SCIENTIFIC VALIDATION OF BRONCHODILATOR, ANTI-HISTAMINE AND ANTI-OXIDANT ACTIVITIES OF SIDDHA HERBO-MINERAL FORMULATION *"NAGARASINGADHI CHOORANAM"* IN IN-VIVO AND IN-VITRO MODELS

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Scientific Validation of Bronchodilator, Anti-Histamine and Anti-Oxidant Activities of Siddha Herbo-Mineral Formulation *"Nagarasingadhi Chooranam*" in In-vivo and In-vitro models** is a bonafide and genuine research work carried out by me under the guidance of **Dr.V.Velpandian M.D(S).,Ph.D.,** 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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CERTIFICATE BY THE GUIDE

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ABBREVIATIONS

ACh	Acetylcholine
ALT	Alanine Transaminase
ANOVA	Analysis Of Variance
AMP	Adenosine Monophosphate
AST	Aspartate aminotransferase
BUN	Blood Urea Nitrogen
cAMP	Cyclic Adenosine Monophosphate
CD4+	Cluster of Differentiation 4
CMC	Carboxy Methyl Cellulose
COPD	Chronic Obstructive Pulmonary Disease
CPCSEA	Committee for the Purpose of Control & Supervision of Experimental Animals
DC	Differential count
DPPH	1-Diphenyl-2-Picryl-Hydraoxzyl
DTNB	Dithionitrobenzoic acid
E	Eosinophil
ECRHS	European Community Respiratory Health Society
ED_{50}	Effective Dose
ESR	Erythrocyte Sedimentation Rate
FEV	Forced Expiratory Volume
FTIR	Fourier Transform Infrared Spectroscopy
FVC	Forced Vital Capacity
GINA	Global Initiative for Asthma
GM-CSF	Granulocyte Macrophage-Colony Stimulating Factor

GOT	Glutamate Oxaloacetate Transaminase
GPT	Glutamate Pyruvate Transaminase
GSH	Glutathione
Hb	Haemoglobin
HL	Human Leukemic cell lines
HPTLC	High Performance Thin Layer Chromatography
IAEC	Institutional Animal Ethical Committee
ICPOES	Inductively Coupled Plasma Optic Emission Spectroscopy
IL	Interleukin
ISAAC	International Study of Asthma and Allergies in Childhood
LTC_4	Leukotriene C4
MCV	Mean Corpuscular Volume
NSAID	Non-Steroidal Anti-Inflammatory Drugs
NSC	*Nagarasingadhi chooranam*
OECD	Organisation for Economic Co-Operation Development
PCT	Pre-Convulsion Time
PCV	Packed Cell Volume
PEFR	Peak Expiratory Flow Rate
PGE2	Prostaglandin E2
RBC	Red Blood Corpuscles
SEM	Scanning Electron Microscope
SGOT	Serum Glutamate Oxaloacetate Transaminase
SGPT	Serum Glutamate Pyruvate Transaminase
TBA	Thiobarbituric acid

TBARS	Thiobarbituric acid Reactive Substances
TNF	Tumour Necrosis Factor
TLC	Thin Layer Chromatography
UV	Ultra Violet
WBC	White Blood Corpuscles
WHO	World Health Organization

1. INTRODUCTION

Siddha system is one of the ancient traditional systems of medicine of our country compiled by the Siddhars. The term "Siddha" is derived from the word "*Siddhi*" that is Attainment of Perfection or Achievement of Heavenly bliss [(1)]. Among all the system of medicine practised all over the world the Siddha system is undoubtedly the ancient,transcending many centuries.The traditional Siddha System of Medicine is not only used to cure, but also to prevent the onset of disease. It is the first and foremost system of medicine to emphasise health as the perfect state of physical, psychological, social and spiritual wellbeing component of human being.

Siddhars were the people who aimed for spiritual perfection to reach the ultimate goal of life. They were the greatest scientists who were supposed to have lived at a very early period. Siddhars were highly spiritual and intellectual personalities combined with supernatural powers who have the knowledge of healing art in curing many diseases of the mankind.

Siddha medicine aims at the perfection of health.Siddhars simplify the concept of how a human body is exposed to various types of diseases. The main principle is that the human body is constituted with three basic humours named as *Vatha, pitha* and *kabam* which on derangement leads to diseased conditions or ill-health. And the deranged *Vatha, pitha* and *kabam* are termed as "*kuttram"* [(2)].

The imbalance of humours especially *Kabam* and *Vatham* in Respiratory system modifies the air passage by secreting inflammatory mediators causing broncho constriction suddenly leading to difficulty in breathing. Siddhars described this condition in literatures and named it as '***Swasakaasam*** or ***Iraippu***'. The symptoms of the disease '*Swasakaasam*' are related to 'Bronchial Asthma' [(3)].

According to World Health Organisation (WHO), Bronchial Asthma is the disease characterised by recurrent attacks of breathlessness and wheezing which vary in

severity and frequency from person to person.Asthma attacks all age groups but often starts in childhood [4].

World Asthma Day is an annual event organized by GLOBAL INITIATIVE FOR ASTHMA (GINA) to improve asthma awareness and care around the world. World Asthma Day takes place on the first Tuesday of May [5].

During an asthma attack, the lining of the bronchial tubes swells, causing the airways to get narrow and thereby reducing the air inflow and outflow of the lungs. The typical symptoms include wheeze, cough, chest tightness and dyspnoea which are accompanied by the presence of airflow obstruction [6]. The factors precipitating Asthma are cold air, tobacco smoke, dust, acrid fumes, emotional stress, respiratory infection,exercise, drugs like Beta blockers, Aspirin, allergens[7].

As per ECRHS (European Community Respiratory Health Society) and ISAAC (International Study of Asthma & Allergies in childhood) the prevalence of Asthma increased steadily over the later part of the 20th century.

About 300 million people around the world widesuffer from Bronchial Asthma. India has estimated 15-20 millionAsthmatics. As per the survey report of WHO, In India rough estimates indicates a prevalence of between 10% and 15 % in 5-11 years old children[4].

Although the development, course of disease and response to treatment are influenced by genetic determination, the rapid rise in the prevalence of asthma implies that environmental factors are critically important in terms of its expression.

Status Asthmaticus, secondary infections such as Bronchitis and Tuberculosis, Emphysema of lungs, Later stage of Right Heart failure called Chronic cor pulmonale, Bronchiectasis, Pneumothorax, Pneumo mediastinum are the complications of Bronchial Asthma. Acute severe Asthma is termed as Status Asthmaticus is a medical emergency condition which is characterised by tachycardia, tachypnoea, sweating, pulsus paradoxus and altered level of consciousness. Life threatening factors of Bronchial

Asthma are central cyanosis, silent chest, severe hypoxaemia and altered consciousness [7].

According to WHO, mortality due to asthma is not comparable in size to the day to day effects of the disease. Although largely avoidable, Asthma tends to occur in epidemics and affects young people. Worldwide the death from this condition has reached over 180,000 annually.

Asthma cannot be cured but could be controlled. Controlling Asthma needs a good bronchodilator and also to control the hypersensitivity reaction. The famous bronchodilator drugs in market are Salbutamol, Theophylline, Isoprenaline. These drugs give only temporary relief to the patient with the reoccurrence of this disease and thereby causing adverse effects. For example. Salbutamol causes some adverse effects such as muscle tremors, palpitation, restlessness, nervousness. Theophylline causes convulsion,shock, insomnia. Isoprenaline produces tachycardia [8].

Some of the popular Siddha drugs given for Bronchial Asthma are *Mahavasantha kushmagaram, Poorna chandrodayam, Swasakudori mathirai, Thalisadi choornam, Pavala parpam, Thalaga parpam,Vasantha kushmagaram, Gowri chinthamani, Kashthoori karuppu, Thalaga karuppu, Karpooradhi chooranam, Sivanar Amirtham, Kandangkathiri chooranam, Shayakulanthaga chendooram, Arakku thailam, Soombu theneer, Adhatodai kudineer, Thooduvalai nei, Nochi Thailam*[3].

To control bronchial Asthma the world requires safety traditional fast acting bronchodilators and potential drugs having anti histamine activity to control the hypersensitivity reaction without any side effects.

Siddha Herbo-Mineral formulations provide biosafe and relieve the broncho constriction and other symptoms by their fast acting properties. They do not possess any adverse drug reactions. The author was interested in administering Herbo-Mineral preparation "***Nagarasingadhi chooranam***" an effective bronchodilator indicated for Bronchial Asthma in the Siddha literature "*Anuboga Vaidhiya Navaneedham* (Part 8)"[9].

In this preparation most of the medicinal plants have Bronchodilator activity. For example: *Zingiber officinale*, *Terminalia bellerica*, *Solanum surattense*, *Justicia adhatoda*, *Solanum trilobatum*, *Nardostachys jatamansi* and *Piper nigrum*.

Some of the ingredients possess Anti-histamine property. For example: *Clerodendrum serratum, Sodium chloride impura, Piper longum*.

The plants which exhibit Antispasmodic property are *Terminalia chebula, Solanum melongena.* Antioxidant plants in this preparation are *Costus speciosus, Phyllanthus emblica* and*Nymphae alba.*

The plants with Expectorant action are *Rhus succedanea, Costus speciosus, Terminalia chebula.* The herbs which are specifically used in treating Bronchial Asthma with Anti Asthmatic property are included in this preparation. They *are Justicia adhatoda, Rhus succedena, Clerodendrum serratum, Terminalia bellerica* and *Terminalia chebula.*

Hence all these enriched plants used especially formany respiratory disease are combined together in this Herbo-Mineral formulation "*Nagarasingadhi chooranam*", will be used as bronchodilator in treating Bronchial Asthma (*Swasakaasam* or *Iraippu*). Still now no scientific research works have been carried out on this Herbo-Mineral preparation. Therefore the author is interested to conduct Bronchodilator, Anti-histamine and Anti-oxidant activity of ***Nagarasingadhi chooranam*** for Bronchial Asthma.

2. AIM AND OBJECTIVES

AIM

The aim of this dissertation is to establish the Scientific Validation of the Bronchodilator, Anti-Histamine and Anti-Oxidant property of ***Nagarasingadhi chooranam*** for Bronchial Asthma.

OBJECTIVES

The main objective of the present study is to high light the efficacy of ***Nagarasingadhi chooranam*** on ***Swasakasam***, the following methodology was adopted to evaluate the drugs and its standardization studies

- Collection of various Siddha and modern literature relevant to the study.
- Identification of the drugs in this formulation.
- Preparation of ***Nagarasingadhi chooranam*** as per the classical Siddha literature.
- Physicochemical and phytochemical investigation of the test drug.
- Evaluate bio-chemical analysis of the test drug to derive acidic and basic radicals.
- To estimate the present of elements, functional groups and particle size through instrumental analysis of the trial drug.
- Evaluation of the Acute and 28 days repeated dose oral Toxicity of test drug according to OECD guidelines.
- Evaluation of pharmacological study of the drug through the following activities
    - Evaluation of Bronchodilator activity
    - Evaluation of Anti- histamine activity
    - Evaluation of Anti -oxidant activity of ***Nagarasingadhi chooranam***.

3. REVIEW OF LITERATURE

DRUG REVIEW

The trial drug "***Nagarasingadhi Chooranam"*** was taken from the Siddha literature "*Anuboga Vaidhiya Navaneedham* (Part 8)" for treating Bronchial Asthma. The ingredients of the drug are

1. *Chukku (Zingiber officinale)*
2. *Milagu(Piper nigrum)*
3. *Thippilli (Piper longum)*
4. *Sadamanjil (Nardostachys jatamansi)*
5. *Siruthaekku (Clerodendrum serratum)*
6. *Karkadaga singhi (Rhus succedanea)*
7. *Koshtam (Costus speciosus)*
8. *Nelli mulli (Phyllanthus emblica)*
9. *Kadukkai thol (Terminalia chebula)*
10. *Thandrikkai thol (Terminalia bellirica)*
11. *Induppu (Sodium chloride impura)*
12. *Sengazhuneer kizhangu (Nymphaea alba)*
13. *Kandangatri vaerpattai (Solanum xanthocarpum)*
14. *Thoodulai vaerpattai (Solanum trilobatum)*
15. *Adathodai vaerpattai (Justicia adhatoda)*
16. *Siruvazhuthalai vaerpattai (Solanum melongena)*

3.1. GUNAPADAM REVIEW

CHUKKU

Scientific name : *Zingiber officinale*

Other names : *Nagaram, Atagam, aartharagum, chonndi, chowpannaum, verkombu, nava suru, ullarntha inji, Vidam moodiya amirtham*[10A, 11A].

Vernacular names

Tamil : *Chukku*

English : Dried ginger

Telugu : *Sonti*

Malayalam : *Shukka*

Kannadam : *Ona shunti or Sunti*

Sanskrit : *Nagaram*

Hindi : *Sonth*

Part used : Dried Rhizome

Properties :

Suvai (Taste) : *Kaarppu*

Thanmai (Nature) : *Veppam*

Pirivu (Bio-Transformation): *Kaarppu*

Actions :

- ❖ Stimulant
- ❖ Stomachic
- ❖ Carminative

General Characters:

"சூலைமந்தம் நெஞ்செரிப்பு தோடமேப் பம்மழலை

மூலம் இரைப்பிருமல் மூக்குநீர்- வாலகப

தோடமதி சாரந் தொடர்வாத குன்மநீர்த்

தோடம்ஆ மம்போக்குஞ் சுக்கு".

- அகத்தியர் குணவாகடம்

Indications :

Dried ginger was used for indigestion, gastric irritation, anal diseases, asthma, cough, diarrhoea, sinusitis, anaemia and fever.

Therapeutic uses:

- Dried ginger powder one pinch dosage with cow milk for loss of appetite.
- Make it into powder form taken with sugarcane juice in early morning for burning sensation in stomach.
- Dried ginger with sugar candy powder taken with tender coconut in morning and evening for dyspnoea and chest pain after heavy working.
- Dried ginger decoction for poisonous type of fever.
- For relieving from toothache chew one piece of dried ginger[(12A)].

MILAGU

Scientific name : *Piper nigrum*

Other names : *Malayali, Masam, Sarumabandam, Kayam, Kalinai, Miriyal.*

Vernacular names

Tamil : *Milagu*

English : Black pepper

Telugu : *Miriyalu*

Malayalum : *Kurumilagu*

Kannadam : *Menasu*

Sanskrit : *Maricha*

Hindi : *Kali-mirch*

Part used : Dried fruit

Properties :

Suvai (Taste) : *Kaippu, Kaarppu*

Thanmai (Nature) : *Veppam*

Pirivu(Bio- Transformation) : *Kaarppu*

Actions :

- ❖ Carminative
- ❖ Stimulant
- ❖ Anti-vadha
- ❖ Antidote

General characters

"சீதசுரம் பாண்டு சிலேத்மங் கிராணிகுன்மம்

வாதம் அருசிபித்தம் மாமூலம்- ஓதுசன்னி

யாசமபஸ் மாரம் அடன்மேகம் காசமிவை

நாசங் கறிமிளகினால்."

- அகத்தியர் குணவாகடம்

Indications:

It cures Anaemia, gastric ulcer, giddiness, diarrhoea, dysentery, vomiting, anal fissure and Cataract [(11B)].

Therapeutic uses:

- Dried unripe fruits are prescribed in cholera, dyspepsia, flatulence and various gastric ailments.
- Powdered form black pepper with onion and salt, apply this mixture on scalp to cure alopecia and to increase the hair growth.
- Black pepper paste for externally applied to boils.
- Powder of black pepper used as tooth powder [(12B)].

