SCIENTIFIC VALIDATION OF SIDDHA HERBO- METALLIC FORMULATION "KANDHA RASA VILLAI" TO EVALUATE THE EFFICACY OF ITS ANTI- CANCER, ANTI- TUMOUR AND ANTI- OXIDANT ACTIVITIES.

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GOVT. SIDDHA MEDICAL COLLEGE,

CHENNAI-106

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **Scientific Validation of Siddha Herbo-metallic Formulation "*Kandha Rasa Villai" to Evaluate the Efficacy of its Anti-Cancer, Anti- Tumour and Anti- Oxidant Activities***is a Bonafide and genuine research work carried out by me under the guidance of **Dr.R.Karolin Daisy RaniM.D(S).,**Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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

Place: Chennai

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ENDORSEMENT BY THE HOD AND PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **Scientific Validation of Siddha Herbo-metallic Formulation "*Kandha Rasa Villai" to Evaluate the Efficacy of its Anti-Cancer, Anti- Tumour and Anti- Oxidant Activities***is a Bonafide work carried out by **Dr.R.Vimala** under the guidance of **Dr.R.Karolin Daisy Rani MD(s),** Post Graduate Department of Gunapadam, Govt.Siddha Medical College, Chennai - 106.

Seal and Signature of the HOD **Seal and Signature of Principal**

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ABBREVIATIONS

ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AST	Aspartate Amino Transferase
ANOVA	Analysis of Variation
BUN	Blood Urea Nitrogen
CT	Computed Tomography
CML	Chronic myelogenoas leukaemia
DMEM	Dulbecco's Modified Eagle's Medium
DPPH	2, 2-diphenyl-1-picrylhydrazyl
DNA	DeoxyRibo Nucleic acid
DC	Differential Count
DSC	Differential Scanning Calorimeter
FDG-PET	F-18 Fluoro-2-deoxy-D-glucose
FAD-Assay	Flavine Adenine Dinucleotide
FTIR	Fourier Transform Infrared Spectrometry
HPV	Human Papilloma Virus

ICPOES	Inductively Coupled Plasma Optical Emission Spectrometry
IAEC	Institutional Animal Ethical Committee
LD50	Lethal Dos
MCV	Mean corpuscular volume
MRI	Magnetic Resonance Imaging
MTT	3-(4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
NCCS	National Centre for Cell Sciences
OECD	Organisation for economic corporation and development
PCV	Packed cell volume
RBC	Red blood cells
SEM	Scanning electron microscope
WBC	White blood corpuscles
WHO	World Health Organization
XRD	X-Ray Diffraction

1. INTRODUCTION

Human beings are gifted persons in the world not only enjoyed all wealth,but also suffered by many diseases prevailing at the time. Some people have special knowledge and special powers are called “SIDDHARS”. Siddha System is an ancient traditional medicine. Siddhars investigate that the body though transient was the one and only instrument for obtaining success in the spiritual development and growth.

In Siddha system both body as well as soul are treated. They wanted to reach the Eight super natural powers which include *Anima* (The faculty of reducing oneself to the size of an atom), *Magima* (The power of increasing one’s bulk without limit),*Lagima* (Becoming very light like feather levity),etc,that they are essential for their goal. From thisconcept body could be made strong and perfect and get rid of birth and death for the ages together. Siddha system of medicine comprises 4 branches -*Vatham, Vaithiyam, Yogam* and *Gnanam.* This system of medicine is known to be fine- tuned withseveral research and development achievements which surprises the modern medicine in most of the medicine domains[(1)].

In India approximately 50% of population are women. Their health status and socio-economical status is very low. If women are educated then only they lead a healthy life. Then only their family will also be healthy. Women are commonly affected by cancer especially cervical cancer.

As per the text of Siddha book represents that our body, there may be any vigorous growth, both externally [skin]or internally[organs] with or without pain,bleeding etc is known to be cancer. It may be classified into two types, i.e. External Cancer and Internal cancer.Cancer present internally in the organs like lungs, brain, nerves,pancreas, prostate gland,cervix and etc [(2)].

This growth should be like a small tumour.There is no symptoms present in early stage. It will produce symptoms in later stage of disease. Then only it will produce some symptoms due to any pressure or structural change over that area.

In our system putru is described in Yugi muni’s Tamil literature as

***கிருமியோனியின்குணம்*:**

"வாறானசையோகமிகுதியாலும்

வல்குலிலேசோரியதுகெட்டுமேதான்

தூறானகிருமிகளுமிகவுண்டாகி

தொடருமேநமைச்சலுடன்விருப்பங்காணும்

நாறானநாற்றமுடனுதிரந்தோன்றும்

நவிலவேமுடியாதுகளையின்வேகம்

காறானயூகிமுனிசிகிச்சாசாரம்

கருதினார்வேகத்துமாண்பருக்கே"

-யூகி முனி[3]

Increased vaginal secretionwith foul smelling, Persist itching, Inflammation of vagina with worm infestations aredue to increased sexual act.

In modern science,putru is related to malignant type which is called Cancer. Malignant neoplasm marked by the uncontrolled growth of cells, often with the invasion of healthy tissues locally or throughout the body.Cancer cells that make proteins for the stimulation of growth. Cancer is the second leading cause of death. The incidence of cancer is expected to increase as the population ages.

Cervical cancer is one of the type which affects women population abundantly. Cervical cancer will occur in the cells of the cervix the lower part of the uterus that connects to the vagina.This disease is caused by Smoking, birth control pills, having many sexual partners, multiple pregnancy, weak immune system,HPV and etc. Symptoms are abnormal vaginal bleeding, pelvic pain or pain during sexual intercourse, loss of appetite, weight loss, fatigue, bleeding after drenching or after pelvic exam. 95% of the cervical cancers are squamous carcinomas and only 5% are adenocarcinomas[4].

The vast majority of cervical cancer is associated with human papilloma virus among which HPV-10 is the most common being found in 60% of all cervical cancers. Specifically E6 and E7 oncoproteins suppressed by high risk HPV can immortalize primary human keratinocytes and cause cancers in transgenic mouse models in a cofactor- dependent manner. HPV oncogene or oestrogen alone is insufficient to cause cervical cancers.

More than 1000 specimens from sequential patients with invasive cervical cancer were collected and store in a frozen at 32 hospitals in 22 countries. HPV DNA was detected in 93% of the tumours with no significant variation on HPV positivity among countries. HPV 16 was present in 50% of specimens.HPV18 in 14%, HPV45 in 8%, HPV31 in 5%. HPV 16 was the predominant type in all countries, the HPV 18 was more common in squamous cell tumours, HPV 16 is predominated in adenocarcinomas (51% of such tumours) and adenosquamous tumours (39% of such tumours) [(5)] .

The incidence of cervical cancer is 15: 100,000 it is the second most common cancer of the female reproduction tract and causes 5% of all cancer death among women.Although it may occur in younger women the average age at diagnosis is 54. The disease is insidious, asymptomatic in the early stage[(6)].

Globally,CervicalCancer is the fourth most common cancer in women and seventh overall with an estimated 528,000 new cases in 2012.

There were an estimated 266,000 deaths from cervical cancer worldwide in 2012,accounting for 7.5% of all female cancer deaths.

Almost nine out of ten(57%)cervical cancer deaths occur in less developed regions.

An estimated one million plus women worldwide are currently living with cervical cancer.

Rural and poorer women living in low and middle income countries,as well as poorer woman living in high-income countries are at an increased risk of invasive

cervical cancer,because they often do not have access to crucial prevention,screening and treatment services.

Cervical cancer mortality rates have fallen in much of the developed world during the past 30 years,largely due to screening and treatment programmes.During the same time, however,rates in most developing countries have risen or remain unchanged,often due to limited access to health services,lack of awareness and absence of screening and treatment programmes.

The peak age of incidence of cervical cancer is 55-59 years,and a considerable proportion of women report in the late stages of disease.

Every year in India,122,844 women are diagnosed with cervical cancer and 67,477 die from the disease.India has a population of 432.2 million women aged 15 years and older who are at risk of developing cancer.

The age standardized cervical cancer mortality rate in Tamilnadu is 35.7 per 100,000 compared to 16.6 per 100,000 nationally in 2010.

In Chennai,the leading cancer sites among females were breast (26.1%) and cervical cancer (21.2%) accounting for over 47% of all cancer in the state.

Adverseeffectscaused by vary with the type of treatment the patient receives. These treatments may cause symptoms such asbone marrow depression leading to leucopenia and thrombocytopenia,neurological symptoms like peripheral neuropathy,mental depression and psychotropic effects[(7)].

In literature,there is anevidence for*Kandha Rasa Villai*which is the best medicine for cervical cancer. So that,I choose this trial drug is going to be tested by HeLa cell lines for cervical cancer. In this medicine metals and herbs are used whichhave the anti- cancerous and anti-tumour activity.

2. AIM AND OBJECTIVES

Aim

To validate the safety and efficacy of the test drug *Kandha Rasa Villai* for its Anti-cancer Activity against Cervical Cancer preclinical in HeLa cell lines.

Objectives

The objectives of this work were done through the following steps.

- Collection of relevant literature from classical Siddha text as well as modern sciences that supported this study.
- Description of pharmacognostic features of the ingredients in this formulation including the identification, collection and purification of them etc.
- Preparation of the drug according to the procedure described in the Classic Siddha literature.
- Standardization of the trial drug by means of Siddha parameters and physico-chemical analysis.
- Revealing the anions and cations present in the drugs through proximate chemical analysis.
- Elucidation of the chemical structure, microscopical structure of the drugs by means of instrumental analysis.
- Getting approval from IAEC for ethical clearance of animal usage.
- Interpreting the results of acute and repeated dose 28-days oral toxicity of *Kandha Rasa Villai* according to OECD guidelines 423 and 407 in Wistar albino rats.
- Detailing the study of pharmacological activities of the trial drug *Kandha Rasa Villai* like anticancer activity in HeLa cell lines, antitumor activity in SIHA cell lines and antioxidant activity by DPPH assay.

3. REVIEW OF LITERATURE

காந்தரசவில்லை

“காந்தரசவில்லைதனைச் சொல்லக்கேளூ
கணவிரத மிலிங்கந்திப் பிலிநற்சுக்கு
நேர்ந்திடுகார் போகரிசி வாய்விளங்கம்
நினைவான மஞ்சிஷ்டி நிகழ்த்துகாரம்
கூர்ந்திடுவால் மிளகுவகைக் கிருவிகாரன்
கொடுமையுள பாஷாண மொன்றுசூடன்
ஆர்ந்தவொன்று காந்தமிரண் டமைந்ததண்ணீ
ரழகுடனே விட்டுநாற் சாமமாட்டே.
ஆட்டியொன்றே முக்கால் விராகன்பில்லை
யன்புடனே செய்துலர்த்தி யோட்டிவிட்டே
ஒட்டினால் மேன்மூடி யிரண்டுசீலை
யொழுங்குறவே செய்துநாற்பத் தைந்தேநாள்
நாட்டியதா னியபுடத்தில் வைத்துப்பின்பு
நலமான சீசாவில் பதனஞ்செய்யே
வேட்டகுன்றி யெடையிற்கால ரைமுக்காவா
மிகுகாலை மாலையெழு தினம்வரைக்கும்.

பனைவெல்லம் சர்க்கரைதே னெய்யில் வாழைப்

பழத்தில்வைத்துக் கொடுத்துவிட்டா யோனிப்புற்று

நினைக்கவொண்ணாக் கிரந்திவகை மேகசூலை

நேர்மையற்ற வெடிசூலை வாய்வும்போகும்

கனைக்குமுயர் வாதமிர ணங்கள்போகும்

கடினமுட்றற வியாதிகட்கோ வுப்பைத்தள்ளி

இனையாமல் பதினாங்கு நாள்வரைக்கு

மேற்றகடும் பத்தியத்தைக் கைகொள்வாயே"

-வீரமாமுனிவர் வாகடத்திரட்டு[7]

For this thesis, the Herbo-metallic formulation *Kandha Rasa Villai* was taken for Anti-cancer activity from the classical Siddha literature.

3.1.DRUG REVIEW

3.1.1SIDDHA ASPECT OF *RASAM*:

Chemical name: Mercury or Quick Silver[9a]

Mercury is comes under the classification of '*Pancha soothaam'*. It has many connotations such has *sootham, punniyam, bharatham, inimai, sivasathi, kesari*etc, according to *dasanga nigandu.*

Mercury is obtained from its ores in countries like Spain, California, Russia, China and Japan. It is separated from its ore chinnaber.

Types of mercury:

Mercury was classified into five types.

1. *Rasam*
2. *Rasendhiran*
3. *Sootham*
4. *Misaragam*
5. *Baaratham*

Properties:

1. Vitalize
2. Tonic
3. Laxative
4. Diuretic
5. Neutralising *pitham*
6. Silagogue
7. Anti- inflammatory
8. Anti- syphilitic

Taste: Six tastes dominated by sweet.

Potency: Hot and cool (both -specialty)

Special properties of mercury:

Unlike other drugs mercuryis useful in the treatment of diseases caused by both heat and cold.

***Dhosam* (Impurities) of Mercury:**

It is considered that there are two types of *Dhosam* of mercury. They are

1. Dhosam

2. Sattai(Kavasam)

In *Dhosam*there are 8 types of impurities in mercury producing various diseases as shown below:

Dhosam	**Disease caused by them**
1.Undheenam	*Soolai*(Throbbing pain)
2.Kowdilayam	*Kapala noi*(Disease of the head)

3.*AnavarthamBiramai*(Manic illness)

4.*SangaramThathu nattum*(Spermatorrhoea)

5.*Sandathvam* Distress

6.*Panguthvam Kuttam*(Leprosy)

7.*SamalathvamMoorchai*(Syncope)

8.*SavisthavamSareeraElaippu*(Loss of weight)

Sattai is an anotherone classification, there are 7 types of impurities in mercury which producing various diseases as shown below:

Impurities	**Disease Caused by Them**
1.*Naagam*	*Moolam*(Haemorrhoids)
2.*Vangam*	*Tholnoikal*(Skin disease)
3.*Malam*	*Arivinmai*(Idiocy)
4.*Vidam*	*Maranam*(Death)
5. *Akkini*	Morbid thrist (Polydypsia)
6.*Giri*	*Sattium*(Distress)
7.*Sabalam*	*Thathunattam*(Spermatorrhoea)

General properties of mercury:

"விழிநோய் கிரந்திகுன்மம் மெய்சூலை புண்குட்
டழிகாலில் விந்துவினால் அத்தை- வழியாய்
புரியு விதி யாதுபுரியினோயெல்லாம்
இரியு விதியாது மில்லை".

- குணபாடம் தாது- சீவ வகுப்பு

Proper use of mercury as a medicine can able to cures the following diseases they are disease in eyes, syphilis, eight types of ulcers (*gunmam*), throbbing pain (*soolai*), chronic ulcers (*perumpun*), leprosy.

Purification and detoxification of mercury:

Requried quantity of mercury is placed in a thick cloth and squeezed for 1000 times. Then it is placed in an earthen pot. Fresh water is poured in the earthen pot upto the level of the mercury level. The pot is heated with low intensity fire. The water level is maintained by adding water. When the water turns into black in colour, the mercury is separated and washed with vinegar for 4 or 5 times to get the purified mercury.

Preparations of Mercury:

- *Soothakaruppu*
- *Rasa mezhugu*
- *Rasa thailam*
- *Megaviranakalimbu*
- *Rasa kuligai*

Toxic symptoms of Mercury:

If the medicinal mercury is not purified properly, the disease like bleeding, dropsy, anaemia, excessive body heat, sweating, diarrhoea, thirst, flatulence, blabbering, skin diseases, burning sensation of the limbs, head diseases, fever, shivering, hiccough, etc, will manifest and finally death will occur.

The whole body will be burnt and all the teeth will fall down.

Antidote for mercury poisons:

If there is burning sensation in limbs, urticarial, dryness of the throat and the patient is unconscious, Barmuda grass root stalk (Cynodondactylon- Arugan kizhangu) is triturated and dissolved in goat's milk or cow's milk or butter milk, or cotton seed milk is filtered and administered.

SIDDHA ASPECTS OF MAGNETIC OXIDE:

***Kaantham* (Magnetic oxide of iron)[9b]**

Siddhars classified the magnet under 11 types of metals in mineral kingdom. It is a one of the important ingredient of *Thiriloga Chendooram*.

According to *Agathiyar Vaidya Rathina Churukkam* text magnet wasused for making *Panchaloga Chendooram.*

Different varieties of Magnet:

- *Kal kanntham*
- *Oosikanntham*
- *Pachaikanntham*
- *Arakkukanntham*
- *Mayirkanntham*

Purification methods:

1. The magnetic oxide of iron is powdered and wrapped in a cloth. It is boiled by vinegar (kaadineer) steam and with the steam of horse gram decoction. Then it is boiled washed and dried.

2. The magnetic oxide of iron is heated in horse gram decoction for 7 to 21 times to get it in purified form.

General Properties:

“காந்தத்தாற் சோபைகுன்மங் காமிலமே கம்பாண்டு

சேர்ந்த்திரி தோடவெட்டை சீதங்கால்- ஒய்ந்தபசி

பேருதரங் கண்ணோய் பிரமியநீ ராமையும்போம்

ஓரினிறை யாயுளுறும் உன்”.

-குணபாடம் தாது- சீவ வகுப்பு

It was very effective in the treatment of swelling, ulcer (*gunmam*), jaundice (*kamalai*), veneral disease (*megam*), diseases of three humours, leucorrhoea, *kaphavatha* diseases. It also increases longevity.

Special properties of Magnet:

- Consumption of milk boiled in a vessel made up of magnetic oxide of iron improves and strengthens the blood haemoglobin. The bolied milk never spill over out in a magnetic vessel.

In general the magnet oxide of iron has got the similar properties as iron. However, it is considered that magnet is superior to iron in many aspects

Preparations:

- *KannthaParpam*
- *Kannthachendooram*
- *AyakanthaChendooram.*

SIDDHA ASPECT:

VELLAI PASHANAM (WHITE ARSENIC):

Vernacular names:

Eng	:	White oxide of arsenic,White arsenic,arsenious acid,Flower of arsenic
San	:	Sankhavisha,Darumucha,Sambalakshara
Arab	:	Sammula far
Pers	:	Margamosha
Hind	:	Sankhya
Duk	:	Safed sambala
Beng	:	Sumbulkhar
Guj	:	Somal khar
Mah	:	Sankhya sambala
Tam	:	Vellai pashanam
Can	:	Sankhya pashana
Sinh	:	Sudu pashanam[10]

Other names in siddha

"வெள்ளை பாஷாணாப் பேர் விளம்பக் கேளு
மிடுக்கான யெலிக்கு நல்ல காலனாகும்
விள்ளையாம் விஷராசன் கொடு விஷமுமாகும்
வெற்பான குடோரியாம் மன்னுயிரின் காலன்
முள்ளையா முஷப் பாஷாணமாகும்
மூர்க்கனாங் கற்பானா மாகும் பாஷாண
முள்ளையா முலோகங் கற்காரனாமு
முரைத்த வெள்ளைப் பாஷாணாப் பேருமாமே"

*-போகர் நிகண்டு*1200[11]

Vellai, Vedam, sangatthallai, samarasabagam, rasidaviddhai,tarapriti.

Source

- Found in arsenic ores as arsenates of iron, nickel or cobalt.
- Commercially it is obtained by roasting the ore in the form of sublimatio.Sublimation increases its penetrating power.

General properties:

Characters

Solid, heavy, white powder or stratified masses or minute transparent and glass like crystals.

Actions:

Stomachic,
Nervine tonic,
Alterative,
Antiperiodic
Respiratory,
Intestinal and sexual stimulant.

Medicinal uses

"வெள்ளைப் பாடாணம் விடங்கடிதீ ரும்பூசக்
கொள்ளைச் சுரதோஷங் கோரசந்நி – தொள்ளையுறு
நாசிப்புண் வாய்ப்புண் நனைகிரந்தி போமுண்ண
ஆசிக்கு கும்பமுலைஆய்".

-பதார்த்த குண சிந்தாமணி[12] .

- It is used in the treatment of epidemic fever,poison bite, deliriumandinfections, ulcer of the nose and mouth, venereal ulcer, skin diseases.
- Externally it is used to remove large growth like cancer and lupus and to kill vermins in head and other hairy parts.
- In unani system of medicine it is used as aphrodisiac.

Method of purification:

Arsenic trioxide 35 gm is powdered and triturated with lemon juice. It is made in to small cakes and dried. This process is repeated for 7 times.

Toxic symptoms of arsenic trioxide:

Acute poisoning:

Blister, ulcer, pain in the hands and toes, swelling of the face, ulcer of the upper lip, vomiting and bad odor , tastelessness, sore throat, burning sensation in the stomach, bleeding diarrhea and vomiting, haemetemesis, excessive sweating, thirst, strangury, syncope, convulsion, loss of memory and anasarca.

Chronic poisoning:

Pruritis, eczema, chronic hepatitis, indigestion, swelling of the face, pain in the throat, gastritis, giddiness and diarrhoea.

Antidote:

1. Cardamom and root ofMusumusukai (*BryoniaScabrella)* (4.2 gm each) are taken and decoction is made with sugar and padikaraum. It is taken twice a day for 40 days.

2. If *Pitha* exceeds with vomiting, give decoction.

3.Give pepper paste for curing the arsenic trioxide poisoning.

4.The *Kalkam* of the indigo plant root can also be used in arsenic poisoning, as twice a day [9c].

SIDDHA ASPECT OF *Lingam* (cinnabar):[9d]

Synonyms: Natural cinnabar, Vermilion.

Chemical name: Red sulphide of mercury.

Other names: *Inkuligam, Raasam, Kadai vanni, Karpam, Kalikkam, Kaanjanam, Kaaranam, Sandagam, Samarasam, Saaniyam, Chendooram, Maniragam, Milechem, Vani*and *Vanni.*

Nowadays,The *Lingam* used by us is called as *Jaathi linga paadanam*, grouped under *Vaippu paadanam.*

Preperation of *Vaippu paadanam:*

Rasam (Mercury)– 280 gm

Gandhagam (Sulphur)–70 gm

Vediuppu(Pottassium nitrate)– 70 gm

Procedure:

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

***Gunam*(Properties):**

It is hard, when it is put into fire it becomes smoke; not soluble in water, has no smell and taste and has hot potency.

General properties:

“பேதிசுரஞ் சந்நி பெருவிரண நீரோடுத

காதகடி காசங் கரப்பான்புண் - ணோத

வுருவிலிங்க சங்கதமா யூறுகட்டி யும்போங்

குருவிலிங்க சங்கமத்தைக் கொள்”.

- -பதார்த்த குண சிந்தாமணி.

It is effective in the treatment of diarrhea, pyrexia, delirium, urticaria, tuberculosis, scabies, unknown insect bites, syphilis, leprosy, eczema, skin diseases, throbbing pain and *vatha* diseases.

Method of purification:

Lime juice, cow's milk and the Indian acalypha juice are mixed together in equal proportion and allowed to fuse cinnabar so as to get it in a purified potency form.

Other preparation:

Padigalinga Chendooram - dysentery, diarrhea, menorrhagia and fever.

SaathiSamberaKuzhambu - diarrhea, nausea, vomiting, syncope, fever and thirst.

Sign and symptoms of cinnabar toxicity:

Dyspepsia, loss of taste, ulcers in the buccal floor, uvula, inner portion of the tongue, larynx and large intestine, foul odour from mouth, whitish saliva,burning sensation are the toxic symptoms of red cinnabar.

Antidote:

Nutmeg (*Myristicafragrans)* - 4.2 gm

Cubeb pepper (*Piper cubeba)*-4.2 gm

Root bark of red cotton tree *(Gossypium arboretum)*-4.2 gm

Sugar candy - 4.2 gm,

These are mixed together and made into decoction and administered twice daily for 48 days.

