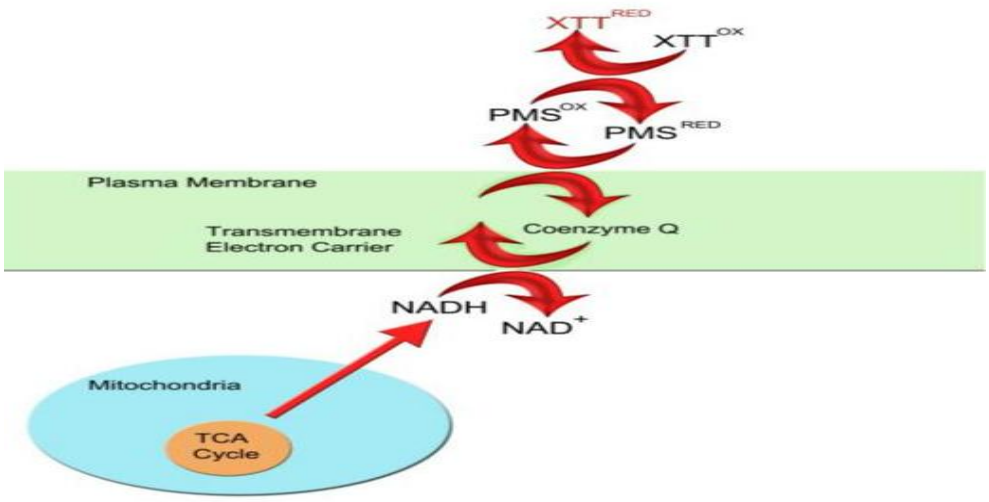


Figure 2. The Colorimetric Reduction of XTT by Cellular Enzyme

XTT Assay Greatly Simplifies Measuring of Cell Proliferation and Viability

- The test procedure includes cultivation of cells in a 96-well plate, adding the XTT reagent and incubating for 2 – 24 hours. During the incubation time (usually within 2 – 5 hours) an orange color is formed, the intensity of which can be measured with a spectrophotometer, in this instance, an ELISA reader.
- The intensity- of the dye is proportional to the number of metabolically active cells, i.e. the greater the number of metabolically active cells in the well, the greater the activity of mitochondrial enzymes, and the higher the concentration of the dye formed. The dye formed is water soluble and the dye intensity can be read without further treatments. The use of multiwell plates and an ELISA reader enables testing- a large number of samples and obtaining rapid results[47]

Advantages of Cell Proliferation Kit XTT

- Save: without using radioactive isotopes
- Accurate: dye absorbance is proportional to the number of cells in each well
- Easy-to-use: 1-step process, results within 2 – 5 hours.
- Includes XTT reagent and activation reagent. Additional reagents or cell washing procedures are not required.
- For use in plate readers

Materials Required

- Inverted Phase contrast microscope
- Biosafety cabinet class-II
- Cytotoxic safety cabinet
- CO_2incubator
- Deep freezer
- ELISA plate reader
- Micropipettes
- XTT cell proliferation kit (Cayman, USA)[48]

List of cells which were tested with Cell Proliferation Kit XTT[49]

- Pancreatic carcinoma cell line
- Monocytes
- Human hepatocarcinoma cells – Hep G2 Ehd-1 embryonic fibroblasts
- Human embryonic 293 kidney cells Mouse fibroblasts
- Human prostate carcinoma cells – CL1, 22RV1 (subclone of CWR22 xenograft) and LNCaP
- Myofibroblasts
- Prostate cancer cell lines DU-145 and PC-3
- Mammary gland breast cancer cell lines MCF-7alpha and MDA-MB231
- Epithelial colorectal adenocarcinoma cell line HT-29
- Small cell lung carcinoma cell line AL-780
- Mouse myeloid cell line
- Primary human umbilical vein endothelial cells
- ALL cells of B cell lineage
- FDCP cell line
- Human monocytic cell lines U937, THP-1 and monomact Mouse macrophage like cell line RAW 264.7
- Freshly isolated human T cells isolated into CD3, CD4 and CD28 populations
- 293T – (embryonic kidney)
- Hela – (cervix carcinoma)
- Hep G2 – (heptocellular carcinoma)
- D 145 – (prostate cancer)
- A375 – (malignant melanoma)
- MCF-7 – (breast adenocarcinoma)
- BXPC-3 – (pancreatic adenocarcinoma)
- Synovial cells
- Monocytic cells (U937)
- CD3 T-cells from 4–6 week mouse spleens
- Human keratinocytes (HaCat) and murine fibroblasts (NIH 3T3)
- Human breast cancer cells (T-47D, MDA-MB-468)

- Murine C3H10T1/2 progenitor cells
- Human embryonic kidney cells (HEK 293)
- CHO cells
- Early and late murine hematopoietic cells

XTT Assay Data Interpretation

The plot of the XTT assay data should provide a curve with a linear portion. This is the area that will show the greatest sensitivity to changes induced by the experimental parameters. Absorbance values that are higher than control conditions indicate an increase in cell proliferation and viability. Absorbance values that are lower than control conditions indicate a decrease in cell proliferation and may be the result of cellular necrosis or apoptosis. It is recommended that assay results be confirmed by assessing microscopic morphological changes using an inverted light microscope to observe the cells.

16.2. ANALGESIC ACTIVITY OF *SIDDHAR KULIGAI*

Aim:

To study the Analgesic activity of ***siddhar Kuligai*** in in Wistar albino mice by Eddy's Hot plate method

Materials and methods:

Test Substance	**:**	***siddhar kuligai***
Animal Source	**:**	TANUVAS, Madhavaram, Chennai.
Animals	**:**	Wistar Albino mice (Male -12, female -12)
Age	**:**	4-5 weeks
Body Weight	**:**	25-30gm.
Acclimatization	**:**	14 days prior to dosing.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	Bycage number, animal number and individual Marking by using Picric acid.
Diet	**:**	Pellet feed
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	24-28°C
Relative humidity	**:**	between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.

Selection of animals:

Healthy Wistar albino mice (25-30g) of both sex were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory. IAEC approved no. NIS/IAEC-II/15/2016

The animals kept in plastic cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water and libitum. They were feed with standard diet and kept in well ventilated animal house they also maintained with alternative dark-light cycle of 12hrs throughout the studies. Rats were allowed an acclimatization period of 14 days before actual experiments.

The rats were closely observed for any infection and if they show signs of infection they were excluded from the study. The animal experiment was performed with accordance legislation on welfare.

Grouping:

Group I	-	2% distilled water (10ml/kg)
Group II	-	Pentazocine (5mg/kg)
Group III	-	*Siddhar Kuligai* (2mg/kg)
Group IV	-	*Siddhar Kuligai* (4mg/kg)

Procedure:

Animals were weighed and placed on the hot plate. Temperature of the hot plate was maintained at 55±1^{o}C. Responses such as jumping, withdrawal and licking of the paws were seen. The time period (latency period), from when the animals were placed and until the responses occurred, were recorded using a stopwatch. To avoid tissue damage of the animals 10 seconds was kept as a cut off time. The time obtained was considered the basal/normal reaction time in all the untreated groups of animals. Increase in the basal reaction time was the index of analgesia. All the animals were screened initially at least three times in this way and the animals showing a large range of variation in the basal reaction time were excluded from the study. A final reading of the basal reaction time was recorded for the included animals. After selecting the animals, the drugs were administered to all the groups at the stipulated doses. The reaction times of the animals were then noted at 0, 30,60,90,120 and 150 min interval after drug administration.

Statistical analysis

Results were expressed as mean ± SEM and analyzed using Graph Pad Prism software. One way analysis of variance (ANOVA) test was applied P value less than 0.05 ($P<0.05$) was considered as statistically significant. The results were tabulated in Table –09

16.3. ANTI-INFLAMMATORY ACTIVITY OF SIDDHAR KULIGAI

Aim:

To study the Anti-inflammatory effect of *Siddhar Kuligai* in Wistar albino rats by Carrageenan-induced rat paw edema.

Materials and methods:

Test Substance	**:**	*Siddhar Kuligai*
Animal Source	**:**	TANUVAS, Madhavaram, Chennai.
Animals	**:**	Wistar Albino Rats (Male -12, Female -12)
Age	**:**	6-8 weeks
Body Weight	**:**	140-160gm.
Acclimatization	**:**	14 days prior to dosing.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	By cage number, animal number and individual marking by using Picric acid.
Diet	**:**	Pellet feed
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	24-28°C
Relative humidity	**:**	between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.

Selection of animals:

Healthy Wistar albino rats (140- 160g) of both sexes were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory. IAEC approved no: NIS/IAEC-II/15/2016.

The animals kept in plastic cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water and libitum. They were feed with standard diet and kept in well ventilated animal house they also maintained with alternative dark-light cycle of 12hrs throughout the studies. Rats were allowed an acclimatization period of 14 days before actual experiments.

The rats were closely observed for any infection and if they show signs of infection they were excluded from the study. The animal experiment was performed with accordance legislation on welfare.

The experimental protocol

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

Group I	-	2% distilled water (10ml/kg)
Group II	-	Indomethacin (10mg/kg)
Group III	-	*Siddhar Kuligai* (24mg/kg)
Group IV	-	*Siddhar Kuligai* (50mg/kg)

Acute inflammation was induced by carrageenan. Carrageenan was administrated by sub-planter injection of 0.1 ml freshly prepared 1% suspension in right hind paw in rats. The paw volume was measured initially and then 0,1,2,3hr after the carrageenan injection by using plethysmo Graphic method.

All the results were reported as mean + SEM. They were further analyzed using Two way analysis of variables (ANOVA) followed by Tukey's multiple comparison test.

The results were tabulated in Table –08

17. RESULTS

Many studies have been carried out to bring the efficacy and potency of the drug *SIDDHAR KULIGAI*. The study includes literary collections, organoleptic character, physicochemical and phytochemical analysis, FTIR, UV, SEM-EDAX, XRF and pharmacological study. The drug ***SIDDHAR KULIGAI*** has been selected from the text ***"VEERAMAMUNIVAR VAGADA THIRATU PART 1".***

- Botanical aspect explains the active principle and medicinal uses of the plants.
- Gunapadam review brings the effectiveness of the drug in the management of cancer.
- The pharmacological review explains about the evaluation Of Anti inflammatory, Anti cancer and analgesic Activities.

Standardization of the test drug

Traditional remedies is advantageous, it does suffer some limitations. The main limitation is the lack of standardization of raw materials, of processing methods and of the final products, dosage formulation, and the non- existence of criteria for quality control. Standardization of the drug is more essential to derive the efficacy, potency of the drug by analyzing it through various studies. Following tables and charts are the results of physicochemical and chemical analysis. Physical characterization and estimation of basic and acidic radicals have been done and tabulated. pharmacological activity of the drug were derived. Its result has been tabulated below.

ANALYTICAL STUDY OF SIDDHAR KULIGAI

1. ORGANOLEPTIC CHARACTER

Table: 1. Organoleptic characters of Siddhar Kuligai

Colour	Dark Brown
Odour	Pleasant
Taste	Characteristic taste
Texture	Solid

2. PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *SIDDHAR KULIGAI*

The preliminary phytochemical studies of aqueous extract of *Siddhar Kuligai* were done using standard procedures. The present study reveals that bioactive compounds carbohydrates, saponins, Phenols, Flavanoid Diterpenes, were present in all extracts of *Siddhar Kuligai* and the results were tabulated in Table number 2

Table: 2-Preliminary Phytochemical Analysis of *Siddhar Kuligai*

S.no	Phytochemicals	Test Name	H_2O ext.
1	**Alkaloids**	Mayer's test	-ve
		Wagner's test	-ve
2	**Carbohydrates**	Molisch's test	-ve
		Benedict's test	**+ve**
3	**Glycosides**	Libermann Burchard's test	**-ve**
4	**Saponins**	Froth test	**-ve**
		Foam test	**+ve**
5	**Phytosterols**	Salkowski's test	-ve
6	**Phenols**	Ferric chloride test	**+ve**
7	**Tannins**	Gelatin test	-ve
8	**Flavonoids**	Alkaline Reagent test	**-ve**
		Lead acetate test	**+ve**
9	**Proteins and Amino acids**	Xanthoproteic test	**-ve**
10	**Diterpenes**	Copper acetate test	**+ve**
11	**Gum & mucilage**	Extract + alcohol	-ve
12	**Quinone**	NAOH + Extract	-ve

+ve/-ve present or absent if component tested

Interpretation

The phytochemical analysis reveals the presence of alkaloids, tannins, flavonoids, anthral glycosides, cardiac glycosides, saponins, phenols, proteins and carbohydrates.