THIPPILI

Scientific name **:** *Piper longum*

Other names **:** *Pippli, Aadhi,Kaaman, Sowndi, Kanam, Saram, Koli, Ambu, Aathimarunthu, Kanai*.

Vernacular names

Tamil : *Thippili*

English : Long pepper

Telugu : *Pippilu*

Malayalam : *Thippili*

Kannadam : *Hippili*

Sanskrit : *Pippali*

Hindi : *Pipar*

Part used **:** Dried fruit and Roots

Properties **:**

Suvai (Taste) : *Kaarppu*

Thanmai (Nature) : *Veppam*

Pirivu (Bio- Transformation): *Kaarppu*

Actions **:**

- Carminative
- Stimulant
- Expectorant
- Antiseptic
- Febrifuge

General characters

"கட்டி யெதிர்நின்று கடுநோயெல் லாம்பணியும்
திட்டி வினையகலும் தேகமெத்த-புட்டியாம்
மாமனுக்கு மாமனென மற்றவர்க்கு மற்றவனாங்
காமமெனுந் திப்பிலிக்கும் கை."

- தேரன் வெண்பா

Indications:

It relief kabham related diseases and strengthen the body [11C].

Therapeutic uses

- Powered long pepper with honey will relieve cough, cold, asthma, hoarseness and hiccough.
- Long pepper powder with honey and betel leaf juice cures fever.
- A mixture of long pepper, long pepper root, black pepper and ginger in equal parts was a useful preparation in colic, flatulence.
- Powered form of long pepper seeds with ghee for aphrodisiac activity [12C].

SADAMANJIL

Scientific Name	**:**	*Nardostachys jatamansi*
Synonyms	**:**	*Nardostachys grandiflora*
Other Names	**:**	*Sadamaasi, Jadamanji, Paisasi, Sadilai, Sadamanji*

Vernacular Names

Tamil	:	*Sadamanji*
English	:	Valerina root
Telugu	:	*Jadamamsi*

Malayalam : *Manij*

Hindi : *Jatamansee*

Parts used : Rhizome

Properties :

Suvai (Taste) : *Inippu, Kaarppu*

Thanmai (Nature) : *Veppam*

Pirivu (Bio- Transformation): *Kaarppu*

Actions :

- ❖ Stimulant
- ❖ Antispasmodic
- ❖ Expectorant

General Characters:

"குட்டஞ் சிலந்திவிடம் கோர புராணசுரம்
உட்டினங்கால் பேதிகண்ணோய் ஒட்டிருமல் - சொட்டிரத்த
பித்தமிரைப் பேகும் பெருங்கோரை என்றுரைக்குஞ்
சுத்தசடா மாஞ்சிலை சொல்."

- அகத்தியர் குணவாகடம்

Indications:

Sadamanjil was used for Cough, Asthma, Hypertension, Diarrhoea[11D].

Therapeutic uses of Sadamanjil:

- Powder of rhizome was used as expectorant for cough with sputum.
- Rhizome decoction is used for spasmodic hysteria
- Oil prepared from rhizome was used for grey hair externally[13A].

SIRUTHAEKKU

Scientific name **:** *Clerodendrum serratum*

Other name **:** *Kanduparangi*

Vernacular names

Tamil : *Siruthaekku*

English : Beetle killer

Telugu : *Gantu bharag*

Malayalum : *Cherutekka*

Kannadam : *Bharangi*

Sanskrit : *Bharangi, Barbara*

Hindi : *Gant- Bharangi*

Part used **:** Root, Leaves

Properties **:**

Suvai (Taste) : *Kaippu*

Thanmai (Nature) : *Veppam*

Pirivu(Bio- Transformation) : *Kaarppu*

Actions **:**

- ❖ Stimulant
- ❖ Expectorant
- ❖ Anti - inflammatory

General characters :

"கண்டுபா ரங்கியெனுஞ் சிறுதேக குண்டேல்,

காலெங்கே பித்தமெங்கே கபந்தா னெங்கே

தொண்டுதொட்டுத் தொடர்சுவாச காச மெங்கே

சுரமெங்கே வெரியெங்கே தொனிநோ யெங்கே

மிண்டுபுரி பீநசநீர்க் கோவை யெங்கே

வெளிநீருண் ணீரெங்கே விறற்கா லெங்கே

அண்டுபடாச் சீதசுரங் கடுப்பு மெங்கே

யழலையக நோயெங்கே யறைகு வீரே!"

- தேரையர் குணவாகடம்

Indications:

It cures Bronchial asthma, fever, sinusitis, myalgia [11E].

Therapeutic uses:

- Root of beetle killer and dried ginger powder are mixed with honey to cure Asthma.
- Leaves of beetle killer are grinded with decoction of triphala and paste was applied on boils and oedema [12D].
- The root decoction was effective in Bronchitis.
- A decoction of root with ginger and coriander is taken for relieving nausea[13B].
- The leaves are used as a vermifuge and as bitter tonic. The anthelmintic property is due to the bitter principle presented in the leaves [14A].

KARKADAGA SINGHI

Scientific Name **:** *Rhus succedanea*

Vernacular Names

Tamil : *Karkadaga singhi*

English : Galls

Hindi : *Kakarasinigi*

Malayalam : *Karkada srungi*

Kannadam : *Karkataka shrinngi*

Sanskrit : *Karkatashring*

Telugu : *Karkataka sringi*

Parts Used : Galls and Fruits

Properties **:**

Suvai (Taste) : *Thuvarppu*

Thanmai (Nature) : *Veppam*

Pirivu (Bio- Transformation) : *Kaarppu*

Actions **:**

- ❖ Astringent
- ❖ Tonic
- ❖ Nutritive
- ❖ Expectorant
- ❖ Digestive.

General Characters :

"கர்க்கடக சிங்கி கபங்காசம் ஈளையொடு

முக்கல் கிராணி முதிரிரைச்சல்-பொக்கெனவே

சாடுகின்ற பேதியையுஞ் சாடும் அரிவையரைக்

கூடுதிறங் கொடுக்குங் கூறு."

- அகத்தியர் குணவாகடம்

Indications :

Galls are useful in treating Cough, Asthma, Irritability of stomach, Fever and Diarrhoea [11F].

Therapeutic Uses:

- Galls are used in decoction form to treat leucorrhoea.
- The powder of galls was administered to infants suffering from diarrhoea resulting from teething and to the infants with Bronchial troubles.
- Gall paste was recommended as external application in psoriasis [13C].

KOSHTAM

Scientific Name : *Costus speciosus*

Other Names : *Kottam, Kura, Oli*

Vernacular Names

Tamil : *Kottam.*

English : Costus root.

Telugu : *Kostam.*

Malayalam : *Kottam.*

Kannada : *Koshtam.*

Duk. : *Pachak.*

Sanskrit : *Koshtam.*

Arabian : *Qusth.*

Persian : *Kosht*

Kottam was the dried root of *Costus speciosus (*Koenig ex Retz), planted around the northwest part of Himalayas. It was cultivated in Himachal Pradesh, Uttaranchal and Sikkim. It grows in *Kurinchi thinai*. The root was used to protect from insect bite and its aromatic property.

Roots collected in September to October

Varieties **:** It has two Varieties in Nature i.e., – white and red.

Trade name **:** Keyu

Parts used **:** Rhizome

Properties **:**

Suvai (Taste) : *Kaippu*

Thanmai (Nature) : *Veppam*

Pirivu (Bio- Transformation): *Kaarppu*

Actions **:**

- ❖ Stomachic
- ❖ Expectorant
- ❖ Tonic
- ❖ Stimulant
- ❖ Diaphoretic

General characters:

"நாட்டிலுறு வெட்டை நடுக்கம் எனுநோய்கள்
கொட்டமெனச் சொன்னால் குலையுங்காண்- கூட்டிற்
சுரதோடந் தொண்டை நோய்தோலாத பித்தம்
பரதேசம் போமே பறந்து."

- அகத்தியர் குணவாகடம்

Indications :

The costus root was used for treating the eye, jaw, abdomen, neck, head, tongue and mouth diseases, fever, puffiness, vatha, piles, ulcers, bronchial asthma, rat poisoning, snake venom, abscess and also the psychiatric disorders (*Paithiyam*) [(11G)].

Therapeutic uses of Rhizome:

Bitter, astringent, acrid, cooling, purgative, aphrodisiac, anthelmintic, depurative, febrifuge, expectorant and tonic, also beneficial in asthma, anaemia, bronchitis, leprosy, flatulence, constipation, fever, skin diseases and inflammation.

- The costus root was mixed with coriander seeds in equal quantity, then grinded and used for external application in scalp ulcers or *kottam* was grinded with butter and applied for the same.
- The preparation, *kotta thylam* was used as external application for piles.
- *Kottam* was mixed with the acorus calamus root and honey, and used for treating *verinoi.*

Chemical constituents:

- Rhizomes - Tiogenin and diosgenin
- Stem - a-amyrin stearate, b-amyrin and lupeol
- Leaves - Palmitates[(15A)]

NELLI

Scientific name : *Phyllanthus emblica*

Other names : *Aambal, Nellimulli, Thathri, Korangam, Amalagam, Alagam,Nellikai*

Other Varieties : *Karunelli, Arunelli*

Vernacular names

Tamil : *Nelli*

English : Emblic myrobalan, Indian gooseberry

Hindi : *Amla, Aonla*

Sanskrit : *Amalaki, Dhatriphala*

Telugu : *Usirika*

Kannada : *Nelliayi*

Part used **:** Leaf, Flower, Bark, Root, Seed, Dried fruit

Properties

Suvai (Taste) : *Inippu, Pulippu, Thuvarppu*

Thanmai (Nature) : *Thatpam*

Pirivu (Bio- Transformation): *Inippu*

Actions **:**

- Coolant
- Laxative
- Diuretic

General characters

"பித்தமன லையம் பீநசம்வாய் நீர்வாந்தி

மத்தமலக் காடும் மயக்கமுமில்-ஒத்தவுரு

வில்லிக்கா யம்மருங்கா மென்னாட்கா லந்தேர்ந்தே

நெல்லிகா யம்மருந் துணீ."

"நெல்லிகாய்க் குப்பித்தம் நீங்கு மதன்புளிப்பால்

செல்லுமே வாதமதிற் சேர்துவரால்- சொல்லுமையம்

ஓடுமியதைச் சித்ததில் உன்ன அனலுடனே

கூடுபிற மேகமும் போங் கூறு"

-தேரையர் குணவாகடம்

Indications :

It cures Sinusitis, Vomiting, Constipation, and Giddiness[(11H)].

Therapeutic uses

- Fresh fruits : It is used in inflammations of the lung.
- Juice of the fruit : Added with *Piper longum* to stop hiccup
- Dried fruit : Useful in haemorrhage and dysentery
- Seeds : Given as a febrifuge and in diabetes
- Root bark : Used in Stomatitis [(13D)].

KADUKKAI

Scientific name : *Terminalia chebula*

Other names : *Anthan, Abhayan, Amudham, Devi, Divya, Rohini, Ammai, Abaranam,Aritaki,*Varikkai, Jeevandhi

Vernacular names

Tamil : *Kadukkai*

English : Myrobalan

Hindi : *Harre, Harad, Harar*

Sanskrit : *Haritaki, Abhaya, Kayastha, Siva, Pathya*

Telugu : *Karaka, Karakkaya*

Kannadam : *Alalekai*

Other Varieties : *Visayan, Arokini, Prithivi, Amrita, Sivanthi, Thiruvirti, Abayan*

Part used : Dried fruit

Properties:

Suvai (Taste) : *Thuvarppu, Inippu, Kaarppu, Kaippu, Pulippu*

Thanmai (Nature) : *Veppam*

Pirivu (Bio- Transformation) : *Kaarppu*

Actions :

- Digestive
- Expectorant
- Laxative
- Appetizer
- Nutrient

General characters

"தாடை கழுத்தக்கி தாலு குறியிவிடப்

பீடை சிலிபதமுற் பேதிமுடம்- ஆடையெட்டாத்

தூலமிடி புண்வாத சோணிகா மாலையிரண்

டாலமிடி போம்வரிக்கா யால்"

- அகத்தியர் குணவாகடம்.

Indications : It cures Obesity, jaundice, constipation, Filariasis[11I].

Therapeutic uses

- Terminalia chebula are used in asthma, fever, urinary diseases
- Used as a gargle in sore mouth and stomatitis, spongy and ulcerated gums
- Paste formed by Rubbing the fruit with a little water mixed with castor oil and applied to burns and scalds[13E].

THAANDRI

Scientific name : *Terminalia bellirica*

Synonyms : *Terminalia puneta* Roxb

Myrobalanus belerica.Gaertn [16]

Other names : *Sakatham, Thanikai, Thirilingam, Aaramam, Kalanthundri,Akkantam, Amutam, Erikatpalam.*

Vernacular Names

Tamil : *Thantri*

English : Beleric myrobalan

Hindi : *Bahera*

Sanskrit : *Bibhitaka, Vibhita, Aksa, Aksaka*

Telugu : *Thanikkaya*

Kannada : *Tarekai, Shantikayi*

Part used : Dried fruit

Properties :

Suvai (Taste) : *Thuvarppu*

Thanmai (Nature) : *Veppam*

Pirivu (Bio- Transformation): *Kaarppu*

Actions :

- ❖ Expectorant
- ❖ Astringent
- ❖ Tonic.

General characters

"சிலந்திவிடம் காமியப்புண் சீழான மேகங்

கலந்துவரும் வாதபித்தங் காலோ- டலர்ந்துடலில்

ஊன்றிக்காய் வெப்ப முதிரபித் துங்கரக்குந்

தான்றிக்காய் கையிலெடுத் தால்."

"ஆணிப்பொன் மேனிக் கழகும் ஒளியுமிகும்

கோணிக்கொள் வாதபித்தக் கொள்கைபோம்- தானிக்காய்

கொண்டவர்க்கு மேகமறும் கூறா அனற்றணியும்

கண்டவர்க்கு வாதம்போம் காண்"

-பதார்த்த குண விளக்கம்

Indications : It cures Hypertension, leucorrhoea, insect bite [11J].

Therapeutic uses

- Along with this dried fruit, Salt and long pepper fruit are given in the form of decoction in cough, hoarseness, sore throat and dyspepsia.
- Kernel was narcotic and astringent and used as an application to inflamed parts [13F].

INDUPPU

Chemical Name : *Sodium chloride impura*

Other Names : *Sainthavam, Sinthooram, Santhiranuppu, Mathiyuppu, Mathikoormai, Minthaachchol, Vaani, Sainthalavanam, Mathilavanam.*

Actions :

- ❖ Laxative
- ❖ Carminative
- ❖ Diuretic
- ❖ Stomachic

General Characters

"அட்டகுன்ம மந்தம் அசிர்க்கரஞ் சூர்சீதபித்தந்
துட்டவையம் நாடிப்புண் டோடங்கள் - கெட்டமலக்
கட்டுவிட விந்தையக் காமியநோய் வன்கரப்பான்
விட்டுவிட விந்துப்பை விள்."

-குணபாடம் தாது சீவம் வகுப்பு

Indications :

It cures Gastric ulcer, Constipation, Cough [17A].

Therapeutic uses :

- As a Digestive, a compound powder made of Rock salt, *Terminalia chebula*, *Phyllanthus emblica* and Long pepper in equal parts, recommended in doses of 10 grains twice a day.
- Used in the treatment of sprains externally
- Beneficial in dyspepsia and other abdominal disorders [18].

SENGAZHUNEER

Scientific Name : *Nymphaea alba*

Other Names : *Kazhuneer, Kuvalai, Urbalam, Kuvalayam*

Vernacular Names

Tamil : *Sengazhuneer*

English : Water Lily

Malayalam : *Chenkazhuneer*

Telugu : *Nirucncha*

Sanskrit : *Kumud*

Kshmiri : *Brimposh*

Parts used : Seeds, flowers and rhizome.