SIDDHA ASPECT OF SOODAN(CAMPHOR)[9e]

Other names: Karpooram, sudarkodiyon, pooram, deepam.

Camphor is found in china, japan, sumithra, California.Natural camphor is present in the plant Cinnamomum camphora. The natural crude camphor may be obtained by steam distillation of chips of the camphora tree; the crude camphor so obtained is purified usually by sublimation.

Properties of camphor:

Camphor sublimation is mixed with lime and purified.It is white in colour, gracy in nature and has pleasant smell.It is not soluble but floats in water, and dissolves in air.It can be powdered. It is soluble in oil, arrack and gum.It is triturted with sugar syrup or white of an egg with water can be mixed with water.

Taste: Salty and pungent.

Potency: Hot

Action:

Carminative,

Anodyne

Antispasmodic

Antiseptic

Anticonvulsants

Expectorant.

General properties:

“கிருமிசல தோடங் கிளைவலிப்பு சந்நி

பொருமுமந்தம் அங்கிபட்ட புண்ணோ-டெரிசுரங்கள்

வாந்திபித்தஞ் சீதமுறு வாதஞ் செவிமுக-

காந்திகருப் பூரமொன்றாற் சாற்றுநோய்.”

-குணபாடம் தாது- சீவ வகுப்பு

It cures worm infection, rhinitis, convulsion, delirium, dyspepsia due to vatha, burns, hyperpyrexia, vomiting, pitha, kaphavatha, diseases of ear and face, postnatal eclampsia, wheezing, cardiac arrest, dysmenorrhea, fissures, burning sensation in the penis due to venereal focus, hysteria, urinary tract infection, arthritis, chronic ulcer,diarrhoea, cholera, cough, tachycardia, whooping cough, spermatorrhoea, aphrodisiac.

Dosage: 65mg to 325mg.

Toxic symptoms:

More than 1 gm of camphor causes toxicity effect in children, camphor poisoning have been record when the dose exceeded 1gm. There has been report of instant collapse in infants fol-lowing the local application of camphor to their nostrils.

In excess dose camphor is toxic. There is a superstitious belief that if camphor is taken in plantain fruit in high doses, it may cause abortion.

Purification:

The impure camphor is soaked in water lily flower juice (Nymphaea Alba) for 24 minutes and taken out dried in the sun shade to get purified.

Chukku (Zingiber officinale)

Dried ginger is called as *chukku.*

Other names: [13a]

Allam, Arukkan, Arthagam, Ubakullam, Kadupathiram, Aundi, Sondi, Sowbannan, Sowarnam, Navasuru, Nagaram, Manowshadam, Vichvibeshajan, VishamoodiyaAmirtham, Vaerkombu

Useful Part : Rhizome (Dried)

Taste : Acrid

Character : Heat

Division : Acrid

Actions

Stimulant

Stomachic

Carminative

General Properties

"சூலைமந்தம் நெஞ்செரிப்பு தோடமேப் பம்மழலை மூலம்

இரைப் பிருமல் மூக்குநீர் வாலக

தோடமதி சாரந் தொடர்வாத - குன்மநீர்த்

தோடம் ஆமம்போக்குஞ் சுக்கு "

-பதார்த்த குண சிந்தாமணி

Chukku is used therapeutically for treating diarrhea, belching problems of respiratory tract, anaemia, ulcer and fever.

Thippili(Piper longum)

Other Names: [i3b]

Argadi, Unsaram, Ulavainasi, Kaaman, Kudari, Kolagam, Koli, Kozhaiyarukki, Saram, Saadi, Thulasi, Maagadi, Kanai, Soundi, Thanduli, Kanam, Kalini, Paanam, Pippili, Vaidegi, Ambu, AathiMarunthu.

Parts used : Fruit, Rice

Taste : Sweet

Character : Cool

Division : Sweet

Actions:

Stimulant,

Carminative

General Properties:

"இருமல்குன்மம் இரைப்பு கயப்பிணி

ஈளைபாண்டு சந்யாசம் அரோசகம்

பொருமல்ஊதை சிரப்பிணி மூர்சைநோய்

பூரிக்குஞ்சல தோடம் பீலிகமும்

வருமலப் பெருக்கோடு மகோதரம்

வாதம்ஆதி மூச்தோடஞ் சுரங்குளிர்

பெருமாலைப் புரிமேகப் பிடகமும்

பேருந்திப் பிலிப்பேரங் குரைக்கவே".

-பதார்த்த குண சிந்தாமணி

Medicinal Properties and Uses

It is used for treating cough wheezing, distention,fever.

MANJITTI (*Rubia cordifolia*)

Other names: [13c]

Pandi, manjugam, manjitti

Part used: Root

Taste: Pungent

Character : Hot

Division : Pungent

Action:

Emmenagogue

Medicinal Properties and Uses

Dried root was much used in dropsy, paralysis, amenorrhoea and visceral obstructions

KARPOKARISI *(Psoralea corylifolia)*

Other name:[13d]

Karpuvaaarisi, Paaguse, Kappuvaarisi

Parts used: Seeds

Taste : Bitter (seeds)

Character : Dry

Division : Pungent

Action:

Laxative

Stimulant

General Properties:

"கார்போக மாமரிசி கண்டாற் கரப்பான்புண்

பீர்சகுவ நஞ்சிவைபோம் பித்தமுண்டாம் - பார்மீதில்

வாத கபநமைச்சல் வன்சொறிசி ர்ங்குமறுஞ்

சீத மலர்க்குழலாய் செப்பு".

-அகத்தியர் குணவாகடம்

Medicinal Properties and Uses

Seeds are useful in bilious affections and are also used to make a perfumed oil, and its power is specially recommended by vaidyas in leprosy and leucoderma internally and are also applied in the form of paste or ointment externally.

VAIVILANGAM (*Embeli ribes)*

Other name:[13e]

Vayuvilangam, Keralam, Varnai

Parts used : Fruit, seeds

Tast : Biteer(seeds)

Character : Hot

Division : Pungent

Action:

Anthelmintic

Carminative

Stomachic

General Properties:

"பாண்டுகுட்டம் குன்மம் பருந்தூல நோய்வாதந்

தீண்டு திரிவிடஞ சிரந்துண்டம் - பூண்டமடி

நோய்விளங்கக் காட்டாத நுண்கிருமி யாசனப்புண்

வாய்விளங்கங்காட்டவிருமார்""

-அகத்தியர் குணவாகடம்

Medicinal Properties and Uses

Seeds are useful as powder expel intestinal worms especially tape-worms.

Berries prevent flatulence and are useful in dyspepsia.

Berries crushed and mixed with butter is an ointment applied to the forehead in headache

VALMILAGU (*Piper cubeba*)

Other name: [13f]

Val milaka, Lankesaha, Kababah

Part used : Unripe fruit

Taste : Pungent

Character : Heat

Division : Pungent

Action:

Stimulant

Carminative

Diuretic

General Properties:

"வாதபித்த ஐயம் வயிற்று வலிதாகஞ்

சீதம் பலநோய் சிதையுங்காண் - போத

அதிதீ பனமாம் அணங்கரசே! நாளுந்

துதிவால் மிளகருந்தச் சொல்".

- *அகத்தியர் குணவாகடம்.*

Medicinal Properties and Uses:

The powdered piper cubeba with milk is used for throat infection and clears the voice.

3.1.2 MODERN ASPECT OF MERCURY:

Mercury:[14]

Mercury should not have less than 99.5 percent of Hg. It occurs naturally as a sulphide ore called cinnabar (HgS). It also occurs in small globules disseminated through rocks and as amalgam of silver and gold.

Preparation:

- It's obtained by roasting cinnabar in a current of air. $HgS+O_2 \rightarrow Hg+SO_2$
- The free mercury gets liberated, it may be either purified by volatilization or chemically by dropping mercury into a column of dilute nitric acid for removing basic impurities

Properties:

It has shinning silvery white in nature. Heavy liquid easily divisible into globules and extremely mobile it easy volatilizes on heating. It boils at 359.58°C

Almost insoluble in water, alcohol and Hcl. It dissolves in cold and dilute nitric acid, giving mercurial nitrate and nitric oxide.

$$6Hg+8HNO_3 \rightarrow 3Hg_2 (NO_3)2+2NO+4H_2O$$

Density: 13.581ml at 25°C

Mercurial preparations:

- Mercury with Chalk(Grew powder)
- Yellow mercuric oxide(HgO)
- Mercuric oxide
- Oleated mercury
- Mercurous chloride(HgCl-Calomel)

Uses:

Formerly metallic mercury found use as such therapeutically as a cathartic and parasticide. But it is more used as such;as it has been extremely poisonous and prolonged inhalation of even very minimal amounts of mercury prove fatal. Almost all the salts of mercury with the exception of the sulphide, has been poisonous.

1. Mercury with chalk (Grew powder)

- It is having 31 -35% w/w of mercury and 62-70% w/w of $CaCO_3$
- It is used as a purgative (Dose 60-300mg)

2. Yellow mercuric oxide (HgO)

- It is having not less than 99.5%HgO
- It is used as a mild antiseptic action and used as anti- infective and anti- bacterial agents.

3. Mercuric Oxide:

- It contains not less than 95% but not more than105% w/w of the stated amount of yellow mercuric oxide
- It is used in ophthalmology, 1% ointment to treat mild inflammatory conditions for the treatment of blepharitis and conjunctivitis.

4. Oleated mercury:

- It has the equivalent of 20% of yellow mercuric oxide
- It is used as an anti- infective.

5. Mercuric chloride (HgCl) (Calomel):

- It is being not less than 99.6% of HgCl
- It has been used for centuries as a cathartic but recently it is replaced by other drugs.

- Calomel has been insoluble in gastric juice and has been not absorbed from the stomach. It gets absorbed in the intestine by the alkaline pancreatic juice where it slowly gets dissociated into mercury and irritant mercuric compounds which have been exerting a cathartic action.

MODERN ASPECT OF MAGNETIC OXIDE:

Chemical Name: Magnetic oxide[15]

Synonyms:

Magnetite / Black Iron Oxide (Fe_30_4), super paramagnetic iron oxide , black iron sand, magnetite sand, beach magnetite sand, iron oxide (Fe_3O_4),magnetic black, magnetic iron ore, ferrous ferric oxide , magnetic oxide,triirontetraoxide, ferroso ferric oxide, iron black, black Iron BM, iron(III) oxide, meramec M 25, river sand, black gold F 89.

Magnetic Oxide Formula:

Fe_3O_4

Magnetic Oxide (Fe_30_4) Description:

a) Magnetite was a natural occurring iron oxide of magnet, consequently the name giving its distinguishing characteristic.

b) Magnetite was a member of spinel group which has the standard formula $A(B)2O_4$. The A and B of this represent different metal ions that occupied in specific sites on its crystalline structure. In magnetite standard formula was Fe_3O_4, in this A metal represent Fe +2 and the B metal represent Fe +3; two different metal ions in two specific sites. This arrangement causes a transfer of electron ions between the different irons in a structured path or vector. This electric vector was responsible for generates a magnetic field.

c) Lustrous black, magnetic mineral occurs on crystals of the cubic system in masses and as loose sand. It was one of the main ores of iron (magnetic iron ore) and

is a common constituent of igneous and metamorphic rocks. It was found in various parts of the United States, Norway, Swedenand the Urals. A variety of magnetite was lodestone or loadstone exhibits its polarity especially interesting for its natural magnetism.

d) Magnetite is sometimes found in large quantities in beach sand. Such mineral iron sands or black sands are found in various places of California and New Zealand west coast lands. The magnetite was carried out from beach to rivers from erosion and it's concentrated via wave action and currents.

Chemical Properties:

- Purity Available: From 96 percent to 99.9 percent
- Super paramagnetic iron oxide was available in 10 microns size and had no magnetic memory.

Physical Properties:

- Lumps, pieces, targets, granules and various powder or particle granulations down to as small as 15 to 20 nanometers
- Black iron oxide nano particles are presently available as smaller size from 15 to 20 nanometers

Nominal Physical Constants:

- Magnetite Luster: Metallic
- Magnetite tenacity: Brittle
- Magnetite ID Mark: Ferromagnetic
- Magnetite Solid Density (gm/cm3): 5.1
- Magnetite pH: 7
- Magnetite Transparency: Opaque

- Magnetite Hardness @20°C -5.5 to 6.5
- Magnetite Specific Gravity: 5.17 to 5.18
- Magnetite Colour: Black to greyish
- Magnetite Crystal System: Isometric
- Magnetite Particle Shape: Irregular
- Magnetite Magnetic Properties: Ferric magnetic

Magnetite ($Fe_3 0_4$) Typical Applications:

- Magnetite was a main ore form of iron. It used mainly in various fields.
- Magnetite was used as a pigment for polishing compounds, cosmetics, medicines, polymer & rubber filler, building & construction, appliances, and magnetic inks.

MODERN ASPECT OF WHITE ARSENIC

Aresenic is a metalloid element. Arsenic containing preparations have been in medical use formore than 2000 years. Arsenic-based therapy was used in the United States and Europe more than 100 years ago to treat leukemia and infections. More recently, interest in arsenic-based therapy was revived by report of the anti-leukemic activity of some traditional chinese preparations.

There is speculation that it works through a variety of mechanisms including cell cycle specific chemotherapy agent, as atargeted therapy and perhaps as an angiogenesis inhibitor.

Name	: Arsenic
Symbol	: As
Atomic Number	: 33
Melting point	: 817.0^0 C
Boiling point	: 613.0^0 C
Number of protons/Electrons	: 33

Number of Neutrons	: 42
Crystal structure	: Rhombohedral

Uses

Paracelsus was the first to document precise directions for the preparation of metallic arsenic as a therapeutic agent and made a balsam from white arsenic, which was a favoured method used by the barber surgeons to treat wounds, buboes, carbuncles, anthrax and other similar ulcer.

In 1878 in Boston city hospital fowelers Solution was discovered to lower the white cell count in two normal people with a more significany decrease in a person with chronic myelogenousleukaemia (CML) and subsequently became an accepted treatment for leukaemia.

Trisenox is the trade name for arsenic trioxide.Arsenic Trioxide is an anticancer (antineoplastic or cytotoxic) chemotherapy drug.This medication is classified as a "natural product".

Arsenic trioxide may also be used for multiple myeloma, chronic myelogenous leukemia and acute myelogenous leukemia[16].

MODERN ASPECT OF CINNABAR:

Cinnabar (red mercury (II) sulphide (HgS), vermilion) is the ordinary ore of Hg. It is normally found in a substantial, granular form and is bright scarlet to brick-red in color. It is a chemical compound composed of the chemical elements mercury and sulphur (Mercury 86.22 %, Sulfur 13.78 %).

Formula	-	Mercury(II) sulfide
Symbol	-	HgS

Properties:

Molecular formula	-	HgS
Number	-	32
Colour	-	brownish red and lead-gray

Specific gravity	-	8.176
SolubiKlity	-	Soluble in water,
Molecular Weight	-	232.66 gm
Melting point	-	580 °C decomp.
Other anion	-	Mercuryoxide,mercuryselenide
Other cations	-	Zinc sulphide, Cadmium sulphide
Fermion Index	-	0.26
Boson Index	-	0. 74

Toxicity:

It caused shivering, loss of sense, and death. Overexposure to mercury is called mercurialism which was seen as an occupational disease to the ancient Romans.

Realgar:

Realgar is an important ore of arsenic and composed of arsenic (70.03%) and sulphur (29.97 %). It is orange-red in colour and when its flame release fumes of Arsenic and Sulphur burns. Its streak is orange coloured. It is a Arabic name *rahj al ghar* which means "powder of the mine."

Other Names: Ruby sulphur, Ruby of arsenic.

Physical Properties:

- Formula - As4S4 or AsS
- Colour - Red to yellow-orange
- Density - 3.56
- Diaphaneity - Transparent
- Specific gravity - 3.56
- Melting point - 320 °C
- Molecular Weight - 106.99 gm

- Refractive index - 2.538
- Luminescence - Non-fluorescent
- Luster - Sub Metallic
- Streak - orange

Electrical Properties:

- Electron Density - 3.30 gm/cc
- Fermion Index - 0.0022478773
- Boson Index - 0.9977521227
- Radioactivity - 0 GRAPI (Gamma Ray American Petroleum Institute Units)

Uses

The Chinese name for realgar is *xionghuang*, literally 'masculine yellow'. It was used to repel snakes, rats, weeds and insects, as well as being used in Chinese medication. The ancient Greeks called it as "*sandaracha*". It is used in combination with potassium chlorate to make a contact explosive known as "red explosive" for some types of torpedoes[17].

MODERN ASPECT OF CAMPHOR

Synonyms

1,7,7-Trimethyl Bicyclo(2,2,1)-Heptan-2-One

2-Bornanone

2-Camphanone

2-Keto-1,7,7-Trimethylnorcamphane

2-Oxo-Bornane

Alcanfor

Camfora

Camphor-Natural

Camphor-Synthetic

Formasa-Camphor

Gum Camphor

Japan Camphor

L,7,7-Trimethylnorcamphor

Laurel Camphor

Matricaria Camphor

Root Bark oil

Spirit of Camphor

Tramfer

Origin of the substance

Camphor may be natural or synthetic.It occurs naturally in the wood of the camphor tree (Cinnamomum camphor), and is extracted by steam distillation and crystallization.Natural camphor is dextrorotatory. Synthetic camphor may be made from pinene which is converted into camphene by treatment with acetic acid and nitrobenzene.Synthetic camphor is optically inactive.

Physical properties

Properties of the substance

Normal state at room temperature:solid, translucent crystals.

Colour : White crystals.

Odour : Penetrating, aromatic.

Boiling point : 204^0 C

Melting point : 176 to 180^0 C

Sublimes appreciably at room temperature and normal atmospheric pressure

Flash point :65^0 C

Autoignition temperature : 466^0 C

Relative density : 5.2

Vapour pressure : 20 PA at 20^0 C

Solubility in water : 0.125 g/100ml(25^0 C)

Soluble in ethanol, ethylether, turpentine and essential oils

Storage conditions

Store in Airtight containers at a temperature not above 25^0 C.

Medicinal properties and uses

Camphor has stimulant antispasmodic, anti-septic, anti-pyretic and aphrodisiac properties.When locally applied it is stimulant and anodyne.

It is useful in adynamic fever, inflammation, choleraidiarrhoea, whooping cough, epilepsy, chorea, asthma, angina pectoris and puerperal convulsions.

It is also used in treating hysteria, palpitation, in affection of genitourinary system as dysmenorrhoea, spermatorrhoea.

It is also useful in irritable conditions of the nasal mucus membrane causing sneezing and frontal headache[(18)].

CHUKKU (Zingiber officinale)

Vernacular names:[(19a)]

Sanskrit : *Adraka*

Hindi : *Adrak, Ada*

Bengali : *Ada*

Telugu	:	*Allamu*
Tamil	:	*Allam, Inji*
Kannada	:	*Hasisunti*

Taxonomic Classification

Kingdom	:	Plantae
Class	:	Liliopsida
Subclass	:	Zingiberidae
Family	:	Zingiberaceae
Genus	:	*Zingiber*
Species	:	*officinale*
Name	:	*Zingiber officinale Roscoe.*

Distribution:

It is cultivated throughout India, run wild in some places in the Western ghats.

Description:

Rhizomes are widely dug in January-February, buds and roots are removed, soaked overnight in water, decorticated and treated with lime and dried.

Macroscopic characters:[20a]

A slender, perennial, rhizomatous herb, linear leaves, sessile, glabrous, flowers yellowish green in oblong cylindrical spikes. The rhizomes are white to yellowish brown in colour, irregularly branched. The growing tips are covered over by a few scales.

The surface of the rhizome is smooth and if broken a few fibrous elements of the vascular bundles project out from the cut ends. The pieces are about 5-15 cm long, 1-1.5 cm wide usually 1-1.5 cm thick; showing longitudinal striations and occasional fibres, odour agreeable and aromatic.

Taste agreeable and pungent.

Microscopic characters

Transverse section of rhizome shows cortex of isodiametric thin walled parenchyma with scattered vascular strands and numerous isodiametric idioblasts, about 40-80 µm in diameter containing a yellowish to reddish brown oleo resin.

Constituents

Gingerols, Shogaols, Dihydrogingerol, Gingerdione, Hexahydrocurcumin and Desmethylhexahydrocurcumin, α-zingiberene, β-sesquiphellandrene, ar-curcumene, lipids.

Properties and Uses

The rhizome is sweet, pungent, appetizer, laxative, stomachic, aphrodisiac, carminative useful in diseases of the heart and throat, dyspepsia, inflammations, bronchitis, asthma, vomiting and aches. Ginger is pungent, stomachic, aphrodisiac, laxative, improves taste, useful in indigestion, vomiting, pains, asthma, bronchitis, diseases of the heart, elephantiasis, piles, eructations, abdominal troubles, scorpion sting, snake bite.InCambodio, the rhizome is given internally as an aromatictonicexternally it is applied to boils and enlarged glands.

THIPPILI (Piper longum)

Vernacular Names: [19b]

Tam	:	*Tippali, Ambu*
Beng	:	*Pipul*
Eng	:	Pippali,Long pepper
Hindi	:	*Pipar*
Tel	:	*Pippalu*
Mal	:	*Pippali*

Taxonomic Classification

Kingdom	:	Plantae
Division	:	Magnoliophyta

Class	:	Magnolipsida
Order	:	Piperales
Family	:	Piperaceae
Genus	:	*Piper*
Species	:	*longum*

Distribution

It is cultivated in hotter parts of India from Central Himalayas to Assam upto hills of West Bengal and evergreen forests of Western Ghats as wild and also in North East and many parts of the South.

Description

MacroscopicCharacters[(20b)]

Fruit greenish-black to black, cylindrical, 2.5 to 5 cm long and 0.4 to 1 cm thick, consisting of minute sessile fruits, arranged around an axis, surface rough and composite, broken surface shows a central axis and 6 to 12 fruitlets arranged around an axis, taste pungent producing numbness on the tongue, odour aromatic.

MicroscopicCharacters

Catkin shows 6 to 12 fruits, each having an outer epidermal layer of irregular cells filled with deep brown content and covered externally with a thick cuticle, mesocarp consists of larger cells, irregular in shape and thin walled,outer layer of this zone composed of thin walled cells and colourless, most of the endocarp cells filled with starch grains, round to oval measuring 3 to 8 in diameter.

Constituents

Piperine,

Piplartine

Alkaloid,

Dihydrostigmasterol,

Properties and medicinal uses

The root and fruits are used in palsy, gout, lumbago. The fruit has a bitter, hot, sharp taste; carminative, tonic to the liver, stomachic, emmenagogue, abortifacient, aphrodisiac.

It appears to partake in a minor degree of the stimulant properties of the fruit. Long pepper in the form of powder is suspended in warm water and given to women after parturition to check haemorrhagic fever.

The fruits are used as a spiceand also in pickles and preservatives. They have pungent pepper like taste and produce salivation and numbness of the mouth. Pellitorine, piplartine, piperine exert medicinal uses. Mainly used in respiratory tract infection diseases[21].

VALMILAGU (*Piper Cubeba*)

Classification:

Kingdom - plantea

Order - Piperales

Family - Piperaceae

Genus - *Piper*

Species -*Cubeba*

Vernacular Names:[22]

Sanskrit : *Kankola*

Assam : *Kakkol, Kababcheni*

Ben : *Kababchini*

Eng : Cubebs,Tailed peper

Guj : *Chanakabab*

Hindi : *Kabalochini*

Kan : *Balamenasu*

Kash :*Kushfal*

Mal : *Kankol*

Punj : *Kababchii, Sardchini*.

Tam : *Valmilagu*

Tel *:Chalavamiriyalu*

Distribution:

A Native of Indonesia cultivated mainly in Karnataka.

Botanical characteristics

Habit – A liana like climber

Stem – Climbing, rooting at the joints

Leaves – 4 to7 inches long, petiolate oblong to ovate.