Flavonoids

- It is the most important group of polyphenolic compounds in plants.
- Flavonoids have potent Anti-Oxidant activity and it is its important function.
- Oxidative strees plays a vital role in the pathogenesis of hepatic diseases.
- Flavonoids can exert their Anti-Oxidant activity by scavenging the free radicals, by chelating metal ions or by inhibiting enzymatic systems responsible for free radical generation.
- It also possesses anti-microbial activity which is confirmed by the various anti-microbial assays.

Tannins.

- They restore the Anti-Oxidant status of the organs to almost normal levels.
- Increases the cellular Anti-Oxidant enzymes.
- Helps in healing of wounds and inflammation of mucous membrane.

Phenols.

- They possess rich Anti-Oxidant property and protect body from oxidative stress.
- Phenol groups are the essential part of many anti-oxidant compounds

3. PHYSICO-CHEMICAL ANALYSIS

Table: 3. Physico-chemical properties of siddhar kuligai

S.No.	Parameters	Results
1	**LOD**	17.35%
2	**Ash value**	
	a. Total ash (w/w)	4.47%
	b. Acid insoluble ash (w/w)	<1%
	c. Water Soluble ash (w/w)	2.58%
3	**Extractive values**	
	a. Alcohol successive soluble (w/v)	15.47%
4	**PH**	7.5

Interpretation

Ash:

Ash constitutes the inorganic residues obtained after complete combustion of a drug. Thus Ash value is a validity parameter describe and to assess the degree of purity of a given drug

Total ash:

Total ash value of plant material indicated the amount of minerals and earthy materials present in the plant material. The total inorganic content (ammonium, potassium, calcium, chloride, iron, etc.,) present in the drug is measured through the Total ash value and it is of 4.47 % for Siddhar Kuligai

Acid insoluble ash:

The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low.It is <1% for Siddhar Kuligai

Water soluble ash:

Water-soluble ash is the part of the total ash content, which is soluble in water. It is 2.58%for Siddhar Kuligai

EXTRACTIVE VALUES

- These are indicating the approximate measure of chemical constituents of crude drug.
- The percentage of soluble matters present in the drug is determined by the values of water extractive and ethanol extractive.
- Based on the extractive value suitable solvent can be selected. It also gives the percentage of drug which will correlate with the metabolism reactions.
- Water-soluble extractive value plays an important role in evaluation of crude drugs
- The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value

Loss on drying

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

pH:

- ❖ It is a measure of hydrogen ion concentration; it is the measure of the acidic or alkaline nature.7.0 is neutral, above 7.0 is alkaline and below is acidic.
- ❖ The pH of the drug *Siddhar Kuligai* is 7.5 which is slightly alkaline in nature and it is essential for its bioavailability and effectiveness.

4. CHEMICAL ANALYSIS

The Chemical analysis shows the presence of Phosphate, sulphate, carbonate and sulphide, Iron, Zinc, Calcium, Magnesium, Potassium, Starch, Reducing sugar, Alkaloids in Siddhar Kuligai

Table: 4. Chemical Analysis of Siddhar Kuligai-Acid Radicals

S.NO	Parameters	Results
1.	**Silicate**	Absent
2.	**Sulphate**	**Present**
3.	**Chloride**	Absent
4.	**Phosphate**	**Present**
5.	**Carbonate**	**Present**
6.	**Nitrate**	Absent
7.	**Sulphide**	**Present**
8.	**Oxalate**	Absent
9.	**Nitrite**	Absent
10.	**Borate**	Absent
11.	**Lead**	Absent
12.	**Copper**	Absent
13.	**Aluminium**	Absent

Interpretation

The acidic radicals test shows the presence of **Phosphate, sulphate, carbonate and sulphide.**

Table: 4.1 Chemical Analysis of *Siddhar Kuligai* –Basic Radicals and Miscellaneous

S.NO	Parameters	Results
14.	**Iron**	**Present**
15.	**Zinc**	**Present**
16.	**Calcium**	**Present**
17.	**Magnesium**	**Present**
18.	**Ammonium**	Absent
19.	**Potassium**	**Present**
20.	**Sodium**	Absent
21.	**Mercury**	Absent
22.	**Arsenic**	Absent
23.	**Starch**	**Present**
24.	**Reducing sugar**	**Present**
25.	**Alkaloids**	**Present**
26.	**Tannic acid**	Absent

Interpretation

The basic radical test shows the presence of Iron, Zinc, Calcium, Magnesium, Potassium, Starch, Reducing sugar, Alkaloids and absence of heavy metals such as lead, arsenic and mercury.

5. HPTLC/TLC RESUL

The TLC photos are presented in the Fig.1 and finger print profiles under short, long wavelength and white light are shown in Fig.2-4.

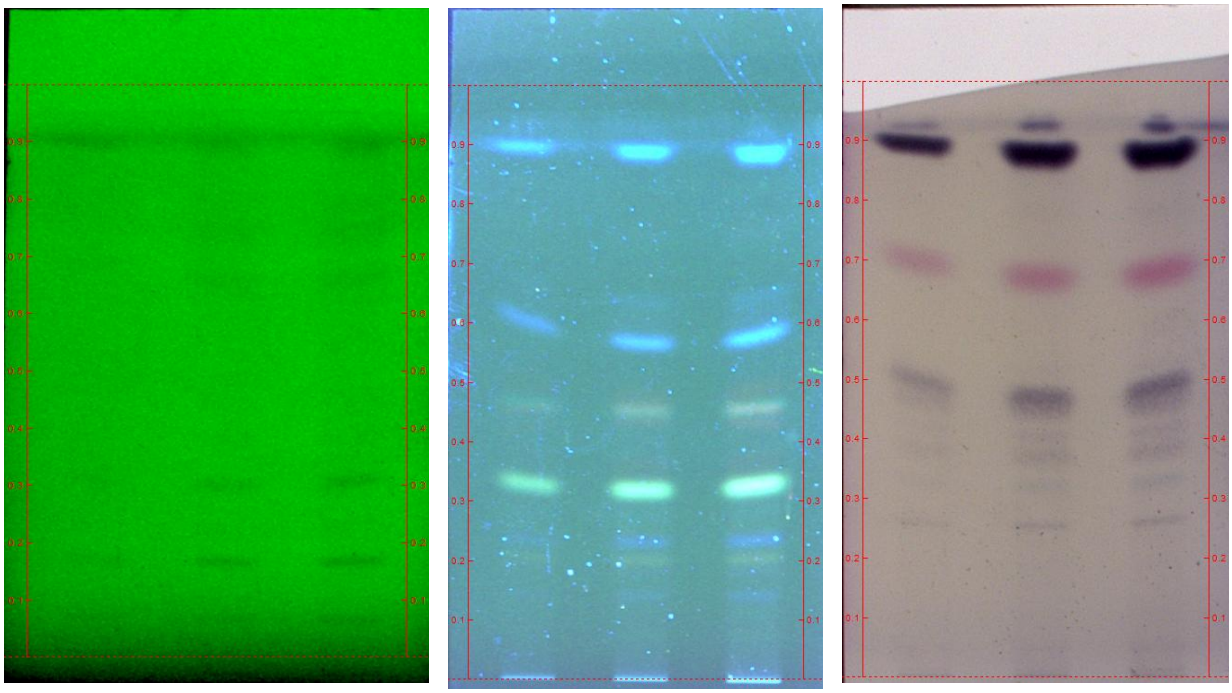
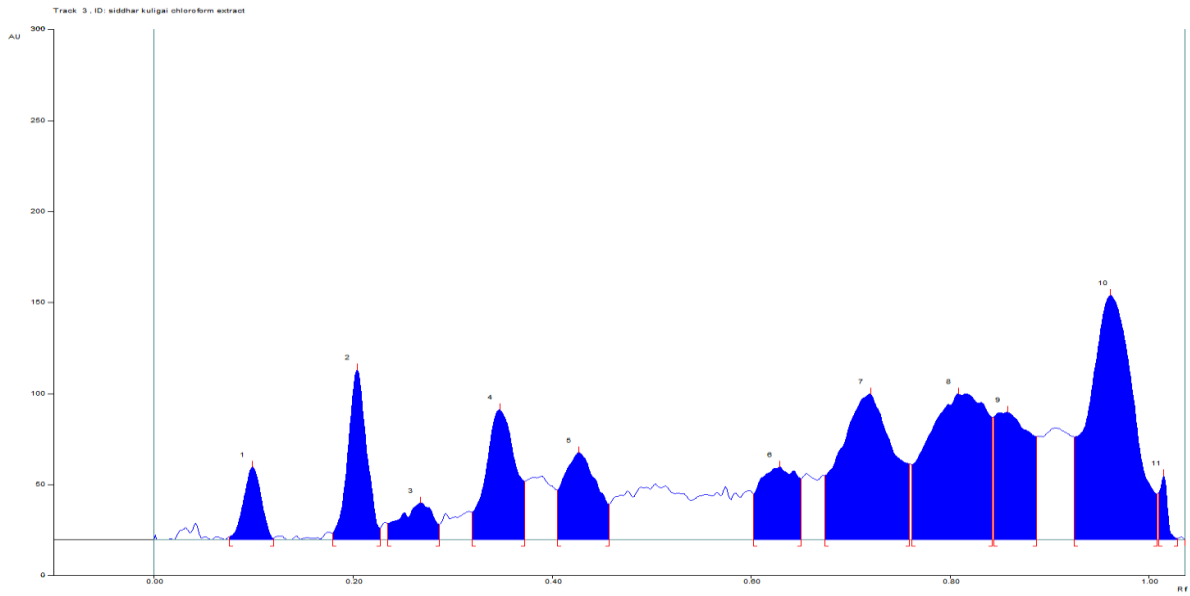


Table 5.R_f and Color of spots of TLC of Siddhar Kuligai

UV 254 nm		**UV 366 nm**		**Derivatized with Vanillin sulphuric acid reagent**	
Color	**R_f value(s)**	**Color**	**R_f value(s)**	**Color**	**R_f value(s)**
Green	0.07	Blue	0.14	Ash	0.25
Green	0.17	Brown	0.21	Ash	0.32
Green	0.30	Blue	0.24	Ash	0.37
Green	0.67	Light Green	0.32	Blue	0.47
		Brown	0.46	Pink	0.68
		Blue	0.57	Blue	0.87
		Blue	0.89	Blue	**0.94**

Interpretation

- In the TLC photo under UV 254 nm, four spots were visible at R_f 0.07, 0.17, 0.30 and 0.67 (all green color); under UV 366 nm, 7 spots appeared in which the spots at R_f 0.32 (light green), 0.46 (brown), 0.57 (blue) and 0.89 (blue) were dominant spots and other spots are minor. The derivatized plate showed seven spots in which the spots at R_f 0.47 (blue), 0.68 (pink) and 0.87 (blue) were predominant spots and other spots were minor.
- The HPTLC finger print profile under UV 254 nm showed 11 peaks among which the peak at R_f 0.93, 0.81 and 0.72 were the major peaks with area of 22.83 %, 18.39 % and 16.35 % and all other peaks individually contributed to less than 10 percent each.
- The HPTLC finger print profile under 366 nm showed 16 peaks among which the peak at R_f0.95 and 0.60 were the major peaks with area of 37.51 % and 16.08 % and all other peaks individually contributed to less than 10 percent each.
- The HPTLC finger print profile at 520 nm showed 12 peaks among which the peak at R_f 0.92, 0.71, 0.51 and 0.97 were the major peaks with area of 35.86 %, 23.69 %, 14.75 % and 10.58 % and all other peaks individually contributed to less than 10 percent each. The 3D chromatogram showed the similarity between the different concentrations of the extract.