Properties :

Suvai (Taste) : *Inippu*

Thanmai (Nature) : *Thatpam*

Pirivu (Bio- Transformation): *Inippu*

Actions :

- ❖ Refrigerant
- ❖ Anti pitha[11K].

General Characters:

"கண்ணுங் குளிர்ந்திருக்குங் காசம்போம் பித்தமறு

மெண்ணரிய மேக மிடையுங்காண் -.பெண்ணேகேள்

பொங்கெழின் மாதைப் புணர்ந்தவழ லுந்தணியுஞ்

செங்கழு நீர்க்கிழங்காற் றேர்."

-பதார்த்த குண விளக்கம் [19A]

Indications :

It cures cough, dyspepsia, burning sensation of the eyes [10B].

Therapeutic Uses :

- Decoction of rhizomes was given in diarrhoea; alcoholic extract has mild sedative and spasmolytic action.
- Seeds are used in diabetes
- Decoction of flowers was valued as a cardiac tonic in palpitation of heart, effective in combating thirst, burning sensation of the body and internal haemorrhage [20].

KANDANGATHRI

Scientific Name : *Solanum xanthocarpum*

Synonyms : *Solanum surattense* .Burm. f.

Solanum virginianum

Vernacular Names

Tamil : *Kandangkattari*

English : Wild eggplant, Bitter sweet woody nightshade, Yellow berried night shade

Telugu : *Nela Mulaka-Vakudu*

Malayalam : *Vellottuvalutina*

Hindi : *Kateli*

Sanskrit : *Kanta-karika*

Kannadam : *Nela-gulla*

Parts Used : Leaf, flower, root, fruit, seed

Properties :

Suvai (Taste) : *Kaarppu*

Thanmai (Nature) : *Veppam*

Pirivu(Bio- Transformation) : *Kaarppu*

Actions:

- Expectorant
- Diuretic
- Carminative

General Characters:

"காச சுவாசங் கதித்தகூய மந்தமனல்

வீசுரஞ் சன்னி விளைதோடம் - ஆசுறுங்கால்

இத்தரையு ணிற்கா எரிகாரஞ் சேர்க்கண்டங்

கத்திரியுண் டாமாகிற் காண்."

"வேரிலைபூ காய்பழமவ் வித்துமதன் பட்டையுமிவ்

வூரி லிருக்க உடற்கனப்பும் - நீராய்

வரும்பீந சங்கயஞ்சு வாசமுந்தங் காதே

அருங்கண்டங் கத்தரியு ளார்."

- அகத்தியர் குணவாகடம்

Indications: It cures Cough, loss of appetite, Asthma[(11L)].

Therapeutic Uses:

- A decoction of the leaves and root with long pepper in dose of half to one ounce with honey was an excellent mixture used in chronic bronchitis, asthma.
- Juice of berries are beneficial in sore throat
- Leaf juice is a combination with black pepper prescribed in Rheumatism.
- Root decoction in combination with *Tinospora cordifolia* was useful in cough and fever [(13G)].

THOODULAI

Scientific name : *Solanum trilobatum*

Other names : *Thuthuvalai, Alarkkam, Singavalli*

Vernacular Names

Tamil : *Thuthuvalai*

English : Climbing brinjal

Sanskrit : *Alarka*

Telugu : *Mullamusti*

Malayalam : *Mullakaththari*

Parts used : Whole plant, Leaves, Flower, Fruit, Root[14B]

Properties :

Suvai (Taste) : *Kaippu, Kaarppu*

Thanmai (Nature) : *Veppam*

Pirivu (Bio- Transformation): *Kaarppu*

Actions :

- Tonic
- Stimulant
- Expectorant

General characters

"தூது பத்திரி யூண்சுவை யாக்கும்பூ
தாது வைத்தழைப் பித்திடும் காயது
வாத பித்தக பத்தையு மாற்றுவேர்
ஓதும் வல்லிபன் நோயுமொ ழிக்குமே"

-தேரையர் குணவாகடம்

Indications :

It cures Cough, Bronchial asthma [11M].

Therapeutic uses

- 7 leaves of this plant and 7 black peppers were taken. Grind the leaves and peppers together into a thick paste. Make it into small balland consume daily as such for 3 days. Stomach pain caused by extreme heat and excessive gas will disappear.
- Thuthuvalai leaves are used as a kaya kalpam (Rejuvenator). The leaves have the medicinal values of haematonic, increasing blood flow [21].

ADATHODAI

Scientific Name **:** *Justicia adhatoda*

Synonyms **:** *Adhatoda vasika*

Adenanthera vasika,

Justicia beddomei (Clarke) Bennet

Other Name **:** *Vaasi, Singam*[22, 23, 24]

Vernacular Names

Tamil : *Adhatodai*

English : Malabar-nut

Telugu : *Addasaram*

Malayalam : *Ata lotakam*

Hindi : *Arusa*

Sanskrit : *Vasaka*

Kannadam : Adasoge

Properties **:**

Suvai (Taste) : *Kaarppu*

Thanmai (Nature) : *Veppam*

Pirivu (Bio- Transformation): *Kaarppu*

Actions **:**

- Antispasmodic
- Expectorant
- Germicide
- Diuretic

General Characters:

"காசமொடு மந்தங் கதித்தபித் தங்கொடுஞ்சு

வாசங் கழுத்து வலிமுதனோய்-கூசியே

யோடா திராதிங் கொருநாளு மொண்டொடியே

யாடாதோ டைத்தூருக் கஞ்சி."

- பதார்த்த குண விளக்கம்.

Indications :

Remedy for cough, fever, dyspnoea, emaciation, asthma [19B].

Therapeutic Uses :

- Whole plant was well known for its beneficial effects in bronchitis.
- Fresh leaf juice infusion, decoction for treating Asthma, chronic bronchitis, cold, cough and whooping cough.
- Root decoction in Asthma [13I].

SIRUVAZHUTHALAI

Scientific Name : *Solanum melongena*

Other Names : *Kaththari,Vazhithunai*

Vernacular Names

Tamil : *Kaththari*

English : Eggplant, Brinjal

Telugu : *Vankaya*

Malayalam : *Valutina, Mulakutakali*

Kannadam : *Badanekayi*

Hindi : *Bhata*

Sanskrit : *Varkatam, Peetaphalam*

Properties :

Suvai (Taste) : *Kaarppu*

Thanmai (Nature) : *Veppam*

Pirivu (Bio- Transformation): *Kaarppu*

Actions :

- ❖ Stimulant
- ❖ Expectorant
- ❖ Hypnotic[11N]

Indications :

It cures Cough, Asthma and Fever

Therapeutic Uses:

- Fruits - Useful in Dysuria, Asthma, Liver compliants,
- Seeds - Causes digestion, stimulant
- Leaves - Beneficial in Asthma , Bronchitis and dysuria
- Root - Antiasthmatic and stimulant.
  Decoction of the root used in syphilis [13J].

3.2. BOTANICAL REVIEW

DRIED GINGER

Botanical name : *Zingiberofficinale*

Family : Zingiberaceae

Part used : Rhizome

Scientific classification [25]

Kingdom : Plantae

Class : Dicots

Order : Zingiberales

Family : Zingiberaceae

Genus : *Zingiber*

Species : *officinale*

Occurrence and distribution

Chukku was the dried rhizome of the perennial herb *Zingiber officinale* was widely cultivated in many parts of India.

Rhizomes dug in January- February, buds and roots removed, soaked overnight in water, decorticated and sometimes treaded with lime and dried.

Description:

Rhizomes are white to yellowish brown in colour, irregularly branched, somewhat annulated and laterally flattened. Pieces about 5 to 15 cm long, 1.5 to 6.5 cm wide, usually 3 to 4 cm or 1 to 1.5 cm thick. They growing tips are covered over by a few scales. The surface of the rhizome was smooth and if broken a few fibrous elements of the vascular bundles project out from the cut ends. Odour was agreeable and aromatic; taste agreeable and pungent [13K].

Chemical Constituents:

α-zingiberene, gingerin, beta-sesquiphellandrene, gingerdione, gingerdiols, gingerdiacetates, 6-gingersulfonic acid, gingerol, shogaol, diterpenes, ginger glycolipids A,B & C, curcumene, fats and starch [26] .

BLACK PEPPER

Botanical name : *Piper nigrum*

Family : Piperaceae

Part used : Dried fruits

Scientific classification [27]

Kingdom : Plantae

Class : Dicot

Order : Microembryeae

Family : Piperaceae

Genus : *Piper*

Species : *nigrum*

Occurrence and distribution

The plant cultivated in the hotter and moist parts of India, in evergreen forest up to 1,500 meters.

Description of the plant

Climbing perennial shrubs, rooting at the nodes, leaves are cordate or round based; flowers minute in spikes usually dioeciously. Fruiting spikes very variable in length, fruits ovoid or globose one seeded berries, bright red when ripe, seeds are globose, albumin hard and testa thin greyish-black to black, perisperm hard, wrinkled and white, 0.4 to0.5 cm in diameter; odour aromatics, taste pungent.

Flowering occurs in the rainy season and fruits ripening in the autumn season (December to April) [28A].

Chemical Constituents:

Piperine, chavicine, Piperettine, Piperoline A&B, Trichostachine, N-trans-feruloyl piperidine, Feruperine, Citrohellol, Arginine, Piperolic acid, Serine, Ascorbic acid, Carotene[13L] .

LONG PEPPER

Botanical name	**:**	*Piper longum*
Family	**:**	Piperaceae
Synonyms	**:**	*Piper sarmentum*
		Piper latifolium
		Chavica roxburghii
		Charvica sarmentosa
Part used	**:**	Dried spikes

Scientific classification [29]

Kingdom	:	Plantae
Class	:	Dicot
Order	:	Microembryeae
Family	:	Piperaceae
Genus	:	*Piper*
Species	:	*longum*

Occurrence and distribution

This plant mostly occurs in hotter parts of the India from central Himalayas to Assam up to lower hills of the west Bengal and evergreen forests of western Ghats as wild and also cultivated in north east and south. It grows in Kurinji nilam.

Description of the plant

A slender aromatic climber and leaves alternative, lower ones broadly ovate cordite, upper ones oblong, oval, all entire 5 to 7 nerved leaves; male spikes longer, slender, 2.5 to 7.5 cm long. Female spikes short, cylindrical, 1.5 to 2.5 cm long, 5 to 7 mm thick. 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. Odour is aromatic; taste is pungent producing numbness of the tongue [28B].

Chemical constituents:

Piperine, piperidine, Piperlongumine, Pipernonaline, Sesamin, Piperundecalidine, Futoamide, Piplasterol, Volatile oil [15B].Pellitorine, Piplartine[30].

VALERINA ROOT

Botanical name : *Nardostachysjatamansi*

Family name : Valerianaceae

Synonyms : Indian spike root, Musk root, Nord

Scientific Classification [31]

Kingdom : Plantae

Class : Dicots

Order : Rubiales

Family : Valerianaceae

Genus : *Nardostachys*

Species : *jatamansi*

Parts used **:** Rhizome

Occurrence & distribution:

These plants are found in the Alpine Himalayas at an altitude of 1000-5000m.

Grown in Sikkim & Bhutan and also found in Deccan plateau.

Description of the Plant:

Root stock woody, long covered with fibres from the petioles of withered leaves, erect perennial herbs, Radial leaves elongated, Spathulate and narrowed into the petiole. Flowers capitate, Fruit & seed obovate & compressed.

Flowers & fruits during April – October.

Chemical constituents :

Jatamansic acid,Jatamansone, sesquiterpene, resin, sugar, starch, volatile oil, hydrocarbon, valaranone, valaranal , β sitosterol , Nordostechone, Hydrocarbon, n-hexa cosanyl, selinidin, Jatamol A and Jatamol B[(32A)] .

BEETLE KILLER

Botanical name : *Clerodendrum serratum*

Family : Verbenaceae

Part used : Root, Leaves

Scientific classification

Kingdom : Plantae

Class : Dicots

Order : Lamiales

Family : Verbenaceae

Genus : *Clerodendrum*

Species : *serratum*

Occurrence and distribution

It has the native of evergreen forest of India.

Description of the plant

A slightly woody shrub with bluntly quadrangular stems and branches leaves usually 3 at a node, sometimes opposite, oblong or elliptic, coarsely and sharply serrate, flowers blue, many in long cylindrical thyrsus with a pair of acute bracts at each branching and flower in the fork, fruit a 4 lobed purple drupes. The roots are bitter, acrid.

Chemical constituents:

Serratagenic acid, Clerodin, D–mannitol, Caryophylleneoxide, stigmasterol, Eugenol, Acetophenone, Benzylsalicylate, Palustrol, luteolin[(13M)] .

GALLS

Botanical Name : *Rhus succedanea*

Synonyms : *Toxicodendron succedaneum*

Family : Anacardiaceae

Scientific Classification

Kingdom : Plantae

Class : Dicots

Order : Sapindales

Family : Anacardiaceae

Genus : *Rhus*

Species : *succedanea*

Occurrence & Distribution:

Found in mixed forest of the Himalayan upto 2500m from Punjab to Assam & in the Khasi hills.

Characters :

- The galls are horn-like excrescences caused by a kind of insects (aphis) on the leaves, petioles and branches of Rhus succedanea.
- They are hard, hollow, thin walled, generally cylindrical, tapering to either extremity.

Description :

A moderate sized deciduous tree about 10m high, leaves imparipinnate, 15 -30cm long, leaflets opposite 3-6 pairs, oblong lanceolate, long accuminate, flowers are small greenish yellow borne in lateral panicles. Drupes on lax drooping panicles, oblique, yellow or light brown, kernel hard, whitish enclosed by a fibrous pericarp. Seeds large and oily. The milky juice of the tree causes blisters.

Flowers& Fruits occurs during May-September.

Parts Used : Fruits and Galls

Chemical constituents:

- Rhusflavone,
- Succedaenins A and B,
- Succedea flavone [33A].

COSTUS ROOT

Botanical name **:** *Costus speciosus*

Family **:** Costaceae

Scientific Classification

Kingdom **:** Plantae

Class	:	Monocots
Subclass	:	Zingiberidae
Order	:	Zingiberales
Family	:	Costaceae
Genus	:	*Costus*
Species	:	*speciosus*

Occurrence and Distribution:

Crepe Ginger is a tall plant with large (up to 20 cm long) pubescent, dark green leaves arranged on the stalk in a spiral. In tropical regions it can growsupto 10 ft (3.1 m) tall, while in cooler regions grows to about 6 ft (1.8 m) tall and it dies back in winter. From each cone 3 or 4 pure white crinkled flowers appear one at a time, hence the common name, Crepe Ginger.

Costus speciosus is native to South East Asia, especially found in India, Srilanka, Indonesia and Malaysia, but it has been naturalized in some tropical areas of the rest of the world like Hawaii.

It is found throughout the country in moist tropical evergreen forests, up to an altitude of 1200m, common along roadsides, streams and in wastelands. It is widely distributed in Kerala, Tamil Nadu Assam, Meghalaya, Bihar, Khasi and Jaintia Hills, Uttaranchal, Orissa, MP, North Bengal while the Himachal sub Himalayan tracts and Western Ghats are the ideal places for its collection

Description:

Costus speciosus Koen. (Keu) is an ornamental, rhizomatous, perennial, erect, succulent herb, up to 2.7 m in height, arising from a horizontal rhizome. Rhizomes clothed with sheaths in the lower parts, leafy upwards, leaves elliptic to oblong or oblong-lancoelate, thick, spirally arranged, 15-35 cm X 6-10 cm, silky beneath, with stem clasping sheaths up to 4 cm.