Flowers – Dioecious, in spikes opposite the leaves, flowers in the rainy season and fruits in the autumn

Part used: Mature, Dried fruit

Action: Stimulant, Carminative, Stomachic, Expectorant

Medicinal uses

It is commonly used in the treatment of gonorrhoea and relieve hoarseness

The fruits are used predominatiy as a digestive aid, carminative and stomachic.It is also used as sialogogue and digestive aid.

Fruits are potent germicidal and chewed to remove mouth ulcer, swellen gums, fruit powder given in dysentery,catarrh,sedative, rheumatism, urethritis.

Smoking cubebs is a popular method of treating nasal catarrh and Hay fever[23].

VAIVIDANGAM (*Embeliribes*)

Classification:

Division : Angiosperms

Class : Dicotyledenae

Sub class : Gamopettalae

Series : Heteromerae

Order : Myrsinales

Family : Mrysenaceae

Genus : *Embelia*

Species *: Ribes*

Vernacular Names:[24]

Tam - *Vaividangam*

Sans - *Vidanga*

Hind - *Wawrung*

Ben - *Biranga*

Punamj - *Babrung*

Guj - *Karkannie*

Tel - *Vellal*

Description

A shrub with climbing habit generally found in scrubs and shols in moist and study localities. It was odserved in Meghalaya that the plant was found growing in association with Myricanagi, Cinnamomum obtusiloba.

Part Used:

Fruit,

Leaves and

Root-bark.

Constituents

Alkaloid- Christembine

Quinone- embelineandvilangin

Miscellaneous compounds- Tannins, resins, volatile oil, quercitol.

Action

Fruits are carminative, anthelmintic, stimulant and alterative.

Pulp is purgative

Fresh juice is cooling, diuretic and laxative.

Properties and uses

It is an important indigenous drug used as an anthelminitic, alternative and tonic.It has been recommended for a number of diseases such as Headache, rhinitis, hemicranias, epilepsy, insomnia and in dyspepsia.

The fruit cures dental, oral and throat double except cancer of lips.Seeds shows antibiotic and anti TB and anthelmintic.Root used as antifertility drug.

MANJITTI (*Rubia cordifolia*)

Vernacular name: [25a]

Sans - *Manjista*

Eng - Indian Madder

Hind&Ben - *Manjit*

Tel -*Mandastic*

Tam - *Manjitti*

Classification:

Kingdom : Plantae

Order : Gontianales

Family : Rubiaceae

Genus : *Rubia*

Species *: cordifolia*

Description

Perennial, herbaceous, climbing.Roots very long, cylindric, flexuose,with a thin red bark.

Distribution

Throughout India in hilly districts, Ceylon, Malay peninsula, Japan, tropical Africa.

Constituents

Root contain resinous and extractivematter-gum,sugar,colouring matter and salt of line.

Glucoside- manjistin, garancin, alizarin and xanthine

Proporties and medicinal uses

The root powder is improvedthe voice and the complexion.

Cure kapha diseases and uterus, vagina diseases.

Increased the appetite

Cure leucoderma, ulcers, and urinary diseases.

The fruit cure diseases of the spleen.

KARPOKARISI (*Psoralea corylifolia*)

Vernacular names:[25b]

Sans - *Aindavi*

Hindi - *Bavanchi*

Ben - *Hakuch*

Punj - *Babchi*

Tel - *Kalaginja*

Tam - *Karpokaraishi*

Classification:

Kingdom : Plantae

Order : Fabales

Family : Fabaceae

Genus : *Psoralea*

Species *: p. corylifolia*

Distribution

Throughout India, Ceylon

Description

Macroscopic:[20c]

Fruits dark chocolate to almost black with pericarp adhering to the seed-cost 3 to 4.5mm.Long, 2 to 3mm.broad, ovoid-oblong or bean shaped, somewhat compressed, glabrous rounded or mucronate, closely pitted.Seeds camphylotropous, non-endospermous, oily and free from starch; odourless, but when chewed smell of a pungent essential oil felt; taste bitter, unpleasant and acrid.

Microscopic

Transverse section of fruit shows pericarp with prominent ridges and depressions, consisting of collapsed parenchyma and large secretory gland containing oleo-resinous matter; testa, an outer layer of palisade epidermis, layer of bearer cells which are much thickend in the inner tangential and basal radial walls and 2 or 3 layers of parenchyma;cotyledons of poiyhedral parenchyma and three layers of palisade calls on the adaxial side.

Constituents

Psoralen, psoralidin, isopsoralen, bakuchiol, corylin,β-sitosterol,linoleic.

Proporties and medicinal uses:

The root is useful in caries of the teeth**.**

The fruit cures leprosy, skin diseases, asthma, piles.

The seeds cure leucoderma,urinary discharges, heals ulcers, scabies.

3.2 LITERATURE REVIEW OF DISEASE

3.2.1 SIDDHA ASPECT OF THE DISEASE:

Siddha system of medicine deals cancer and its treatment widely. In ancient Siddha literature cancer is explained as in the name of putru which gives the direct meaning and as Arpudham and vanmeegam.For the purpose of diagnose and treatment following volumes contributes great ideas about Cancer.

Agathiya vaithihiya vallathi 600[(26)]

Pulipani 500[(27)]

Anuboga vaidhya navanetham

Agatthiya vaidhya gandam

Anuboga vaidhya brahma ragasiyam

YogiVaidhya sindhamani

The great siddha Agathiya in his vaidhya vallathi 600 had explained cancer and its different categories.

"போக்குமே திமிர்வாத மண்டைசூலை புற்றுடனே

கேட்குமே அரையாப்பு பவுத்திரத்தைக் கேள்

தண்டு சூலையொடு லிங்கப் புற்றே"

- அகத்தியர் வைத்திய வல்லாதி

"நாமப்பா கருங்கிரந்தி யோனிப்புற்று

ஆமப்பா கருவழிக்குங் கிரந்தி லிங்கப்புற்று"

- யூகி வைத்திய சிந்தாமணி

“இருபுடரி நுனி நாசி சிலந்தி புற்று

தடர்சிலந்தி படர்சிலந்தி அல்குல் புற்று

பின்கரப்பான் முங்கரப்பான் அண்டப் புற்று

துணிவாத உந்திப்புண் துடையில் புற்று

கீழ்நாக்கு மேல்நாக்குப் புற்றுப் போமே”

-புலிபாணி 500

The unique saint Pulipani also dealt with different type of cancer in his Pulipani 500.

“ஓமேனி குழிப்புற்று யோனிப்புற்று

ஒளிவான இடிப்புற்று கன்னப்புற்று”

In this medical system of life, the cancerous growth and tumors are headed as Arputha viranangal and Arputha katikal.

According to Yugi Mamunivar Vaidhya Sindhamani 800 I part, some kinds of cancer clarified under different systemic diseases.

Yugi classifications of disease are compared with Westernsystem of medicine by means of symptoms for quick and easy approach.[28]

For example,

Ukkara soolai is understand as prostatic cancer

Vil peruvayiru is known as Testicular cancer

Mamisa magotharam and Kal peruvayiru as cancerous growth within the abdomen.

To handle cancer effectively it is considered as Vippuruthi.[3b]

Appearance

Cancer of various classes looks like one or more following appearance such as Kazhalaikatti Spreading ulcer

Initially like warts then grows and develops as turtle shell with oozing

Hyper pigmentation of skin, affects hair follicles and destroys entire body

Classification

Cancer classified into 3 types under its spreading nature

- Skin and its structures
- Muscles
- Blood vessels and bones

Causes

- Vitamins and minerals deficiency
- Frequent sexual activities
- Prolonged starvation
- Excessive use of tobacco nicotiana
- Rich intake of hot and spices
- Taking excessive amount of salt and pungent.
- Taking large quantity of fish and meat.
- Making sleep in day time

Symptoms

Symptoms are varying depending on the particular type of cancer.

Treatment

Siddha medical system delivers huge line of treatments for different kinds of life threatening diseases including Cancer To kill and destroy the severity of cancer.

Siddha listed a lot of medicines. They include herbs, minerals and metals

YONIPUTRU

Yoni means birth passage. This is cervix of uterus. So the cancer of cervix is known as yoniputru. And also it is called Karuppai kazhuthu putru.

Siddha highlights of yoniputru as follows

- Small grain like growths in cervix
- Hardening of surface
- Burning sensation and irritation
- Honey like discharges
- Profuse bleeding
- If untreated within a year, this cancerous growth pushes rectum.
- Oliguria and anuria, Administration of diuretics causes haematuria
- In some patients discharges with intolerable foul smell.
- Bleeding while urination, intercourse and vaginal douche.

Cervical cancer discharges classified into 3 types

- Viscous yellowish discharge due to infections.
- Yellowish discharge with mucous due to irritation of cervical os and cervical not healing ulcers.

The clever Siddhar Yogi in his Vaidhya Sindhamani mentioned the symptoms of yoniputru in different ways as follows.

Kuruthi yoni

"திறமான வுபத்திரவ மதிகங் காணும்

தெளியாத ரத்தமுடன் சீழ்நீர்ப் பாய்ச்சல்

கறமான நுரையுடனே நோயுண் டாகும்

கடினமாஞ் சதையுடனே குத்தல் காணும்

நிறமான மஞ்சளுடன் கசரோ கந்தான்

நிலையாது வல்குலிலே புழுவோ மெத்த

மலமான சொல்லதுவு முளுத்தாற் போல

மஞ்ஞையா னிறம்போல் மசக்கும் பாரே."[(3c))]

Profuse bleeding with mucous, micro ulcers like pits on the wall of cervix, discoloration of os.

Kuruthicheezh yoni

"பாரேதான் வேதனை மிகவுண்டாகும்

பாங்கான சீழுடன் ரத்தங் காணும்

சீரேதான் ஒழுக்குடன் நாற்றமாகும்

சிதறியே பலபேத வண்ணங் காட்டும்

நேரேதா நிதம்பத்தின் ஸ்தனந் தன்னில்

நெடிதான ரோகத்தை மேவச் செய்யும்

வேரேதான் சொன்னபடி சிகிச்சா சாரம்

விரித்திட்டர் யூகிமுனி விளக்கந் தானே."[(3d)]

Bleeding with mucous sometimes in

Multicolor,

Bad odour discharge,

Spread to whole uterus.

Mamisa magotharam

“போக்கான மாமிசந்தான் வளர்ந்து மீறி

பொருமியே அடிவயிற்றில் கல்லைப் போலத்

தாக்கான சடந்தானு முலர்ந்து வற்றி

தவிக்குமே யடிக்கடிதான் கண்ணீர் தேடி

வாக்கான மதுரமொழி குளறிப் பேசி

வாய்வுதா னடிக்கடிக்கு மேலே நோக்கும்

நீங்கான மலசலமிதில் மாமிசங் காணும்

நேரான மாமிச மகோதரத்தி னேரே.”[31]

There are plenteous treatments available especially for yoniputru in Siddha medicine.

3.2.2 MODERN REVIEW

CANCER

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Other terms used are malignant tumors and neoplasms [30].

Normal cells in the body follow an orderly path of growth, division and death Programmed cell death is called apoptosis,[31] and when this process breaks down, cancer begins to form. Unlike regular cells, cancer cells do not expetience programmatic death and instead continue to grow and divide.

This leads to a mass of abnormal cells that grow out of control beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. This process is referred to as metastasis.Metastases are the major cause of death from cancer [32].

Further mutation occurs, selecting a subset of cell for more rapid growth which is mediated by increased growth factor production, constitutive activation of signaling pathways that stimulate cell division and failure of apoptosis.

Abnormal regulation of cell growth in cancer can occur as the result of several mechanisms.

They are,

- Activation of cell growth
- Inhibition of tumor suppressor genes
- Maintenannce of telomeres
- Angiogenesis
- Immune surveillance
- Invasion and metastasis
- Anatomical spread of tumor

Predisposing environmental factors

- Occupational exposure
- Dye and rubber manufacture unit, asbestos mining, construction work and petroleum industry.
- Cigarette smoking
- Viral infection- Epstein-Barr virus and HPV
- Bacterial infection-Helicobacter pylori
- Parasitic infection
- Radiation-UV

There are five broad groups that are used to classify cancer

1. Carcinomas-malignant tumors derived from epithelial cells common forms of breast, prostate, lung and colon cancer.
2. Sarcoma- malignant tumors derived from connective tissue, or messenchymal cells.
3. Lymphomas are cancers that begin in the lymph nodes and immune system tissues.
4. Leukemia are cancer that being in the bone marrow and often accumulate in the bloodstream.
5. Adenomas are cancers that arise in the thyroid, the pituitary gland, the adrenal gland, and other glandular tissues.

Investigation

Histology

- Light microscopy
- Immunohistochemistry
- Electron microscopy
- Cytogenetic analysis

Imaging

- Radiography
- Ultrasound
- Computed tomography
- Magnetic resonance imaging
- Position emission tomography

Biochemical markers

- Alphafet protein (AFP)
- Calcitonin
- Cancer antigen 125 (CA 125)
- Carcinoembryonic antigen (CAE)
- Human choeionicgonodotrophin (HCG)
- Placental alkaline phosphatase (PLAP)
- Prostate specific antigen (PSA)
- Thyroglobulin
- B-2-microglobulin

Present problems in oncology

- Weight loss and fever
- Palpable mass
- Finger clubbing
- Ectopic hormone production
- Neurological paraneoplastic syndromes

Emergency complications of cancer

- Spinal cord compression
- Superior vena cava obstruction
- Hypercalcaemia
- Neutropenia sepsis

Metastatic diseases

- Brain metastases
- Lung metastases
- Liver metastases
- Bone metastases
- Malignant pleural effusion

Therapeuties in oncology

- Palliative- to produce an improvement in quality of life
- Adjuvant- administered after surgery to increase the disease free and overall survival
- Neo adjuvant- chemotherapy, radiotherapy or hormonal treatment before surgery.
- Surgery
  - Biopsy
  - Excision
  - Palliation
- Systemic chemotherapy
- Radiation therapy
  - Teletherapy
  - Brachytherapy
  - IV radioisotope
- Hormone therapy
- Immunotherapy
- Biological therapy
  - Gefitinib/ erlotinib
  - Imatinib
  - Trastuzumab[33a]

CERVICAL CANCER

Cervical cancer occurs when abnormal cells on the cervix grow out of control. The incidence is decreasing in developed contries but continues to rise in developing nations. Cervical cancer is the leading cause of death from gynaecological cancer.The cervix is the lower part of the uterus that opens into the vagina [34].

Pathogenesis

There is strong association between Cervical Cancer and sexual activity that include sex at young age and multiple sex partners[35].

Infection with HPV is an important causal role and this has underpinned the introduction of programmes to immunize teenagers against HPV in an effort to prevent the later development of cervical cancer.

Causes

- Followed by smoking
- Immunodeficiency virus
- Weakened immune system
- Long term oral contraceptive use
- Human papillomavirus (HPV) probably is the cause of almost all cervical cancer worldwide HPV DNA is present in 93% of cervical cancer and its precursor lesions.

There are many types of the HPV virus.A all types of HPV will not cause cervical cancer. Some of them cause genital warts, but other types may not cause any symptoms.

Mucosal and genital HPVs are divided into

Low risk (HPVs 6,11,42,43,44) and

High risk (HPVs 16,18,31,33,35,45,51,52,56)[36].

Symptoms of cervical cancer:

Abnormal cervical cell changes rarely cause symptoms[37].

- Bleeding from the vagina that is not normal, such as bleeding between menstrual periods, after sex or after menopause.
- Pain in the lower belly or pelvis.
- Pain during matting.
- Rarely vaginal mass
- Very rarely leakage of urine or faeces from vagina.

Classification[38]

There are several types of cervical cancer, classified on the basis of where they develop in the cervix.

- Cancer that develops in the ectocervix is called squamous cell carcinoma, and around 80-90% of cervical cancer cases (more than 90% in India) are of this type[WHO/ICO Information Centre on HPV and Cervical cancer].
- Cancer that develops in the endocervix is called adenocarcinoma.
- In addition, a small percentage of cervical cancer cases are mixed versions of the above two, and are called adenosquamous carcinomas or mixed carcinomas.
- There are also some very rare types of cervical cancer, such as small cell carcinoma, neuroendocrine carcinoma etc.

Stages of Cervical Cancer

If the diagnostic tests indicate the presence of cancer, additional imaging (CT,MRI,etc.) may be performed to determine the location and extent of the disease.This is called staging and affects how the cancer will be treated.

There are five types of staging. They are classified as Stage 0, Stage I,Stage II, Stage III, Stage IV & Stage V.

Stage 0: The cancer is found only in the top layer of cells in the tissue that line the cervix. Stage 0 is also called carcinoma in situ.

StageI: The cancer has invaded the cervix beneath the top layer of cells. It is found only in the cervix

StageII: The cancer extends beyond the cervix into nearby tissuse. It extends to the upper part of the vagina. The cancer dose not invade the lower third of the vagina or the pelvic wall.

StageIII: The cancer extends to the lower part of the vagina. It also may have spread to the pelvic wall and nearby lymph nodes.

StageIV: The cancer has spread to the bladder, rectum, or other parts of the body.

Recurrent cancer: The cancer was treated, dut has returned after a period of time during which it could not be detected. The cancer may show up again in the cervix or in other parts of the body.

Screening methods

- The Pap test is the best way to find cervical cell charges that can lead to cervical cancer. Regular Pap test almost always shows these cell changes before they turn into cancer (1) The
- Regular Pap test, as we talked about above; and (2) the liquid- based Pap test, when the cells are placed in a special liquid first, before being looked at for abnormalities.
- Vinegar test- swab cervix with diluted vinegar then make abnormal cells briefly with colour changes.
- Dilation and curettage is also used diagnostically, with cystoscopy and rectosigmoidoscopy.
- MRI is often used to characterize the primary tumour.
- CT of the abdomen and pelvis is performed to look for metastasis in the liver and lymph nodes and to exclude hydronephrosis and hydroureter.

- A routine chest X-Ray should be obtained to help rule out pulmonary metastasis.

Prevention

The biggest problem is lack of primary prevention of cervical cancer. Should be Vaccinated from the age of 9 to 45 years.

Cervarix

Gardasi l(HPV 6, 11, 16, 18)[39]

Treatment:

The treatment for most stages of cervical cancer includes

Surgery such as a hysterectomy and removal of pelvic lymph nodes with or without removal of both ovaries and fallopian tubes.

Chemotherapy.

Radiation therapy.

Brachytherapy-ninternal radiation

Radial trachelectomy- cervix is removed with part of vagina.Here pregnancy is possible

Even with treatment, cervical cancer is leading cause of death in women[33b].

3.3 PHARMACEUTICAL REVIEW

Chendhooram

Concept and terminology:

Chendooram is category of medicines made from metals or minerals by grinding them with specified juices or distillates or extractives and subjecting them to

a process of sublimation or calcination of burning or frying or exposing to insolation till the characteristic reddening of the product takes place.

Method of preparation:

Usually two method of preparation are adopted in their processing through there are some exceptions and variants.

1. Sublimation by the sand – bath process

2. Calcination.

1. Sublimation by the sand - bath process *(KuppiErippu)*:

In the conventional set up of the sand –bath sublimation contrivance, a heat resistant glass flask with a long neck is used as the container for the drug ingredients. Ceramic ware had also been in use. Before being put to use, these container are wound around with clay Smeared cloth ribbons so as to give seven superimposed layers, leaving open the mortar of the flask. The flask thus encased should be kept for perfect drying of the covering.

It has been found in recent times that one could make use of the enameled iron bowls instead of glass flasks.

When using enameled iron bowls, two identical bowls of appropriate dimensions and capacity should be selected and checked for neat contact of rims when juxtaposed. Then small holes should be punched along the margins so that the two bowls could be fastened with a bonding wire (metallic). Then a perforation is made in the centre of the bottom of one of the bowls. Having prepared the bowls thus, they should be secured and bound by pasting the binding wire through the marginal holes. This would produce a capsule with a top orifice.

Clay smeared cloth tape is wound around as would be done for the glass flask, leaving the central opening uncovered. This opening is the one through which the reaction going on inside is inspected by inserting a probe.

The sand – bath is set up by taking a wide earthern trough and spreading fine gravel or coarse sand at the bottom to a depth of two centimeters.

The capsule into which the drug ingredients are put is placed on the gravel or sand and is properly centered. Then the sides packed with sands, leaving the top two centimeters unpacked and exposing the capsule. When using glass flasks, the neck should be just out of the sand. This setup is placed on the oven and heat is applied, by burning fire wood.

In the application of heat, there gradations are recognized. These three stages, mild, moderate and intense are best understood and mastered with some experience.

It is said that, if the flames are convergent and resemble a single tongue of flame as in a lamp, it is mild fire (*Deepakkini*). If several such tongues of flame lick the vessel and diverge like the flower of lotus, it is moderate (*Kamalakkini*). If the multiple tongues of flame fill the oven and enrich the sand bath. It is the intense stage of fire (*Katakkini*).

These stages of fire should be manipulated and followed as prescribed in the method of preparation. In general, the heating is spread over three continuous days. In such cases, mild, moderate and intense stages are maintained for 24 hours each, in that order of succession.

When the setup has cooled down, the capsule containing the medicine is taken out and the clay tape winding cut out. The material that has sublimed in upper bowl is gently tapped with suitable beater or lifted with a spatula. The sublimate collected should be finely ground in a mortar.

If the glass flasks had been used, the flask is carefully broken, open to collect the medicine that has sublimed in around the neck.

2. Calcination *(Pudam)*: (Thaniyapudam)

The powder is ground in a *Kalvam*with specified fluids for a specified time. The paste is made into small discs and dried. They are put in earthern saucers (*man agal*) covered with another and the edge well sealed with mud cloth. It is allowed to

dry. For *Putams,* generally pits of various depths and circumferences are made in the ground.

All the metals and other ingredients are taken after the usual purification. In specified cases, specific purification (*Suddhi*) is mentioned; otherwise, it is to be taken as general method of purification for the drug as mentioned in Materia- Medica books.The medicine is prepared and pellets were placed in the Earthern crucible and closed with other Earthern crucible, then it was sealed with 2 mud pasted plaster and kept in thaniyapudam for specific number of days.

Other method of preparations:

1. Prepared without heating *(Araippu Chendooram)*

2. Prepared by open heating *(Erippu or Varuppu Chendooram)*

3. Prepared by applying heat in the range close to 100^{o}C *(LaguPuda Chendooram).*

SHELF LIFE

The chendooram are said to retain their potency for 75 years

PRESERVATION AND STORAGE

To be stored in a clean, dry and air tight glass containers.

SPECIAL EXPERXIMENT

In a glass bowel contains water, sprinkle pinch of Chendooram and place a whole black gram on it.

If Chendooram bear the entire weight of grain and shouldn't sink into the water, its preparation method was perfect [(40)].

Tab. No: 1 ANALYTICAL SPECIFICATIONS OF SINDURA/ CHENDOORAM (CALX) [41]

S. NO	TEST
1	Description Colour Odour
2	Identification – chemical
3	Particle size – 200 to 400
4	Loss on drying 105^0 C
5	Total ash
6	Acid – insoluble ash
7	Water soluble ash
8	Assay of element
9	Ayurvedic specification
10	Lusterless
11	Fine enough to enter the crevices of finger (Rekhapurnatva)
12	Floats on water (Varhara)
13	Smokeless (Nirdhoora)
14	Tasteless (Niswadu)
15	Irreversible (Apunanbhav)

3.4 PHARMACOLOGICAL REVIEW

SIDDHA ASPECT

Siddha medicen system gave us so many drugs for different type of cancer.Limited medicines for cervical cancer only listed her,

Pills

Chithramoolakuligai[42a]

Chooranam

Karanthi chooranam[43]

Parpam

Thambiraparpam[44a]

Rasa parpam[45]

Gandhagaparpam[46]

Karuvangaparpam

Chendhooram

Pancha padanachendhooram[47]

Swarnapushpa rasa chendhooram[48]

Muthuchendhooram[49]

Nei

Chitramoolanei[44b]

Ennai

Perungayaennai[50]

Gandhagathylam[42b]

Mezhugu

Rasagandhimezhugu[51]

Gandhagamezhugu[52]

Gorosanaimezhugu

Kattu

Poorakattu[53]

Pathangm

Linga pathangam[54]

ANTICANCER DRUGS- MODERN ASPECT

The drugs which prevent neoplasm areknow as anticancer drugs. They also called antineoplastic drugs

They may be divided into two classes

- Cycle specific

Cycle specific drugs act only at specificpoints of the cell's duplication cycle, such as anaphase or metaphase

- Non- cycle specific

Drugs may act any point in the cellcycle, In order to gain maximum effect, antineoplastic drugs are commonly used in combinations.