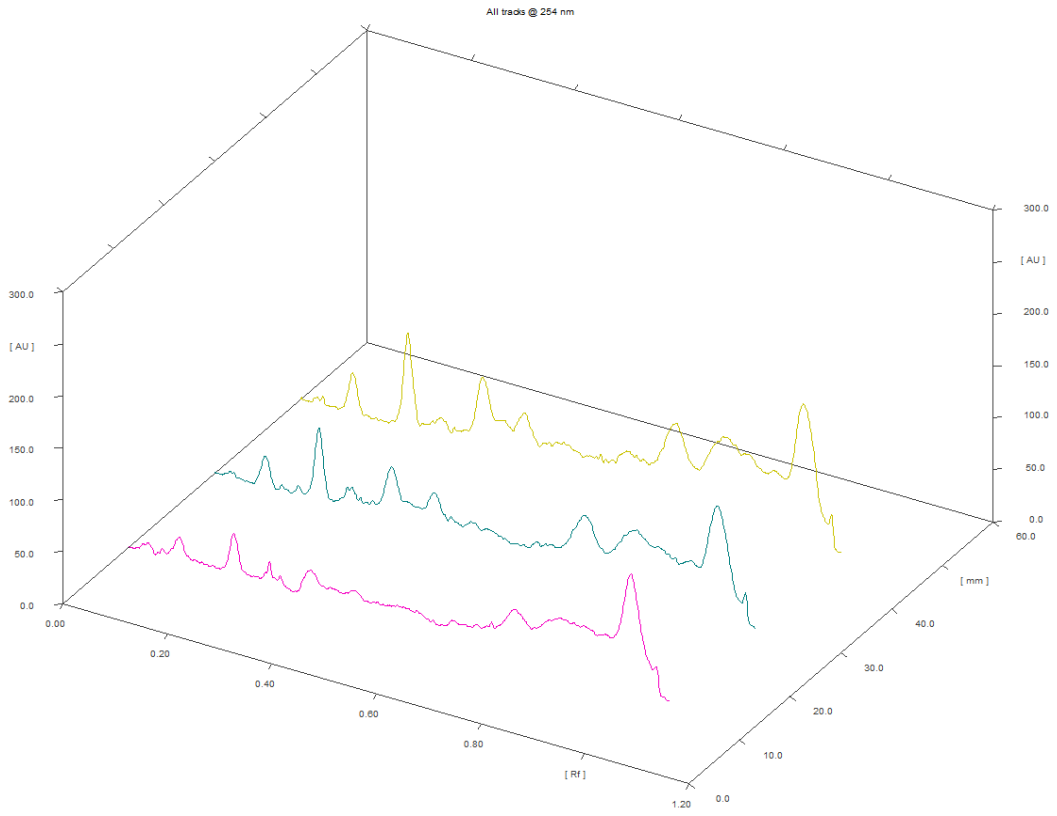


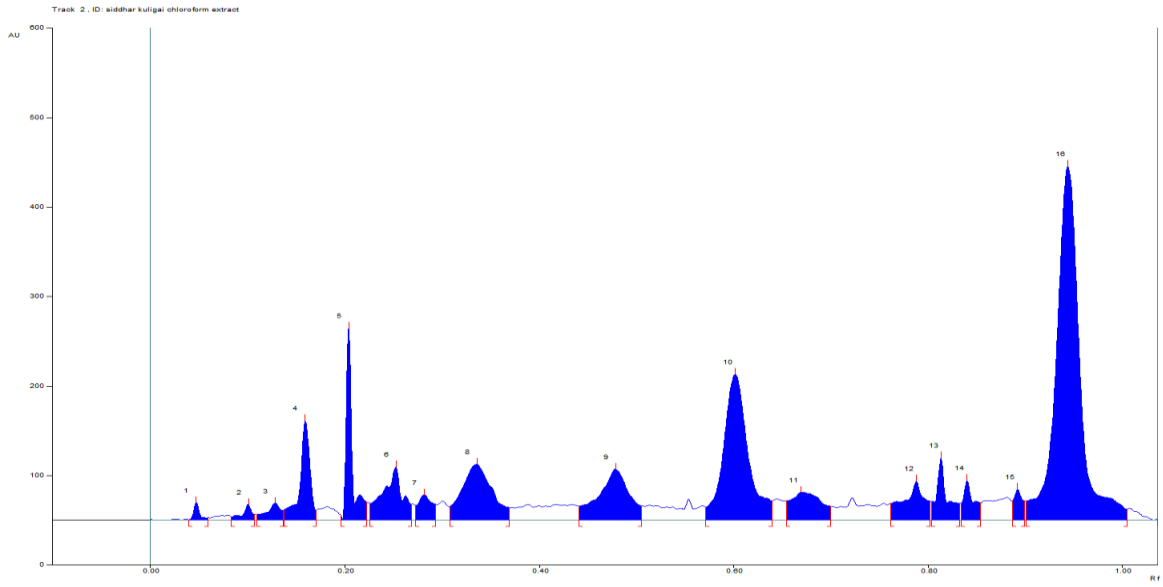
Track 3, ID: siddhar kuligai chloroform extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.08 Rf	1.6 AU	0.10 Rf	39.9 AU	5.60 %	0.12 Rf	0.5 AU	638.3 AU	2.67 %
2	0.18 Rf	3.5 AU	0.21 Rf	93.2 AU	13.09 %	0.23 Rf	6.2 AU	1576.8 AU	6.60 %
3	0.24 Rf	9.0 AU	0.27 Rf	20.1 AU	2.83 %	0.29 Rf	8.2 AU	587.9 AU	2.46 %
4	0.32 Rf	14.9 AU	0.35 Rf	71.3 AU	10.03 %	0.37 Rf	32.0 AU	1932.7 AU	8.09 %
5	0.41 Rf	27.1 AU	0.43 Rf	47.8 AU	6.71 %	0.46 Rf	19.3 AU	1492.5 AU	6.25 %
6	0.60 Rf	25.2 AU	0.63 Rf	40.0 AU	5.62 %	0.65 Rf	33.5 AU	1383.8 AU	5.79 %
7	0.68 Rf	35.2 AU	0.72 Rf	79.9 AU	11.23 %	0.76 Rf	41.6 AU	3905.8 AU	16.35 %
8	0.76 Rf	41.1 AU	0.81 Rf	80.1 AU	11.26 %	0.84 Rf	67.2 AU	4391.1 AU	18.39 %
9	0.85 Rf	67.3 AU	0.86 Rf	69.9 AU	9.82 %	0.89 Rf	56.8 AU	2265.5 AU	9.49 %
10	0.93 Rf	56.3 AU	0.96 Rf	134.3 AU	18.88 %	1.01 Rf	25.1 AU	5452.7 AU	22.83 %
11	1.01 Rf	25.4 AU	1.02 Rf	35.1 AU	4.93 %	1.03 Rf	0.6 AU	257.0 AU	1.08 %

Figure 2. HPTLC Chromatogram and peak table at 254 nm

Figure 2b. 3D Chromatogram @ 254 nm





Track 2, ID: siddhar kuligai chloroform extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.4 AU	0.05 Rf	19.4 AU	1.42 %	0.06 Rf	2.6 AU	107.6 AU	0.47 %
2	0.08 Rf	3.9 AU	0.10 Rf	17.6 AU	1.28 %	0.11 Rf	6.4 AU	154.8 AU	0.67 %
3	0.11 Rf	6.1 AU	0.13 Rf	18.8 AU	1.37 %	0.14 Rf	10.6 AU	243.9 AU	1.05 %
4	0.14 Rf	11.4 AU	0.16 Rf	111.0 AU	8.10 %	0.17 Rf	10.9 AU	1057.1 AU	4.57 %
5	0.20 Rf	5.6 AU	0.21 Rf	214.6 AU	15.66 %	0.22 Rf	20.3 AU	1229.1 AU	5.31 %
6	0.23 Rf	18.9 AU	0.25 Rf	59.5 AU	4.34 %	0.27 Rf	17.6 AU	1075.4 AU	4.65 %
7	0.27 Rf	16.3 AU	0.28 Rf	28.3 AU	2.07 %	0.29 Rf	17.6 AU	367.7 AU	1.59 %
8	0.31 Rf	14.8 AU	0.34 Rf	62.1 AU	4.53 %	0.37 Rf	14.0 AU	1747.8 AU	7.56 %
9	0.44 Rf	15.1 AU	0.48 Rf	56.8 AU	4.15 %	0.51 Rf	15.9 AU	1607.2 AU	6.95 %
10	0.57 Rf	14.5 AU	0.60 Rf	163.0 AU	11.90 %	0.64 Rf	21.4 AU	3718.9 AU	16.08 %
11	0.66 Rf	21.5 AU	0.67 Rf	31.1 AU	2.27 %	0.70 Rf	15.3 AU	903.4 AU	3.91 %
12	0.76 Rf	18.4 AU	0.79 Rf	43.6 AU	3.18 %	0.80 Rf	21.1 AU	827.1 AU	3.58 %
13	0.81 Rf	20.7 AU	0.82 Rf	69.5 AU	5.08 %	0.83 Rf	18.4 AU	685.7 AU	2.96 %
14	0.84 Rf	18.2 AU	0.84 Rf	44.6 AU	3.25 %	0.86 Rf	19.3 AU	438.3 AU	1.90 %
15	0.89 Rf	21.6 AU	0.89 Rf	34.5 AU	2.51 %	0.90 Rf	21.2 AU	290.6 AU	1.26 %
16	0.90 Rf	20.9 AU	0.95 Rf	395.5 AU	28.87 %	1.01 Rf	11.9 AU	8676.0 AU	37.51 %

Figure 3a. HPTLC Chromatogram and peak table at 366 nm

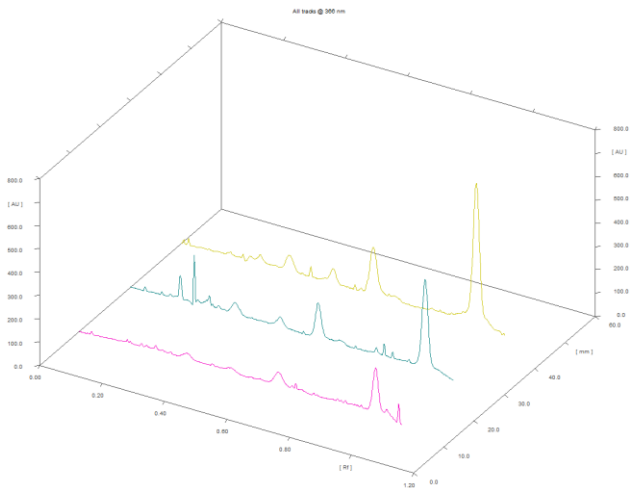
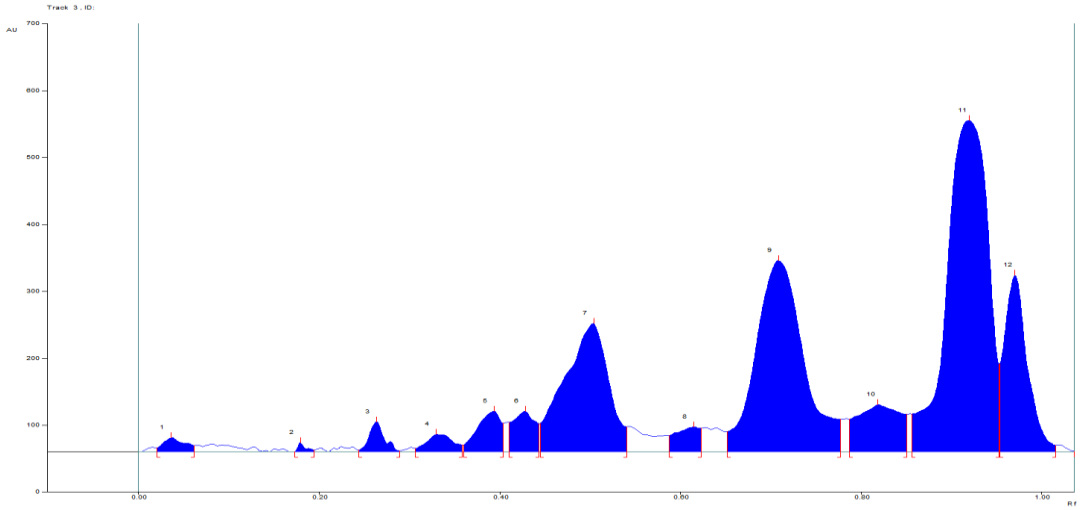


Figure 4b. 3D Chromatogram @ 366 nm



Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	5.7 AU	0.04 Rf	21.1 AU	1.34 %	0.06 Rf	9.3 AU	463.7 AU	0.80 %
2	0.17 Rf	0.1 AU	0.18 Rf	13.5 AU	0.86 %	0.19 Rf	2.7 AU	103.0 AU	0.18 %
3	0.24 Rf	2.4 AU	0.26 Rf	44.7 AU	2.85 %	0.29 Rf	1.2 AU	681.4 AU	1.18 %
4	0.31 Rf	4.6 AU	0.33 Rf	25.8 AU	1.64 %	0.36 Rf	9.9 AU	697.5 AU	1.20 %
5	0.36 Rf	10.2 AU	0.39 Rf	60.2 AU	3.84 %	0.41 Rf	42.3 AU	1448.5 AU	2.50 %
6	0.41 Rf	43.9 AU	0.43 Rf	60.4 AU	3.85 %	0.44 Rf	41.9 AU	1360.2 AU	2.35 %
7	0.45 Rf	42.0 AU	0.51 Rf	191.4 AU	12.20 %	0.54 Rf	37.2 AU	8544.3 AU	14.75 %
8	0.59 Rf	24.2 AU	0.62 Rf	37.0 AU	2.36 %	0.62 Rf	34.9 AU	925.7 AU	1.60 %
9	0.65 Rf	29.9 AU	0.71 Rf	285.5 AU	18.21 %	0.78 Rf	48.7 AU	13726.8 AU	23.69 %
10	0.79 Rf	48.8 AU	0.82 Rf	70.2 AU	4.48 %	0.85 Rf	56.2 AU	3080.7 AU	5.32 %
11	0.86 Rf	56.4 AU	0.92 Rf	495.1 AU	31.57 %	0.95 Rf	30.7 AU	20775.0 AU	35.86 %
12	0.96 Rf	131.5 AU	0.97 Rf	263.4 AU	16.80 %	1.02 Rf	9.6 AU	6130.2 AU	10.58 %