Flowers large, white, in thick, cone-like terminal spikes, with bright red bracts, lip with yellowish throat; fruits globosely trigonous, red capsules, 2 cm in diameter, seeds black, with white aril. The attractive red coneshaped bracts remain even after the flowers are withered. The fruits are red capsules and they contain black seeds with a white fleshy aril.

The dried rhizome is curved or somewhat straight, cylindrical, branched piece, 10-30 cm in length and 3-5 cm in diameter in dried condition, upper surface marked with circular nodal scars with remnants of leaf bases, lower and lateral surfaces exhibit small circular scars of roots or few wiry rootlets fracture fibrous and fractured surface is yellowish brown.

Flowering occurs during September-October.

Chemical constituents

Diosgenin is the major constituent isolated from Costus speciosus.

Other constituents are Tigogenin, dioscin, resinoids, saussurine, resin, gracillin beta-sitosterol, glucoside. The seed contains pale yellow sweet smelling fatty oil[(13N)].

INDIAN GOOSEBERRY

Botanical name : *Phyllanthus emblica*

Family : Euphorbiaceae

Synonyms : *Emblica officinalisGeartn.*

Scientific classification [(34)]

Kingdom : Plantae

Class : Dicots

Order : Malpighiales

Family : Euphorbiaceae

Genus : *Phyllanthus*

Species : *emblica*

Occurrence and distribution

A deciduous tree with the native as India, Wild or cultivated throughout tropical India from the foot of the Himalayas. It grows in kurinji and marudham thinai.

Parts used : Fruits, seeds, flowers, leaves, barks, roots

Description of the plant

A large handsome deciduous tree with greenish-grey or red bark: peeling of scales and long stripes. Leaves pinnate distichously close set, linear - oblong; Feathery small leaves are fine and delicate. The tree has a peculiarity of shedding its twigs along with the leaves attached; Flowers are very small, greenish densely fascicled along the branchlets, yellowish; males on slender pedicels, females sub sessile.

Flowering occurs during March to May. Fruits ripen from November to February.

A dried fruit consists of pericarp. Colour grey to black, pieces showing a broad, external convex surface shrivelled and wrinkled; external surface shows a few whitish specks; taste sour and astringent[33B] .

Chemical constituents

- It was a good source of vitamin C.
- Fruit -Anti-Oxidant due to Gallic Acid.
- Fruits, Leaves - Tannins
- Alkaloids - Phyllantidine And Phyllantine
- Polyphenolic Compounds - Terchebin, Corialgin and Ellagic Acid [35].

MYROBALAN

Botanical name : *Terminalia chebula*

Family : Combretaceae

Part used : Fruits and bark

Scientific classification [36]

Kingdom : Plantae

Class : Dicot

Order : Mytales

Family : Combretaceae

Genus : *Terminalia*

Species : *chebula*

Occurrence and distribution:

It was distributed in chiefly in deciduous forests and areas of light rainfall, but occasionally in moist forests, up to about 1500 m throughout India. Abundant in northern India; also occurs in Bihar, West bengal, Assam. It grows in kurinji thinai.

Description of the plant

A large tree; young branchlets, leaf buds and leaves with long, soft, shining, rust coloured, sometimes silvery hairs. Leaves are mostly sub -opposite; distant, ovate or oblong ovate, 8 to 20 cm long, deciduous in the cold season. Flowers dull white or yellowish, with a strong offensive smell, borne in spikes from the upper axils and in small terminal panicles. Bark 6mm thickness, dark brown with shallow vertical cracks; wood very hard, brownish grey with a greenish or yellowish tinge. Fruit yellowish -brown, ovoid, 20 to 55 mm long, 13 to 25 mm wide, wrinkled and ribbed longitudinally. Non adherent to the seed; taste astringent [37].

Chemical Constituents

- Fruits and bark contains Gallic acid, Terminoic acid, Ferulic acid, Vanillic acid and Tannin.
- In leaves the characteristic compounds are Tannins, Triterpenes, saponins and mucous substances. The tannins are esters of different phenol-carbonic acids.
- The fruit of Terminalia chebula contains three hydrolysable Tannins, Chebulinic acid, Chebulagic acid [13O].

BELERIC MYROBALAN

Botanical name : *Terminalia bellirica*

Family : Combretaeceae

Synonyms : Myrobalanus bellirica

Parts used : Leaves, fruit, seed

Scientific classification [38]

Kingdom : Plantae

Class : Dicot

Order : Mytales

Family : Combretaceae

Genus : *Terminalia*

Species : *bellirica*

Occurrence and distribution

Found in the plains and lower hills throughout India and along the foot of the Himalayas. It grows in kurinji and marutam thinai.

Description of the plant

A large deciduous tree, 10 -20 m height; leaves gathered about the extremities of the branches, alternate, coriaceous, elliptic or obovate; both surfaces puberulous when young, glabrous and reticulate when old, the margins entire, pellucid, base narrowed; nerves 6-8 pairs, midrib prominent on both surfaces. Flowers pale greenish yellow, offensive odour, bracts linear, early caduceus; calyx pubescent outside, inside woolly with long brown hairs; when mature the leaves are glabrous and punctate on the upper side. Bark is bluish grey with fine vertical cracks; the wood is yellowish grey, hard, no heartwood; annual rings indistinct. Fruit shape spherical to ovoid, ripe fruits slightly silvery; Mature fruits grey or greyish brown. Taste astringent.

Chemical constituents

Gallicacid, Ellagicacid, Chebullagicacid, Belericoside, Arjunglucoside, Belleric acid.

Medicinal uses

Useful in cough, dropsy, hoarseness of voice and eye diseases[(13P)].

ROCK SALT

Chemical Name **:** *Sodium chloride impura or Sodium chloridum impure*

Chemical Formula **:** NaCl (impure sodium chloride)

Vernacular Names

English : Rock salt, Sea salt, Bay salt, Sodium chlorate

Hindi : *Sendhalon, sedhalon*

Malayalam : *Intu-Uppu*

Sanskrit : *Saindhava*

Telugu : *Saindhalavanam*

Tamil : *Indu-Uppu*

Characters:

- Found in small white crystalline grains or transparent cubes.
- Brownish white externally and white internally.
- It is a soluble compound having a characteristic salty taste.
- Pure salt – colourless and transparent, but often variously coloured as tinged grey or blue or brown or pink due to impurities.

Specific gravity:

2.1 – 2.6

Composition of Rock salt:(% dry basis)

Sodium chloride	-	68.85
Manganese chloride	-	0.55
Calcium chloride	-	0.53
Magnesium chloride	-	0.43
Sodium bicarbonate	-	0.74
Insoluble matter	-	30.34
Moisture	-	1.54

Solubility:

- 35.7 g/100g of water at 0ºC
- 39.8 g/100g of water at 100ºC

Actions:

- Rock salt possesses stronger purgative properties than Cream of Tartar.
- Laxative – dosage : 4.2 to 8.4gm
- Purgative – dosage: 16.8 to 21gm
- Carminative
- Diuretic
- Stomachic

Therapeutic uses:

- Used in Dyspepsia and abdominal disorders
- Promotes digestion and cell formation,
- Acts as a stimulant, increases flow of saliva in the human.
- Used in the treatment of sprains, prevention of goitre [39].

WATER LILY

Botanical Name **:** *Nymphaea alba*

Family **:** Nymphaeaceae

Scientific Classification [40]

Kingdom : Plantae

Class : Dicotyledons

Order : Ranales

Family : Nymphaeaceae

Genus : *Nymphaea*

Species : *alba*

Parts Used **:** Seeds, flowers and rhizome

Occurrence and Distribution:

A native of Europe and North Africa now found in the lakes of Kashmir and in high altitudes below 1800m.

Description **:**

A perennial aquatic herb with black rhizomes. Leaves entire orbicular, cordate, glossy. Flowers floating, solitary, 5 – 11cm diam. Fruits a spongy berry, ripening under water. Seeds punctuate, striate, buried in pulp.

Chemical Constituents:

The plant contains Nuparine, Nymphaeine and the cardiac glycoside,nymphalin.The presence of β sitosterol, gallic acid and myricitrin have also been reported [28C].

WILD EGG PLANT

Botanical Name **:** *Solanum xanthocarpum*

Synonyms **:** *Solanum surattense* Burm.f

Solanum virginianum.

Family **:** Solanaeceae

Parts Used **:** Fruits, seeds, flowers, leaves, stem and root

Scientific Classification [41]

Kingdom : Plantae

Class : Dicots

Order : Solanales

Family : Solanaceae

Genus : *Solanum*

Species : *xanthocarpum*

Occurrence and Distribution :

Grown abundantly in India. Common in road sides, waste places and along railway lines throughout India.

Description :

A prickly diffuse perennial herb. Numerous branches. Leaves ovate or elliptic sinuate or subpinnatifide glabrescent, with many straight spines. Flowers

borne in few flowered lateral cymes. Corolla blue, lobes shallow. Fruits globose, glabrous berries, whitish and green blotched, yellow when ripe. Seeds glabrous.

Flowers and fruits during March – July [42A].

Chemical Constituents :

Solancarpine, solacarpidin, carpesterol[14C]

CLIMBING BRINJAL

Botanical name : *Solanum trilobatum*

Family : Solanaceae

Parts used : Leaves, Flower, Fruit, Root

Scientific classification [43]

Kingdom : Plantae

Class : Dicot

Order : Solanales

Family : Solanaceae

Genus : *Solanum*

Species : *trilobatum*

Occurrence and distribution

Found in waste places, swampy areas throughout India[42B].

Description of the plant

A climbing shrub, 1.8 -3.6 m long, subscanded by numerous hooked prickles; stems slender; branches long and divaricate. Prickles from a broad triangular base, very sharp compressed and recurved. Leaves 2.5-5 cm, ovate or rotund ovate, obtuse, irregularly 3 - 5 lobed, sparsely stellately hairy or glabrous, with or without 2

or 3 prickles on the mid rib, base not cordate; petioles 1.3-3.8 cm long, prickly Flowers are large and showy, violet purple in colour. Peduncles are very short. Calyx: cyathiform, stellate hairy. Corolla lobes oblong lanceolate, acute, stellately hairy outside, reflexed. Filaments are narrowly oblong, opening by small pores. Berry scarlet when ripe. Seeds slightly pitted.

Flowers and fruits during March – July [44, 45, 46, 47].

Chemical constituents

- Whole plant contains β-solamarine, Solannolide, Solasodine, Solanine, Carbohydrates, saponin, phytosterol, tannin, Phenolic compounds, Tannin, alkaloids, Flavonoids, Cardiac glycosides.
- Seed oil - Linoleic acid, palmitic acid, oleic acid, stearic acid [13H].

MALABAR-NUT

Botanical Name : *Justiciaadhatoda*

Synonyms : *Adhatoda vasika*

Adenanthera vasika,

Justicia beddomei (Clarke) Bennet

Family **:** Acanthaceae

Parts Used **:** Leaves, Roots, Flowers and bark

Scientific Classification [48]

Kingdom : Plantae

Class : Dicots

Order : Lamiales

Family : Acanthaceae

Genus : *Justicia*

Species : *adhatoda*

Occurrence and Distribution:

Common almost throughout India, particularly in plains of tropical parts.

Description :

A dense shrub,young parts tomentose. Leaves elliptic or elliptic-lanceolate,acuminate at apex.Flowers white,borne in dense spikes.Bracts ovate or obovate,subacute,puberulous. Calyx deeply 5 lobed; lobes equal, lanceolate. stamens glabrous.ovary and style base minutely hairy. Fruits 4 seeded capsules. Seeds glabrous.

Flowers and fruits during February-May.

Chemical Constituents :

Vasicine, vasicinine, quinazoline, linoleic acid, oleic acid, kaempferol, quercetin, β-sitosterol, vasicinol, adhatodine, adhavasinone[32B].

EGG PLANT

Botanical Name : *Solanum melongena*

Family : Solanaceae

Parts Used : Fruits, Leaves and Root

Scientific Classification [49]

Kingdom : Plantae

Class : Dicots

Order : Solanales

Family : Solanaeceae

Genus : *Solanum*

Species : *melongena*

Occurrence and Distribution:

Cultivated throughout India

Description :

An undershrub , prickly. Leaves ovate, sinuate or lobed, stellately woolly beneath. Flowers borne in few flowered lateral cymes, blue. Calyx lobes lanceolate, corolla shortly lobed. Berries glabrous, often large ellipsoid, globular or elongate, white , dark-purple or yellow. Seeds many discoid [42C].

Chemical Constituents :

Solasodine,Arginine, Leucine, isoleucine, choline, aspartic acid, oleic, palmitic & stearic acid,β sitosterol, stigmasterol[14D] .

3.3. SIDDHA ASPECT OF THE DISEASE

***Swasakaasam*(Bronchial asthma)**

OTHER NAMES :

- *Iraippu*
- *Izhuppunoi*
- *Swasam*
- *Thoivu*
- *Eelai*
- *Suram*
- *Iraippirumal*

NATURE OF THE DISEASE:

Swasakasam arises with severe chest tightness leading to difficulty in inspiration and expiration of the air (i.e. dyspnoea). In addition to difficulty in breathing while exhaling the air, expiratory noise will be produced resembling the sounds of musical instruments like flute, veena, lute etc., are heard obviously. Further if hard attempts are made to expel the phlegm, it results in vain.

GENESIS OF THE DISEASE:

"கால்பெருக் குணவுபொருள் தண்ணீர் மாறல்

கருதிருமல் மிகல்வாந்தி குளிர்ந்த காற்று

மால்செய்து நாள்தோறும் வருந்துங் காய்ச்சல்

மந்தன முயிர்நிலை யிலடிகள் தாங்கல்

ஏல்சீத பேதிவிட பாண்டு புகைகள்

இலகிய நெல்லாதிமணிச் சுனையுட் செல்லல்

மேல்வழியிற் சிலவரினு மிரைப்பாம் நோயு

முனிவர்கள் விளம்பினாரே"

- கையெழுத்துப்பிரதி

Swasakasam is considered to be arised due to the following factors such as

- ❖ Ingestion of allergic food stuffs
- ❖ Allergy inducing activities such as wandering in cool climates.
- ❖ Immunity deprivation
- ❖ Ingestion of diet which increases kapha and activities which increases kapha.
- ❖ Grass, plantage, rice and ragi also triggers the symptoms
- ❖ Symptoms may also develop due to inhalation of foul smelling substances.

PRODROMAL SYMPTOMS:

"மார்பில் விளாவிரண்டில் மண்ணுமிகு நெரியில்

சேர்ந்து வலித்தல் திணறல் - தார்மூச்சு

உப்பல் வயிற்று லுருவது முற்குறியாச்

செப்பிரைப்பு நோய்க்குதனைத் தேர்"

-யூகி வைத்திய சிந்தாமணி[50A]

Generally the prodromal symptoms and intensity of the disease will be recognised earlier by chronic asthmatics. While taking unsuitable food and while inhaling the chill air the patient develops rhinitis, sneezing, chest discomfort, chest tightness, pain, difficulty in normal breathing, pain in paravertebral region with dyspnoea, distension of abdomen and excess sweating.

TYPES OF SWASAKASAM:

Swasakasam (Iraippu) has been classified into 5 types.

Of these, first four types are based on *kuttram* and the final one is based on intensity of breath. They are as follows

1. ***Vali Iraippu***
2. ***Iya Iraippu***
3. ***Iyavali Iraippu***
4. ***Mukkutra Iraippu***
5. ***Melnokku Iraippu***

Apart from this further it is also classified into another 5 types.