Classification[55]

1. Alkylating agent
2. Antimetabolites

3. Natural origin
   From plants
   From micro organisms
4. Hormones
5. Miscellaneous

Precautions

Because antineoplastic agents do not target specific cell type, they have a number of common adverse side effects.

- Hair loss is common due to the effects on hair follicles
- Anemia
- Immune system impairment
- Clotting problem are caused by destruction of the blood forming organs, leading to reduction in the number of red cells, white cells and platelets.

Interactions

Anticancer drugs may interact with a number of other medicines. When this happens, the effects of one or both of the drugs may change or the risk of side effects may be greater.

Table: No: 2 Anticancer Drugs[56]

Generic (Brand Name)	**Clinical Uses**	**Common Side Effects To Drug**
Altretamine (Hexalen)	Treatment of advanced ovarian Cancer	Bone marrow depression, nausea and vomiting
Asparaginase (Elspar)	Commonly used in combination with other drugs; refractory acute lymphocytic leukemia	Liver, kidney, pancrease,CNS abnormalities,

Bleomycin (Blenoxane)	Lymphomas, Hodgkin's disease, testicular cancer	Hair loss, stomatitis, pulmonary toxicity, hyperpigmentation of skin
Busulfan (Myleran)	Chronic granulocytic leukemia	Bone marrow depression, pulmonary toxicity
Carboplatin (Paraplatin)	Palliation of ovarian cancer	Bone marrow depression, nausea and vomiting
Carmustine	Hodgkin's disease,brain tumors, multiplemyeloma,malignant melanoma	Bone marrow depression,nausea and vomiting
Chlorambucil (Leukeran)	Chronic lymphocytic leukemia, non-Hodgkin's lymphomas, Breast and ovarian cancer	Bone marrow depression, excess uric acid in blood
Cisplatin (Platinol)	Treatment of bladder,ovarian, uterine, testicular, head and neck cancers	Renal toxicity and ototoxicity
Cladribine (Leustatin)	Hairy cell leukemia	Bone marrow depression, nausea and vomiting, fever
Cyclophosphamide (Cytoxan)	Hodgkin's disease,non-Hodgkin's lymphomas,neuroblastoma.Often used with other drugs for breast, ovarian, and lung cancer;acute lymphoblastic leukemia in children;multiple myeloma	Bone marrow depression, hair loss,nausea and vomiting, inflammation of the bladder.
Cytarabine (Cytosar- U)	Leukemias occurring in adult and children	Bone marrow depression, nausea and vomiting, diarrhea,

		stomatitis
Dacarbazine (DTIC-Dome)	Hodgkin's disease, malignant melanoma	Bone marrow depression, nausea and vomiting
Diethylstilbestrol (DES) (Stilbestrol)	Breast cancer in post-menopausal women, prostate cancer	Hair loss, nausea and vomiting, edema, excess calcium in blood; feminizing effects in men
Ethinyl estradiol (Estinyl)	Advanced breast cancer in post-menopausal women, prostate cancer	Excess calcium in blood, anorexia, edema, nausea and vomiting; feminizing effects in men
Etoposide (VePesid)	Acute leukemias, lymphomas, testicular cancer	Bone marrow depression, nausea and vomiting, hair loss
Mitomycin (Mutamycin)	Bladder,breast,colon, lung, pancreas, rectum cancer,head and neck cancer, malignant melanoma	Bone marrow depression, nausea and vomiting, diarrhea, stomatitis, possible tissue damage
Mitotane (Lysodren)	Cancer of the adrenal cortex (inoperable)	Damage to adrenal cortex, nausea, anorexia
Mitoxantrone (Novantrone)	Acute nonlymphocytic leukemia	Cardiac arrhythmias, labored breathing, nausea and vomiting,diarrhea, fevar,anorexia

Paclitaxel (Taxol)	Advanced ovarian cancer	Bone marrow depression, hair loss, nausea and vomiting, hypotension, allergic reactions, slow heart action, muscle and joint pain
Pentastatin (Nipent)	Hairy cell leukemia unresponsive to alpha-interferon	Bone marrow depression, fever, skin rash, liver damage, nausea and vomiting
Pipobroman (Vercyte)	Chronic granulocytic leukemia	Bone marrow depression
Plicamycin (Mithracin)	Testucular tumors	Toxicity/damage to bone marrow, kidneys, and liver
Prednisone (Meticorten)	Used in adjunt therapy palliation of symptoms in lymphomas, acute leukemia Hodgkin's disease	May be toxic to all body systems
Procarbazine (Matulane)	K2 Hodgkin's disease	Bone marrow depression, nausea and vomiting
Streptozocin (Zanosar)	Islet cell carcinoma of pancreas	Nausea and vomiting, toxicity to kidneys

Tamoxifen (Nolvadex)	Advanced breast cancer in post-menopausal	Nausea and vomiting, ocular toxicity, hot flashes
Teniposide (Vumon)	Acute lymphocytic leukemia in children	Bone marrow depression, nausea and vomiting, hair loss.
Vinblastine (Velban)	Breast cancer,Hodgkin's disease, metastatic testicular cancer	Bone marrow depression, neurotoxicity

PHARMACOLOGICAL REVIEW OF CANCER:

The pharmacological screening of plants, minerals and animals is an essential mean for the invention of new, harmless and effective drugs. Over 50,000 plants have therapeutic virtues in the world, and around 80% of human use medicines based on plants and salts at least once in their life,Medicinal plants and mineral shave diversified chemical constituents which are important for the discovery of new active molecules against many types of cancer. Active compounds from many medicinal plants and minerals with effective cytotoxic properties were developed in to anti-cancer drugs.

Nowadays it has become mandatory to monitor the quality of life of patients while in treatment of cancer. It is healthy aware that the quality of life of cancer patients treated with chemotherapeutic drugs are very much affected even long time after withdrawal of drugs. Therefore, the challenging task at this moment is to identify the quick and novel methods that can identify and develop molecules, which can be of therapeutic value in human cancers. This urgently necessitates screening of a large number of compounds. For this purpose both, the *in vitro* and *in vitro* models are employed for systematic screening of an anticancer drug.

INVITRO METHODS:

In studies in vitro cytotoxicity on cell line, various cell staining methods are used in order to indirectly estimate the number of viable cells present after treatment. An ideal test in assessing cell proliferation and cytotoxicity should have as main feature in vitro: be simple, fast, efficient, economical, reproducible, sensitive, safe, effective as far viable cell population and do not show interference with to evaluate the compound.

ADVANTAGES:[57]

- Reduce the usage of animals
- Testing the ability of the compound to kill the cells by taking the advantage of various properties of cell
- Able to process the large number of compounds quickly with minimum of quantity.
- Range of concentrations used is comparable to that expected for in vivo studies.

DISADVANTAGES

- Difficulty in maintaining of culture
- Show negative results for the compounds which gets activated after metabolism and vice versa
- Impossible to ascertain the Pharmacokinetics.

How to culture cell line

- Tumor cell line derived from several cancer types.
- Adaptable to a suitable growth medium.
- Show reproducible profile for growth and drug sensitivity.
- The lines were prepared and preserved using regents such as DMSO during freezing.
- Thawing- bringing the freezed ampoule to room temperature by slow agitation.

Cell lines for cancer

There are plenty of cell lines are available for research purpose.Only very few are listed.[58]

Table no: 3 Cancer cell lines

S.NO	Cell name	Tissue	Species
1	UM-UC	Bladder	Human
2	FM3A	Breast	Mouse
3	C 170	Colon	Human
4	SHP 77	Lung	Human
5	RAG	Kidney	Mouse
6	HF 1	Liver	Rat
7	MEWO	Skin	Human
8	TT	Thyroid	Human
9	OV	Ovary	Human
10	C 6	Nueral(Glial tumor)	Rat

ASSAY[59]

For energy metabolism and autophagy

- FAD assay
- ATP assay
- Lysosome detection
- Mitochondrial membrane potential assay
- Reactive oxygen species test

For nuclear signaling, DNA damage and cell proliferation

- P^{53}assay
- Topoisomerase II assay

- P^{21}assay
- Cell proliferation assay
- Mdm2 assay

For inflammation, angiogenesis and metastasis

- Cytokine and chemokine assay
- STAT 1,2,3,6 assay
- COX-2 activity assay
- LDL uptake assay

For apoptosis, pyroptosis and necrosis

- Caspase 1 assay
- Bax assay
- Cytolysis assay

For cancer signaling pathway and phenotype

- ERK assay
- c- AMP assay
- c- Jun test

IN VIVO MODELS:

Many animal species develop cancers spontaneously and are valuable for understanding the biology of sporadic cancer development in humans. The major use of spontaneous cancer models is to compare the biology with human, in these animals are increasingly valuable for cross- comparison of response or resistance to clinical agents used for patients[(60)]

Animal models

I. Mouse cancer models
   a. GEM – Genetically Engineered mouse Models
   b. Inbred mice (systematic sibling mating)
   c. Transplantation models

- Allograft models (syngeneic tumor tissuse derived from same genetic mouse)
- Xenograft models (actual human cancer cells or solid tumors are transplanted into host mouse)

d. Carcinogen induced and spontaneous models

- Digestive system cancer induced by polycyclic aromatic hydrocarbons
- Chemically cancer induced by Cadmium an Arsenic.
- Radiation-skin cancer by ultraviolet radiation;leukemic changes by ionizing radiation.

II. Rat cancer models

a. Genetically altered rats

i) Treat embryos with DNA damage causing chemical mutagen.Frequently N-ethyl-N-nitrosourea(ENU) is used.

ii) Insertion of mutagenesis strategies (Retro viruses)

iii) Transgenic strategies (pronuclear injection of DNA)-quickly developed and more effective models

b. Inbred rats.

III. Other laboratory animal models

a. Hamster
b. Rabbits
c. Zebrafish

IV. Other animal models

a. Dogs
b. Cats
c. Goats

d. Horses
e. Pigs

There is also work done with various species, such as baboons, chimpanzees, macaques, marmosets and tamarins.

Cervical cancer cell lines

- HeLa (HPV 16)
- SIHA (HPV 18)
- C 33A (HPV Negative)
- Ca Ski

Induction of cervical cancer in animal models:

Cervical neoplasia is induced in mouse by an extract of varicella zoster virus infected cells (HPV or Herpes simplex virus type 2 DNA)

Genomic HSV 2 DNA was isolated from infected HE p2 cells and separated from host cell DNA by cesium chloride density gradient centrifugation. The DNA was applied to mouse cervix for period of 80 to 100 weeks. Should examine monthly to detect abnormalities.

4. MATERIALS AND METHODS

4.1 PREPARATION OF THE DRUG:

KANDHA RASA VILLAI

SELECTION OF THE DRUG:

For this thesis, the Herbo-metallic formulation *Kandha rasa villai* was taken as the compound drug preparation for Cervical Cancer were taken from the customary Siddha literature VEERAMAAMUNIVAR VAAKADATHTHIRATTU, Written by S.P.Ramachandiran, Page no:102.

INGREDIENTS:

Mineral and Metal ingredients:

Rasam(Mercury)	: 30 gm
Saathilingam(Cinnabar)	: 30 gm
*Vellai pashanam(*White arsenic*)*	: 20 gm
*Soodan(*Camphor)	: 20 gm
Kaantham(Magnetic oxide of Iron)	: 30 gm

Combined Plant Materials:

Thippili(Piper longum)	: 30 gm
Chukku(Zingiber officinale)	: 30 gm
Manjitti(Rubia cordifolia)	: 30 gm
Karpokarisi(Psoralea corylifolia)	: 30 gm
Vaivilangam(Embelia ribes)	: 30 gm
Valmilagu(Piper cubeba)	: 30 gm
Water	:1/2 Litre

COLLECTION OF THE DRUG:

The herbal ingredients, *Thippili (Piper longum), Chukku (Zingiber officinale), Manjitti* (*Rubia cordifolia*), *Karpokarisi* (*Psoralea corylifolia*),*Vaivilangam* (*Embelia ribes*),*Valmilagu* (*Piper cubeba*) were purchased from Tampcol Raw drug store, Anna Hospital Campus, Arumbakkam, Chennai.

The metal and mineral ingredient Rasam (Mercury), *Saathilingam*(Cinnabar), *Vellai pashanam (*White arsenic*), Soodan (*Camphor*), Kaantham*(Magnetic oxide of Iron)were purchased from Gopalasan raw drug stores, Nagarkoil, Tamailnadu.

IDENTIFICATION AND AUTHENTICATION OF DRUG:

The Herbal, metal and mineral were identified and authenticated by botanist andsiddha experts, PG Department of Gunapadam, Government Siddha Medical College, Arumbakkam,Chennai. A specimen sample of each raw materials hane been kept in the department for future reference.

PURIFICATION PROCESS:

Done as per various siddha classical literature

Purification of Rasam (Mercury):

Materials Required:

Mercury	- 30gm
Brick powder	- 100gm
Turmeric powder	- 100gm
Indian Acalypha juice(Acalypha indica)	-1.3litre

Procedure:

Mercury was triturated with brick powder and turmeric powder for one hour respectively and washed with water. Then the mercury is boiled with the juice of Indian Acalypha.

Purification of Saathilingam(Cinnabar):

Materials Required:

Cinnabar - 30gm

Lime juice - 100 ml

Cow's milk - 100 ml

Indian Acalypha juice - 100 ml

Procedure : Equal proportion of lime juice, cow's milk, Acalyphajuice are mixed with each other. Cinnabar was processed with above juice and cow's milk to get purified form.

Purification of Vellai pashanam(White arsenic):

Materials required:

White arsenic - 20gm

Lime stone water - 100 ml

Bitter guard fruit - 1

Procedure : In a pot,containing lime stone dissolved water,Arsenic is placed as a kizhi and boild for 2 hours.It is then place'd inside the bitter guard fruit(Kombupagal)and sealed with 2 clay smeared ribbon and cooked well with the rice husk pudam.

Purification of Kantham(Magnetic oxide of iron)

Materials required:

Kantham - 30gm

Horse gram tecoction - 100 ml

Procedure

In the boiling horse gram decoction,kantham is dipped and taken this process is repeated for 5 times.

Purification of Soodan(Camphor):

Materials required:

Camphor - 20gm

Water lilly juice - 100 ml

Procedure : It is soaked in water lilly juice for 24 mints and placed in the sunlight for purification.

Purification of raw drugs

Thippili(*Piper longum*): Soaked in lemon juice and it was dried.

Chukku(*Zingiber officinale*):Skin of dried ginger was peeled off.

Manjitti(*Rubia cordifolia*):Dust and odd materials were removed and slightly fried.

Karpokarisi(*Psoralea corylifolia*):Dust and odd materials were removed and slightly fried.

Vaivilangam*(Embelia ribes*):Dust and odd materials were removed and slightly fried.

Valmilagu*(Piper cubeba*) :Dust and odd materials were removed and slightly fried.

Preparation for *KANDHA RASA VILLAI:*

Purified Mercury	-20gm
Purified Cinnabar	-20gm
Purified White arsenic	-10gm
Purified Camphor	-10gm
Purified Magnetic oxide of Iron	-20gm

Plant materials:

Purified Thippili*(piper longum*)	-20gm
Purified Chukku(*Zingiber officinale*)	-20gm
Purified Karpokarisi (*Psoralea corylifolia*)	-20gm
Purified Vaivilangum(*Embelia ribes*)	-20gm
Purified Valmilagu(*Piper cubeba)*	*-20gm*
Water	-1/2 Litre

Procedure:

- Mercury and white arsenic were mixed thoroughly and grounded,at the end of this process the Hydragyrum changed into powder state.
- The other drugs were made into fine powder.
- All the powders were mixed along with the medicine which was placed in the stone mortar and grinded for 1 hour, by adding small quantity of water the medicine was grounded well for 12 hours.
- The above prepared medicine was made into pellets of 13/4 varaganedai.

- The pellets were placed in the Earthern crucible and closed with other Earthern crucible, then it was sealed with 2 mud pasted plaster and kept in thaniyapudam for 45 days.
- After 45 days, the pellets were taken from the Earthern crucible and made into powder. Labelled as Kantha Rasa Villai (KRV)

Storage **:** The drug was preserved in a clean,air tight glass container.

Dosage **:** *Kundrimanialavu(130mg)*

Form of Medicine **:** Chendooram

Route **:** Enteral

Time of Administration : Two times a day

Adjuvant **:** Honey,Ghee.

Indications **:** Linga putru,Yoni putru,Kanna putru,Kabala kirandhi.

INGREDIENTS

Fig:1.1 Vellai pashanam (White arsenic)

Fig:1.2.Saathilingam(Cinnabar)

Fig: 1.3 Rasam (Mercury)

Fig:1.4Kaantham(Magnetic oxide of Iron)

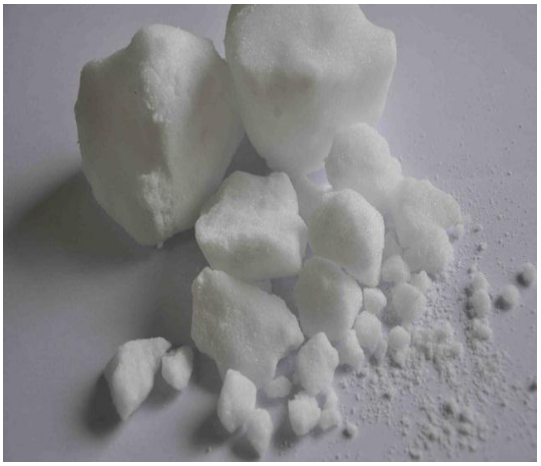


Fig: 1.5 Soodan (Camphor)

Fig: 1.6 Karpokarisi (*Psoralea corylifolia*)

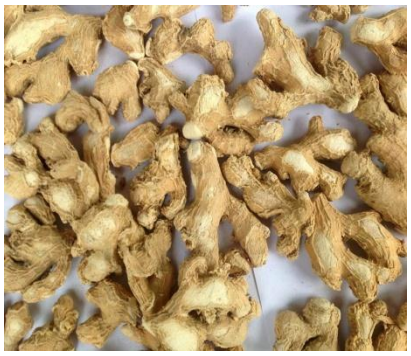


Fig: 1.7 Chukku (*Zingiber officinale*)

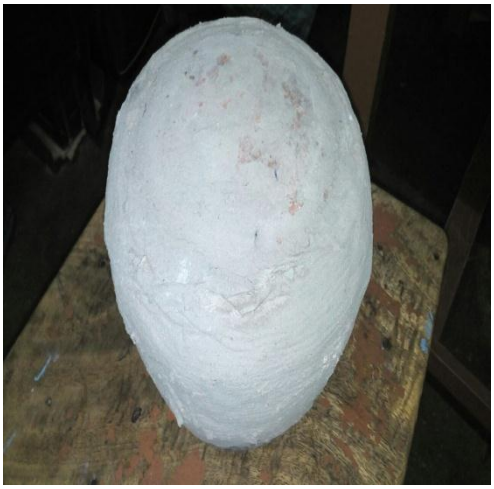
Fig: 1.8 Vaivilangam (*Embelia ribes*)

Fig: 1.9 Thippili (*Piper longum*)

Fig: 1.10 Valmilagu (*Piper cubeba*)

Fig: 1.11 Manjitti (*Rubia cordifolia*)

pellets



sealed with 2 mud pasted plaster

Fig: 2 KANDHA RASA VILLAI PREPERATION

:

Fig: 2.1 END PRODUCT

4.2 STANDARDIZATION OF THE DRUGKRV*:*

Standardization of drugs helps to prove its identity and determination of its quality and potency. Standardization of the Herbo- metallic formulation was based on the qualitative and quantitative analysis through physico-chemical investigations and instrumental analysis. The physico-chemical analysis of the prepared Herbo- metallic drug have been done at Central Research Institute, Arumbakkam, Chennai and elemental analysis have been done at IIT, Chennai (FTIR,SEM,ICP-OES,XRD).

4.2.1Analysis as per classical Siddha literature:

1.Floating on Water:

A pinch of KRV gently placed on the still surface of water in a vessel, did not sink immediately. It was found that the *Chendooram* particles floated over the surface of water indicated lightness of the trial drug.

2.Lines on fingers:

Chendooram in well prepared form should be fine. When taken between thumb and index finger, the fine powder will fill up the lines of the finger print. A pinch of *KRV* was taken in between the thumb and index finger and rubbed. It was found that the *KRV* entered into the lines of the finger, and was not easily washed out from the lines, confirmed its fineness.

3. Irreversible reaction:

The well prepared *chendooram* does not reversible to its metallic state when heated with a mixture of cane jaggery, hemp powder, ghee and honey. A pinch of *KRV* was taken and mixed with cane jiggery, ghee and honey. It was observed that the *KRV* did not reversible to its metallic state.

4. Tasteless:

The well prepared *Chendooram* should be completely tasteless.Presence of any tastelike sweet or bitter indicate incomplete preparation which needed anothercalcination process. When a small amount of *KRV* was kept on the tip of the tongue, no specific taste was found

.

5. Lusterless:

If any shining particles present in *Chendooram*, it indicates that the *chendooram*was not manufactured properly and contains unchanged substances like minerals, metals and other toxic substances. There should be no shining particles present in the wellmanufactured *Chendooram*. The *KRV* was taken in a Petri bowl and observed for any lustre in daylight through magnifying glass. No lustre was observed in the *Chendooram*.

4.2.2Physico-Chemical Investigations:

Physico-chemical investigations like pH value, Loss on drying at 105°C, Action on heat, Flame test and Ash test have been done at Central Research Institute,Arumbakkam,Chennai as per the guide lines of AYUSH.

pH value:

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

Loss on Drying:

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

Action on heat:

A small amount of the KRV was taken in a dry test tube and heated gently. If strong white fumes evolve indicate the presence of Carbonate.

Flame test:

A small amount of KRV was made into a paste with con.Hcl in a watch glass and introduced into non-luminous part of the Bunsen flame. Appearance of bluish green flame indicates the presence of Copper.

Ash Test:

A filter paper was soaked into a mixture of KRV and cobalt nitrate solution and introduced into the Bunsen flame and ignited. Appearance of yellow colour flame indicates the presence of Sodium.

4.2.3PHYTOCHEMICAL SCREENING

The Phytochemical screening of the extract gives general idea regarding the nature of chemical constituents present in the crude drug. The phytochemical tests were done as the method illustrated.[61]

Test for Alkaloids:

A small portion of solvent free KRV extracts were stirred separately with adding few drops of dilute hydrochloric acid and filtered carefully& tested with various alkaloidal reagents.

Mayer's reagent - Cream precipitate colour

Test for Carbohydrates and Reducing Sugars

The little amount of KRV extracts were dissolved in 5ml of distilled water & filtered. The filtrate was subjected to conduct the test for carbohydrates & glycosides.

a) Molisch's test: The KRV filtrate 1 ml was treated with 2-3 drops of 1% alcoholic alpha naphthol solutionand 2ml concentrated sulphuric acid was added along the sides of test tube slowly. Presence or absence of violet ring was observed at the junction of two layers.