Figure 3a. HPTLC Chromatogram and peak table at 520 nm

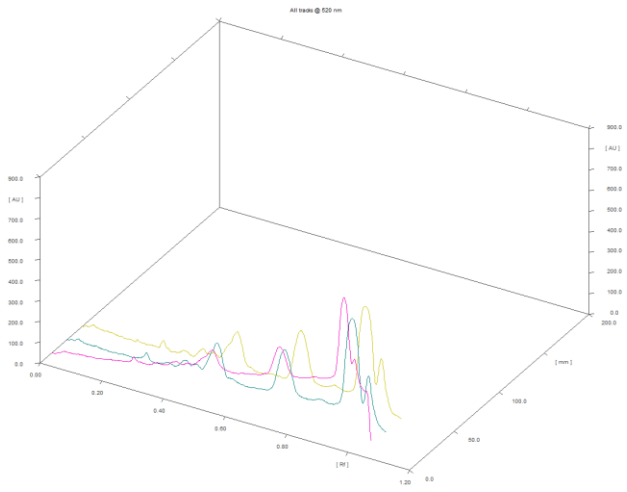


Figure 4b. 3D Chromatogram @ 520 nm

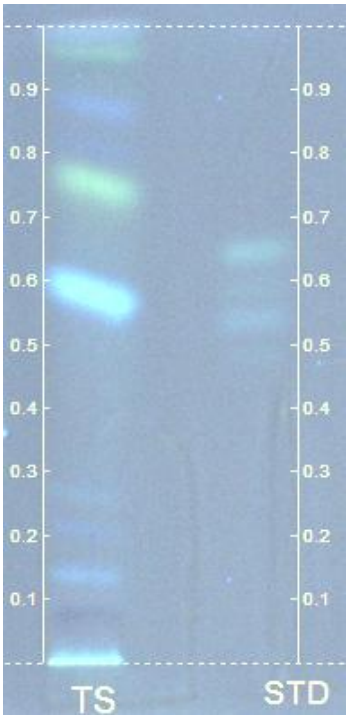
6. MICROBIAL LOAD:

Table .6.

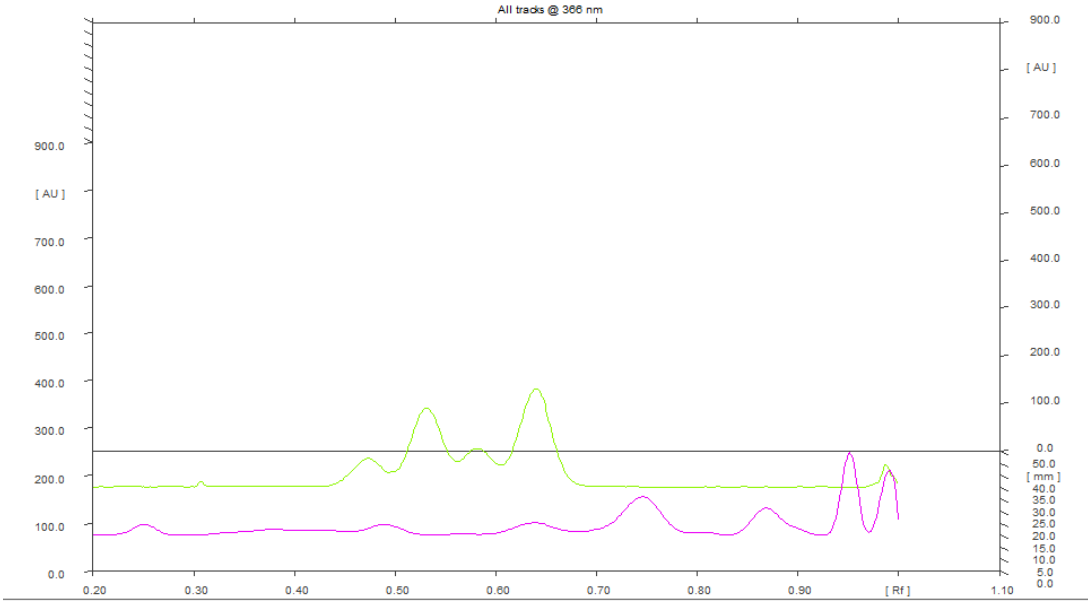
S. No.	Parameters	Reference Limits as per WHO (2007)	Results	Remarks
1	**Total Bacterial Count (TBC)**	10^5CFU/gm	Less than 1 cfu/g	Within permissible limits
2	**Total Fungal Count (TFC)**	10^3CFU/gm	Less than 1 cfu/g	
3	**Enterobacteriaceae**	10^3	Absent	
4	**Escherichia coli**	10	Absent	
5	**SalmonellaSpp**	Absent	Absent	
6	**Staphylococcus aureus**	Absent	Absent	
7.	**Pseudomonas aeruginosa**	Absent	Absent	

7. AFLATOXIN:

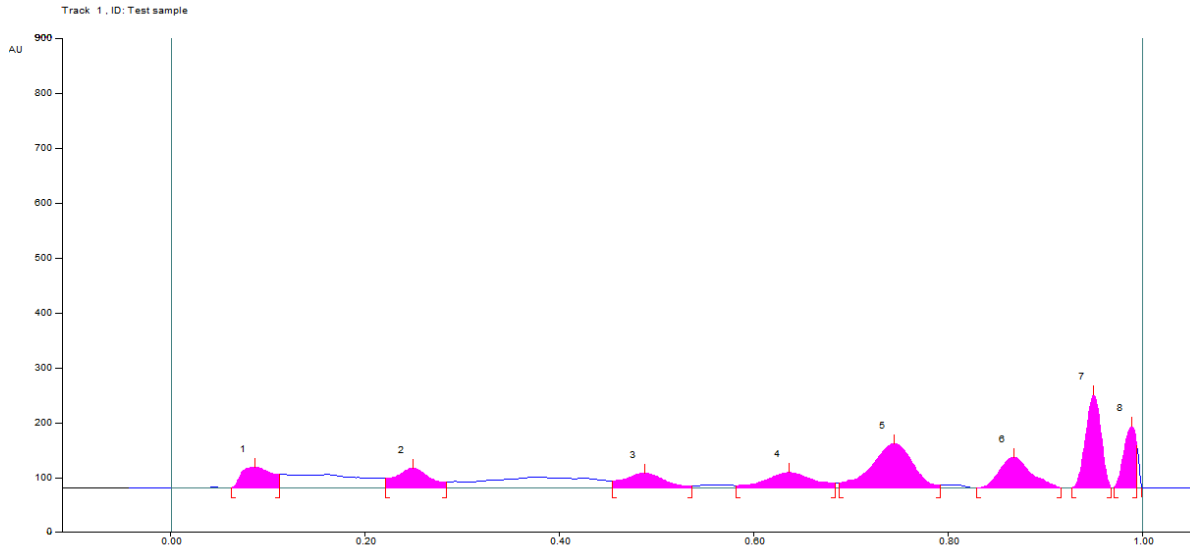
The test sample – Siddhar Kuligai (20µl) and Standard - G2, G1, B2 and B1 (15µl) were applied on TLC aluminium sheet silica gel 60 F 254 (E.MERCK) and plate was developed using the solvent system Chloroform : Acetone: Water (14 : 2 : 0.2).After development the plate was allowed to dry in air and examined under UV 366 nm



UV-366nm



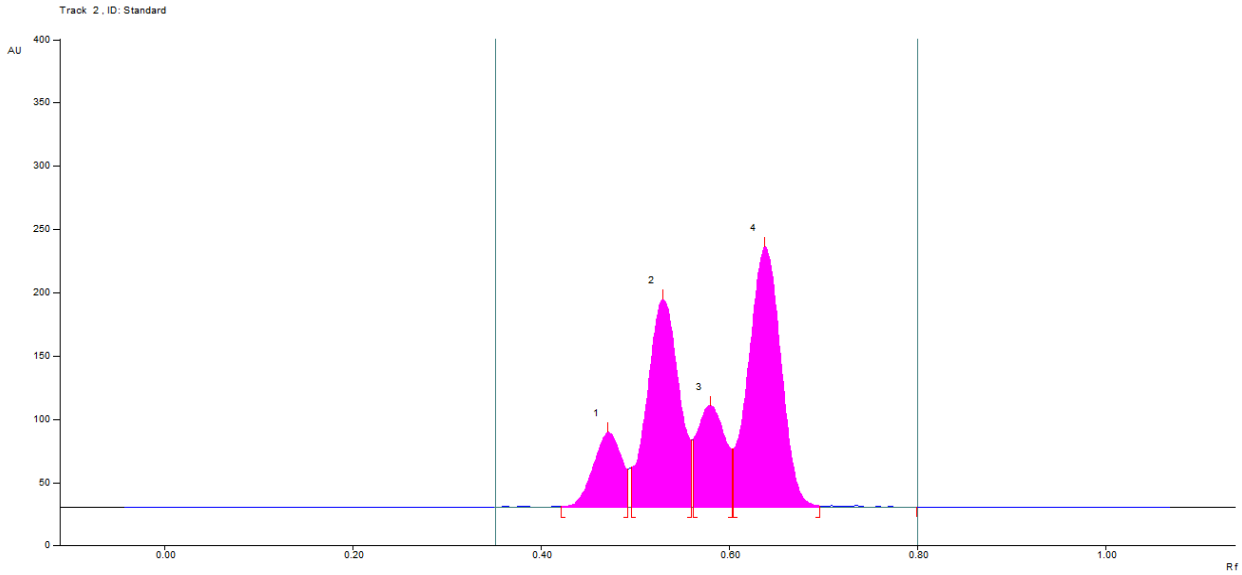
Densitometric chromatogram at UV-366nm; Test sample ; Standard – G2, G1, B2 & B1



HPTLC finger print of Test sample at 366nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.06 Rf	0.5 AU	0.09 Rf	38.0 AU	6.93 %	0.11 Rf	24.4 AU	991.2 AU	8.37 %
2	0.22 Rf	17.6 AU	0.25 Rf	36.1 AU	6.58 %	0.28 Rf	10.7 AU	1046.0 AU	8.84 %
3	0.46 Rf	13.1 AU	0.49 Rf	27.2 AU	4.96 %	0.54 Rf	3.7 AU	931.0 AU	7.86 %
4	0.58 Rf	4.4 AU	0.64 Rf	28.0 AU	5.11 %	0.68 Rf	9.2 AU	1136.3 AU	9.60 %
5	0.69 Rf	9.5 AU	0.75 Rf	80.9 AU	14.74 %	0.79 Rf	4.8 AU	2838.5 AU	23.98 %
6	0.83 Rf	0.0 AU	0.87 Rf	56.3 AU	10.26 %	0.92 Rf	0.2 AU	1506.5 AU	12.72 %
7	0.93 Rf	0.2 AU	0.95 Rf	169.1 AU	30.83 %	0.97 Rf	0.4 AU	2210.8 AU	18.67 %
8	0.97 Rf	0.8 AU	0.99 Rf	112.9 AU	20.58 %	1.00 Rf	77.7 AU	1178.9 AU	9.96 %

Rf value of Test sample at 366nm



HPTLC finger print of Standard at 366nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.42 Rf	0.5 AU	0.47 Rf	59.3 AU	11.62 %	0.49 Rf	29.9 AU	1476.4 AU	10.53 %
2	0.50 Rf	32.1 AU	0.53 Rf	164.7 AU	32.29 %	0.56 Rf	52.9 AU	4629.3 AU	33.02 %
3	0.56 Rf	53.3 AU	0.58 Rf	80.3 AU	15.75 %	0.60 Rf	45.9 AU	2024.3 AU	14.44 %
4	0.61 Rf	46.2 AU	0.64 Rf	205.7 AU	40.33 %	0.70 Rf	1.1 AU	5889.8 AU	42.01 %

Rf value of Test standard at 366nm

Interpretation

- Similar Rf values were seen in the standard and Siddhar kuligai, upon derivatization with isopropyl alcohol and conc. H_2SO_4 (9:1).
- The band colour changed from bluish green to yellow in Track 2 (standard).
- As no colour change was seen in the Track 1 in siddhar kuligai which indicated the absence of aflatoxins in the siddhar kuligai.