"சிறுபே றிரைப்பு திணறல் மந்தாரம்

வருமே லிரைப்புந் தின்மாண்பு"

They are as follows

1. *Sitriraipu*
2. *Periraippu*
3. *Thinaraliraippu*
4. *Mandhara Iraippu*
5. *Maeliraipu*

SIGNS AND SYMPTOMS:

"வன்மையாய் கோழைகட்டி இருமி வீழும்
மாநாகம் போலவே வாங்குஞ் சுவாசம்
திண்மையாய்ச் சேருமலுண்டா மடிக்க டிக்குஞ்
சீரண மிலாமலே வயிறு மூதும்
நன்மையாய் நாசியது தணல்போ லாகும்
நலிந்துடம்பு வற்றி வருங் குரலுங் கம்மும்
உண்மையா யுண்ணாக் கிலுாறுங் கேணி
யுழந்துமே சுவாசகா சத்தி னொப்பே"

-யூகி வைத்திய சிந்தாமணி(50B)

VALI IRAIPPU NOI ORARPA SWASAM:

Vatha dosha gets aggravated due to ingestion of food that is not easily digested, wandering under hot sun rays, eating tubers. Due to increased *vatha dosha* the patient may feela condition as if nothing is inside the chest. Inspite of all these conditions patient doesn't experience severe illness and this condition is curable. ***Vali iraippu*** is also mentioned as "***Soothira swasam".***

KABHA SWASAM (IYA IRAIPPU NOI OR MANTHARA SWASAM):

Kapha swasam is caused due to increased *kapha dosha* because of taking foods which increase *kapha* and also roaming in chill air. It produces nasal congestion, rhinitis, chest tightness, inability to breathe.

Sometimes constricted type of chest pain may aggravate to the extent as if the patient dies of inability to breath. When the patient mildly attempts to cough and expectorates some mucus relief occurs for some extent. When the patient does not cough and expel the mucus dyspnoea occurs and patient is unable to lie on bed, makes him to stand.

Sweating on forehead, blackening of face, chillness of limbs, dryness of tongue, shivering of body, dyspnoea, inability to sleep are the associated symptoms of this disease. It is also known as '*Thamaraga suvaasam'.*

IYAVALI IRAIPPU OR PERIAPPU:

In this condition both *kapha dhosam* and *vatha dhosam* are dearranged together and causes the following symptoms. Symptoms of this type will be very severe and the derangement of *vatha dosha* combines along with *udhana vaayu*. Clinical features of this type are dyspnoea, inability to inspirate and expirate air, constipation, abdominal distension, dryness of tongue, redness and painful eyes, shivering of body, giddiness, excessive sleep, incoherent talk, etc., this condition is also called as "***Vichinna swasam".***

MUKKUTTRA IRAIPPU OR MAGAASWASAM:

In this condition, all the three doshas gets deranged at once and *udhana, abanan, viyanan, samanan* get deranged one by one which in turn affects the seven major elements of the body. It is life threatening type of asthma. The prodromal symptoms are Shivering of body, dyspnoea, depression, breathing like cow's breathing, chest tightness and pain, constipation, oliguria, pain all over the body, stammering and excessive sweating over the forehead. This is also called as "***Thinaraliraippu***".

MAELNOKKU IRAIPPU:

If any of the above mentioned disease continues for many days without response to treatment, then the upward directional *udhana vaayu* loses its strength and in such a situation, expiration may not be possible. The patient tends to develop dyspnoea with prominent eyeball. There may be dryness of mouth. Patient may be unable to speak; he may appear astonished and will not lie down on the bed; he may look upward; he may also attempt to exhale by his opened mouth. If proper treatment is given at this stage he may survive. Otherwise he may fall unconscious with darkening of face and may die with mouth open [51].

Other factors affecting the disease:

- Eating foods which will induce excessive *kapha.*
- Exposure to chill air.
- Living in the mountains.
- Walking in the cold climate.

Pulse:

"கபமல்லாது காச சுவாசம் வாராது"[2B]

- *Kaba Nadi*
- *Vathakaba Nadi*
- *Kapthpitha Nadi* are the classical pulse for *Swasakasam*.

Sputum:

- If the sputum is found excessive in quantity, light weight and foamy, it is considered that the disease gets developed due to *kapha dosham*.
- If the sputum is black in colour, hard and with smell of flesh, it will denote *kapha dosham.*
- If it is found white like pus and mixed with yellow colour, it will denote *pitha dosham*[3B].

3.4.MODERN ASPECT OF THE DISEASE

BRONCHIAL ASTHMA

Introduction

Asthma, the word was derived from Greek word. The term **"ASTHMA"** in Greek means breathless or breathe with open mouth.

Asthma is defined as a chronic inflammatory disorder of the airways, characterised by reversible airflow obstruction causing cough, wheeze, chest tightness and shortness of breath.

Inflammation of the bronchial wall involving eosinophils, mast cells and lymphocytes, together with the cytokine and inflammatory products of these cells, induces hyper-responsiveness of the bronchi so that they narrow more readily in response to a wide range of stimuli. Narrowing of the airway in usually reversible, but in some patients with chronic asthma the bronchial wall inflammation may lead to irreversible obstruction of airflow.

Epidemiology

- The prevalence of asthma increased steadily over the later part of the century first in the developed and then in the developing countries. Current estimates suggest that asthma affects 300 million people world-wide and additional 100 million persons will be diagnosed by 2025. In India, 15-20 millions are asthmatics.About 2,50,000 annual deaths. The socio- economic impact is enormous, particularly when poor control leads to days lost from school or work, unscheduled health-care visits and hospital admissions. [(6)]
- Epidemiological studies suggest that the multiple genetic and environmental factors contribute to the causation of asthma, a clinical condition that is viewed as a cluster of related disorders to smooth muscle hypertrophy [(52)].

Factors that triggers asthma

- Smoking
- Infections like cold.
- Allergens such as food, pollens, dust mites and pet dander.
- Exercise
- Air pollution and toxins
- Emotional stress and anxiety
- Weather, especially extreme changes in temperature

- Drugs (such as aspirin, NSAID and beta blockers)
- Perfumes and fragrances
- Acid reflux

Allergens are the most causative factor for 50 to 70% for adults in asthma. In children under 3 years of age, viral infections (respiratory syncytial virus) are the most common trigger. After 3 years of age, the allergies also begin to play an increasing role as a trigger. After 20 years of age, occupational exposure to any toxic substances and allergens also can be important triggers.

Dietary deficiency of antioxidants may predispose to development of asthma from childhood days [(6).]

PATHOLOGY

- Inhaled allergens stimulate sensory nerve endings called irritant receptors lying below the airway epithelium.
- Stimulation of these irritant receptors causes parasympathetic nerves to release acetylcholine (ACh). When acetylcholine binds to M3 muscarinic receptors on airway smooth muscle, a series of events is initiated which results in an increase in intracellular calcium and smooth muscle contraction (bronchoconstriction or bronchospasm).
- Some inflammatory mediators such as histamine can also increase intracellular calcium and cause bronchospasm.
- Inflammation of the airways is brought on the several factors like eosinophil, T-lymphocytes (CD4+), macrophages, and mast cells infiltrate the bronchial wall.
- The epithelium is vacuolated and the ciliated cells desquamate. Several cellular factors play their roles in the inflammatory process.
- Neuropeptides such as bradykinins, substance P and neurotension are lead to bronchoconstriction and excessive secretion of mucous.
- Mast cells initiate the response on exposure to allergens, excessive osmatic changes and variations in temperature.

- Macrophages produce cytokines, which are either bronchoconstriction or bronchodilator. Presence of eosinophil in the inflammatory exudate is characteristic of asthma.
- Eosinophils are derived from bloodstream. Major basic proteins and cationic proteins of eosinophil lead to destruction of mucosal surface.
- T-lymphocytes, especially CD4+ produce cytokines IL-3, IL-4, IL-5 and GM-CSF which modify the inflammation.
- TNF which is an inflammatory cytokine is expressed in greater amounts by mast cells. The bronco-alveolar secretions contain higher levels of TNF.
- The main chemical transmitters, which alter the airways, are histamine, prostaglandin and leukotriene. These lead to contraction of bronchial muscle, increase in vascular permeability and excessive secretion of abnormal mucous. Airway inflammation persists for several years. Its severity correlates with the severity of asthma. Hyper responsiveness of the inflamed airways is aggravated by autonomic and neural mechanisms.
- The final result is obstruction of the small and medium sized airways brought about by mucosal oedema, tenacious mucous and bronchoconstriction [6].

Classification:

Bronchial asthma classified into 2 groups

- Extrinsic asthma (Atopic)
- Intrinsic asthma (Cryptogenic)

Extrinsic

- IgE was raised at least 70%
- Atopic subjects
- Onset was early (10-15 years)
- Intermittent in nature
- Family history of atrophy

Intrinsic

- IgE was normal or low
- Usually Non-atopic subjects
- Onset in middle age (30years)
- Constant in nature
- Family history of asthma

Clinical Features:

➢ Asthma classically displays a diurnal pattern, with symptoms and lung function being worse in early morning.

Typical symptoms are

- Recurrent episodes of wheeze
- Chest tightness
- Breathlessness
- Occasionally Cough

➢ Cough may be a dominant symptom in some asthmatic patients and the lack of wheeze or breathlessness may lead to a delay in reaching the diagnosis of called "cough variant asthma".

➢ The classical aspirin-sensitive patient is female and presents in middle age with asthma, rhino-sinusitis and nasal polyps. Aspirin sensitive patients may also report symptoms following alcohol (white wine) and foods containing salicylates.

Diagnosis

- Diagnosis of bronchial asthma is clinical. The history of sudden attack of paroxysmal dyspnoea, cough and auscultator hallmark of expiratory wheeze heard all over the chest are diagnostic.
- Long duration of complaints history of allergy and positive family history are other helpful clinical points.
- Objective assessment of the severity of airways obstruction and response to bronchodilator therapy can be made by use of bedside peak flow meter.
- Respiratory function tests reveal gross reduction in FEV1, FEV1/FVC ratio and PEFR and increase in the time taken for forced expiration.
- It is important to assess the severity of airways obstruction. Confirmation of the diagnosis of asthma is usually achieved by serial PEFR

monitoring. PEFR in the majority of cases shows a diurnal variation of more than 15 % and improvement with therapy.

- When it is necessary to investigate for provocative factors bronchial challenge testing or BPT may be desirable.

Clinical features which indicate severe ventilator impairment are

1. Inability to narrate history continuously or severe distress even on mild exertion
2. Cyanosis, flapping tremors
3. Mental confusion
4. Respiratory rate above 25/min
5. Heart rate persistently above 110/min
6. Inspiratory fall in blood pressure exceeds 16 mm Hg
7. PEFR less than 40 % of predicted value
8. Feeble breath sounds

Differential diagnosis:

- Chronic bronchitis
- Cardiac failure
- Pulmonary embolism
- Pulmonary eosinophilia
- Metabolic acidosis
- Emphysema
- Foreign body aspiration [53].

3.5. PHARMACEUTICAL REVIEW

CHOORANAM

Definition

Chooranam are fine dry powders of drugs. The term *"Chooranam"* may be applied to the powders of a single drug or a mixture of two or more drugs, which are powdered separately prior to their being mixed to homogeneity.

Method of preparation

Equipment required

1. The drug enumerated in the recipe in clean and dried state.
2. A mortar and pestle.
3. A fine sieve or fine cloth of close mesh.

Process of preparation

- The drugs which are to be used in the preparations should be taken from recently collected material. Drugs which are aged by prolonged storage or changed in colour, taste and scent and those that are insects infected or attacked by fungi should be positively rejected.
- The raw drugs are chopped and dried in sun or shade completely and the drugs are pounded in stone mortar then sieved through a fine mesh.
- If the raw drugs are pounded together it will not yield a beneficial result.
- Some substances before pounding are roasted .The substances to be roasted are kept in an earthen ware and under minimal heat separately .In case if the different substances to be roasted together ,some drugs are roasted earlier and some are burnt under the same heat. So they should not be roasted together.
- In general, the aromatic drugs are slightly fried in order to enhance their aroma and milling properties. Any extraneous material, organic or inorganic, should be removed from the drugs by close inspection.
- The Chooranam should be as fine as to be called amorphous and should be never damp. The fineness of the sieve should be 100 mesh or still finer.

Purification of the prepared *Chooranam*

“தானென்ற சூரணத்தின் சுத்திக்கேளு
தப்பாதே சரக்கெல்லாஞ் சூரணித்து
நானென்ற வாவின் பாலாற் பிசைந்து
நலமான சட்டியிலே பாலைவிட்டு
வானென்ற சுத்தசலம் பாதிவிட்டு
வளமாக மேற்சீலை கோடுகட்டிப்
பானென்ற சூரணத்தைப் பிட்டுபோல் வைது
பதறாதே வெந்தெடுக்கச் சித்தியமே!”

-அகத்தியர் வைத்திய இரத்தினச் சுருக்கம்

The prepared *Chooranam* is mixed with the milk in pot half quantity milk and half quantity water is taken. The mouth of the pot is covered with a thin cloth material. Above this cloth the mixed *Chooranam* is placed. The pot is covered with lid and heated.

“ஆமப்பா ரவியுலர்த்திப் பொடிதான் செய்து
அப்பனே சமனாய்ச் சர்க்கரையைச்சேர்த்து
நாமப்பா கொண்டுவர தோஷம் போச்சு
நன்றாகச் சுத்தி செய்யாச் சூரணந்தான்
தாமப்பா ரோகத்தை வெல்லா தப்பா
தளமான வியதி யெல்லாம் பாரிக்கும்பார்
வேமப்பா சுத்தி செய்து கொண்டாயனால்
வெகுசுறுக்காய் தீருமா வியாதி கேளு.”

-அகத்தியர் வைத்திய இரத்தினச் சுருக்கம்.

Then the *Chooranam* is placed in the sunlight and powdered. Equal amount of sugar is added and taken internally. Many types of disease get cured. If the drug is

taken without purification the diseases does not cure. If taken after purification the disease gets cured easily.

Storage

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

The *Chooranam* to facilitate easy handling and to assure exact dosage of administration could be pressed into tablets with the addition of a suitable binder. These tablets could be packed in bottles or tubes made either of glass or packed in strip of metal foil or plastic sheets.

Shelf life of medicines

Medicines can be classified into internal and external medicines .They are each in 32 types. *Chooranam* comes under the category of internal medicines. The shelf life of medicines indicates the potency of medicines. The medicine even though seems to be fresh is not efficacious after sometime. So the medicines should not use after certain period.

As per siddha literature *Agamarunthu padal* in *Gunapadam Thathu-seevam* text

"உயர்சூர ணம்பிட்டு வடகம் வெண் ணெய்நான்கி

னுயிர்மூன்று திங்களெண்ணெய்....." (17B)

From the above quote, the shelf life of *Chooranam* (powder) is 3 months But According to AYUSH guidelines the shelf life of Chooranam (powder) is 1 year (54).

The *Chooranam is* said to retain its potency for three months and then gradually deteriorate. However, if properly packed and stored they keep good for a year as per AYUSH guidelines.

3.6. PHARMACOLOGICAL REVIEW

A. REVIEW OF DRUG (Modern Medicine)

BRONCHODILATOR DRUGS USED

- A bronchodilator is a substance that dilates the bronchi and bronchioles, decreasing resistance in the respiratory airway and increasing airflow to the lungs.
- Bronchodilators may be endogenous (originating naturally within the body), or they may be medications administered for the treatment of breathing difficulties.
- They are most useful in obstructive lung disease of which asthma and COPD are the most common conditions.