Test for Glycosides:

The KRV extract was hydrolyzed with dil. HCl and subjected to test for glycosides.

a) Modified Borntrager's test: To the hydrolysateKRV extract of the sample mixed with 1 ml of Ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volume of benzene. The benzene layer of the sample was separated and treated with ammonia solution. Formation of rose pink colour in the ammonical layer was noted.

Test for Saponins

The extract of KRV was taken into 0.5 ml and it was shaken well with 5 ml distilled water. The presence of formation of copious lather was noted.

Test for Phenolic compounds

To 0.5 ml of KRV extract, 1 ml of alcoholic ferric chloride solution was added. Formation of bluish green or bluish black was noted.

Test for Phytosterol:

Ferric chloride – acetic acid test:1 ml of KRV extract was treated with 1 ml of chloroform and then, 2 ml of ferric chloride acetic acid reagent was added followed by 1 ml of conc. sulphuric acid. Appearance of reddish pink colour presence or absence recorded.

Test for Triterpenes

Salkowski's test: 1 ml of KRV extract was titrated with 1 ml of chloroform followed by 1 ml of conc.sulphuric acid, shaken slowly and allowed to stand. Appearance of golden yellow colour noted and recorded in results column.

Test for Flavonoids:

a) Alkaline reagent test: To 1 ml KRV of extract, 1 ml of 10% sodium hydroxide solution was added. Formation of dark yellow colour was noted.

Test for Proteins and Free Amino Acids:

a) Xanthoproteic test: To 1 ml of KRV extract, 3-4 drops of conc. nitric acid was added. Formation of yellow precipitate recorded.

Test for Quinones

Sodium hydroxide test: To 0.5 ml of KRV extract, 1 ml of 10% sodium hydroxide was added. Appearance of blue or green or red colour recorded in results.

4.2.4BIO-CHEMICAL ANALYSIS

The bio-chemical analysis was done to identify the acid and basic radicals present in the KRV.

Preparation of extract

5g of KRV was taken in a 250 ml clean beaker and 50 ml of distilled water was added, boiled well and allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water.

Preliminary Basic and Acidic radical studies

Test for basic radicals

1. Test for Potassium

To a pinch of the KRV 2 ml of sodium nitrate and 2 ml of cobalt nitrate solution in 30% glacial acetic acid was added and observed for the presence of yellow precipitate.

2. Test for Calcium

To 2 ml of KRV extract, 2 ml of 4% ammonium oxide solution was added and observed for the formation of white precipitate**.**

3. Test for Magnesium:

To 2ml of KRV extract, drops of sodium hydroxide solution was added and watched for the appearance of white precipitate.

4. Test for Ammonium:

To 2ml of KRV extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added for theappearance of brown colour.

5. Test for Sodium

Hydrochloric acid was added with a pinch of the KRV, made as paste and introduced into the blue flame of Bunsen burnerand observed for the appearance of intense yellow colour.

6. **Test for Iron (Ferrous)**

The KRV extract was treated with Conc. HNO_3 and ammonium thiocyanate and waited for the appearance of blood red colour**.**

7. Test for Zinc

To 2 ml of the KRV extract drops of sodium hydroxide solution was added and observed for white precipitate formation.

8. Test for Aluminium

To the 2m1 of the KRV extract sodium hydroxide was added in drops and changes are noted.

9. Test for Lead

To 2 ml of KRV extract 2ml of potassium iodide solution was added and noted for yellow coloured precipitate**.**

10. Test for Copper

a. A pinch of KRV was made into a paste with con. Hcl in a watch glass and introduced into the non-luminous part of the flame and noted for blue colour appearance.

b. To 2 ml of KRV extract excess of ammonia solution was added and observed for the appearance of blue coloured precipitate**.**

11. Test for Mercury

To 2m1 of the KRV extract sodium hydroxide solution was added and noted for yellow precipitate formation**.**

12. Test for Arsenic

To 2 ml of the KRV extract 2ml of sodium hydroxide solution was added and brown or redprecipitate formation was noted.

Test for acid radicals

1. Test for Sulphate

To 2 ml of the KRV extract 5% of barium chloride solution was added and observed for the appearance of white precipitate.

2. Test for Chloride

The KRVextract was treated with silver nitrate solution and observed for the appearance of white precipitate.

3. Test for Phosphate

The KRVextract was treated with ammonium molybdate and conc. HNO_3 and observed for the appearance of yellow precipitate.

4. **Test for Carbonate**

The KRVextract was treated with conc.Hcl and observed forth appearance of effervescence.

5. Test for Fluoride & Oxalate:

To 2ml of KRV extract 2ml of di.acetic acid and 2ml calcium chloride solution was added and heated and watched for cloudy appearance.

6. Test for Nitrate:

To 1 gm of the KRV, copper turnings was added and again conc.H_2SO_4 was added, heated and the test tube was tilted vertically down and observed for any changes.

TLC/ HPTLC finger print studies

HPTLC finger printing was carried out as per the reference.[62]

Preparation of spray reagent-vanillin-sulphuricacid reagent

Vanillin (1g) was dissolved in ice cold ethanol (95ml). Add to 5ml of cooled concentrated sulphuricacid.Ice was added and stirred well. The solution was stored in refrigerator.

Chromatographic conditions

Instrument : CAMAG (Switzerland).

Sample Applicator : CamagLinomat - IV applicator with N_2 gas flow.

Photo documentation System : Digi store - 2 documentation system with Win Cat & video scan software.

Scanner : Camag HPTLC scanner - 3 (030618), Win Cats - IV.

Development Chamber : Camag HPTLC 10X10, 10 X 20 twin trough linear Development chamber.

Quantity applied : 5, 10 µl for extracts and 5 µl for standards

Stationary phase : Aluminium plate pre-coated with silica gel 60(E. Merck)

Plate thickness : 0.2 mm.

Mobile Phase : For Chloroform extract - Toluene: Ethyl acetate (9:1) and ethanol extract - Toluene: Ethyl acetate (1:1).

Scanning wavelength : 254 nm

Laboratory condition : 26 ± 5^{o}C and 53 % relative humidity

The plate was developed up to a height of 8 cm, air dried, spots were observed under the UV light at 254 and 366 nm. Finally the plates were derivatized using vanillin-sulphuric acid reagent heated at 105° till colour spots appeared.

4.2.5AVAILABILITY OF BACTERIAL LOAD: [63]

Enumeration of bacteria by plate count – agar plating technique

The plate count technique is one of the most routinely used procedure because of the enumeration of viable cells by this method.

Principle:

This method is based on the principle that when material containing bacteria is cultured, every viable bacterium develops into a visible colony on a nutrient agar medium. The number of colonies, therefore are the same as the number of organisms contained in the sample.

Dilution:

A small measured volume are mixed with a large volume of sterile water or saline called the diluent or dilution blank. Dilution are usually made in multiples of ten. A single dilution is calculated as follows:

$$\text{Dilution} = \frac{\text{Volume of the sample}}{\text{Total volume of the sample and the diluent}}$$

Requirements:

- Sample or Bacterial suspension
- K9 ml dilution blanks (7)
- Sterile petri dishes (12)
- Sterile 1 ml pipettes(7)
- Nutrient agar medium (200 ml)
- Colony counter

Procedure:

1. Label the dilution blanks as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}.
2. Prepare the initial dilution by adding 1 ml of the sample into a 9 ml dilution blank labelled 10^{-1} thus diluting the original sample 10 times.
3. Mix the contents by rolling the tube back and forth between hands to obtain uniform distribution of organisms.

4. From the first dilution transfer 1 ml of the suspension while in motion, to the dilution blank 10^{-2} with a sterile and fresh 1 ml pipette diluting the original specimen to 100 times.
5. From the 10^{-2} suspension, transfer 1 ml of suspension to 10^{-3} dilution blank with a fresh sterile pipette, thus diluting the original sample to 1000 times.
6. Repeat this procedure till the original sample have been diluted 10,000,000 times using every time a fresh sterile pipette.
7. From the appropriate dilutions transfer 1ml of suspension while in motion, with the respective pipettes, to sterile petri dishes. Three petri dishes are toused for each dilution.
8. Add approximately 15 ml of the nutrient medium, melted and cooled to 45^0c, to each petri dish containing the diluted sample. Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.
9. Allow the plates to solidity.
10. Incubate these plates in an inverted position for 24-48 hours at 37^0c.

Observation:

Observe all the plates for the appearance of bacterial colonies. Count the number of colonies in the plates.

Calculate the number of bacteria per ml of the original suspension as follows:

$$\text{Organisms per millimetre} = \frac{\text{Number of colonies (average of 3 replates)}}{\text{Amount of plated} \times \text{dilution}}$$

4.2.6. SOPHISTICATED INSTRUMENTAL ANALYSIS

FT-IR (Fourier Transform Infra-Red)

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 was the preferred method of infrared spectroscopy. FT-IR was an important and more advanced technique. It was 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.(64)

In FT-IR infrared was passed from a source through KRV.This infrared was absorbed by the sample KRV 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 was no two unique molecular structures producing the same infrared spectrum. It was recordedas the wavelength and the peaks seen in the spectrum indicates the amount of material present. (65)

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

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated

SEM (SCANNING ELECTRON MICROSCOPE)

In scanning electron microscope high-energy electron beam was focused through a probe towards KRV. Variety of signals was produced on interaction with the surface of the sample. This results in the emission of electrons or photons and it was collected by a appropriate detector.(66)

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.[67]

ICP- OES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Manufacturer: Perkin Elmer

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

Principle:

An aqueous KRV samplewas converted to aerosols via a nebulizer.The aerosols are transported to the inductively coupled plasma which was a high temperature zone (8,000– 10,000ºC). The analysts are heated (excited) todifferent (atomic and/or ionic) states and produce characteristic optical emissions (lights). These releases are separated based on their respective wavelengths and their strengths are measured (spectrometry). The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification was an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample.

Multi-element calibration standard solutions are prepared from single- and multielement primary standard solutions. With respect to other kinds of analysis where chemical speciation was relevant (such as the concentration of ferrous iron or ferric iron), only total essential concentration wasanalysed by ICP-OES.[68]

Application:

The analysis of major and minor elements in solution samples.

Objectives:

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

Mechanism:

In plasma emission spectroscopy (OES), a sample solution was presented into the core of inductively coupled argon plasma (ICP), which generates temperature of

approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths.

This light was collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light was then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardizationvalues[69]

The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was done in SAIF, IIT MADRAS,Chennai-36 using Perkin Elmer Optima 5300 DV.

Sample preparation:

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

100 mgKRVwas occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowedfor few minutes untill the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution. The digested sample solution was shifted into plastic containers and labeled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106

XRD (X-RAY POWER DIFFRACTION)[70]

Precautions:

The XRD uses x-ray radiation.

Procedure:

Sample Preperation:

XRD can be done on a number of different kind of samples.The KRVwas a crystalline powder that had pressed into the sample holder, and it has hold at an angle of 45 degrees. Solid small volumes of sample taped on the microscope slide glass or thin films deposited on a substrate but will have varying degree of effectiveness.

The result of the sample was based on the sample's crystalline nature .Check the alaram light on the right hand side of the instrument and fill the XRD spreadsheet on desktop.

FTIR (Fourier Transform Infrared Spectroscopy)

Fig: 3.1 FTIR Instrument

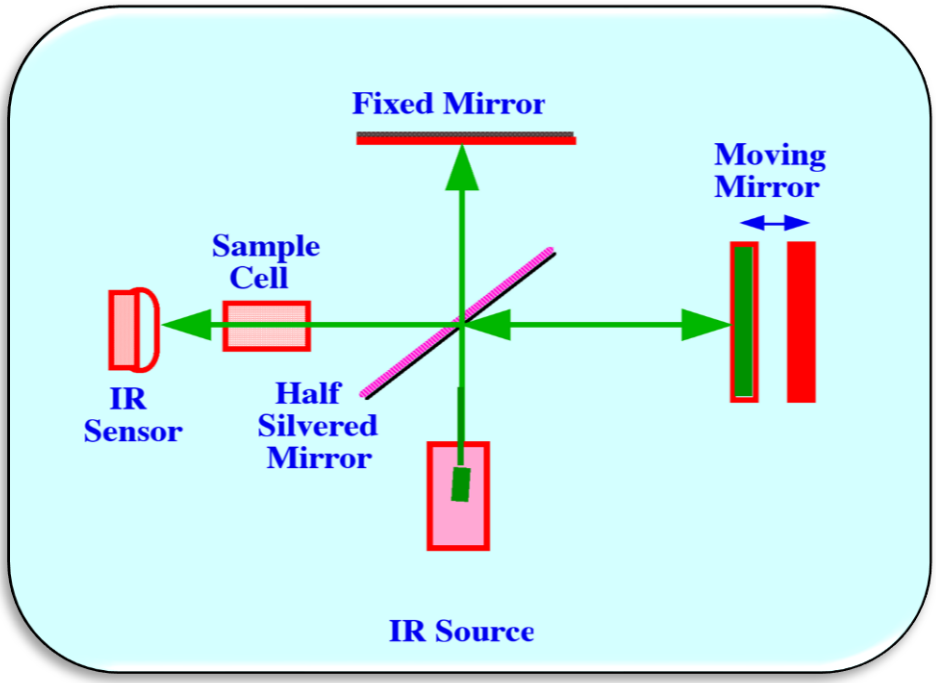


Fig: 3.2 FTIR Mechanism

SEM- SCANNING ELECTRON MICROSCOPE

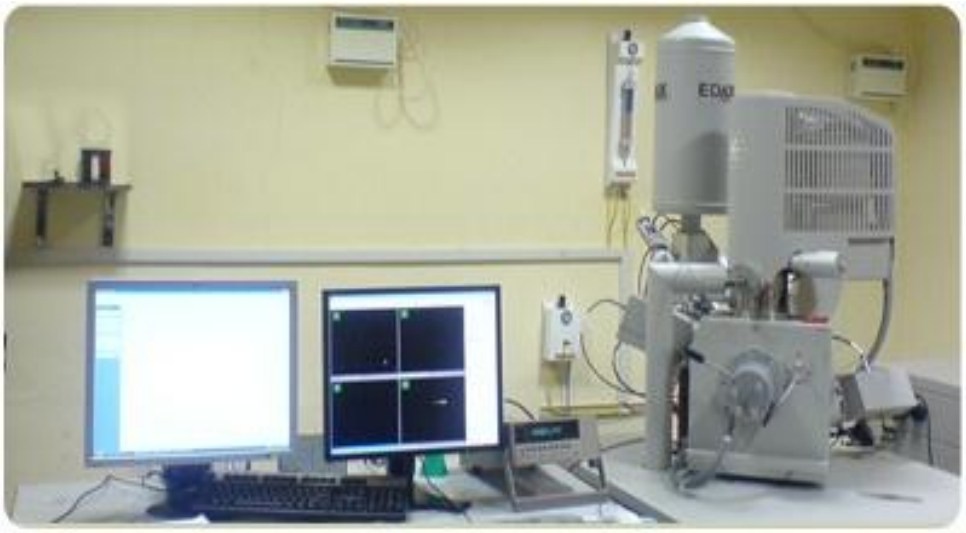


Fig: 3.3 SEM Instrument

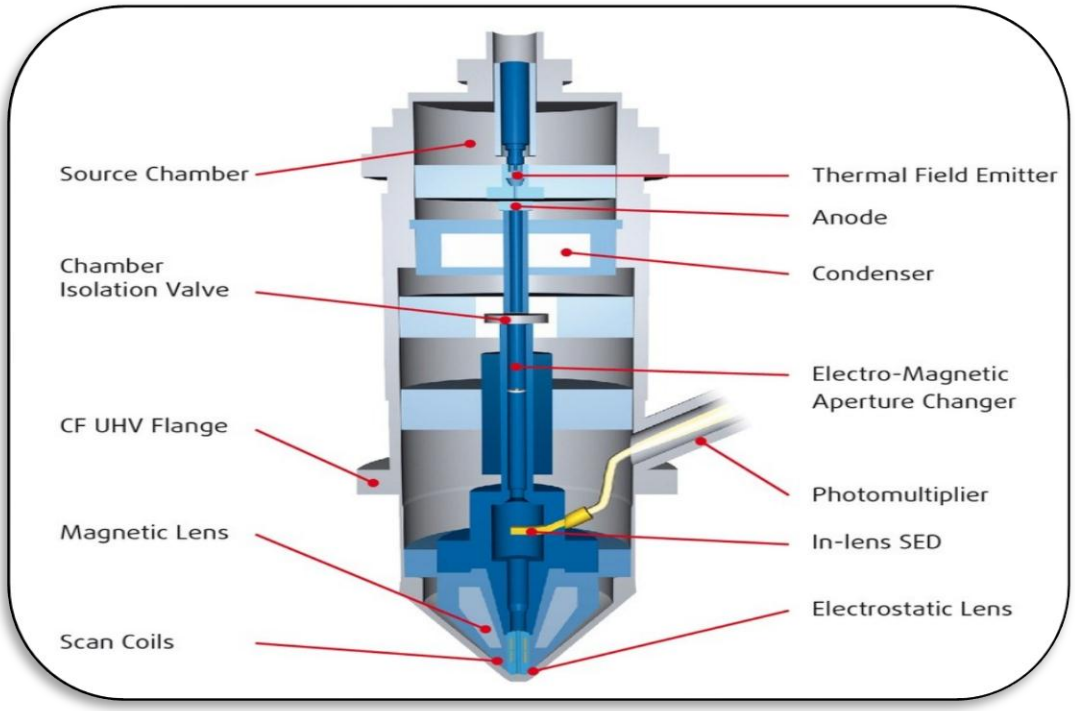


Fig: 3.4 SEM Mechanism

ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Fig: 3.5 ICP-OES Instrument

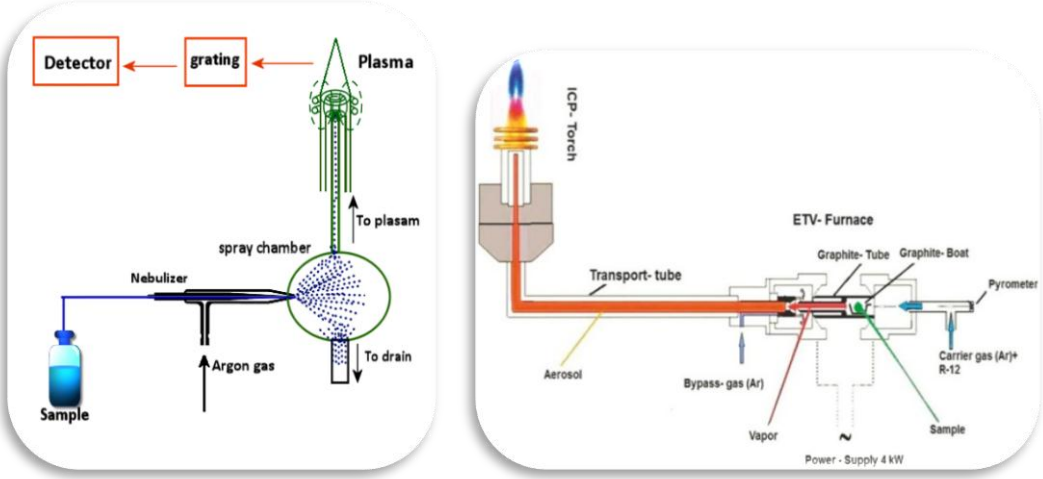


Fig: 3.6 Mechanism of ICP-OES Analyser

Fig: 3.7 X-Ray Diffraction Analysis(XRD)

XRD-Instrument

4.3. TOXICOLOGICAL STUDIES

INTRODUCTION:

The acute toxic class method was a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. Morbid animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.

4.3.1ACUTE ORAL TOXICITY – OECD GUIDELINES - 423

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co - operation and Development, Guideline-423.

The experimental protocol was approved by the institutional ethical committee (IAEC) under CPCSEA (approval no: IAEC/XLIV/25/CLBMCP/2014)C.L.Baid Metha college of pharmacy Thuraipakkam,Chennai.

PRINCIPLE: [71]

It was the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information was obtained on the acute toxicity of the test substance to enable its classification. The substance was administered orally to a group of experimental animals at one of the defined doses. The substance was tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; − no further testing wkas needed − dosing of three additional animals with the same dose − dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

Animal: Healthy wistar albino female rat weighing 200–220 gm.

Studied carried out at three female rats under fasting condition, signs of toxicity was observed for every one hour for first 24 hours and every day for about 14 days from the beginning of the study.

METHODOLOGY

Selection of animal species:

The preferred rodent species was rat, although other rodent species may be used. Health young adult animals of commonly used laboratory strain Swiss albino rat was obtained from Animal house of king's institute, Guindy, Chennai. Females should be nulliparous and non-pregnant. Each animal at the commencement of its dosing should be between 8 and 12 weeks old and its weight should fall in an interval within±20 % of the mean weight of the animals. The studies were conducted in the animal house of C.L.Baid Metha collage of pharmacy Thuraipakkam,Chennai.

Housing and feeding conditions:

The temperature in the experimental animal room should be 22ºC (+3ºC). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be grouped and tagged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals:

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions.

EXPERIMENT PROCEDURE:

Administration of doses

KRVprepared as per the classical Siddha literature was suspended in 2% CMC with uniform mixing and was administered to the groups of Wistar albino rats. It was given in a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration.

The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 hours prior to the administration of the test suspension. Finally, the number of survivors was noted after 24 hours and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Number of animals and dose levels

Since this test drug has been under practice for long time and likely to be non-toxic, a limit test at one dose level of 2000 mg/kg body weight will be carried out with 6 animals (3 animals per step).

Duration of Study : 48 hrs

Evaluation : 14 Days

Limit test

The limit test was primarily used in situations where the experimenter has information indicating that the test material was likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 200 mg/kg body weight was carried out with three animals per step. The test substance-related

mortality was not produced in animals, so further testing at the next lower level need not be carried out.

Observations

- The animals were observed individually after dosing at least once during the first 30mins and periodically during the first 24 hours.
- Special attention: First 1-4 hours after administration of drug, and
- It is observed daily thereafter for a total of 14 days, except when they needed to be removed from the study and killed humanely for animal welfare reasons or are found dead.

a. Mortality

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hour following drug administration on day 1 of the experiment and daily twice thereafter for 14 days.

b. Body weight

Body weights will be recorded at day: -1, day 1, 2, 7 and 14 of the study

c. Cage-side observation

These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour patterns. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

d. Gross necropsy

All animals (including those which die during the test period are removed from the study) will be subjected to gross necropsy. Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents, brain, eye, thymus, lungs, heart, spleen, liver, kidneys, adrenals, testes and uterus of all animals

Histopathology

Microscopic examination will be carried out in organs to show the evidence of any toxicity in gross pathology.

Data and reporting

All the data were summarised in tabular form showing the animals used, number of animals displaying signs of toxicity, the number animals found dead during the test or killed for humane reasons, a description and the time course of toxic effects and reversibility, and microscopic findings.

Test substance and Vehicle

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing KRV 2% CMC solution and it was found suitable for dose accuracy.

Justification for choice of vehicle

The vehicle selected as per the standard guideline was pharmacologically inert and easy to employ for new drug development and evaluation technique [(72)].

4.3.2 REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY OF *KANDHA RASA VILLAI* ON RATS – (OECD-407 guidelines)[(73)]

Justification for Dose Selection

The results of acute toxicity studies in Wistar albino rats indicated that*Kandha rasa villai* was non-toxic and no behavioral changes was observed up to the dose level of 200 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected for the study were100mg/kg, 200 mg/kg and 400 mg/kg body weight. The oral route was selected for use because oral route was considered to be a proposed therapeutic route.

Preparation and administration of dose

KRVat three doses respectively was suspended in 2 ml of 2% CMC in distilled water. It was administered to animals at the dose levels of 100, 200 and 400 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

METHODOLOGY

Randomization, Numbering and Grouping of Animals

Ten rats (Five Male and Five Female) were in each group randomly divided into four groups for dosing up to 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

OBSERVATIONS

Experimental animals were kept under observation throughout the course of study for the following:

Body Weight*:* Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

Clinical signs: All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality*:* All animals were observed twice daily for mortality during entire course of study.