8. FT-IR (Fourier Transform Infra-Red)Spectroscopy

3270, 2917, 2851, 1625, 1544, 1409, 1317, 1239, 1017, 773

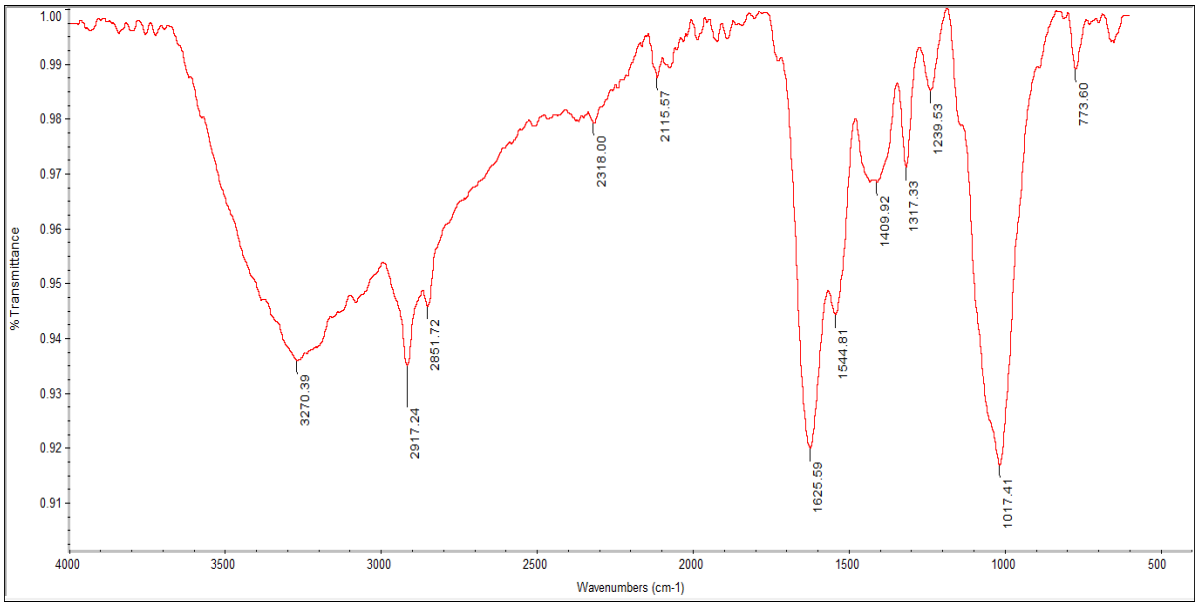


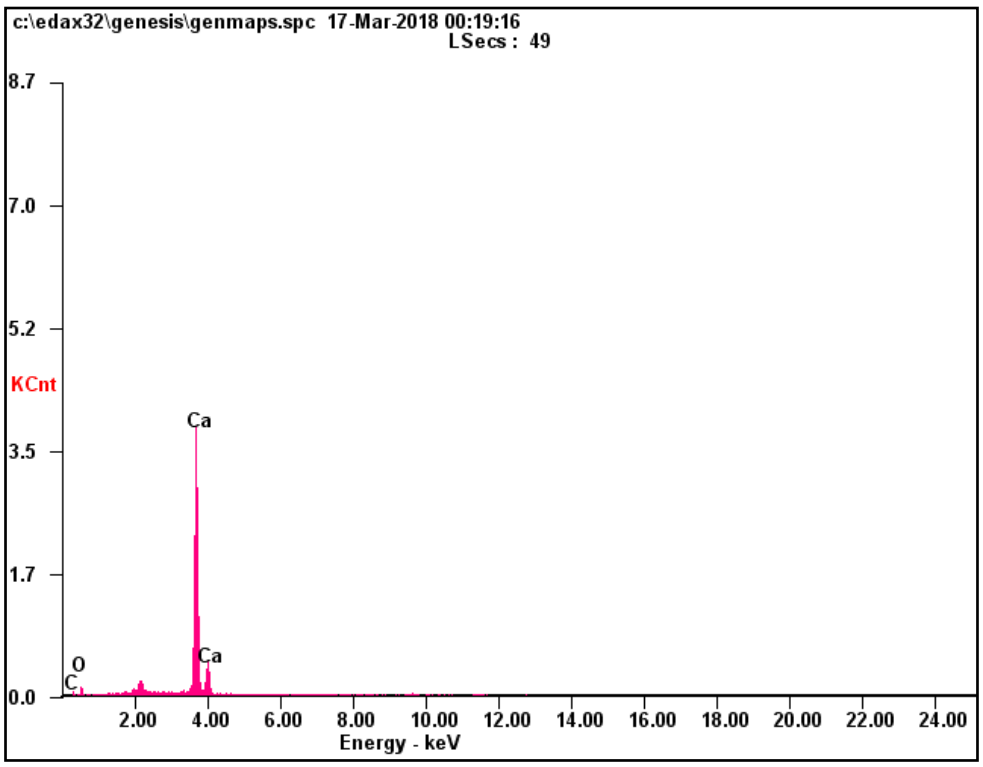
Figure 1. FT-IR Spectrum of Siddhar Kuligai

Interpretation

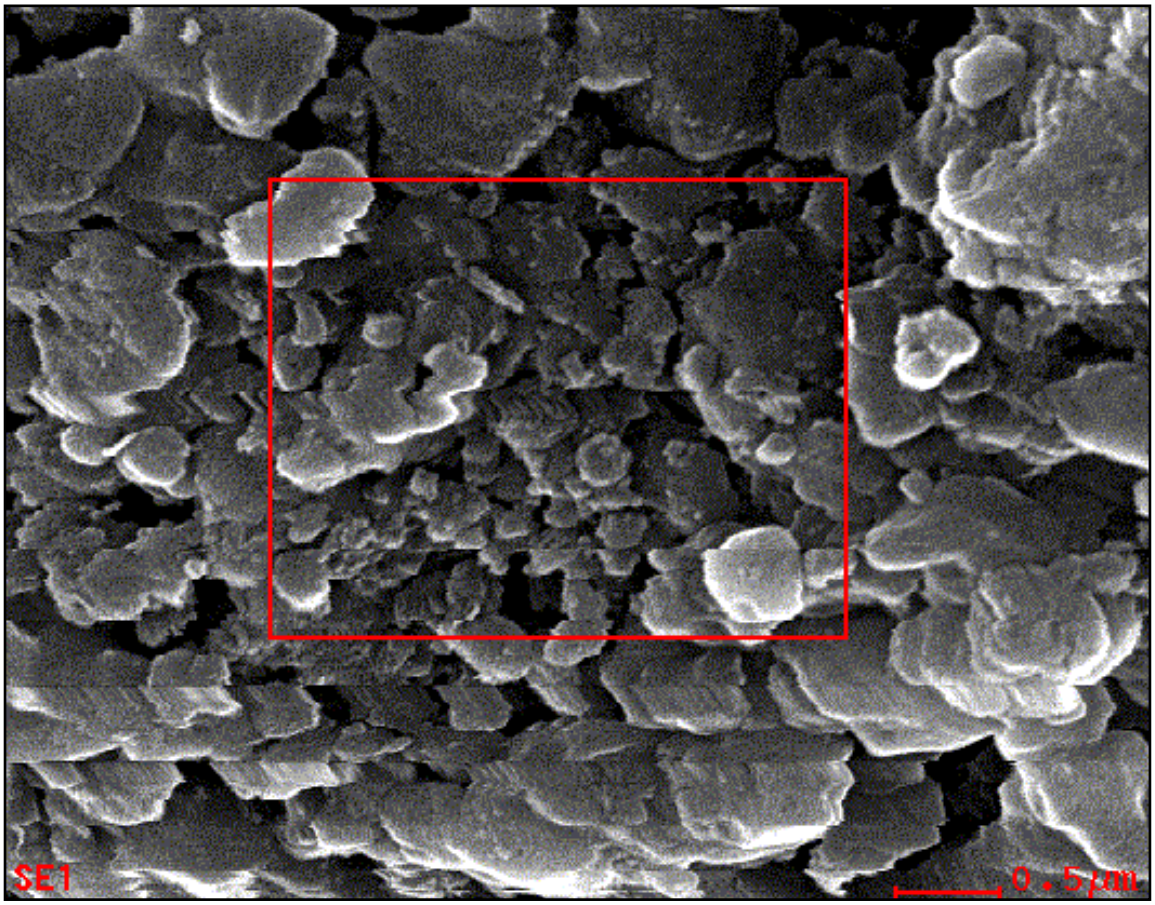
- The FT-IR Spectrum (Fig.1) of Siddhar Kuligai were showed eleven significant bands. The very intense band at 1625 cm^{-1} was assigned to C=O stretching of carbonyl group present in hemicelluloses and lignin.
- The strong band appeared around 1544 cm^{-1} is due to C=C stretching frequency of aromatic moiety present in the lignin.
- The peak at 1317 and 1239 were assigned to alcoholic C-O functional group present in celluloses and phenolic C-O group present in lignins.
- The strongest peaks at 2851and 2917 cm^{-1} refer to the C-H stretching of methyl (CH3) and methylene (CH2) group present in the lignin and cellulose.

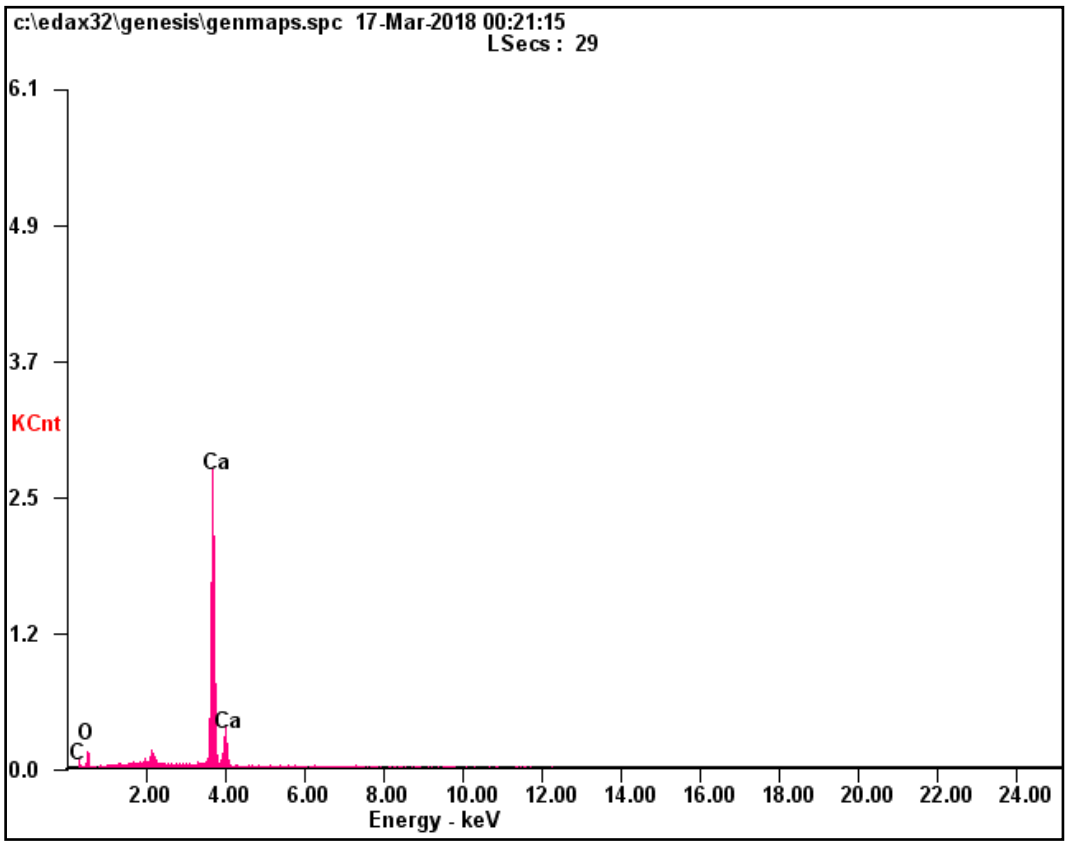
- The absorption at 3200−3500 cm^{-1}were due to the stretching of O-H groups present in the fibres where they can be found in their main chemical composition: celluloses, hemicelluloses, and lignin.
- In the FT-IR Spectrum of Siddhar Kuligai is expected the domination of signals from jaggary and signals from other constituents were merged with strong signals from jaggary.