Types of bronchodilator drugs:

Bronchodilators are either short-acting or long acting. Short-acting bronchodilators are used for relief of bronchoconstriction, while long-acting bronchodilators are predominantly used as preventers.

There are three types of bronchodilators namely

1. β2-agonists (short and long-acting)
2. Anticholinergic (short and long- acting)
3. Theophylline (long-acting)

1. β2-agonists:

(a) Short-acting β2-agonists:

- This medication is providing quick or “rescue” relief from acute bronchoconstriction.
- These medications usually take effect within 20 minutes or less, and can last from 4 to 6 hours.
- These inhaled medications are best for treating sudden and severe or new asthma symptoms.

- ❖ Taken 15 to20 minutes ahead of time, these medications can also prevent asthma symptoms triggered by exercise or exposure to cold air.

  Examples:

  - Salbutamol
  - Levosalbutamol
  - Pributerol
  - Terbutaline
  - Epinephrine
  - Ephedrine

(b) Long –acting β2- agonists:

- ❖ These are long term medications taken routinely in order to control and prevent bronchoconstriction.
- ❖ These medications may take longer to begin working, but relief airway construction for up to 12 hours.
- ❖ Commonly taken twice a day with an anti-inflammatory medication, they maintain open airways and prevent asthma symptoms, particularly at night.

  Examples:

  - Salmeterol
  - Fenoterol
  - Pirbuterol
  - Clebuterol
  - Formoterol
  - Bambuterol
  - Indacaterol[(55)].

(2) Anticholinergics:

- ❖ Anti cholinergics or Anti muscarinic drugs relax the smooth muscles but response is slower than Beta-2 agonist.
- ❖ Some examples of anticholinergics are Tiotropium bromide and Ipratropium bromide.

- Tiotropium bromide is long acting, a single inhalation can have effect lasting for 24 hours. It reduces the frequency and severity of episodes.
- Ipratropium bromide is short acting, given by inhalation which has effect for 4-6 hours.

(3) Theophylline:

- Theophylline is long acting bronchodilator that prevents asthma episodes.
- Available in oral and injectable form.
- It is prescribed in severe cases of Asthma or those that are difficult to control.
- This medication must be taken 1-4 times daily and doses should not be missed.
- Blood tests are required to monitor therapy and to indicate when dosage adjustment is necessary [56].

MECHANISM OF ACTION OF BRONCHODILATOR DRUGS

- Beta-2 agonist drugs bind to beta-2 receptors on airway smooth muscle relaxation. When airway smooth muscle relaxes, the diameter of the air passages is enlarged.
- Bronchodilator drugs blocks the action of phosphordiesterases and prevents the breakdown of cAMP to 5-AMP. This also has the effect to relaxing smooth muscle and allowing the airways to dilate.
- The bronchoconstriction effects of acetylcholine can be blocked by muscarinic antagonists. Muscarinic antagonists bind to muscarinic receptors and prevent acetylcholine from binding.
- Bronchodilator can also be achieved by alpha-2 agonist drugs that bind to alpha 2 receptors on parasympathetic nerves and prevent acetylcholine from being released [57].

B. PHARMACOLOGICAL STUDY IN ANIMAL MODELS

BRONCHODILATOR ACTIVITY

In vitro methods

Spasmolytic activity on guinea pigs isolated tracheal chain

The isolated tracheal chain of guinea pigs can be used for testing compounds which inhibit bronchospasm. It detects sympathomimetic, H_1-histamine receptor antagonist properties of test drug.

Methodology

Guinea pig of either sex weighting between 300-500 g are sacrificed using ether anaesthesia. The entire trachea is dissected out and cut into individual rings. Twelve to fifteen rings are tied together with silk threads and mounted in the organ bath containing Krebs-Henseleit solution and maintained at 37^oC, under a tension of 0.5 g and gassed with carbon. Isometric contractions are recovered via a strain gauge transducer on a polygraph. Forty five minutes are allowed for equilibration before the addition of the spasmogen. The following spasmoegns used Histamine, Carbachol, LTC_4 or LTD_4. It takes about 10-12 min for reaching the contraction to a maximum. At this stage, standard and test drugs are administered. The bronchial response is allowed to plateau and recorded. The tissue is rinsed thoroughly and the control contractions are induced again by adding spasmogen. The percent inhibition of spasmogen induced contractions is calculated. From dose response curve ED_{50} is calculated [58].

Isolated Frog Rectus Abdominis Muscle Preparation:

A frog is pithed and laid out on frog dissection board. The skin of the anterior abdominal wall is cut by a midline incision which is extended laterally up to the anterior aspects of the limbs. This exposes the flat whitish muscle of the anterior abdominal wall from their pubic origin to their sternal insertion. The two recti are removed and placed in frog ringer solution in a shallow dish. They are carefully cleaned and one of them is trimmed to the desired size and mounted in an organ bath of 5ml capacity, at room temperature, aerated with oxygen. For recording purposes, an isotonic lever with a sideways writing point is used tangential to the smoked drum,

balanced for a tension of 2.5gm with an extra load of 1gm on the long arm. A standard solution of Ach is added to the bath and a slow contraction is recorded on the slow moving drum for exactly 90sec. The drum is stopped and the bath fluid is replaced by fresh Frog-Ringer. An extra 1gm load is used to extend the muscle to its original length [59].

In vivo methods

Histamine induced bronchospasm in guinea pig

Guinea pigs subjected to inhibition of aerosols containing histamine or other bronchospasm inducing agents, exhibits the symptoms of asphyxiating convulsions resembling acute attack of bronchial asthma. These challenging agents are administered in the form of aerosols through a nebulizer to individual guinea pigs placed in a histamine chamber. The initial symptoms are increased frequency of breathing, forced breathing and finally asphyxiating convulsions. The occurrence of these symptoms can be delayed by antagonistic drugs and bronchodilators. Pre-convulsion time is noted as the end point.

Methodology

Male guinea pigs weighing around 400 g are used in groups of 8-10 animals. The animals are treated with test / standard drugs orally or subcutaneously. The animals are then placed in the standard Histamine chamber, 30 min after the administration of drug and exposed to an aerosol of 0.1 % solution of histamine dihydrochloride through a nebulizer. Time required for the onset of asphyxiating convulsions is recorded. The animal is immediately withdrawn from the inhalation box and placed in a well-ventilated area for revival from the convulsions. This method has been further improvised using an ultra-sound nebulizer which provides the steady exposure to histamine solution at a pre-determined rate. Percent of increase of pre-convulsion time is calculated from the average values of treated and control groups of guinea pigs. ED_{50} values denoting 50% increase in the pre-convulsion time can also be calculated. Histamine aerosol exposure is a very commonly used and dependable method for screening the bronchodilator activity of novel compound [60].

Egg albumin induced anaphylaxis in guinea pig:

Guinea pig was sensitized by two intra peritoneal injections of 0.5 ml and 10% w/v solution of egg albumin at a 48 hours interval. After sensitization, the animals were divided into two groups. Animals of group I received 0.5% CMC and severed as control group. Animals of group II received ethanolic extract trial drug (500 mg/kg. once daily) dissolved in distilled water for 14 days. On day 14, two hours after treatment, the animals were challenged with 0.5 ml of 2% w/v solution of egg albumin into the saphenous vein. Guinea pigs were observed for onset of symptoms such as dyspnoea and cyanosis, duration of persistence of symptoms and mortality [61].

ANTI-HISTAMINE ACTIVITY:

Effects of diphenhydramine in experimentally produced asthma in guinea pigs

Aim

To demonstrate the antagonistic effects of diphenhydramine against histamine induced bronchospasm in the guinea pig.

Principle

Guinea pig is very sensitive to histamine. When guinea pig is exposed to histamine vapour it exhibits bronchospasm, difficulty in breathing and convulsion. These effects of histamine are mediated through the action of histamine on H_1 receptors. Diphenhydramine is a H_1 receptors blocker. Therefore, diphenhydramine prevents the bronchospasm induced by histamine.

Equipments and other materials required

Histometer, stop watch, disposable needle and syringes.

Animal: Guinea pigs

Drug solutions required

1. Normal saline
2. Diphenhydramine 5 mg/ml
3. Histamine diphosphate 30µg/ml

Procedure

Select 4 guinea pigs having body weight between 250-350 g. Fast the guinea pigs for 12 hours before the experiment. Divide the guinea pigs into 2 groups of 2 animals each. Weigh the guinea pigs in each group and mark them for identification. Administer the drug solutions as Group I: Normal saline 1 ml/kg, Group I: Diphenhydramine 5 mg/kg. After one hour place each guinea pig in histamine chamber and replace the cover. With the help of compressor, spray a finely atomized mist of histamine diphosphate from nebulizer in both comparments. Using a stop watch record the time of histamine administration. Observe the signs of respiratory distress and the animal falling on its side and record the observations [(62)].

Isolated Guinea Pig Ileum

Overnight fasted guinea pigs of either sex weighing 400-600gram were sacrificed using cervical dislocation method. The lower most 10cm of ileum was removed from the abdomen and placed in a shallow dish containing warm Triode solution. Ileum lumen was cleaned by passing through warm 0.9% saline and then segments about one inch in length, were made. The mesenteric attachment and blood etc. were carefully cleaned and tissues was mounted in a thermostatically controlled Dale's organ bath containing 20ml Triode's solution under basal tension of 500mg. the composition of solution in was Nacl, 137; Cacl2, 1.8; Kcl, 2.7; glucose, 5.55; NaHco3, 11.9; Mgcl2, 1; NaH2Po4, 0.4. The solution was continuously bubbled with air. The responses to drug were recorded on a student physiography using isotonic transducer, which exerted a basal tension equivalent to 500mg load tissue. The issue was allowed to equilibrate for 30 min, during which, the bathing solution was changed at every 10 min. Increasing concentration of histamine were added to the bath and the control cumulative concentration-response curve was constructed[(63)].

C. REVIEW OF SIDDHA DRUGS

List of Siddha drugs used in Bronchial Asthma

- *Swasakudoori Mathirai*[(64A)]
- *Vasantha kushmagaram*[(64B)]
- *Gowri chinthamani*[(64C)]

- *Thalisadi Chooranam*[11P]
- *Pavala parpam*[64D]
- *Kashthoori karuppu*[64E]
- *Adathodai Chooranam*[65A]
- *Swasakas amatthirai*[65B]
- *Kodasoori kuligai*[64F]
- *Melagu Chooranam*[66]
- *Thalaga karuppu*[64E]
- *Karpooradhi Chooranam*[67B]
- *Swasa krudhum*[67A]
- *Sivanar Amirtham* [64H]
- *Kandangkathiri Chooranam*[68]
- *Thooduvalai nei*[64I]
- *Mahathalisapatthira Chooranam*[69]
- *Arakku thailam*[64J]
- *Soombu theneer*[64K]
- *Adhatodai kudineer*[64L]
- *Nochi Thailam*[64M]

4. MATERIALS AND METHODS

Drug selection

In this dissertation "***Nagarasingadhi Chooranam"*** was taken as a trial drug for Bronchodilator activity from the Siddha literature "*Anuboga Vaidhiya Navaneedham* (Part 8)" Author:Hakeem P.Mohammed Abdullah Sayub. Page no: 8. Published by *Arul migu. Pazhani Dhandayuthapaani Swamy Thirukoil*, Siddha Medical books publishing committee, 1975.

Ingredients of the Drug

Table: 1. Ingredients

NAME OF DRUGS	BOTANICAL NAME	QUANTITY
Chukku	*Zingiber officinale*	35 gm (1 palam)
Milagu	*Piper nigrum*	35 gm (1 palam)
Thippili	*Piper longum*	35 gm (1 palam)
Sadamanjil	*Nardostachys jatamansi*	35 gm (1 palam)
Siruthaekku	*Clerodendrum serratum*	35gm (1 palam)
Karkadagasinghi	*Rhus succedanea*	35gm (1 palam)
Koshtam	*Costus speciosus*	35gm (1 palam)
Nelli mulli	*Phyllanthus emblica*	35gm (1 palam)
Kadukkai thol	*Terminalia chebula*	35gm (1 palam)
Thandrikkai thol	*Terminalia bellerica*	35gm (1 palam)

Induppu	*Sodium chloride impura*	35gm (1 palam)
Sengazhuneer kizhangu	*Nymphae alba*	35gm (1 palam)
Kandangkathri vaerpattai	*Solanum xanthocarpum*	35gm (1 palam)
Thooduvalai vaerpattai	*Solanum trilobatum*	35gm (1 palam)
Aadathodai vaerpattai	*Justicia adhatoda*	35gm (1 palam)
Siruvazhuthalai vaerpattai	*Solanum melongena*	35gm (1 palam)

Collection of the raw materials

All the raw materials were bought from the Ramasamy mudhaliyar store, Parry's corner, Chennai.

Identification and Authentication of the drug

All the raw materials were identified and authenticated by the Botanist and experts of *Gunapadam*, Government Siddha Medical College, Arumbakkam, Chennai – 106.

The specimen sample of all the raw drugs have been preserved in PG *Gunapadam* department individually for future reference.

4.1. PREPARATION OF THE DRUG

Purification of the drugs

Purification Process for all the drugs mentioned here were done as per various classical Siddha literatures [70, 71].

Chukku : Skin of dried ginger was peeled off.

Milagu : Soaked in 250ml of buttermilk for 3 hours and allowed to dry

Thippili	:	Soaked in 250ml of lemon juice and it was dried.
Sadamanjil	:	Dust and odd materials were removed.
Siruthaekku	:	Roots were washed in water and dried in the sunlight.
Karkadagasinghi	:	Dust and odd materials were removed.
Koshtam	:	Dust and odd materials were removed.
Nelli mulli	:	Boiled in 250 ml of milk and then dried in sun light.
Kadukkai	:	Roasted in pan,inner seed was removed and rind was used
Thaandrikkai	:	Roasted in the pan, inner seed was removed and rind was used
Induppu	:	Soaked in 250ml of rice water for 3 days then dried in sunlight
Sengazhuneer kizhangu	:	Roots are washed in water and dried in the sunlight.
Kandangathri vaerpattai	:	Dust and odd materials were removed.
Thooduvalai vaerpattai	:	Dust and odd materials were removed.
Adathoda vaerpattai	:	Dust and odd materials were removed.
Siruvazhuthalai vaerpattai	:	Dust and odd materials were removed.

Preparation of the drug

Procedure

The purified ingredients were grounded separately as powder. The powder was sieved through a white cloth and all the powders were mixed well . It was kept in an air tight container and was labeled as "***Nagarasingadhi Chooranam"*** (*NSC*)**.**

Purification of the *Chooranam*:

***Pittaviyal murai* (Milk Steaming process):**

The ***Nagarasingadhi Chooranam*** was purified by *Pittaviyal* method (milk steam cooking) as per Siddha classical literature. A mud pot was taken and it was quarter filled by milk and quarter filled by water. The mouth of the pot was

sealed by a cloth. This Chooranam was then placed over the cloth and the pot was covered with lid and heated. The same drug was later dried and powdered then sieved again. It was used for the further study [69B].

Preservation of the drug

The prepared test drug was stored in a clean, air tight glass container.

Administration of the drug

Form of the medicine	:	*Chooranam* (Powder)
Route of Administration	:	Enteral
Dose	:	2 – 4gm (1/2 – 1 *varahan*)
Vehicle	:	Honey
Time of Administration	:	Twice per day
Indication	:	*Swasa kasam* (Bronchial asthma) *Irumal* (Cough) *Ulai maandham* (Intestinal TB) *Vaayu vahaigal*.