Functional Observations: At the end of the 4^{th} week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' were assessed.

Laboratory Investigations: Following laboratory investigations were carried out on day 29 in animal's fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes. On 28th day of the experiment, 24 hours urine samples were collected by placing the animals in the metabolic cage with free access to tap water but no feed was given.

The urine was free from fecal contamination. Toluene was used as a preservative while collecting the sample. The sediments present in the urine were removed by centrifugation and the collected urine was used for biochemical estimations. On 29th day, the animals were fasted for approximately 18 hours, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Haematological Investigations: Blood samples of control and experimental rats was analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

Biochemical Investigations: Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Urine analysis: Urine samples were collected on end of treatment for estimation of normal parameters. The estimations were performed using appropriate methodology.

Necropsy*:*All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, spleen, brain, heart, and lungs were recorded. The relative organ weight of each animal was then calculated as follows;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of animal on sacrifice day (g)}} \times 100$$

Histopathology: Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 400 mg/kg were preserved and were fixed in 10% formalin for 24 hours and washed in running water for 24 hours. Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin. The organs included heart, kidneys, liver, ovary, pancreas, brain, spleen and stomach of the animals were preserved they were subjected to histopathological examination.

Statistical analysis: Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by Dunnet's multicomparision test using a computer software programme GRAPH PAD INSTAT-3 version.

4.4 PHARMACOLOGICAL STUDIES

4.4.1INVITRO ANTICANCER ACTIVITY DETERMINATION BY MTT ASSAY

HeLa(cervical cancer cells) were initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium (Gibco, Invitrogen).

The HeLa cell lines was cultured in 25 cm2 tissue culture flask with DMEM (Dulbecco's modified Eagle medium) supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO2 incubator (NBS Eppendorf, Germany). The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

Cells seeding in 96 well plate:

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension (5x104 cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO2 incubator.

Preparation of plant extracts and compound stock:

1 mg of compound/sample was added to 1ml of DMEM and dissolved completely by cyclomixer. After that the extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

Cytotoxicity Evaluation:

After 24 hours the growth medium was removed, freshly prepared each plant extracts in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 100µl of 5% MEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO2 incubator.

Cytotoxicity Assay by Direct Microscopic observation:

Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Cytotoxicity Assay by MTT Method: Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization.

After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5%

CO2 incubator for 4 hours. After the incubation period, the supernatant was removed and 200µl of MTT Solubilization Solution (DMSO) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico et al., 2004).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples} \times 100}{\text{Mean OD of control group}}$$

4.4.2 ANTI-TUMOUR ACTIVITY:

Cell Culture

The human cervical carcinoma cell lines, SiHa (HPV-16), HeLa(HPV-18) and C33A (HPV-negative) were obtained from National Center for Cell Science (NCCS), Pune, Maharashtra, India. The cells were grown in DMEM supplemented with 10% FBS, 2m ML-glutamine, and antibiotics (100 units/ml penicillin and streptomycin). The cells were incubated in a humidified 5% COincubator at 37^0 C.

Cell Growth Analysis

The assay was performed as described by Koppikar SJ et al [(74)]. Briefly, SiHa and HeLa cells were seeded at a density of $1x10^5$ and $1.5x10^5$ cells/ml respectively in 24-well plates in triplicates. Nextday, the cells were treated with different concentrations of test drug *Kandha Rasa Villai*(0–80 mg/ml) for 24, 48 and 72 h. The cells were harvested and counted for viability using trypan blue dye exclusion method [(75)].

Cell Growth in Monolayer

The assay was performed as described previously. Briefly, SiHa and HeLa cells were plated at a seeding density of $1x10^3$cells/ml in 6-well plates. After 24 h, the cells were exposed to various concentrations of test drug Kandha rasa villai(0–80 mg/ml) followed by incubation at 37^0 C in a 5% CO_2 incubator for one week in presence of the extract. This wasfollowed by fixing the colonies with 4% para formaldehyde and staining with 0.5% crystal violet.

Cell Growth in Soft Agar Assay

The assay was performed as described previously. Briefly, SiHa and HeLa cells ($5x10^3$cells/ml) along with different concentrations of Kandha rasa villai(0–80 µg/ml) were mixed with 0.35% agarose in complete DMEM medium at 40^0C and gelled at room temperature for 20 min over a previously gelled layer of 0.5% agarose in complete medium in 6wellplates. After incubation for two weeks, the colonies were counted in 10 different fields using an Axiovert 200 M microscope and the average value was calculated.

Assessment of Cell Cycle Arrest

For cell cycle analysis, HeLa, SiHa and C33A cell lines were plated at a seeding density of $5x10^5$cells/well in 6-well plates and allowed to adhere for 24 h at 37^0 C inCO_2 incubator. Next day, the cells were treated with test drug Kandha rasa villai(0–80 µg/ml) for 24 h. The cells were harvested by trypsinization and fixed in ice-cold 70% ethanol at -20^0 C for 30 min. Following washing with 1xPBS, the cells were treated with RNAse A (100 mg/ml) at room temperature for 30 min and stained with PI (20 µg/ml). Stained cells were analyzed for DNA-PI fluorescence using a flow cytometer (FACS Calibur, BD). Software (Becton Dickinson) for the proportions of cells in G/G1, S phase and G2/M phases of the cell cycle.

Assessment of Apoptosis

To determine the number of cells undergoing apoptosis upon Kandha rasa villai treatment, HeLa, SiHa and C33A were plated at a seeding density of $5x10^5$ cells/well in 6-well plates and allowed to grow overnight at 37^0 C inCO_2 incubator. Next day, the cells were treated with various concentrations of Kandha rasa villai (0–80 mg/ml) and incubated for 24 h. Cells were stained with Annexin V-FITC according to manufacturer's instructions. A total of 10,000 events were acquired and dual parameter dot plot of FL2-H(X-axis; PI fluorescence, linear scale) versus FL1-H (Y-axis; Annexin V-FITC-fluorescence, linear scale) was recorded. The data was analyzed using the FACS Calibur Cell Quest software.

Statistical Analysis :

All the experiments were performed in triplicates and repeated at least three times and the data has been presented as mean ± SD. Statistical analysis was

conducted with the Sigma Stat 3.5 program (Systat Software, Inc.) using one-way ANOVA with $\alpha = 0.05$.

4.4.3 FREE RADICAL SCAVENGING ACTIVITY: (AntioxidantAssays)

DPPH assay (2, 2-diphenyl-1-picrylhydrazyl)

Theradicalscavengin gactivity of Kandha rasa Villai extracts was determinedby using DPPH assay according to Changetal. (2001). The decreaseinthe absorption of the DPPHsolution after the additionof anantioxidant was measured at 517nm. Ascorbic acid (10mg/mlDMSO) was used as reference.

Principle

1,1Diphenyl2- Picryl Hydrazyl is a stable (inpowder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH)and an antioxidant (H- A) can be written as,

(DPPH)+ (H-A)→DPPH-H +(A)

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Reagent preparation

0.1mM DPPH solution was prepared by dissolving 4mgof DPPH in 100mlof ethanol.

Working procedure

Different volumes (1.25-20µg/µl) of *Kandha Rasa Villai* extracts were made upto 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. There action mixture was in cubated in dark condition at room temperature for 20min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The %r adical scavenging activity of the *Kandha Rasa Villai* extracts was calculated using the following formula

$$\% \text{ inhibition} = \frac{control - test}{control} X100$$

5. RESULTS AND DISCUSSION

One of the unique Formulation of Siddha, *Kandha Rasa Villai* had been exposed to several modern scientific studies to establish its fruits to scientific people and public. Literary collection, Physicochemical and elemental analysis, toxicological studies and pharmacological studies are done to justify the anticancer activity of *KRV* against cervical cancer.

The desirable consequences of *Kandha Rasa Villai* are displayed and discussed for its anticancer nature.

From review of literature

Discussion on Gunapadam review

- The poem for general properties of processed quicksilver directly indicates its anticancer nature
- Application of white arsenic kill certain cancerous growth is mentioned as its general property.
- Kantham used in treatment for tumour and it also increases the life span
- Lingam has properties of tonic and in treatment of syphilis.

Discussion on modern drug review

- The tannin content which present in the Embelia ribes naturally which helps to destroy the cancer cells and reduces the tumour growth [(76)].
- The piper cubeba contains cytotoxicity effect.
- The bioactive compounds from the seeds of Psoralea corylifolia, which are potential to inhibit cancer cell proliferation[(77)].
- Rubia cordifolia contains anticancer activity and antitumor activity [(78)].
- Zingiber officinale contains anticancer activity [(79)].
- White arsenic, mercury and cinnabar possess anti-tumor and anti-cancer activity.

Discussion on pharmaceutical review

“வேர் பாரு தழை பாரு மிஞ்சினக்கால்

மெல்ல மெல்ல பற்ப செந்தூரம் பாரே”

These lines stressed about potentiality and medicinal values of red chemical medicinal (Chendooram) preparation.

75 years of shelf life denotes its long time efficacy.

Discussion of pharmacological review

The cell lines for my anticancer activity were HeLa and SIHA. They are the genomes of HPV 16 and HPV 18 respectively.These HPV 16 and HPV 18 are the responsible for 93% of Cervical Cancer

So, the analysis of pharmacological activity through HeLa and SIHA cell lines are the novel methods for validation. They explained effective anticancer character of *KRV*.

Discussion on materials and methods

The selection of trial drug was taken from the book *VEERAMAA MUNIVAR VAAKADA THIRATTU*, Written by S.P.Ramachandiran, was approved by the Department of AYUSH as Per Classical Siddha literature.

This illustrates that *KRV* is one of the best medicine in Siddha system.

The ingredients were bought from the authenticated vender and they were identified and authenticated by the experts in Post Graduate Department of Gunapadam, GSMC, Chennai. So the ingredients were perfect and original.

The preparation of medicine was done at the well-equipped lab of the Post Graduate Department of Gunapadam.So the principles of GMP were adhered during the process.

The analytical parameters were conducted at registered and licensed laboratories only. Thus the result of *Kandha Rasa Villai* under various analytical procedures shows accuracy of it.

Discussion on Standardization techniques

Siddha parameters

The system of regenerationstandardizes its medicinal preparations itself by some exclusive ways to ensure the safety and efficacy of them.For the preparatory medicine Chendhooram, the perfect Siddha system handled the following procedure as the part of standardization.

The outcome of *Kandha Rasa Villai* according to Siddha standardization techniques are represented here.

Table no: 4 Results of Siddha standardization

S.No	**Parameter**	**Results of ideal *Chendooram***	**Results of KRV**	**Interpretation**
1.	Colour	Reddish	Brown in colour	Chendooram colour.
2.	Floating on Water	Floats on water	Floats on water	Lightness of drug.
3.	Finger Print Test	Impinged in the furrows of fingers	Impinged in the furrows of fingers	Indicates fine particles of powder.
4.	Lustre	Lustreless	Lustreless	Change of specific metallic character of raw material after incineration.
5.	Taste	No specific taste	No specific taste	Change of specific metallic character of raw material after incineration.

Colour:

It is brown in colour. The absence of shining indicates there is no free form of metals.

Floating on water:

Kandha Rasa Villai floats on water. It is due to its less specific gravity. So, it possess theproperty of Chendooram.

Finger print test:

Kandha Rasa Villai impinged on the crevices of finger. This indicates the particles are fine are fine and it is in micro size.

Lustreless& tasteless:

It is lustreless and tasteless

Table no: 5Physical characterization of *Kandha Rasa Villai*

S.no	Parameter	Result
1.	Colour	Brown in colour
2.	State of the drug	Powder
3.	Consistency	Fine powder
4.	Solubility	Sparingly soluble in water, DMSO. Well soluble in acids (Hcl and H_2So_4)
5.	Sense on touch	Fine
6.	Sense on taste	Tasteless
7.	Sense of smell	No significant smell is observed
8.	Specific gravity	0.976
9.	Ph	3.7
10.	Flame test	+
11.	Ash test	-
12.	Particle size	Completely passes through sieve no.120
13.	Loss on drying at 105 degree Celsius	8.44%
14.	Total ash	24.12%
15.	Water soluble ash	13.68%

Discussion on physico- chemical parameters

Solubility

- Solubility is one of the important parameters to attain desired concentration of drug in systemic circulation the required pharmacological response
- The oral bioavailability depends on several factors including aqueous solubility, drug permeability etc.
- The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability[80].
- *KRV* is soluble in major solvents,sparingly soluble in some of the solvents thereby it proves its efficiency of solubility in the stomach indirectly, increased in bio-availability.

pH (potential hydrogen):

- *Kandha Rasa Villai* shows acidic pH.
- This pH level plays a role in enzyme activity by maintaining the internal environment thus regulating the homeostasis.
- It is also an important factor for drug absorption. Because of the acidic nature, the drug is more readily absorbed in an acid medium like stomach which enhance the bioavailability of the drug[81].

Specific gravity

- The trial drug *Kandha Rasa Villai* shows specific gravity which is lesser than water. It shows its nature of absorption.

Flame test

- Water blue colour flame was found which indicates presence of amount of arsenic[82].

Loss on drying

- The low moisture content of *KRV* indicates that it has long shelf life.

- Moisture increased can adversely affect the active ingredient. But,*Kandha Rasa Villai* moisture doesn't damage it.So the low moisture content of *KRV* offers maximum microbial stability.

Ash Values

Total Ash value

- Low total Ash value of*KRV*indicates the richness of organic substanes.
- These organic compounds are responsible for the mineral supplements and therapeutic effect of *Kandha Rasa Villai*

Acid insoluble ash

- Lower the acid insoluble ash value better will be the drug quality. The drug possesses a low value(0.89%) of acid insoluble ash indicating that the preparation did not contain any sand, dust and stones.

Water soluble ash

- Decreased water soluble ash value(13.68 %) indicates easy facilitation of diffusion and osmosis mechanisms[(83)].

PHYTOCHEMICAL ANALYSIS:

Table no:6 Phytochemical screening test result

Phytochemicals	**Test**	**Result**
1. Alkaloids	Mayer's test	+
2. Carbohydrates	Molisch's test	-
3. Reducing sugars	Benedicts test	-
4. Glycosides	Modified Borntrager's test	+
5. Cardiac glycosides	Legal's test	-
6. Saponins	Froth test	-

7. Tannins	Gelatin test	+
8. Phenols	Alcoholic Ferric chloride test	+
9. Phytosterols	Ferric chloride acetic acid test	+
10. Diterpenes	Copper acetate test	-
11. Flavanoids	Alkaline reagent test	+
12. Proteins and amino acids	Xanthoproteic test	+

DISCUSSION:

Alkaloids

- Alkaloids play major role as anticancer agents by inhibiting the enzyme topoisomerase which is involved in DNA replication.
- Their mechanisms of action in uncontrolled proliferation of cells would help in formulating drugs [(84)].

Tannins:

- The tannins blocking virus adsorption to the target cells and inhibition of reverse transcriptas activity of the virus.
- Tannins are effective against cancer and tumorus[(85)].

Amino acids:

- The blended amino acids have a number of negative influences on cancer cells.
- Elastin a known protein which induce the growth of cancer cells. Amino acid cause a reduction in the production of elastin, in such a way for cancer the new blood vessels, formed by elastin is decreased [(86)].

Flavanoids:

- Flavanoids greatly influence the cascade of immunological events associated with the development and progression of cancer.

- Flavanoids have the potential of modulating many biological events in cancer such as apoptosis, vascularizatiing, cell differentitation, cell proliferation etc [87].
- They may be responsible for the presence of anticancer action of the *KRV.*

Bio chemical analysis

Table no: 7 Results of basic and acidic radicals studies:

S.no	Parameter	Result
1.	Test for Potassium	+
2.	Test for Calcium	+
3.	Test For Magnesium	-
4.	Test For Ammonium	-
5.	Test For Sodium	-
6.	Test for Iron (Ferrous)	-
7.	Test For Zinc	-
8.	Test For Aluminium	-
9.	Test For Lead	-
10.	Test for Copper	-
11.	Test For Mercury	+
12.	Test for Arsenic	+
13.	Test for Sulphate	+
14.	Test for Chloride	+
15.	Test for Phosphate	-
16.	Test for Carbonate	-
17.	Test for fluoride &oxalate	-
18.	Test For Nitrate	-

DISCUSSION:

The biochemical analysis for basic radicals of *Kandha Rasa Villai* shows the presence of Calcium, Mercury and Arsenic.

The biochemical analysis for acidic radicals of *Kandha Rasa Villai* shows the presence of Sulphate and Chloride.

The presence of these radicals helps *KRV* for its accurate therapeutic effect.

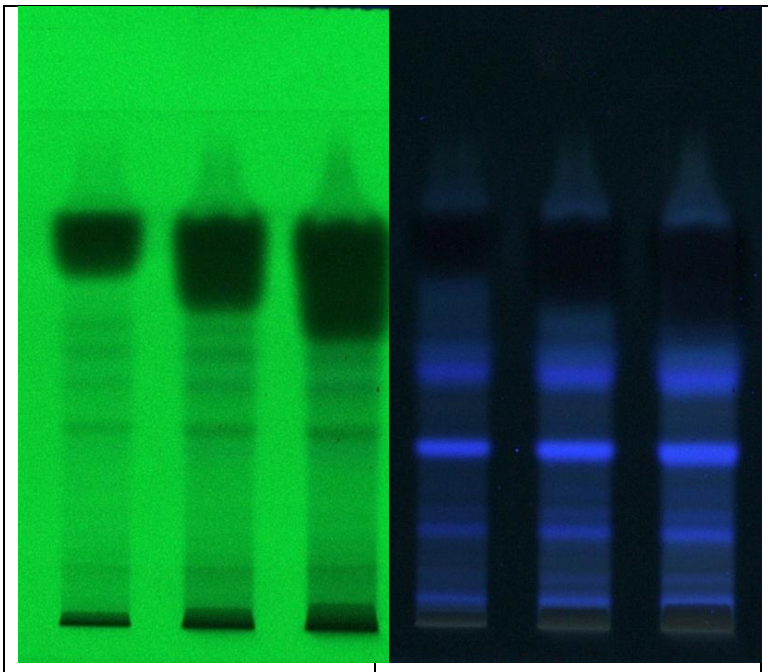
TLC/HPTLC analysis of chloroform extract

HPTLC analysis

Chloroform extract was applied in TLC aluminum sheet silica gel 60 F 254 (E. MERCK) and plate was developed using the solvent system Toluene: Ethyl acetate (5:2:0.1). After development, the plate is allowed to dry in air and examined under UV - 254nm, 366 nm 575 nm and Visible light (Vanillin - Sulphuric acid).

Sample Name/ID – Kantha Rasa Villai

Fig: 4.1 HPTLC RESULT



□ = 254 nm	□ = 366 nm	□= 575 nm (Derivatized)

Table no: 8 TLC Photo Documentatation of chloroform extract of KRV

TLC photo documentation of chloroform extract of *Kandha Rasa Villai*					
Color	**R_f value(s)**	**Color**	**R_f value(s)**	**Color**	**R_f value (s)**
Green	0.09	Light Blue	0.05	Black	0.09
Green	0.38	Light Blue	0.18	Grey	0.36
Green	0.42	Bright Blue	0.32	Grey	0.48
Green	0.50	Light Blue	0.47	Grey	0.56
Green	0.55	Light Green	0.76	Grey	0.63
Green	0.66			Grey	0.72

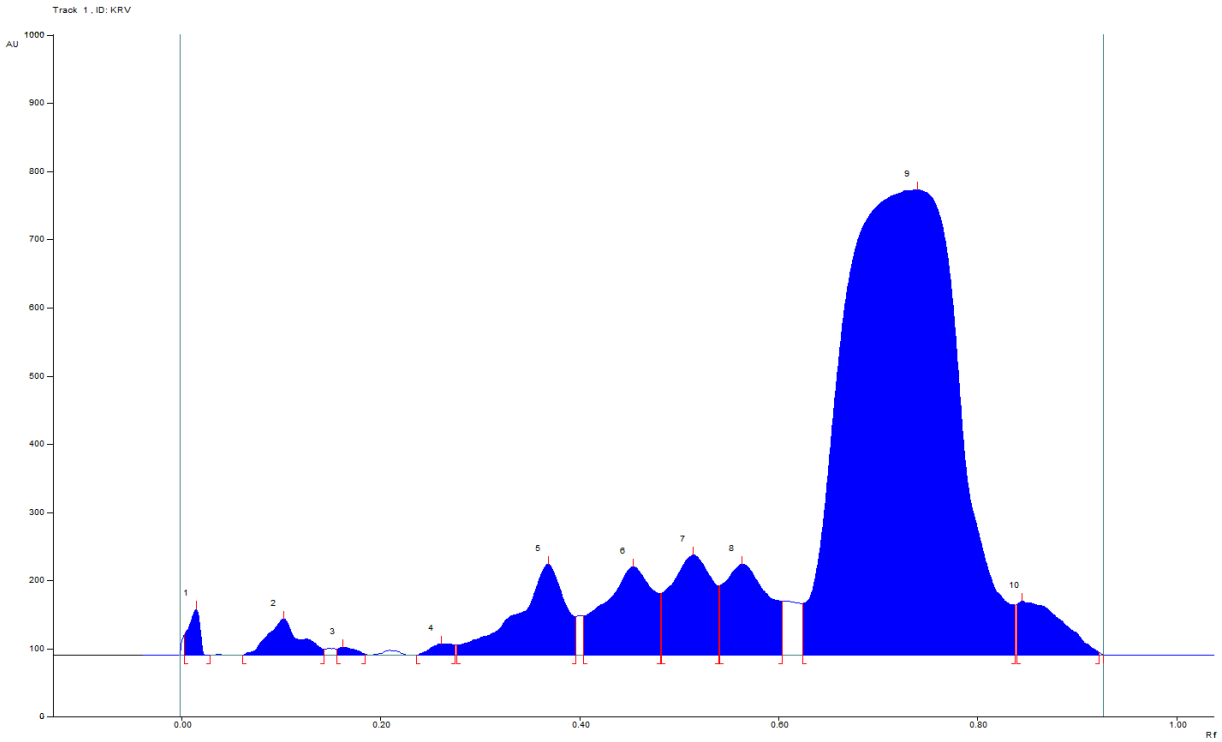


Fig: 4.2 HPTLC Chromatogram @ 254 nm:

Table no: 9 Peak Table @ 254 nm:

Track 1, ID: KRV

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	19.7 AU	0.01 Rf	63.1 AU	4.36 %	0.03 Rf	0.0 AU	586.9 AU	0.59 %
2	0.06 Rf	0.2 AU	0.10 Rf	53.3 AU	3.68 %	0.14 Rf	8.6 AU	1524.6 AU	1.54 %
3	0.16 Rf	9.1 AU	0.16 Rf	11.9 AU	0.82 %	0.18 Rf	1.7 AU	184.4 AU	0.19 %
4	0.24 Rf	0.5 AU	0.26 Rf	16.8 AU	1.16 %	0.28 Rf	14.6 AU	344.6 AU	0.35 %
5	0.28 Rf	14.8 AU	0.37 Rf	133.4 AU	9.21 %	0.40 Rf	56.9 AU	5671.9 AU	5.73 %
6	0.40 Rf	57.4 AU	0.45 Rf	129.1 AU	8.92 %	0.48 Rf	90.3 AU	5732.2 AU	5.79 %
7	0.48 Rf	90.6 AU	0.51 Rf	146.8 AU	10.13 %	0.54 Rf	02.0 AU	5441.2 AU	5.50 %
8	0.54 Rf	102.3 AU	0.56 Rf	133.3 AU	9.20 %	0.60 Rf	78.8 AU	5266.5 AU	5.32 %
9	0.62 Rf	75.9 AU	0.74 Rf	682.0 AU	47.08 %	0.84 Rf	73.4 AU	70957.6 AU	71.72 %
10	0.84 Rf	73.9 AU	0.85 Rf	78.9 AU	5.45 %	0.92 Rf	3.4 AU	3226.7 AU	3.26 %

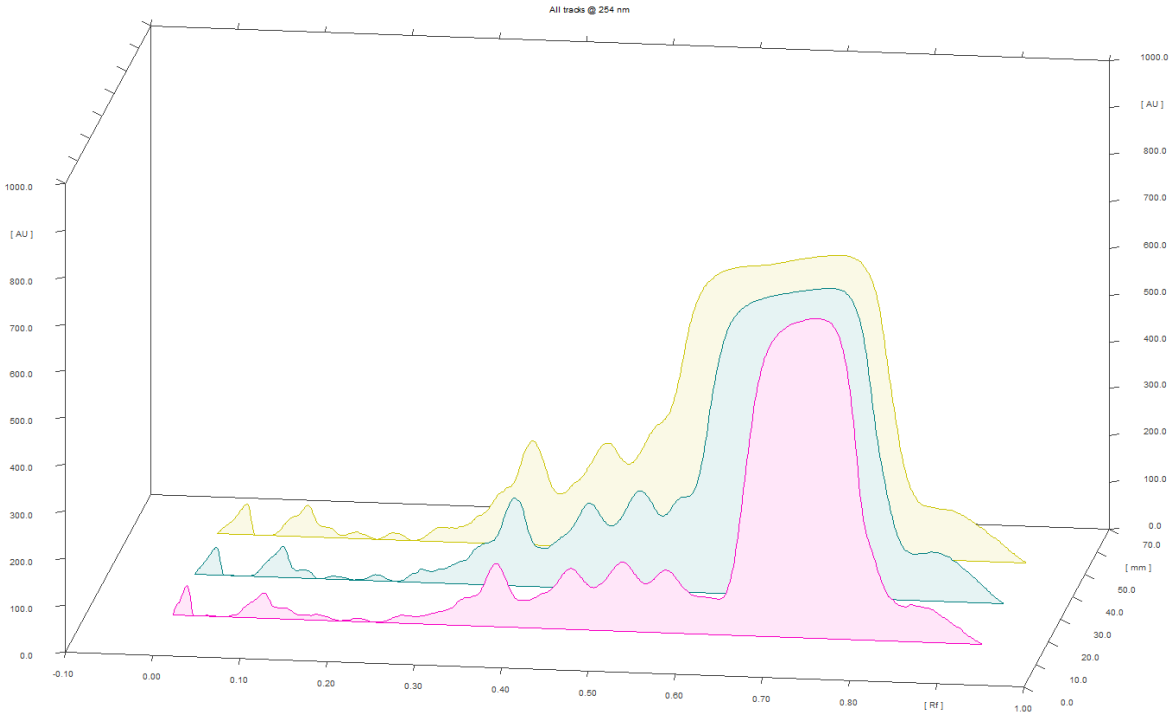


Fig: 4.3, 3D Chromatogram @ 254 nm:

DISCUSSION:

A qualitative fingerprinting of *Kandha Rasa Villai* has been performed by HPTLC method, which provide qualitative insights into the bioactive constituents present in the drug. HPTLC shows separation of components present in the Chloroform extract of *Kandha Rasa Villai*. The method may be applied to identify the *Kandha Rasa Villai* from other manufacturing process.