9. SEM WITH EDAX

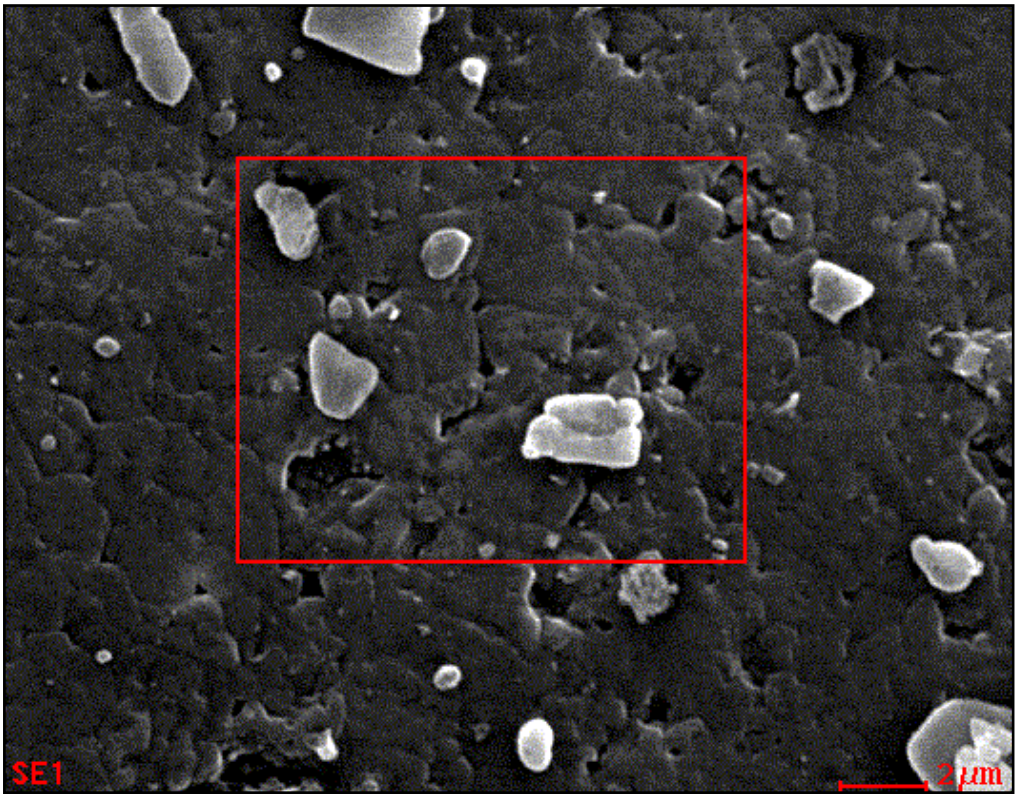


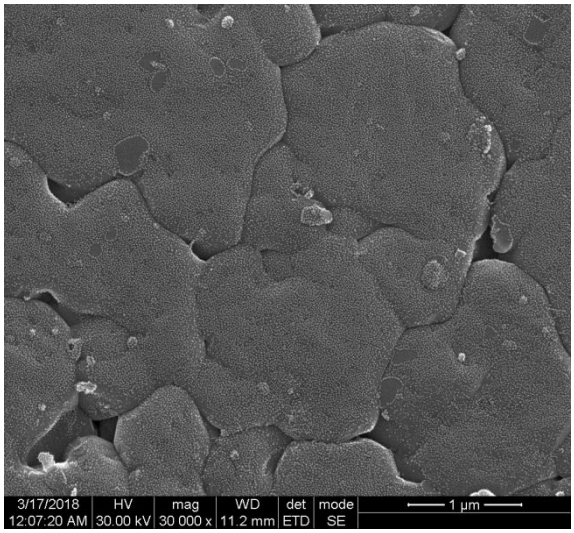
Element	*Wt%*	*At%*
CK	09.46	19.21
OK	28.06	42.78
CaK	62.47	38.01
Matrix	Correction	ZAF

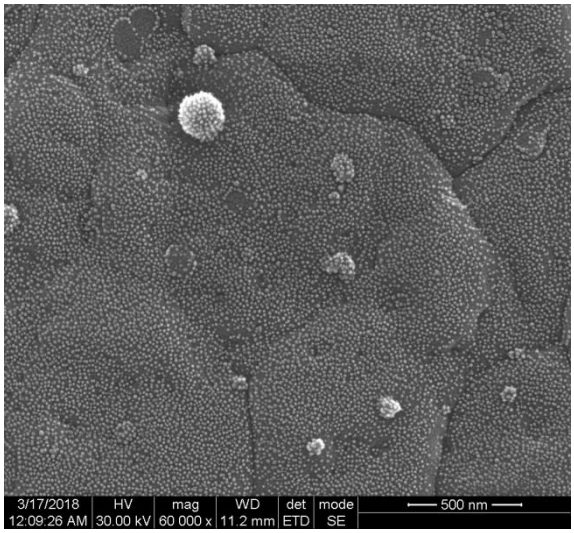

SE1
0.5µm

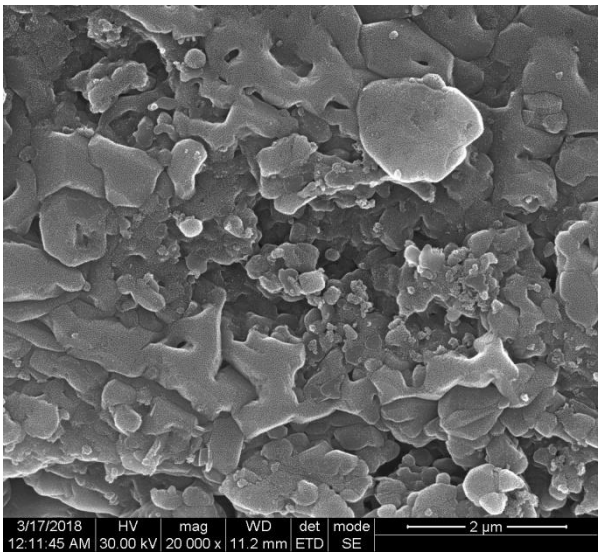

c:\edax32\genesis\genmaps.spc 17-Mar-2018 00:21:15
LSecs : 29
6.1
4.9
3.7
KCnt
2.5
1.2
0.0
Ca
O
C
Ca
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
Energy - keV

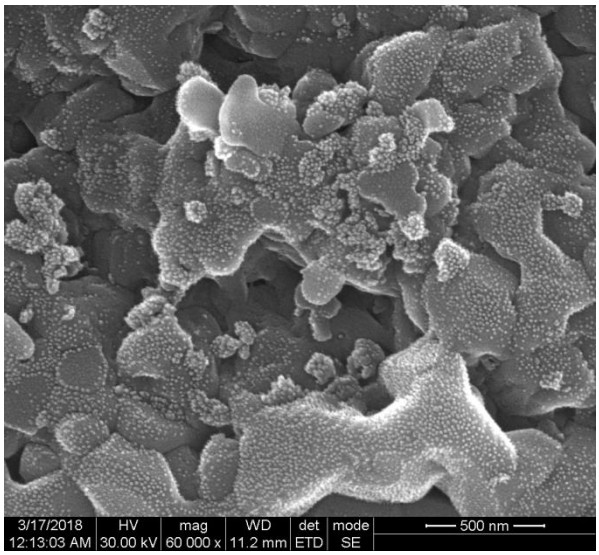
Element	*Wt%*	*At%*
CK	11.91	22.10
OK	34.51	48.09
CaK	53.59	29.81
Matrix	Correction	ZAF

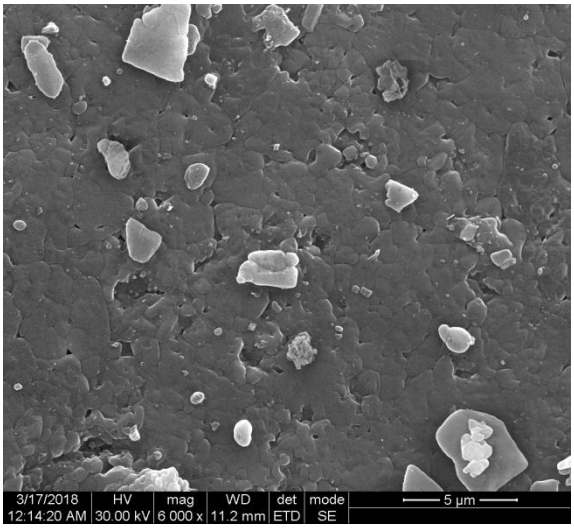


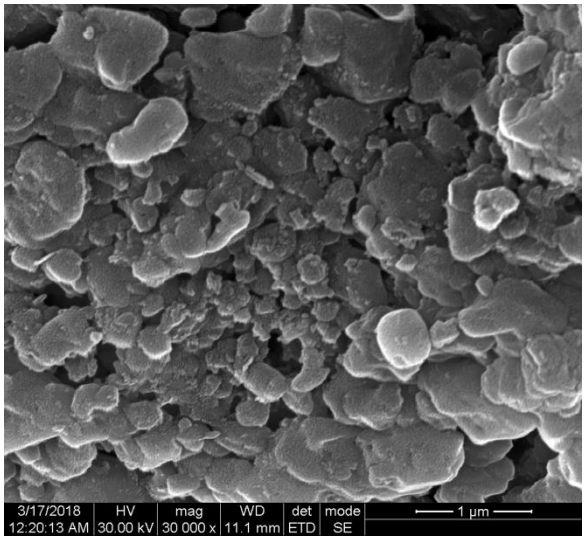












INTERPITATION

SEM-EDAX

The SEM photographs shows that the size of the particle is in nanometers which will promotes the easy or quick assimilation of the drug and thereby improving the efficacy. Also the EDAX results shows that the drug SK is detected with calcium which would have come from palm jaggery used in the drug. The other elements such as carbon and oxygen, detected in the drug are commonly present in all the herbal drugs originating from the primary metabolites.

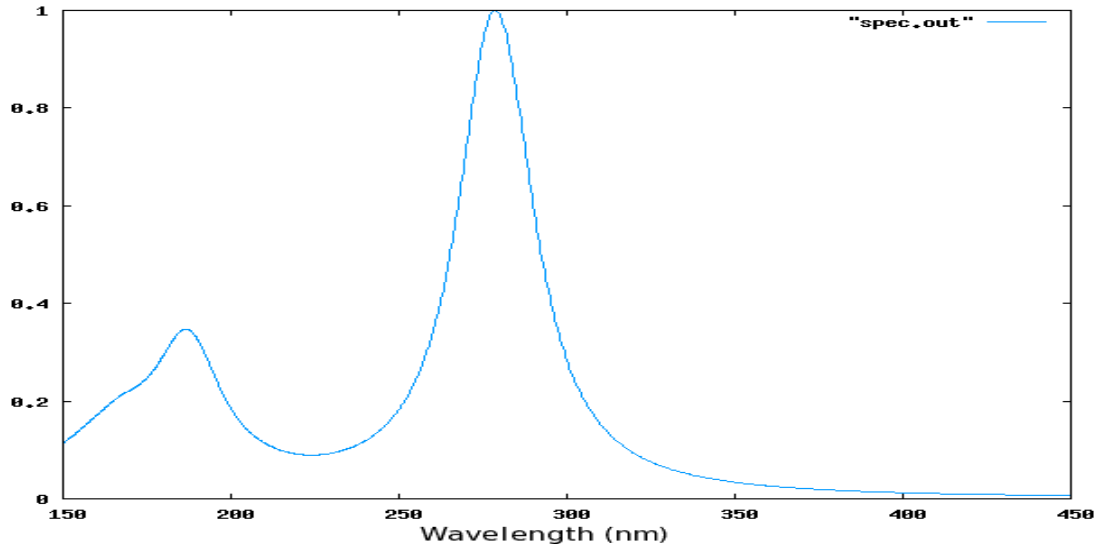
10. XRF (X-RAY Fluorescence Spectroscopy) Table- 7