INGREDIENTS OF *NAGARASINGADHI CHOORANAM*:

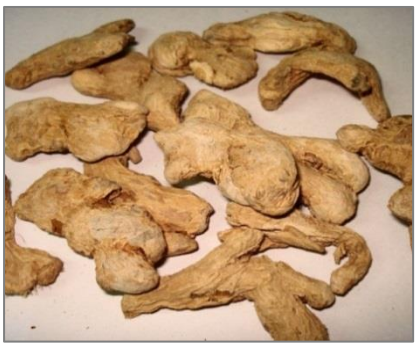


Fig.1.1. *Zingiber officinale*

Fig.1.2.*Piper nigrum*

Fig.1.3 *Piper longum*

Fig.1.4.*Nardostachys jatamansi*

Fig.1.5.*Clerondendrum serattum*

Fig.1.6.*Rhus succedanea*

Fig.1.7.*Costus speciosus*

Fig.1.8.*Phyllanthus emblica*

Fig.1.9.*Terminalia chebula*

Fig.1.10.*Terminalia bellerica*

Fig.1.11.*Sodium chloride impura*

Fig.1.12.*Nymphae alba*

Fig.1.13.*Solanum xanthocarpum*

Fig 1.14.*Solanum trilobatum*

Fig.1.15.*Justicia adhatoda*

Fig.1.16.*Solanum melongena*

Fig.2.*Nagarasingadhi Chooranam*

4.2. STANDARDIZATION OF THE DRUG

Standardization of the drug brings the validation to be used as a medicine by subjecting the drug *NSC* to many analysis and determining its quality and effectiveness. Standardization includes many studies such as its organoleptic properties, physical characteristics and phytochemical properties and also to assess the active principles and elements present in the drug. Thus standardization brings the efficacy and potency of the drug.

4.2.1. ORGANOLEPTIC EVALUATION

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

4.2.2. PHYSICOCHEMICAL ANALYSIS

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

Determination of Ash Values

Total Ash

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

Water Soluble Ash

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

Acid insoluble Ash

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

Determination of Extractive Value

Alcohol Soluble Extractive Value

3g of the *NSC* was weighed and macerated with 100ml of Ethanol in a closed container for 24 hours. The resulting solution was shaken continuously for 6 hours. It was then allowed to stand and soak for 18 hours. The solution was filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105^{o}C. Then the content was cooled and weighed.

Water soluble Extractive value

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

Loss on Drying

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

Physical characterization

Solubility:

- A pinch of *NSC* was shaken well with distilled water.
- A little amount of the *NSC* was shaken well with conc.HCl and Con H_2SO_4. Test sample *NSC* solubility was observed.

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

4.2.3. PHYTOCHEMICAL ANALYSIS

The Phytochemical screening of the extract gives general idea regarding the nature of chemical constituents present in the crude drug.

The *NSC* was subjected to the following phytochemical screening [72].

Preparation of the extract

5g of *NSC* 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.

Test for Alkaloids

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

Mayer's reagent - No Cream precipitate was formed.

Test for Carbohydrates and Reducing Sugars

The minimum amount of *NSC* extract was dissolved in 5ml of distilled water & filtered. The filtrate was subjected to test for carbohydrates & glycosides.

Molisch's test

1 ml of *NSC* filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol & 2ml concentrated sulphuric acid was added along the sides of test tube. Violet ring was observed at the junction of 2 layers which showed the presence of carbohydrate was noted.

Test for Glycosides

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

Modified Borntrager's test

To the hydrolysate *NSC* extract, 1 ml of Ferric chloride solution was added and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal voulme of benzene. The benzene layer was separated and treated

with Ammonia solution. Formation of rose pink colour in the Ammoniacal layer indicates the presence of Anthranol glycosides was noted.

Test for Saponins

The extract of *NSC* was taken into 0.5 ml was shaken with 5 ml distilled water. The presence of saponins was indicated by formation of copious lather was noted.

Test for Phenolic compounds

To 0.5 ml of *NSC* extract, 1 ml of alcoholic Ferric chloride solution was added. Formation of bluish green or bluish black indicates the presence of Phenolic compounds.

Test for Phytosterol

Ferric chloride – acetic acid test

1 ml of *NSC* 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 shows the presence of phytosterol.

Test for Triterpenes

Salkowski's test

1 ml of *NSC* extract was treated with 1 ml of chloroform followed by 1 ml of conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour shows the presence of triterpenes.

Test for Flavonoids

Alkaline reagent test

To 1 ml of *NSC* extract, 1 ml of 10% Sodium hydroxide solution was added. No formation of dark yellow colour indicates the absence of flavonoid.

Test for Proteins and Free Amino Acids

Xanthoproteic test

To 1 ml of *NSC* extract, 3-4 drops of Conc. Nitric acid was added. Formation of yellow precipitate was recorded.

Test for Quinones

Sodium hydroxide test

To 0.5 ml of *NSC* extract, 1 ml of 10% Sodium hydroxide was added. No formation of blue or green or red colour indicates the absence of quinones.

TLC/ HPTLC finger print studies

HPTLC finger printing was carried out as per the reference [73].

Preparation of spray reagent-vanillin-sulphuric acid reagent

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

Chromatographic conditions

Instrument : CAMAG (Switzerland).

Sample Applicator : Camag Linomat - 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

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.

4.2.4. BIO-CHEMICAL ANALYSIS

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

Preliminary Basic and Acidic radical studies

Preparation of extract

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

Test for basic radicals

1. Test for Potassium

To a pinch of the *NSC* 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 *NSC* 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 *NSC* extract, drops of sodium hydroxide solution was added and watched for the appearance of white precipitate.

4. Test for Ammonium:

To 2ml of *NSC* extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added and observed for the appearance of brown colour.

5. Test for Sodium

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

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

NSC 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 *NSC* extract drops of sodium hydroxide solution was added and observed for white precipitate formation.

8. Test for Aluminium

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

9. Test for Lead

To 2 ml of *NSC* extract 2ml of potassium iodide solution was added and observed for the appearance for yellow coloured precipitate**.**

10. Test for Copper

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

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

11. Test for Mercury

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

12. Test for Arsenic

To 2 ml of the *NSC* extract 2ml of sodium hydroxide solution was added and observed for brown or red precipitate.

Test for acid radicals

1. Test for Sulphate

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

2. Test for Chloride

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

3. Test for Phosphate

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

4. **Test for Carbonate**

The *NSC* extract was treated with conc.Hcl and observed for froath appearance of effervescence.

5. Test for Fluoride & Oxalate:

To 2ml of *NSC* extract 2ml of dil.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 *NSC* 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.

4.2.5. AVAILABILITY OF MICROBIAL LOAD

BACTERIAL LOAD [74]

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 *NSC*.

Dilution:

A small measured volume of *NSC* are mixed with a large volume of sterile water or saline called the diluent or dilution blank. Dilution is 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
- 9 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 *NSC* extract 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 these procedures till the *NSC* 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 to used 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 *NSC* extract. 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:

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

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 was an excellent tool for quantitative analysis [(75)].

In FT-IR infrared was passed from a source through the sample *NSC*. This infrared was absorbed by the sample *NSC* 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 *NSC*. Like the finger print there was no two unique molecular structures producing the same infrared spectrum. It was recorded as the wavelength and the peaks seen in the spectrum indicates the amount of material present [(76)].

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 the sample material *NSC*. Variety of signals was produced on interaction with the surface of the sample *NSC*. This results in the emission of electrons or photons and it was collected by a appropriate detector [(77)].

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 *NSC* and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample *NSC* [78].

ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Manufacturer **:** Perkin Elmer

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

Principle:

An aqueous sample of *NSC* was 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) to different (atomic and/or ionic) states and produce characteristic optical emissions (lights). These releases are separated based on their respective wavelengths and their strengths are measured (spectrometry). The intensities are proportional to the concentrations of analyses in the aqueous sample *NSC*. 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 multi element 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 was analysed by ICP-OES [79].

Application:

The analysis of major and minor elements in *NSC* solution.

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), the *NSC* 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[80].

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

Sample preparation:

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

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

FTIR (Fourier Transform Infrared Spectroscopy)

Fig.3.1 FTIR INSTRUMENT

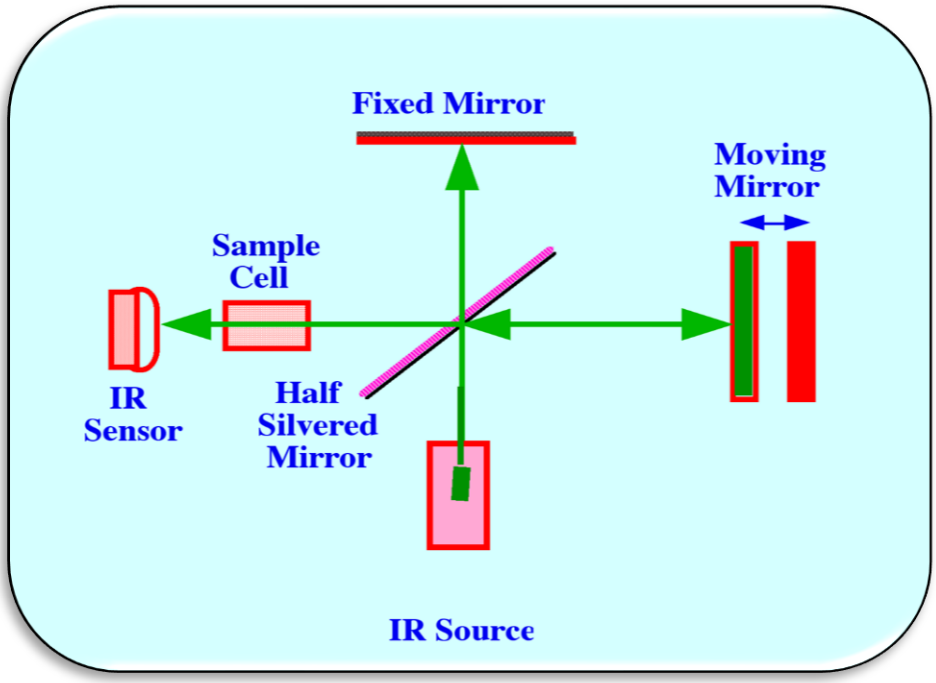


Fig.3.2 FTIR MECHANISM

SEM- SCANNING ELECTRON MICROSCOPE

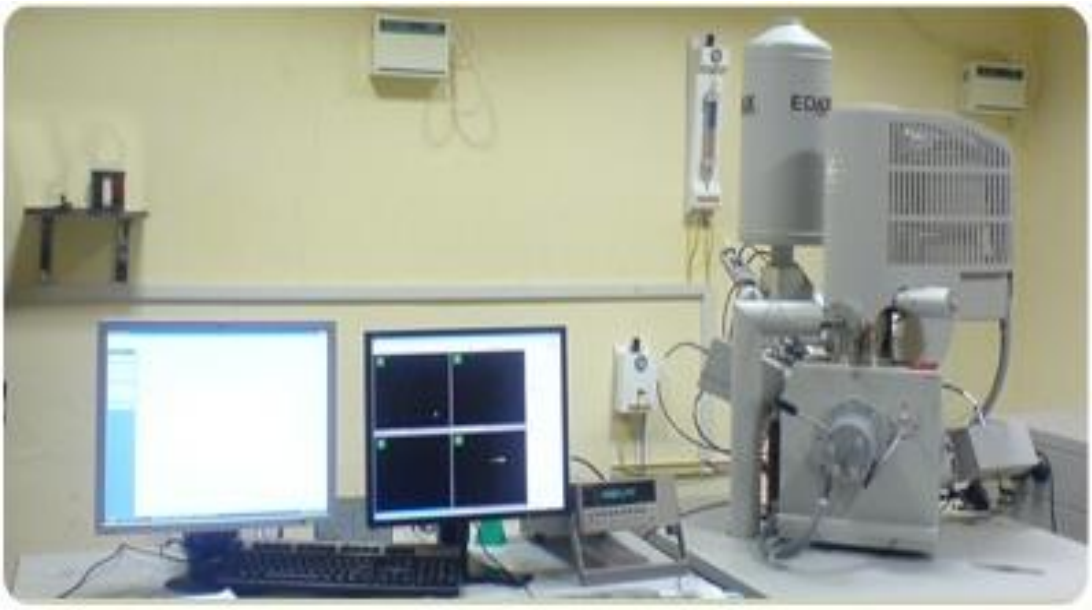


Fig.3.3 SEM INSTRUMENT

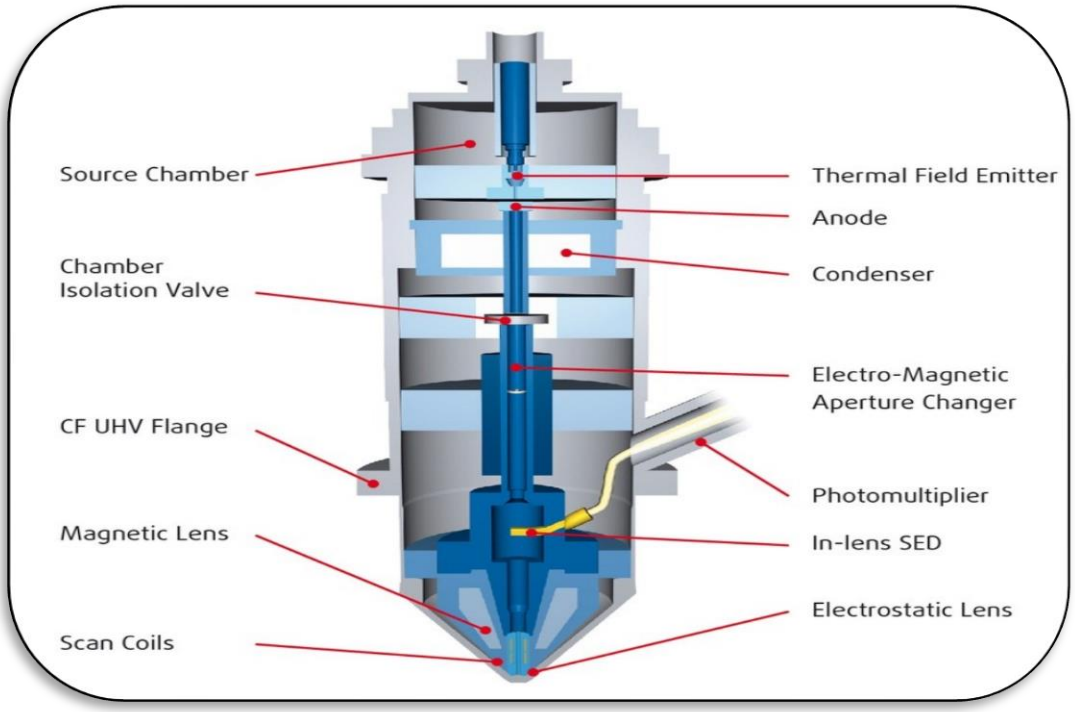


Fig.3.4 SEM MECHANISM

ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Fig.3.5 ICP-OES ANALYSER (Perkin Elmer Optima 5300 DV)

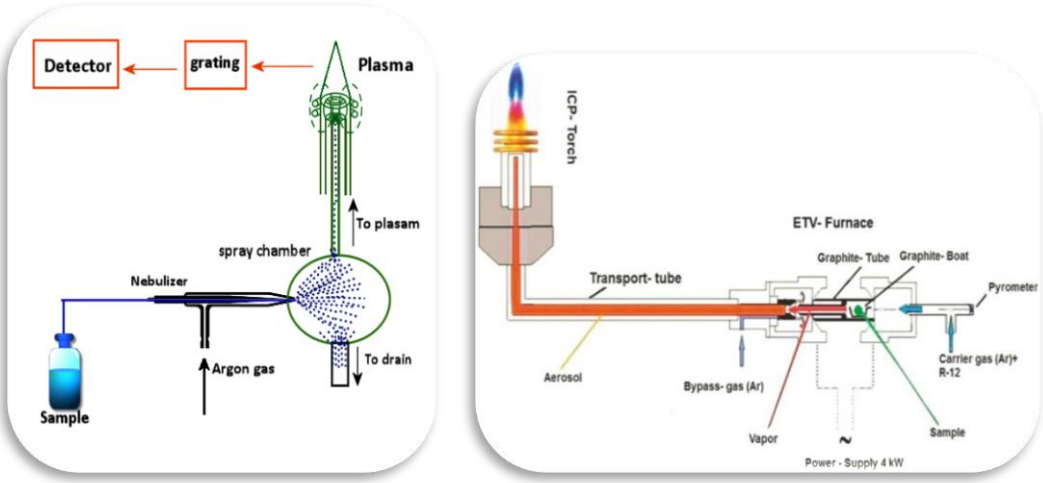


Fig.3.6 Mechanism of ICP-OES analyser

4.3. TOXICOLOGICAL STUDIES

4.3.1. ACUTE ORAL TOXICITY – OECD GUIDELINES – 423

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%.