The present study revealed that *Kandha Rasa Villai*showed best results in Toluene: Ethyl Acetate: Formic acid: 5:2:1 solvent system. After scanning and visualizing the plates in absorbance mode at both 254 nm, 366 nm and 575 nm and visible light range, best results were shown at 575 nm.

TLC plate showed different colour Phytoconstituents of chloroform extract of *Kandha Rasa Villai*. The bands revealed presence of six greenish, three light blue, one light blue, one light greenish, one black and five grey and bands showing the presence of steroids, terpenoids, alkaloids, flavonoids, tannins and saponins.

The results from HPTLC finger print scanned at wavelength 575 nm for chloroform extract of *Kandha Rasa Villai*. There are ten polyvalent phyto constituents and corresponding ascending order of Rfvalues start from 0.00 to 0.84 in which highest concentrations of the phyto constituents was found to be 9.21% and 9.20 % with its corresponding Rf value were found to be 0.28 and 0.54 respectively.

AVAILABILITY OF BACTERIAL AND FUNGAL LOAD:

BACTERILA LOAD

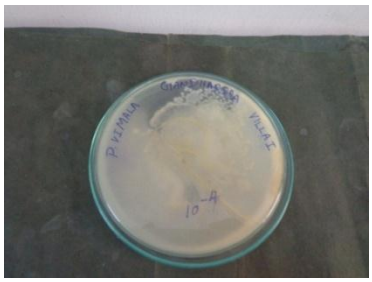


Fig: 5.1$10^{-4}$ dilution

Fig: 5.2$10^{-6}$ dilution

FUNGAL LOAD

Fig: 5.4 10^{-3} dilution

DISCUSSION:[88]

These Herbo-metal drug was prepared from plant material they are prone to contamination. The contamination of herbal drugs by microorganism not only cause bio deterioration but also reduces the efficacy of drugs.

The toxin produce by microbes makes herbal drugs unfit for human consumption because the contaminated drug may develop unwanted disease instead of disease being cured. Here, the contamination of *chendhooram*have been examine for bacterial and fungal load.

- Total bacterial load in 10^{-4} dilution is 15 and in 10^{-6} dilution is 7.
- Total fungal load in 10^{-2} dilution is nil and in 10^{-3} dilution is nil.

Here, the contamination of *KRV* is within the WHO norms. Hence, the drug is collected, prepared, stored and packed and decontaminated prior to formulation.

Instrumental analysis:

Fig: 6 Fourier Transform Infrared Spectroscopy Analysis (FT-IR)

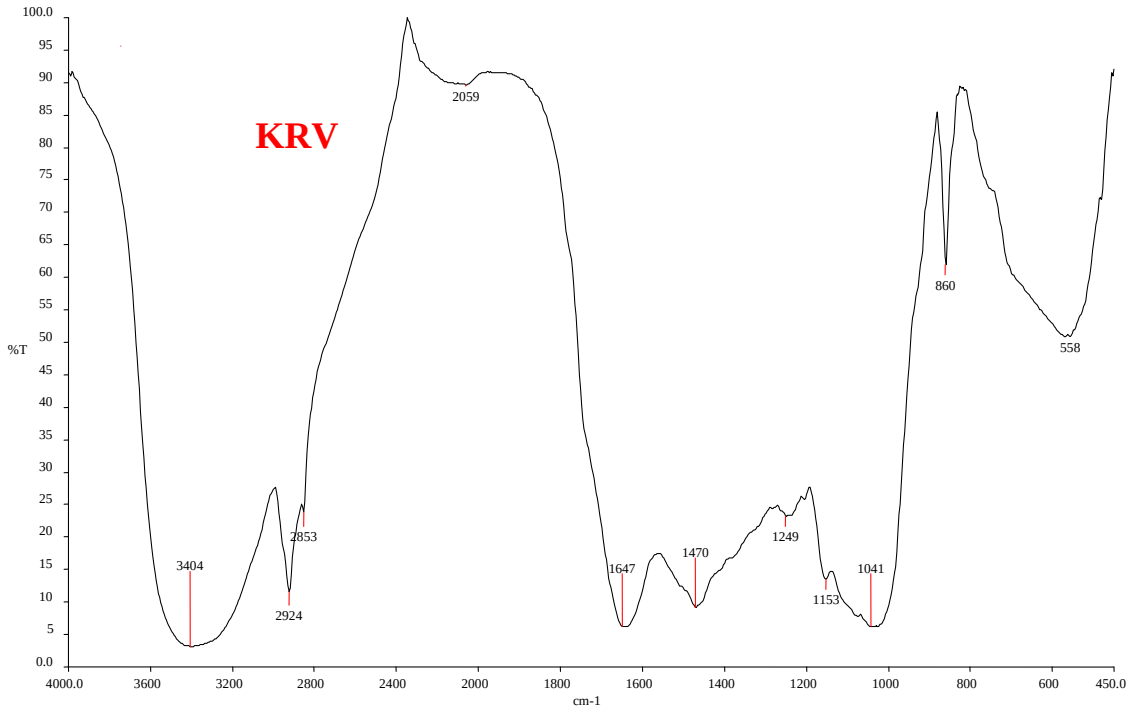


Table no: 10 Result of FT-IR

S.NO	Group frequency Wavenumber (cm^{-1})	Section	Assignment
1	3404	O-H	Alcohol
2	2924	C-H	Alkane
3	2853	C-H	Alkane
4	2059	C-O	Carboxylic acid
5	1647	C=C	Alkene
6	1470	-C-H	Alkane
7	1249	C-F	Alkyl Halide
8	1153	C-N	Amine
9	1041	C-F	Alkyl Halide
10	860	=C-H	Alkene
11	558	C-Br	Alkyl Halide

DISCUSSION:

- The wavenumbers from 4000 cm-1 to 1500 cm-1 gives details for identification of functional group.
- The wavenumber from 1500 cm-1 to 400 cm-1 provides particulars about molecular fingerprint.
- The above result showed the presence of functional group like alcohols, alkanes, amides in *Kandha Rasa Villai*.
- They may be responsible for the presence of anticancer action of KRV in cervical cancer

Amides[(89)]

- Amide derivatives of benzene-suffonanilide, pharmaceutical composition are used in cancer treatment
- The lead molecule of these compounds was methane sulfonamide, a cyclooxygenase (COX) inhibitor. They act as efficient anti-tumour agents.

Alcohol

- OH group of *Kandha Rasa Villai* has higher potential towards inhibitory activity against microorganisms

Phenols[(90)]

- Phenols of KRV possess highly Anti-Oxidant property which enhances its effect against the disease
- The effect of phenols is currently of great awareness due to their anti-oxidative and possible anti-carcinogenic activities.
- Free radicals react easily with phenols to abstract the hydrogen atom from the OH group. Phenolic acids and flavonoids also work as reducing agents, free radical scavengers and quenchers of single oxygen formation (Ali Ghasemzadeh et al.2011)
- Phenolic acids 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. Hydroquinone is one of the phenolic group inhibits the free radical reactions. (cho7 Alchohol HTI) They are also an antioxidant substance capable of scavenging free superoxide radicals, anti-aging and reducing the risk of cancer.
- Phenolic and flavonoids possess diverse biological activities, for example, antiulcer, anti-inflammatory, antioxidant, cytotoxic and antitumor,antispasmodic and antidepressant activities.

Alkanes[91]

- Alkane derivative like bis(4-amino-5-mercapto-1,2,4-triazol-3-yl) possess anti- cancer activity.

Carboxylic acid[92]

- Benzene-poly-carboxylic Acid Complex (BP-CI) is a novel anticancer complex against human cancer cells.
- Docosahexaenoic acid (DHA) is an omega-3 fatty acid. Its structure is a carboxylic acid (-oicacid) with a 22- carbon chain (Docosa-is Greek for 22) and six (hexa-) cis double bounds.
- DHA was revealed toincrease the efficacy of chemotherapy in prostate cancer cells and a chemo protective effect in a mouse model was reported.
- It may also be used as a non- toxic adjuvant to increase the efficacy of chemotherapy.
- In mice,DHA was found to reduce growth of human colon carcinoma cells
- The cytotoxic effect of DHA was caused by decrease in cell growth regulators.

Ether:

- Certain ether lipids such as 1-0-octadecyl-2-0 methyl-rec-glycero-3-phosphocholine represent a new class of antineoplastic agents. These ether lipids have been shown to be cytotoxic for a wide variety of tumors.

SEM:

The following image is done by 10000X magnification via 500µm aperture shows maximum depth focused. Zoom magnification denotes rapid surveying of the specimen.

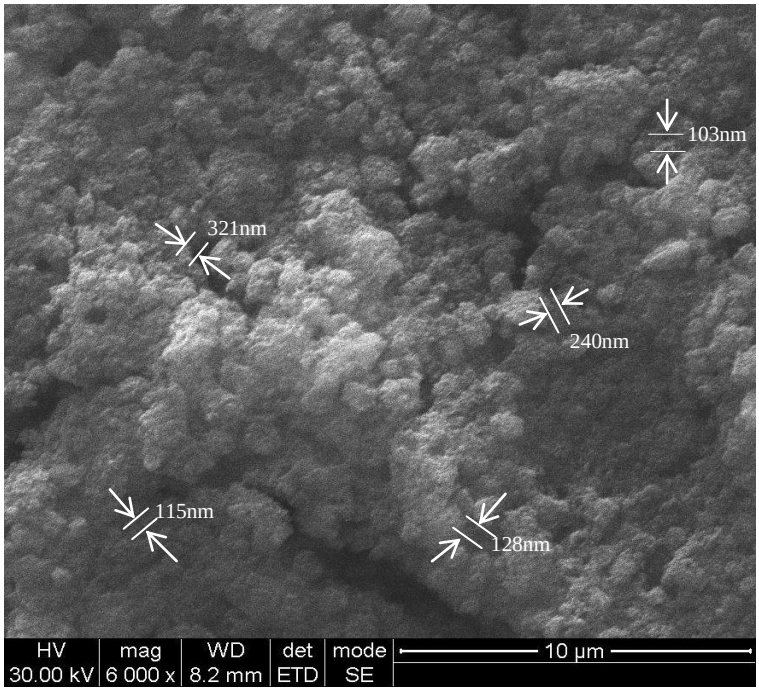


Fig: 7.1 SEM image of 10µm

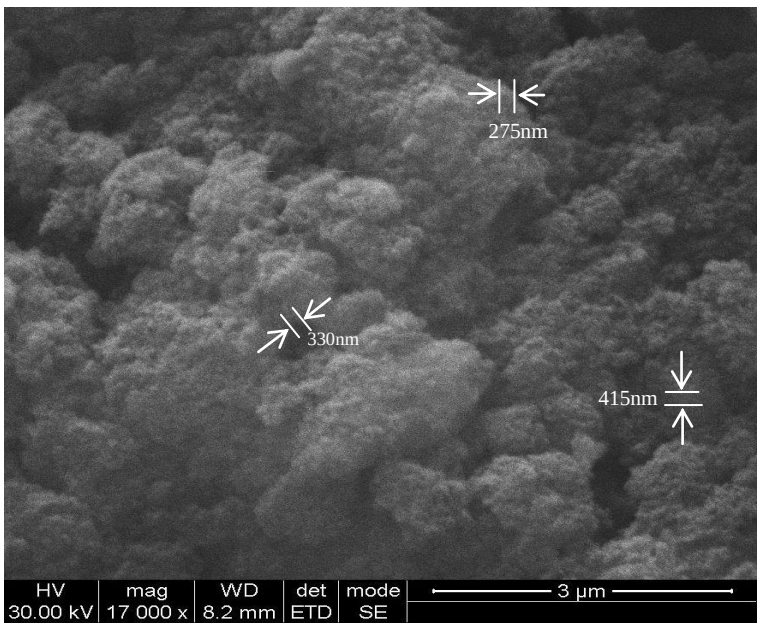


Fig: 7.2 SEM image of 3µm

Discussion on SEM reports

- Nanoparticles, according to the American Society for Testing and Materials (ASTM) standard definition, are particles with lengths that range from 1 to 100 nm in two or three dimensions.

Advantages of nanoparticles:[93]

- Enhancing solubility of hydrophobic drugs,
- Prolonging circulation time,
- Minimizing nonspecific uptake,
- Preventing undesirable side effects,
- Improving intracellular penetration,
- Specific cancer targeting
- The test drug *KRV* contains nanoparticles.
- The presence of nanoparticles in the drug result in a better bioavailability and facilitates absorption.
- Nanotechnology a promising way from cancer management towards cancer elimination.

Table no: 11 ICP-OES RESULTS OF *KANDHA RASA VILLAI*

S. no	Elements	Detected levels
1.	Phosphorus	28.54 mg/L
2.	Sulphur	12.514 mg/L
3.	Calcium	24.150 mg/L
4.	Iron	43.380 mg/L
5.	Mercury	0.994 mg/L
6.	Potassium	110.821 mg/L
7.	Sodium	03.110 mg/L
8.	Nickel	BDL
9.	Lead	BDL
10.	Arsenic	BDL
11	Cadmium	BDL

DISCUSSION:

ICP-OES result shows about the heavy metals like Ar, Pb, Ni, Cd are below detectable limit. And also Hg is in permissible limit.

Here, the result showed that Ca, K, Na, P and S were also present in the drug, which gives synergistic activity.

Iron:

- Several pieces of evidence indicate that iron deprivation could be an excellent therapeutic approach, dietary iron restriction markedly decreases tumour growth in rodents [(94)].

Arsenic:[(95)]

- Arsenic trioxide is a cancer medication that interferes with the growth and spread of cancer cells in the body.
- Arsenic trioxide is used to treat a type of leukemia (acute promyelocytic leukemia- APL) when other types of treatment (e.g chemotherapy) have not worked well or no longer work

Mercury:

Royle (1964) described alleged mercury intoxication after using 200 ml of 1: 500 perchloride of mercury solution as an anti- cancer agent in renal surgery. It is therefore concluded that mercury perchloride is safe anti- cancer agent when used as described in large bowel surgery.

XRD:

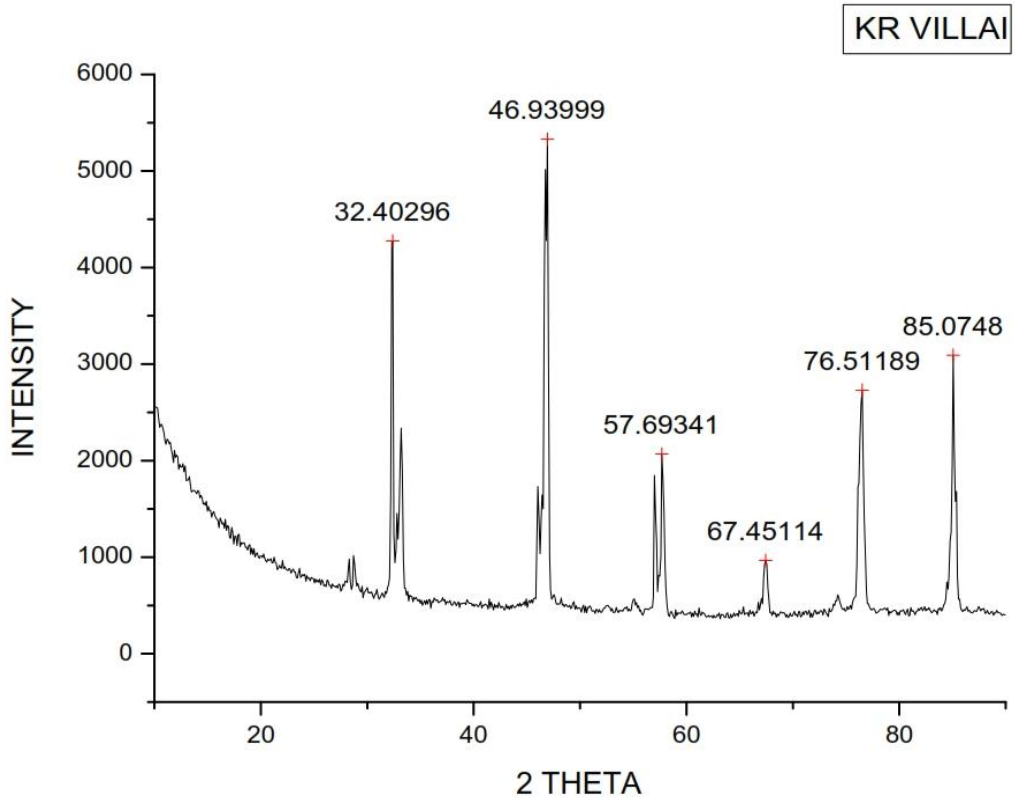


Fig: 8 XRD Image of *Kandha Rasa Villai*

DISCUSSION:

The crystalline structure, the size and shape of the particles are highly dependent on the route of synthesis and highlights the efficacy of the drug. The nanomaterial characteristics may enhance bio absorption of the drug.[96]

XRD pattern of *Kandha Rasa Villai* shows the good crystallinity after calcinations process. The major diffraction peaks are identified after XRD analysis KRV concluded that HgS in nano crystalline range (31-56) is association with organic molecules probably plays an important role in making it biocompatible and non toxic at therapeutic doses.

Otherelements present in *KRV* act as additional supplement and possible helps in increase the efficacy of the formulation [97].

TOXICOLOGICAL STUDIES

Acute oral toxicity in rats

Table no: 12 Dose finding experiment and its behavioral Signs of Toxicity for *Kandha Rasa Villai*

Observation done:

Group	Day
Body weight	Normal
Assessments of posture	Normal
Signs of Convulsion Limb paralysis	Absence (-)
Body tone	Normal
Lacrimation	Absence
Salivation	Absence
Change in skin color	No significant colour change
Piloerection	Normal
Defecation	Normal
Sensitivity response	Normal
Locomotion	Normal
Muscle gripness	Normal
Rearing	Mild
Urination	Normal

Table no: 13 Gross behavior of animals

Dose mg/kg	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
200	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

1.*Alertness 2.Aggressiveness 3. Pile erection 4. Grooming 5.Gripping 6. Touch Response 7. Decreased Motor Activity 8.Tremors 9 Convulsions 10. Muscle Spasm11. Catatonia 12.Musclerelaxant 13.Hypnosis 14.Analgesia15.Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19 Respiration 20. Mortality*

DISCUSSION:

In the acute toxicity study, the rats were treated with different concentration of*KANDHA RASA VILLAI* from the range of 5mg/kg to 200mg/kg.

This dose level did not produce signs of toxicity, behavioral changes, and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period.

These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract.

In acute toxicity test the*KANDHA RASA VILLAI*was found to be non toxic at the dose level of 200mg/ kg body weight.