Formula	Z	Concentration	Status	Line 1	Net int.	Calc. concentration	Stat. error	LLD	Analyzed Layer
G	80	72.73 %	XRF 0	Hg LA1 – HR – Tr	2776	72.73	0.11 %	972.9 PPM	17.0 um
O	8	12.67 %	XRF 0	O KA1 – HR	1.064	11	15.40 %	1.47%	0.180 um
S	16	7.77%	XRF 0	S KA1 – HR – Tr	206.6	7.769	0.40 %	118.1 PPM	1.73 um
CI	17	4.27%	XRF 0	CI KA1 – HR – Tr	38.63	4.27	0.92 %	282.9 PPM	0.89um
As	33	1.07%	XRF 0	As KA1 – HR – Tr	142.6	1.068	0.50 %	146.1 PPM	19.6 um
Si	14	0.24%	XRF 0	SI KA1 – HR – Tr	3.236	0.238	3.80 %	219.8 PPM	1.13 um
K	19	0.20%	XRF 0	K KA1 – HR – Tr	1.846	0.201	4.56 %	163.0 PPM	1.11 um
TI	81	0.18%	XRF 0	TI LA1 – HR – Tr	6.995	0.18	9.95 %	332.8 PPM	18.2 um
Mg	12	0.16%	XRF 0	Mg KA1 – HR – Tr	3.472	0.16	10.60 %	513.4 PPM	0.58 um
Mn	25	0.16%	XRF 0	Mn KA1 – HR – Tr	4.688	0.16	3.23 %	132.2 PPM	4.4 um
Fe	26	0.14%	XRF 0	Fe KA1 – HR – Tr	5.886	0.142	2.91 %	110.2 PPM	5.4 um
Ca	20	0.09%	XRF 0	Ca KA1 – HR – Tr	0.918 7	0.093	7.20 %	186.3 PPM	1.38 um
Pb	82	0.09%	XRF 0	Pb LB1 – HR – Tr	2.705	0.088	22.80 %	427.2 PPM	13.0 um
AI	13	0.08%	XRF 0	AI KA1 – HR – Tr	0.996 9	0.082	6.62 %	122.8 PPM	0.80 um
Se	34	0.06%	XRF 0	Se KA1 – HR – Tr	5.608	0.064	12.40 %	184.7 PPM	23.1 um
Pt	78	0.05%	XRF 0	Pt LA1 – HR – Tr	1.954	0.049	11.80 %	235.8 PPM	14.7 um
V	23	0.04%	XRF 0	V KA1 – HR – Tr	0.633 2	0.036	12.10 %	154.2P PM	2.84 um

INTERPITATION

XRF study has shown the presence of *siddhar kuligai* contains elements of G, O,S,Cl . As, Si, K, TI, Mg, Mn, Fe, Ca, v, Pb, Al, Se, Pt.. The presence of, calcium, iron and other element elevates its therapeutic value of siddhar kuligai

11. UV ULTRAVIOLET – VISIBLE SPECTROSCOPY:-



12. Anticancer activity (In-Vitro HeLa cell Line) of Siddhar Kuligai is Screened by XTT kit method

Materials Required

- Inverted Phase contrast microscope
- Biosafety cabinet class-II
- Cytotoxic safety cabinet
- CO_2incubator
- Deep freezer
- ELISA plate reader
- Micropipettes
- XTT cell proliferation kit (Cayman, USA)

Procedure

HeLa cells were grown at 2 × 10^5cells/ml in T25 flask contains culture medium for 24 hours at 37°C and then seeded in 96 well tissue culture plates for 24 hours.

↓

Siddhar Kuligai were treated with HeLa cells and incubated for upto 48 hours in CO_2 incubator and samples were analyzed with XTT proliferation kit by ELISA.

↓

10 µl of the reconstituted XTT mixture to each well using repeating pipettes

↓

Mixed with one minute on an orbital shaker

↓

Cells were incubated for four hours at 37°C in a CO_2 incubator

↓

Plate were mixed gently on an orbital shaker for one minute to ensure homogeneous distribution of color

↓

Siddhar Kuligai were read at 450 nm wavelength in a Microplate reader. Values were calculated and analyzed as percentage of inhibition

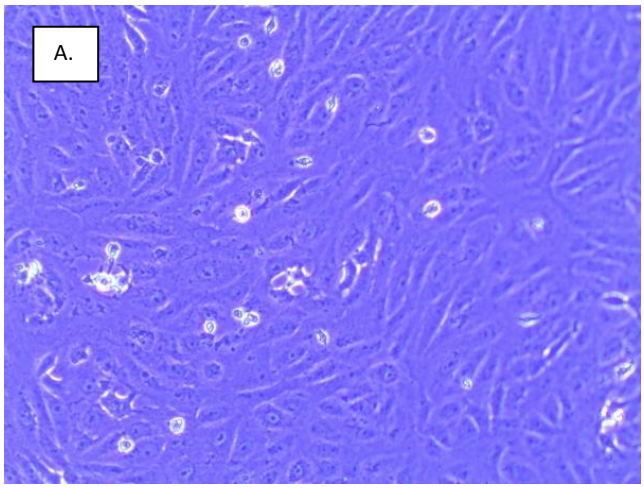


Fig.A; HeLa cells – Control group

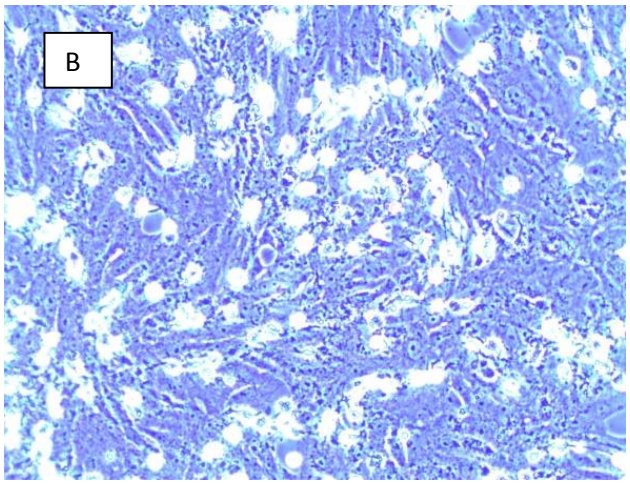


Fig. B; HeLa cells treated with Siddhar Kuligai

Result:

Siddhar Kuligai was found to inhibit the proliferation of HeLa cells (HeLa-Cancer cell line) after 24 hours of treatment. In this assay it was found that inhibition was 71.6 %, when compare with untreated group

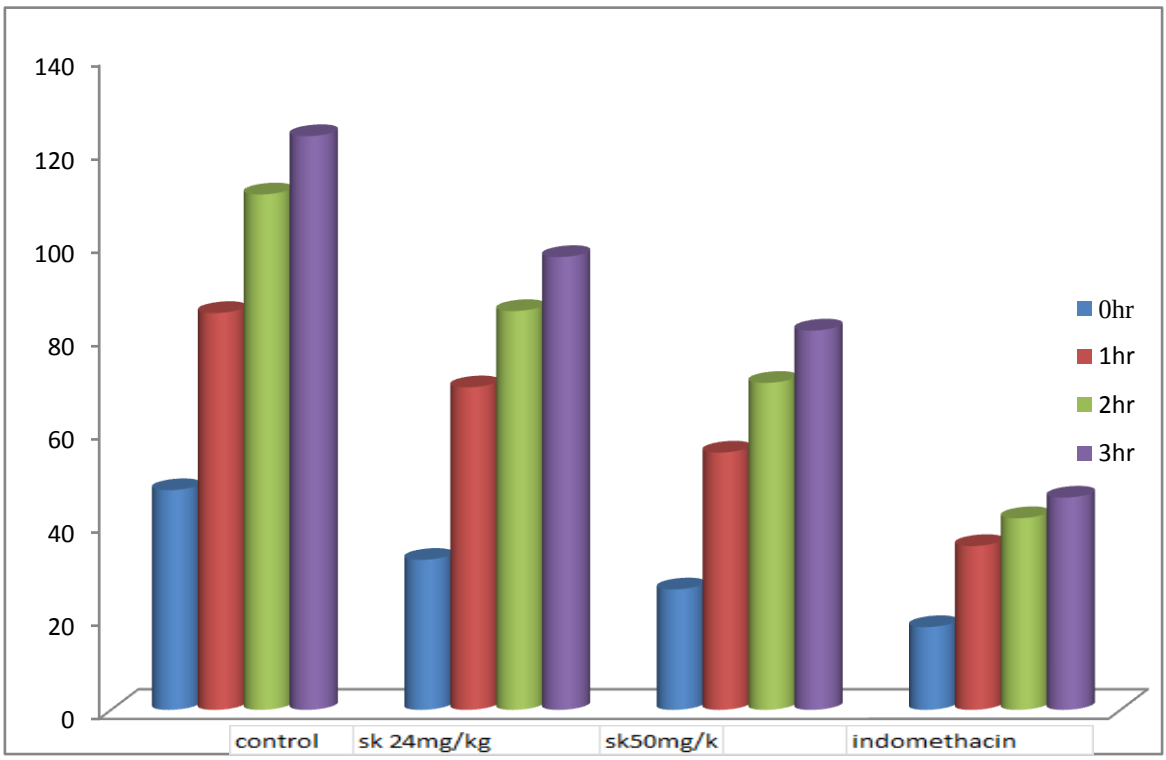
13. Anti inflammatory of Siddhar Kuligai carrageenan induced paw oedema in albino Wistar rats. Table -8

Treatment	**Percentage inflammation after carageen an injection at hr**			
	0	1	2	3
Control	30.10± 2.82	74.12± 10.14	131.14± 11.18	138.50± 4.50
Siddhar Kuligai 24mg/kg	29.00± 3.34	69.28± 1.14	99.34± 1.78 **	76.12 ± 8.08 **
Siddhar Kuligai 50mg/kg	20.00± 2.16	69.16± 3.14	77.50± 7.50 ***	68.00±12.18 ***
Indomethacin 10mg/kg	27.18± 1.28	40.18±8.98 **	48.07± 8.18 ***	39.00±5.26 ***

Values are Mean ± SEM; n = 6 animals in each group: * P<0.05, ** P< 0.01, ***P<0.001 is considered significant when compared with control rats and followed by Two way ANOVA.

Result

Siddhar Kuligai the dose of 50mg/kg/ showed significant Anti inflammatory (p<0.001) activity when compare to the control group.



14. Analgesic activity of Siddhar Kuligai by eddy's hot plate method.

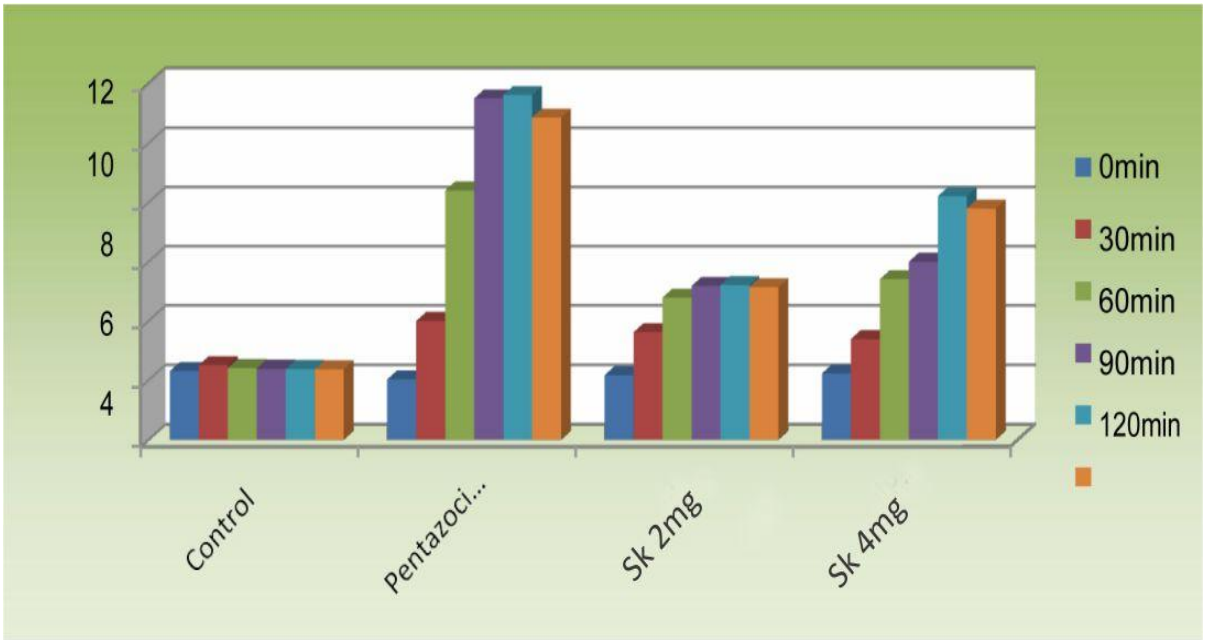
Table -9

Groups	Treatment	Reaction time in sec					
		0min	30min	60min	90min	120min	150min
I	Control	2.06±0.05	2.01±0.04	2.1±0.09	2.21±0.10	2.10±0.09	2.01±0.04
II	Pentazocine (5mg/kg)	2.20±0.21	4.23±0.47	8.13±0.31	10.23±0.38**	11.03±0.08**	12.56±0.37**
III	SK dose-I (2mg/kg)	2.23±0.23	3.16±0.16	4.10±0.09	5.2±0.14	6.2±0.25	6.86±0.05*
IV	SK Dose-II 4mg/kg)	2.03±0.05	4.15±0.15	5.05±0.08	7.23±0.27**	8.33±0.37**	9.11±0.16**

Values are Mean ± SEM; n = 6 animals in each group: * P<0.05, ** P< 0.01, ***P<0.001 is considered significant when compared with control rats. The results were analyzed by ANOVA followed by Dunnet's test.

Result

Siddhar Kuligai the dose of 4mg/kg showed significant Analgesic ($p<0.01$) activity when compared with control rats



18. DISCUSSION

The drug ***Siddhar Kuligai*** was selected from the Siddha literature "Veerama Munivar Vagada Thirattu-part 1" to validate the (anti inflammatory, analgesic activity) in an animal model and anti-cancer activity in in-vitro cell line model

The ingredients of the test drug was identified and authenticated by Siddha experts. The drug was prepared as per the procedure and subjected to various studies such as qualitative, quantitative, Standardization and pharmacological activities. Qualitative analysis includes Chemical analysis, Physicochemical properties and Phytochemical analysis of Siddhar Kuligai. From the above analysis we came to know the presence of active ingredients responsible for its activity.

Literary collections:

Literary collections include drug review, which consist both Botanical aspect, Gunapadam aspect and pharmacological review are support this study.

Drug review:

Botanical aspect:

- Drug review about the ingredients of Siddhar Kuligai from various text books was done.
- Botanical aspect explains the identification, description, active principle and medicinal uses of the plants. Siddha literatures related to the drug bring the evidence and importance of its utility in treating the cancer

Gunapadam aspect

- Gunapadam review brings the effectiveness of the drug in treating cancer.
- Pooram by its formulation as pills directly used as an anti cancer Drug..
- General property of kodiveli used to kill certain cancer growth.

Pharmaceutical aspect

Pharmaceutical review describes about the kuligai and its properties.

Pharmacological aspect

The pharmacological review explains about the methodology of anti inflammatory and analgesic Activity the drugs used and the analysis of pharmacological activity through HeLa and cell lines are the novel methods for validation. They explained about the effective anticancer activity of Siddhar Kuligai.

Physico chemical analysis

- In physico chemical analysis, the pH of *Siddhar Kuligai* was found to be in the range of 7.5.The pH of the drug *Siddhar Kuligai* is 7.5 which is slightly alkaline in nature and it is essential for its bioavailability and effectiveness.
- The loss on drying value at 105ºC of *Siddhar Kuligai* was found to be 17.35%w/w, hence the drug will not lose much of its volume on exposure to the atmospheric air at room temperature. It shows that the drug has more stability.
- Ash value 4.47 % it is the residue remaining after incineration that determines the inorganic substances present in the drug. Similarly it can also detect the nature of the material, whether it is adulterate or not. Hence, determination of the ash value provides an idea for judging the identity and purity of the drug.
- Decreased water soluble ash value (2.58%) indicates easy facilitation of diffusion and osmosis mechanisms.

Phytochemical analysis

Phytochemical analysis of the drug *Siddhar Kuligai* revealed the presence of carbohydrates, saponins, Phenols, Flavanoid, Diterpenes.

Flavonoids

- Flavonoids have potent Anti-Oxidant activity .
- Flavanoids possess diverse biological activities, for example, antiulcer, anti-inflammatory, antioxidant, cytotoxic and antitumor, antispasmodic and anti depressant activities.

Tannins.

- Tannins restore the Anti-Oxidant status of the organs to almost normal levels.
- It increases the cellular Anti-Oxidant enzymes.
- Tannins helps in healing of wounds and inflammation of mucous membrane.

Phenols

- Phenols possess rich Anti-Oxidant property and protect body from oxidative stress.
- Phenol components take part important roles in the control of cancer and other human diseases.
- Phenols are the most important groups of secondary metabolites and bioactive compounds.

Carbohydrates

Carbohydrate in the form of starch is emollient and it produces softener effect on skin and mucous membrane.

Saponin

Satavarin IV, a steroidal saponin found in drug exhibits significant anticancer activity and it also possesses hematinic activity

Chemical analysis:

Chemical analysis of the drug *siddhar kuligai* revealed the presence of Phosphate, sulphate, carbonate and sulphide, Iron, Zinc, Calcium, Magnesium, Potassium, Starch, Reducing sugar, Alkaloids.

Microbial Load

The microbial load analysis confirms *Siddhar Kuligai* was free from microbial organisms and fungal infections.

Instrumental analysis

Based on the result Siddhar kuligai is preferably non –toxic to human in its therapeutic dose. the standardization of the drug was evaluated by chemical analysis characterization with elemental analysis determination of particle size by FTIR ,XRF, and SEM-EDAX respectively.

FTIR

In the FT-IR Spectrum of Siddhar Kuligai is expected the domination of signals from jaggary and signals from other constituents were merged with strong signals from jaggary. The absorption at 3200−3500 cm^{-1}were due to the stretching of O-H groups present in the fibres where they can be found in their main chemical composition: celluloses, hemicelluloses, and lignin. OH group of has Siddhar Kuligai potential towards inhibitory activity against microorganisms

SEM-EDAX

The SEM photographs shows that the size of the particle is in nanometers which will promotes the easy or quick assimilation of the drug and thereby improving the efficacy. Also the EDAX results shows that the drug SK is detected with calcium which would have come from palm jaggery used in the drug. The other elements such as carbon

and oxygen, detected in the drug are commonly present in all the herbal drugs originating from the primary metabolites.

XRF

XRF study has shown the presence of *siddhar kuligai* contains elements of G, O,S,Cl . As, Si, K, TI, Mg, Mn, Fe, Ca, v, Pb, Al, Se, Pt.. The presence of , calcium, iron and other element elevates its therapeutic value of siddhar kuligai

Pharmacological studies:

The pharmacological study was carried out in the animal model Wistar albino rats and mice and Anti-cancer activity in in-vitro cell line model. Three activities were seen in the drug of Siddhar Kuligai. The activities were

- Anti Inflammatory
- Analgesic
- Anti Cancer

Anti Inflammatory Activity:

The anti-inflammatory activity was evaluated using carrageen an-induced paw edema models in Wistar albino rats. The anti-inflammatory activity was found to be dose dependent in carrageenan-induced paw edema model. The aqueous extract of *Siddhar Kuligai* has shown significant ($P< 0.001$) inhibition of paw edema on 3rd hour at the doses of 50 mg/kg, respectively.

Analgesic Activity

Analgesic activity was carried out by eddy's hot plate method analgesic effect lasted for a period of 120 min was found to be possess significant ($p<0.01$) analgesic activity at the dose level of 4 mg/kg by increase in reaction time (increase threshold potential of pain). from these result it was obvious that Siddhar Kuligai has significant analgesic activity

Anti-Cancer Activity

The cell lines for anticancer activity (In-Vitro)were HeLa Cell line Siddhar Kuligai was found to inhibit the proliferation of HeLa cells (HeLa-Cancer cell line) after 24 hours of treatment. In this assay it was found that inhibition was 71.6 %, when compare with untreated group So, the analysis of pharmacological activity through HeLa cell lines are the novel methods for validation. They explained about the effective anticancer activity of Siddhar Kuligai.

19. SUMMARY

- The test drug ***Siddhar Kuligai*** was selected from the siddha literature "Veerama Munivar Vagada Thirattu-part1 its *Standardization and Pharmacological screening* (anti inflammatory, analgesic activity) in an animal model and anti-cancer activity in in-vitro cell line model. The dissertation started with an introduction explaining about the siddha concept and role of the test drug in treating cancer.
- The test drug was prepared properly by the given procedure. All the ingredients were identified and authenticated by the respective field experts.
- Review of literature in various categories was carried out. Siddha aspect, botanical aspect and pharmaceutical review disclosed about the drug and the disease. Pharmacological review was done to establish the methodologies.
- The drug was subjected to analysis such as, physicochemical, chemical analysis, phytochemical, Instrumental and pharmacological analysis which provided the key ingredients present in the drug thus it accounts the efficacy of the drug.
- Identification of functional groups was engaged by using Fourier Transform Infra Red spectroscopy [FTIR]
- The partical size and identification and quantitative analysis of chemical elements of siddhar kuligai were assessed by SEM with EDAX and XRF
- The Siddhar Kuligai does not show the evidence for the presence of any of the aflatoxins.
- The Instrumental analysis report reveals that the heavy metals like Lead, Cadmium Arsenic and Mercury are within the permissible limits.
- Pharmacological study was done. It revealed the Anti-infalmmatory, Analgesic activities of trail medicine in animal model viz., Wistar albino rats and swiss albino mice and anti-cancer activity in in-vitro cell line model This study suggests Siddhar Kuligai has remarkable medicinal value in the treatment of cancer.
- Results and discussion gives the necessary justifications to prove the potency of the drug.

- Conclusion gives a complied form of the study and explains the synergistic effect of all the key ingredients and activities that supports the study.
- Thus the herbo mineral formulation **Siddhar Kuligai** is validated for its safety and efficacy for treating cancer it would be a great drug of choice.

20. CONCLUSION

From the literature evidence, Physico Chemical analysis, chemical analysis, Elemental analysis and Pharmacological studies, the author concludes that the drug ***Siddhar Kuligai*** is safe and it has significant effect in Anti-inflammatory, Analgesic and Anti cancer activities. It was concluded that the ***Sidhhar Kuligai*** can be used in the management of cervical cancer which is cost effective and easy to prepare.

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This Certificate is awarded to Dr/Mr/Mrs....KEERTHANA.R.....

For participating as ~~Resource Person~~ / Delegate in the Twenty First Workshop on

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*For **AYUSH Post Graduates & Researchers***

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University From 25th to 29th April 2016.

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Tambaram Sanatorium, Chennai- 600 047

Workshop on

"BASIC RESEARCH TECHNIQUES AND PRACTICES INVOLVED IN LABORATORY ANIMAL CARE"

06 -10 February 2017

CERTIFICATE

This is to certify that Dr.....R.Keerthana..... has participated as Delegate/~~Resource~~ Person in the workshop on "Basic Research Techniques and Practices involved in Laboratory Animal Care" held on 06-10 February, 2017 at National Institute of Siddha, Chennai-47, Tamilnadu.

Dr. V. Suba
Organizing Secretary

Dr. P. Muthusamy
Veterinary Consultant

Prof. Dr. V. Banumathi
Director / Chairperson

CERTIFICATE

This is certify that the project title..... Standardization and Pharmacological Screening of Siddhar kulipai (SK)'

24 Rats (12M+12F)
24 Mice (12M+12F)

has been approved by the IAEC.

Approval No: NIS/IAEC-II/15/2016

Prof. Dr. V. Banumathi	Prof. Dr. K. Nachimuthu
Name of Chairman/Member Secretary IAEC:	**Name of CPCSEA nominee:**
V. Banumathi 28/03/	29/3/2016
Signature with date	
Chairman/Member Secretary of IAEC:	**CPCSEA nominee:**

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

CERTIFICATE OF AUTHENTICATION OF SAMPLE DRUG

Certified that the sample drug submitted for identification by Dr. R.Keerthan 2nd year PG scholar ,Department of Gunapadam ,National Institute of Siddha, Tambaram, Sanatorium, Chennai-47, is identified as (Mercurous chloride(Hg_2Cl_2) l(Rasakarpooram) with below microscopic and macroscopic characteristics based on Rutly and Danas mineral description.

Date:

Place:

M. Suresh Gandhi

Dr.Suresh Gandhi

Assitant Professor

Department of Geology

Guindy Campus Chennai -25

Dr. M. SURESH GANDHI, M.Sc.,M.Phil.,Ph.D.,
Assistant Professor
Department of Geology
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Guindy Campus, Chennai - 600 025

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drugs used in the Siddha formulation **Siddhar Kuligai** (Internal) taken up for Post Graduation Dissertation studies by **Dr.R.Keerthana** M.D.(S), II year, Department of Gunapadam, 2017, is identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology, Micromorphology and Taxonomical methods as

Plumbago zeylanica Linn. (Plumbaginaceae), Root

Carum copticum Benth. & Hook. f. (Apiaceae), Fruit

MEDICINAL BOTANY CHENNAI 600 047 National Institute of Siddha

Certificate No: NISMB2712017

Date: 20-01-2017

Authorized Signatory

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