SUB-ACUTE ORAL TOXICITY 28 DAYS REPEATED DOSE STUDY IN RATS

Table no:14 Body weight (g) changes of rats exposed to *Kandha Rasa Villai*

Dose (mg/kg/day)	***Days***				
	0	**7**	**14**	**21**	**28**
Control	120.59±0.92	122.79±0.87	123.52±1.18	127.24±1.12	131.25±1.05
20	124.75±2.37	128.28±0.61	130.07±1.35	128.42±2.24	130.16±2.03
40	125.94±1.57	128.86±2.05	133.38 ±0.63	132.85±1.87	137.89±1.42

Values are expressed as mean ± S. E. M (Dunnett's test).*p<0.05, **p<0.01, ***p<0.001 vs control: N= 3

Table no: 15 Effect of *Kandha Rasa Villai* on Organ weight in rats

Organ	**Control**	**20 mg/kg**	**40 mg/kg**
Liver (g)	3.07±0.20	3.36±0.17	2.63±0.18
Heart (g)	0.32±0.04	0.36±0.04	0.41±0.04
Lung (g)	0.28±0.05	0.36±0.03	0.40±0.02
Spleen (g)	0.25±0.06	0.3±0.05	0.35±0.05
Brain (g)	0.37±0.05	0.4±0.05	0.44±0.05
Kidney (g)	0.76±0.05	0.92±0.01	0.65±0.01

*Values are mean of 10 animals ± S.E.M. (Dunnett's test). *P<0.05; **P<0.01;***P<0.001vs control N=3*

Table no: 16 Effect of *Kandha Rasa Villai*on Haematological parameters in rats

Parameter	Control	20 mg/kg	40 mg/kg
RBC ($\times 10^6/mm^3$)	8.29±0.43	8.98±0.61	10.14±0.89
PCV (%)	49.66±0.77	50.08±0.48	51.82±0.52
Hb (%)	15.13±0.39	15.54±0.34	15.30±0.14
WBC ($\times 10^3/mm^3$)	11.75±0.85	12.63±0.60	12.11±0.4
Neutrophils (%)	23.29±0.73	24.66±0.33	27.27±0.82
Eosinophills (%)	4.1±0.23	5.13±0.33	6.4±0.41
Lymphocyte (%)	85.5±0.46	86.19±0.39	87.1±0.39
Platelets ($\times 10^3/mm^3$)	425.73±1.35	451.52±10.9	501.48±6.10

*Values are mean of 10 animals ± S.E.M. (Dunnett's test). *P<0.05; **P<0.01;***P<0.001 N=3*

Table no: 17Effect of *Kandha Rasa Villai*on Biochemical parameters in rats

Parameters	Control	20 mg/kg	40 mg/kg
Glucose (mg/dl)	108.63±0.81	109.21±1.04	110.9±0.63
BUN (mg/dl)	22.06±1.55	22.7±1.30	24.8±1.09
Creatinine (mg/dl)	0.85±0.07	0.93±0.03	1.17±0.17
SGOT (U/L)	74.35±1.23	76.54±1.30	74.36±0.04
SGPT(U/L)	27.07±0.84	32.67±1.14	29.64±0.49
ALP (U/L)	104.63±1.14	108.4±1.43	108.32±1.46
Protein (g/dl)	8.58±0.68	10.14±0.42	9.3±0.33
Albumin (g/dl)	5.34±0.40	6.18±0.52	6.09±0.54
Total Cholesterol (mg/dl)	93.21±1.16	95.83±1.45	93.47±0.29
Triglycerides (mg/dl)	52.58±1.56	57.15±1.10	54.06±0.97

*Values are mean of 10 animals ± S.E.M. (Dunnett's test). *P<0.05; **P<0.01;***P<0.001 N=3*

Table no: 18Effect of *Kandha Rasa Villai* on Urine parameters in rats

Parameters	Control	20 mg/kg	40 mg/kg
Colour	Yellow	Yellow	Yellow
Transparency	Clear	Slightly turbid	Slightly cloudy
Specific gravity	1.01	1.02	1.04
pH	7.2	7.8	8.1
Protein	Nil	Nil	Nil
Glucose	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve
Ketones	-ve	+ve	+ve
Blood	Absent	Absent	Absent
RBCs	Nil	Nil	Nil
Epithelialcells	Nil	1-2 cells/HPF	Nil
Casts	Nil	Nil	Nil

DISCUSSION:

- The dose selected for the Sub acute toxicity study was 20mg, 40mg/kg of *KANDHA RASA VILLAI*.
- All the animals were free of intoxicating signs throughout the dosing period of 28 days.
- No physical changes were observed throughout the dosing period. No mortality was observed during the whole experiment.

- No abnormal deviations were observed. No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits.
- The weights of organs recorded that shows mild differences in the treatment when compared control group. This indicates that *Kandha Rasa Villai* induce mild changes in liver and kidney but not toxic to rest of the organs.
- There was slight changes were observed in hemoglobin (Hb), red blood cell (RBC). No significant changes in white blood cell (WBC), packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups.

HISTOPATHOLOGY EXAMINATION:

- Histopathology studies were carried out on liver, kidney and spleen and recorded. Blood samples for hematological and blood chemical analyses were taken from common carotid artery.
- All rats were sacrificed after the blood collection. The internal organs and some tissues were observed for gross lesions. All tissues were preserved in 10% neutral buffered formaldehyde solution for histopathological examination.

HISTOPATHOLOGY SLIDES:

Control KRV 20mg KRV 40mg

KIDNEY

LIVER

SPLEEN

Fig:9.1-9.9

Control KRV 20mg KRV 40mg

HEART

LUNGS

OVARY

Fig:9.10- 9.18

PHARMACOLOGICAL ACTIVITIES

Anticancer activity:

Table no: 19CELL LINE: HeLa

Sample Concentration (µg/ml)	Average OD at 540nm	Percentage Viability
Control	**0.9808**	
6.25	0.6847	69.8103
12.5	0.4645	47.3593
25	0.4178	42.5979
50	0.3233	32.9629
100	0.2750	28.0383

LD_{50} values –21.84ug/ml (ED50plus software V 1.0)

Results and Discussion

Cytotoxicity Assay by MTT

MTT colorimetric method, also known, is a method for detecting cell survival and growth methods. This assay is based on the metabolic reduction of 3- (4, 5-dimethylthiazol-2-yl) -2, 5-difeniltetrazol (MTT) by mitochondrial enzyme succinate dehydrogenase in a colored compound blue (formazan), allowing to determine the functionality of the mitochondrial treated cells. This method has been widely used to measure survival and cell proliferation. The amount of living cells is proportional to the amount of formazan produced. Cell lines derived from NCCS, Pune were free from any kind of bacterial and fungalcontamination

Kandha Rasa Villai at different doses (6.25-100 µg in100 µl of 5% MEM) was administeredfor 24 hrs. It was found that the number of cells decreases as the dose increases and at approximately 50 µg/mldose of extract, 50% of the cells (HeLa cells) were less as compared to normal control asshown in figure 10. The percentage of cells viability was determined by calculating the O.D of treated against thecontrol. Reading optical density (OD) is performed in a spectrophotometer at a wavelength of 540 nm. Comparison values are made on a basis of 50% inhibition of growth (IC_{50}) in treated cells with specific agents. Results are tabulated in Table 19 and graphically represented in Graph 1

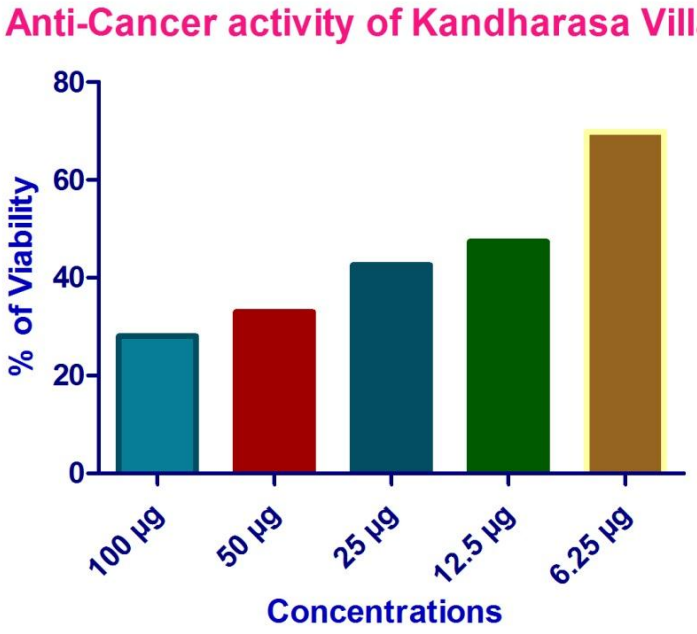


Chart no: 1 Anti- cancer activity

Graph 1shows the drug dose and % of Inhibition of HeLa cells after the *Kandha Rasa Villai* extract treatment. It can be observed by the result of MTT assay that the IC dose of *Kandha Rasa Villai* is 50µg/ml. As the dose increases the HeLa cell viability decreases. It was found that the % growth inhibition increasing with increasing concentration of *Kandha Rasa Villai* steadily up to 6.25 µg/ml on *HeLa* cell line (Table 19 and Graph 1) and that IC value on *HeLa* cell line was 50 and R value was 0.9808.

Analysis of Membrane Morphological Characteristics by Haematoxylin /Eosin (H/E) Staining

Morphological changes such as changes to the cell membrane, loss of membrane asymmetry and cell shrinkage, are the early stage of apoptosis was analyzed by H/E staining. The IC dose (50µg/ml) treated cancer cells show features of apoptosis whereas treated with same amount of dose, to normal treated cells appeared without any significant changes.

Since the discovery of the cisplatin antitumor activity, great efforts have focused on the rational design of metal-based anticancer agents that can be potentially used in cancer chemotherapy. Over the last four decades, a large number of metal complexes have been extensively investigated and evaluated *in vitro* and *in vivo* [(98)].

The key focuses of these studies lie in finding novel metal complexes which could potentially overcome the hurdles of current clinical drugs including toxicity, resistance and other pharmacological deficiencies.

Metals and metal compounds have been used in medicine for several thousands of years. The medicinal uses and applications of metals and metal complexes are of increasing clinical and commercial importance. Monographs and major reviews, as well as dedicated volumes, testify to the growing importance of the discipline [(99)].

Relevant reviews are to be found throughout annual series, for example Metal Ions in Biological Systems [(100)].

The field of inorganic chemistry in medicine may usefully be divided into two main categories: firstly, ligands as drugs which target metal ions in some form, whether free or protein-bound; and secondly, metal-based drugs and imaging agents where the central metal ion is usually the key feature of the mechanism of action [(101)].

Arsenic trioxide, As_2O_3(Trisenox, Cell Therapeutics Inc, Seattle, USA) which was approved by the FDA in September 2000 for treatment of Acute promomyelocyticleukemia (APL) in patients who have relapsed or are refractory to retinoid and anthracyclinechemotherapy. An estimated 1,500 new cases of APL are diagnosed yearly in the US, of which an estimated 400 patients will not respond to, or will relapse from, first-line therapy. The approval ofarsenic trioxide as a chemotherapeutic agent invokes the pioneering work of Ehrlich and thedevelopment of Salvarsan for use in syphilis—the foundation stone for the science of chemotherapy.The use of chelating agents in medicine may even be traced to a collaboration between Werner (thefather of coordination chemistry) and Ehrlich (the father of chemotherapy) to find less toxic arseniccompounds for the treatment of syphilis[(102)].

Arsenic has been used therapeutically for more than 2,000 years and was used in the 1930s for treatment of chronic myelogenous leukemia until supplanted by newer chemotherapies[(103)].

The past, present and future of medicinal arsenic has been described as a story of ‘‘use, dishonor, and redemption’’. Recent interest in arsenic trioxide initially arose through Chinese reports of its efficacy and use [(104)]. Side effects are cardiotoxicity, skin rashes, and hyperglycemia[(105)].

Arsenic trioxide apparently affects numerous intracellular signal transduction pathways and causes many alterations in cellular function. Thus, the mechanisms of cell death induced by arsenic trioxide are multiple: induction of apoptosis, inhibition of proliferation, and even inhibition of angiogenesis have all been reported[(106)].

In cellular studies, arsenic trioxide inhibits glutathione peroxidase, possibly through generation of arsenic–GSH conjugates and increases cellular hydrogen peroxide content[(107)].

Several pieces of evidence indicate that iron deprivation could be an excellent therapeutic approach: (i) dietary iron restriction markedly decreases tumour growth in

rodents and antibodies which block transferrin-binding to ellular receptors inhibit cancer cell growth *in vitro* and *invivo* [108].

Oncologists and scientists engaged in the research of cancer treatments should conduct a comprehensive study on the efficacy of mercury which is being used as an anti-cancer drug in the age old Siddha system. Three years of research has shown that metal (mercury, arsenic and copper) based Siddha drug is a safe alternative for cisplatin therapy or arsenic trioxide in selected cases of cancer treatments wherein the patients cannot bear the adverse effects.

He found that mice treated with Siddha drugs showed better health than what did in cisplatin therapy in terms of appetite, haemoglobin, red blood cells and white blood cells[109].

Studies have shown that phenols present in herbal plants such as Piper longam, Zingiber officinale, Rubia cordifolia, Psoralea corylifolia, Embelia ribes and Piper cubeba have cytotoxic effects on different tumors. Mechanisms of these compounds are carried out through apoptosis. Thus from the above study, it is evident that the cytotoxic property of *Kandha Rasa Villai* may be due to the synergistic interactions between the metal complex and plant derivatives.

C-Normal control D- Normal*KRV* treated

Figure 10: *KRV* significantly altered the morphology of HeLa cancer cells (B) as compared to control; however at the same dose of *KRV* extract, normal skin cells (HaLa) does not show morphological alteration (D). (A) is HeLa control and (C) is (HaLa) control cells. Simultaneously the cell density is also low in HeLa treated section B in comparison to normal treated, normal control and cancer control section Figure D, C and A respectively.

ANTI-TUMOUR ACTIVITY:

Results

Cell Growth Analysis

We have previously reported that *Kandha Rasa Villai* exhibited significantanti-oxidant potential as well as cytotoxicity in cervical cancer cell line HeLa. Based on it, we chose non-cytotoxicconcentrations of test drug *Kandha Rasa Villai*(0–80 µg/ml) for SiHa (HPV-16) andHeLa (HPV-18) in our assays. It was observed that *Kandha Rasa Villai*decreasedthe growth of the cells in a dose and time-dependent manner. InSiHa, *Kandha Rasa Villai*decreased the cell growth at 80 µg/ml concentrationby ~ 4.782($p = 0.008$), ~ 4.722($p = 0.001$) and ~ 3.42-fold($p = 0.053$) at 24, 48 and 72 h, respectively, compared to theuntreated control cells (Figure 11A). Similarly, at 80 µg/mlconcentration of *Kandha Rasa Villai*, HeLa cells exhibited ~ 5.532($p = 0.001$), ~ 5.942($p = 0.010$) and ~ 6.37-fold ($p = 0.001$) decrease in the cellgrowth at 24, 48 and 72 h, respectively, compared to thentreated control cells (Figure 11B). This was further supportedby colony formation and soft agar assays wherein a dosedependentdecrease in the number of colonies was observed in both the cervical cancer cell lines (Figure 11C and D, respectively). Interestingly, at 80 µg/ml concentration, *Kandha Rasa Villai* significantly reduced the number of colonies in HeLa (~ 4.97 fold; $p \leq 0.001$) and SiHa (~2.95 fold; $p \leq 0.001$) compared to their respective untreated control cells (Figure11D). Thus *Kandha Rasa Villai* regulated the growth kinetics of cervical cancer cells in a significant manner. As a negative control, we took C33A (HPV negative) cell line and analyzed the cytotoxicity of *Kandha Rasa Villai*in it. *Kandha Rasa Villai* did not induce any cytotoxicity up to 160 mg/ml concentration in C33A cells, which was similar to that observed in SiHa and HeLa (Figure 11). However, at higher concentrations, *Kandha Rasa Villai* induced cytotoxicity in all the three cell lines, wherein HeLa and C33A cells showed similar cytotoxic effect.

Figure11. Kandha Rasa Villai regulates the growth of cervical cancer cells. SiHa ***(A)*** *and HeLa.* ***(B)*** *were treated with KRV (0–80 µg/ml) for 24–72 h and the number of viable cells were counted using the trypan blue dye exclusion method. Data represent mean ± SD of three independent experiments.* ***(C)*** *The cervical cancer cell lines (SiHa and HeLa) were treated with KRV (0–80 µg/ml) for one week. The colonies were stained with crystal violet andphotographed. The experiments were repeated three times.* ***(D)*** *Both SiHa and HeLa (*$5x10^3$ along with *Kandha Rasa Villai(0–80 mg/ml) were grown in soft agarfor two weeks. Colonies were counted from at least 10 different areas and the average of each has been plotted. The data represents mean ± SD offive independent experiments.*

Apoptosis

We found that in HeLa, *Kandha Rasa Villai* treatment resulted into increase in the number of cells in sub-G phase, indicative of apoptotic population (Figure 12). On staining with Annexin V-FITC, the cells showed a dose-dependent increase in both early as well as late apoptotic cell population (Figure 12). Interestingly, at 80 µg/ml of *Kandha Rasa Villai* concentration, there was ~ 4.4-fold ($p \leq 0.050$) and ~5.5-fold($p \leq 0.050$) increase in both early as well as late apoptotic cellpopulation, respectively, compared to the untreated control cells.On the other hand, no apoptosis was observed in *Kandha Rasa Villai*treatedSiHa or C33A cells (Figure 12).

Fig No. 12. Apoptosis assay of *Kandha Rasa Villai*staining with Annexin V-FITC

*Kandha Rasa Villai*has been extensively used in the management of various disorders. In the present study, we have further elucidated the anti-neoplastic potential of the aqueous extract of *Kandha Rasa Villai*in cervical cancer cells with the possible underlying mechanisms. It was observed that *Kandha Rasa Villai* regulated the growth kinetics of the cervical cancer cells lines in a statisticallysignificant manner and thus, *Kandha Rasa Villai*exhibited a promisinganticancer potential.

RESULT AND DISCUSSION

AntioxidantActivity

Table no:20 DPPHAssayof *Kandha Rasa Villai*

Concentration (µg/µl)*	**Absorbance**		**Percentage of inhibition**	
Kandha Rasa Villai	Drug	Standard	Drug	Standard
Control	0.9857	0.341	-	-
1.25	0.9693	0.299	20.66	40.89
2.50	0.9516	0.232	31.45	51.25
5.00	0.8839	0.114	43.32	74.07
10	0.7567	0.092	54.23	83.33
20	0.6392	0.054	61.15	89.62

***µg/ml: microgram per millilitre. Drug: Kandha Rasa Villai(1.25-20µg/µl). Standard:*

Ascorbic acid(10mg/mlDMSO)

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of *Kandha Rasa Villai* extract. The antioxidant molecules can quench DPPH free radicals by providing hydrogen atomor byelectron donation and a colorless stable molecule 1, 1 diphenyl-2-picrylhydrazil is formed and as a result of which the absorbance at 517 nm of the solution is decreased. In the present study, the *Kandha Rasa Villai* extract was analyzed was able to decolorize DPPH and the free radical scavenging activity was expressed as the percentage decrease in absorbance.

The results show that the extracts of *Kandha Rasa Villai* and the standard drug Ascorbic acid (10mg/mlDMSO) have a concentration-dependent anti-radical activity which was tabulated in Table No. 20

A maximum of 61.15% and 89.62% anti-radical effects are exercised by *Kandha Rasa Villai* and standard drug ascorbic acid at concentrations of 20 µg / ml respectively. Minimum percentage of inhibit ion 20.66% and 40.89% % anti-radical effects are manifested by *Kandha Rasa Villai* and standard drug ascorbic acid at concentrationsat 1.25µg/ml.

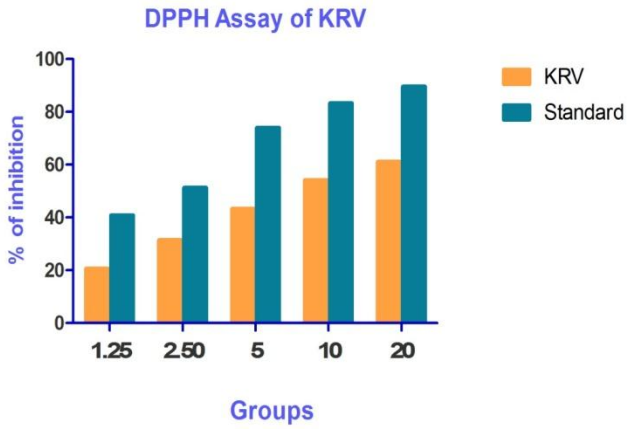


Chart no: 2 Anti- Oxidant activity

This indicated that % of inhibition increased with increase in concentration of both the standard and *Kandha Rasa Villai*extract. But the *Kandha Rasa Villai* extract has lower DPPH scavenging activity than that of standard. From the present study, it was concluded that the *Kandha Rasa Villai* extract has good antioxidant activity at higher concentrations.

It is known that oxidative stress induced cell damage not only through damage to proteins, lipids and DNA. It may also alter signaling pathways redox sensitive to changes involved in the response of apoptosis. The antioxidants are currently the subject of many studies because, in addition to some interest in the preservation of comestibles, they could be useful in the prophylaxis and treatment of diseases in which oxidative stress is implicated. Many studies realized on natural products have proven that they are especially phenolic compounds who are responsible for their antioxidant activity.

Several studies have shown the link between the traditional drug formulations rich in antioxidants and the incidence of certain diseases such as **cancer**, heart disease, diabetes and other diseases related to aging.Phenolic compounds could prevent cancer by the action antioxidant and / or the modulation of several functions of proteins. Phenolic compounds can prevent carcinogenesis by affecting the molecular events in the triggering, promotion and progression stages.

Some phenolic compounds (phenolic acids, flavonoids, quinones, coumarins) have proved an effective antioxidant activity and also had anticancer activities/ anticarcinogenic/ antimutagenic.

Here, the reactive oxygen species (ROS) may be the triggers apoptotic process. In recent years they have been described numerous properties of these compounds such as the ability to inhibit cell cycle, proliferation cellular and oxidative stress, and induce detoxification enzymes, apoptosis, and stimulate the immune system. It is therefore hypothesized that *Kandha Rasa Villai* of its antioxidant power could “to repair" Cancer cells.

6. CONCLUSION

Cervical cancer, which accounts for the second most common malignancy among women worldwide, is highly radio-resistant. The other chemotherapy drugs also deliver intolerable side effects which are worsening than disease. This made the urgency for a movel anticancer drug which cures cervical cancer in human friendly approach.

The trial drug *Kandha Rasa Villai* was selected from the Siddha literature "VeeramaamunivarVaakadaththirattu", written by S.P. Ramachandiran, which was categorized by the Department of AYUSH as classical text.

The trial drug KRV fulfilled all parameters of testing protocol for Chendooram which was assigned by AYUSH. It showed the accurate production and potency of *Kandha rasavillai.*

Analysis for physico-chemical characters exposed better bio-availability and richness of its mineral content. The experiments for analyzing acid and basic radicals exhibited presence of inorganic matters, favor this study.

Various instrumental analysis of *Kandha rasa villai* like FT-IR spectroscopy, XRD, SEM demonstrated its chemical constituents, functional groups and particle size to support its indication counter to cervical cancer.

The microbial load of trial drug was also considered for its potential.

Under OECD guidelines, the acute and 28 days repeated oral toxicity studies said about safety of *Kandha Rasa Villai* at particular dose level.It is very useful in therapeutic dose determination.

The pharmacological activities are justified by anticancer effect on HeLa cell line, antitumor effect on SIHA cell lines and quantitative measurement of antioxidants by DPPH assay.

KRV could be a scientifically validated and proven drug for its anticancer effect.

7. FUTURE SCOPE

Trial drug for the study *KANDHA RASA VILLAI* (KRV) was taken from the classic Siddha literature *Veeramaamunivar VaakadathThirattu*, Written by the S.P.Ramachandiran. Its validation for its Anti-cancer nature was completed at preliminary level. The result enhanced and assured its Anti-cancer property against cervical cancer.

More specific experiments on animal models and also clinical trails are required to understand the exact molecular mechanisms of action. So it could be used worldwide in treatment of cervical cancer and satisfy the hunger for the non-violent antineoplastic medicines.

8. SUMMARY

Trial drug *KANDHA RASA VILLAI (KRV)* was selected from the classic Siddha literature "Veeramaamunivar Vaakadaththirattu", written by S.P. Ramachandiran for anti-cancer, anti-tumour, anti-oxidant activities.

The dissertation started with an introduction explaining about the Siddha concept, prevalence of cervical cancer and root of the test drug in treating cancer cervix.

- Review of literature in various categories was carried out. It was elaborated under Gunapadam and modern aspect of ingredients, Siddha and modern aspect of that disease, pharmaceutical aspect and pharmacological aspect in both Siddha and modern.
- All the ingredients were identified and authenticated by the experts.
- The compound was prepared properly by given procedure in an appropriate situation.
- The end product underwent standardization parameters in Siddha.
- The drug was subjected to analysis such as physicochemical, biochemical and instrumental analysis which provided the ingredients present in the drug thus it accounts the efficacy of the drug.
- The sample was also analyzed for antimicrobial activity to ensure its accuracy.
- For the study protocol, required animals were approved by the IAEC under CPCSEA.
- Toxicological study was made recording to OECD guidelines comprising both acute and repeated oral dose 28days toxicity studies in Wistar albino rats. It showed the safety of the drug which attributes its utility in long time administration.
- Pharmacological studies were completed. It revealed the anti-cancer, anti-tumor and anti-oxidant activities of *Kandha Rasa Villai*.
- Result and discussion gives the essential validations to prove the potency of the drug.

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