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

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) under CPCSEA (Approval no: IAEC/XLIV/23/CLBMCP/2014) at C.L.Baid Metha college of Pharmacy, Thuraipakkam, Chennai.

PRINCIPLE:

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 was 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[81].

Animal: Healthy Wistar albino female rat weighing 200–220 gm. Studied carried out at three female rat 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. Healthy young adult animals of commonly used laboratory strain Swiss albino rat was obtained from Animal house of king's institute, Guindy, Chennai. Female 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 college 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

NSC prepared 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 *NSC* 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 hours

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 2000 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 was 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 necroscopic findings.

Test substance and Vehicle

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing *NSC* with 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 [82].

4.3.2. REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY OF *NAGARASINGADHI CHOORANAM* (*NSC*) ON RATS – (OECD- 407 guidelines)

Justification for Dose Selection

The results of acute toxicity studies in Wistar albino rats indicated that *NSC* was non-toxic and no behavioral changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected for the study were 100mg/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 [(83)].

Preparation and administration of dose

NSC at 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 4th 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 animals 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, haematology 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 ACTIVITIES:

4.4.1. Evaluation of Bronchodilator activity:

Overnight fasted guinea pigs were divided into four groups each containing 6 animals.

- Group 1 was treated as control,
- Group 2 received standard drug chlorpheniramine maleate (2 mg/kg).
- Groups 3 *NSC* 200 mg/kg
- Group 4 *NSC* 400mg/kg.

All the doses were given orally once a day for 5 days. Prior to drug treatment each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The pre convulsive time (PCT) was determined from the time of exposure to onset of convulsions. As soon as the PCT were noted, the animal were removed from the chamber and placed in fresh air. Group 2 received Chlorpheniramine maleate. These animals were again subjected to histamine aerosol after 1hr.of drug administration and PCT was determined. The protection offered by treatment was calculated by using the formula [84].

Percentage Protection = (1 - T1/T2) X 100 Where,

T1 = the mean of PCT before administration of test drugs.

T2 = the mean of PCT after administration of test drugs.

4.4.2. Evaluation of Antihistamine activity:

Vascular permeability test in rats : Immediately after an i.v. injection of 1 ml of 1 % Evans blue in physiological saline, two sites on one side of the shaved back of animals were injected intradermally with 0.1 ml of physiological saline containing 0.1 µg histamine, contralateral sites were injected intradermally with an equal volume of physiological saline (the control skin areas). Nagarasingadhi Chooranam was given orally 30 min in rats prior to the injection of phlogistics . Thirty minutes later, the

animals are sacrificed by overdose of anesthesia, and the skin was removed. Exudation of dye was calculated by subtracting the amount determined in the control skin area and expressed as the mean of two values obtained in each animal [85].

Calculation:

Area of protection = control area – area of exudation of dye

Grouping: Wistar rats were used for the study n=6nos

Group I ---------- Control group

Group II --------- Standard drug Cetirizine 20mg/kg

Group III --------- Nagarasingadhi Chooranam 100mg/kg

Group IV ---------- Nagarasingadhi Chooranam 200mg/kg

Experimental Procedure

Guinea pig was sacrificed and a segment from ileum (2 cm) was dissected from the terminal ileum and mounted in an organ bath containing Tyrode solution (10 ml) between two stainless steel hooks under 0.5 to 1 g initial tension. The lower hook was fixed at the bottom of the organ bath and upper one was connected to an isotonic transducer. The Tyrode solution composition (pH 7.4) was NaCl-8.0, KCl-0.2, $CaCl_2$-0.2, $MgCl_2$-0.1, $NaHCO_3$ .1.0, NaH_2PO_4-0.05, and Glucose-10.0gms/liter.

It was continuously aerated and maintained at 37 ± 0.5ºC. The equilibrium period was 60 min and the bath solution was refreshed every 15 min. After equilibrium period, a dose response curve for histamine in variant molar concentrations, by maintaining 45 min time cycle was taken separately. Results are provided in the table

Statistical Analysis

Ileum contractions induced by agonist were assumed as 100% and reductions induced by test drug calculated. Percentage of ileum contraction was expressed as mean ± SEM. Results were analyzed using one-way analysis of variance (ANOVA). Probability value less than 0.05 were considered as significant. Results are discussed in table.

Method:

After the end of sub-acute toxicity study, the intermediate dosage group of animals were sacrificed and organs such as liver and kidneys were excised out and analyzed for oxidative stress markers. The concentration of oxidative stress markers such as Lipid peroxide, Glutathione, Glutathione peroxidase and Catalase were analyzed. Lipid peroxides (Thiobarbituric Acid Reactive Substances – TBARS) in tissues were assayed by the method of Yagi (Yaki K, 1978). The colour formation with Thiobarbituric acid (TBA) was used as index. Reduced glutathione (GSH) was estimated by the method of Ellman in which yellow colour developed when dithionitro-bis-benzoic acid (DTNB) added to the compounds sulfhydryl groups (Ellman GL, 1959). Glutothione peroxidase (GPx) estimated by the method of Rotruck et al, 1973 in which H2O2 reduced to water whereas organic hydroperoxides reduced to alcohol at the expense of GSH (Rotruck JT et al. 1973). The activity of Catalase (CAT) was determined by the method of Sinha (Sinha AK, 1972). In this assay, Dichromate in acetic acid heated in the presence of hydrogen peroxide converted to perchromic acid and then to chromic acetate. The formed chromic acetate was measured at 620 nm.

4.4.3. Evaluation of Antioxidant activity (In-Vitro Model):

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

The radical scavenging activity of *NSC* extracts was determined by using DPPH assay according to Chang et al. (2001). The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

Principle

1, 1 Diphenyl 2- Picryl Hydrazyl is a stable (in powder 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,

$$(DPPH) + (H\text{-}A) \rightarrow DPPH\text{-}H + (A)$$

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 4mg of DPPH in 100ml of ethanol.

Working procedure

Different volumes (1.25-20µg/µl) of *NSC* extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of the *NSC* extracts was calculated using the following formula,

$$\% \text{ inhibition} = \frac{\text{Control} - \text{test}}{\text{Control}} \times 100$$

5. RESULTS AND DISCUSSION

Many studies have been carried out to bring the efficacy and potency of the drug *Nagarasingadhi Chooranam.* The studies includes Literary collections, Organoleptic character, Physicochemical and Phytochemical analysis, Microbial load, Instrumental analysis, Toxicological studies and Pharmacological studies. The drug *Nagarasingadhi Chooranam* has been selected for Bronchodilator activity in reference with the text ***"Anuboga Vaidhiya Navaneedham (Part 8)".***

- Literary collections about the drug from various text books were done. Siddha literatures related to ingredients of the drug bring the evidence and importance of its utility in treating Bronchial Asthma.
- Botanical aspect explains the identification, description, active principle and medicinal uses of the plants.
- Gunapadam review brings the effectiveness of the drug in treating Bronchial Asthma.
- Pharmaceutical review describes about the *Chooranam* and its properties.
- The Pharmacological review explains about the methodology of Bronchodilator Activity, Anti histamine activity.
- Modern and Siddha aspect of the disease was also reviewed.

Most of the herbs included in this preparation exhibit bronchodilator effect which is useful in the treatment of bronchial asthma.

Zingiber officinale relaxes the airway smooth muscle and potentially serve as novel bronchodilator [(86)]. It also possesses anti-oxidant activity [(87)].

Piper nigrum has contains a pungent alkaloid ''Piperine'' which is known to possess many pharmacological actions like antioxidant, anti-asthmatics, anti-inflammatory, anti-diarrheal, antispasmodic, immunomodulatory activity. All these activity primarily favours the treatment of bronchial asthma [(88)].

Piper longum also have Piperine, which is the prime constituent of fruit which have significant anti-inflammatory and antioxidant activity [(89)]. It also possesses Expectorant effect and is commonly used for Bronchial asthma [(90)].

Nardostachys jatamansi exhibits bronchodilator and antispasmodic activity [(91)].

Clerodendrum serratum have saponin which is responsible for Anti-inflammatory and Anti-oxidant activity [92]. It also possesses anti-asthmatic property [93].

Rhus succedanea exhibit Expectorant action which is useful for cough, fever and other ailments of respiratory tract [94].

Costus speciosus contains Anti-inflammatory [95], Anti spasmodic [96] and Expectorant effects [97].

Phyllanthus emblica have various actions such as Anti-oxidant, anti-inflammatory, anti-tussive, immune modulatory actions [98]. It improves immunity and fight against the chronic inflammatory diseases [99].

Terminalia chebula exhibit strong antioxidant and immune modulator effects [100]. It is antiasthmatic and used for cough, dyspnoea and asthma [101]. It has laxative action and is beneficial in treating asthma associated with constipation [102].

Terminalia bellirica is used for the treatment of asthma due to its antispasmodic and bronchodilator activities [103].

Sodium chloride impura produces Anti-inflammatory, Anti-oxidant and Antihistamine properties. It provides relief from respiratory symptoms [104].

Nymphaea alba have Anti-inflammatory activity [105] and hence used in bronchial asthma [106].

Solanum xanthocarpum exhibits expectorant and anti-inflammatory actions. It produces diminishing effect in the intensity of cough and dyspnoea [107].

Solanum trilobatum possess Mast cell degranulation inhibition property. It causes low release of histamine from the mast cell resulting in reduction of airway secretions. It also has antioxidant and anti-inflammatory activities [108].

Justicia adhatoda have bronchodilator, expectorant and anti-spasmodic activity [109]. It supports the entire respiratory system and its bronchial functions. Used for the treatment of chest congestion. Its expectorant property is valued for treating asthma, fever. The primary constituents namely Vasicine and vasicinone are potent bronchodilators that eases the breathing process, reduces wheezing due to asthma [110]. It also has anti-tussive effect which relief cough [111].

Solanum melongena is reported to have anti-asthmatic, antioxidant and anti-inflammatory activities [112]. Used in the treatment of asthma [113].

Toxicological results of the drug and pharmacological activity of the drug are derived. Its result has been tabulated and interpretation is made below. Thus, it is to give a complete justification, to bring the effectiveness of the trial drug Nagarasingadhi *Chooranam*.

STANDARDIZATION OF THE TEST DRUG

Standardization of the drug is more essential to derive the efficacy, potency of the drug by analysing it by various studies. The standardization of the drugs was achieved through various procedures like analysing the organoleptic characters, physico-chemical characters, elements present in the drug and the results and discussion of standardization parameters is described below. The following characters have been noted in *Nagarasingadhi Chooranam.*

ORGANOLEPTIC CHARACTER

Table: 2.Results of Organoleptic Characters

Colour	Brown
Odour	Characteristic odour
Taste	Pungent
Texture	Fine powder
Particle size	Completely pass through sieve no 88

PHYSICOCHEMICAL ANALYSIS

Table: 3. Results of Physicochemical analysis

S.No	Parameter	Result
1.	pH	6.5
2.	Total Ash	17.83
3.	Acid Insoluble ash	0.905 %
4.	Water soluble ash	9.03 %
5.	Loss on drying at 105^0C	6.19%
6.	Water soluble Extractive	17.09%
7.	Alcohol soluble Extractive	13.69%

DISCUSSION:

pH value

- The pH of *NSC* is 6.5. It is weak acidic in nature.
- This pH level plays a role in enzyme activity by maintaining the chemical environment thus regulating the homeostasis.
- It is also an important factor for drug absorption. Being weak acidic, the drug is more readily absorbed in an acid medium like stomach which enhance the bio availability of the drug.

Total ash

- Total ash value will determine the amount of minerals and earthy materials present in the drug
- The total ash value of *NSC* is 17.83% which determines the presence of inorganic content.

Acid insoluble ash

- The acid insoluble ash value of the drug denotes the amount of siliceous matter (dust, sand etc.,) present in that drug.
- The quality of the drug is better if the acid insoluble ash value is low.
- Here, acid insoluble ash value of *NSC* is 0.905%. Hence, it represents the superior quality of the *NSC*.

Water soluble ash

- Water soluble ash is a part of total ash value, which denotes the colloidal or crystalline nature of the drug.
- Here, the water soluble ash value of *NSC* is 9.03%, which represents easy facilitation of diffusion and osmosis mechanism.

Loss on Drying (LOD)

- It indicates the amount of volatile substance and moisture present in the drug.
- This also indicates the stability and shelf life of the drug.
- The loss on drying percentage of *NSC* is 6.19.
- Being a Chooranam, without incineration process, the moisture content is slightly high. So the stability and shelf life of *NSC* is about 1 yr.

PHYTOCHEMICALS ANALYSIS

Table: 4.Results of Phytochemicals screening test

Phytochemicals Tested	***NSC* Aqueous extract**
Alkaloids	Absent
Glycosides	Present
Saponin	Present
Carbohydrate	Present
phytosterol	Present
Phenol	Present
Triterpene	Present
Flavonoid	Absent
Quinone	Absent
Protein	Present

DISCUSSION:

Phytochemicals are natural bioactive compound, found in plants and fibres, which act as a defence system against diseases and more accurately to protect against diseases**.** The phytochemical analysis reveals the presence of Glycosides, Saponin, Carbohydrate, Phytosterol, Phenol, Triterpenes and Protein.

Glycosides

- Many plants store chemicals in the form of inactive glycosides, such plant glycosides are used as medications.
- Glycosides inhibit eosinophil accumulation in tissue and allergic inflammation.

- ❖ They can be effectively used for preventing or treating allergic diseases associated with inflammation and eosinophil accumulation such as COPD, Bronchial asthma and Allergic rhinitis [114].

Carbohydrates

- ❖ Carbohydrates provide energy for physical activity and functions of the body.
- ❖ Repair of epithelial tissue injury in asthma was made by carbohydrates [115].

Phytosterol

- ❖ Plants steroids have potential anti-inflammatory effect. They are important to cure the chronic inflammatory diseases like bronchial asthma [116].
- ❖ Phytosterol exerts anti-oxidant effect [117].

Saponins

- ❖ It has anti-spasmodic, anti-inflammatory, expectorant and anti-oxidant property.
- ❖ Saponins quicker the expulsion of mucus from the lungs [118].

Phenols

- ❖ Phenol groups are the essential part of many anti-oxidant compounds.
- ❖ They possess rich Anti-Oxidant property and protect body from oxidative stress.
- ❖ It has anti-inflammatory property [119].

Triterpene [120]

- ❖ They possess Anti-oxidant, Anti-inflammatory and mucolytic activity.
- ❖ Suppress the inflammatory response
- ❖ They are often expectorant
- ❖ It will aid absorption of nutrients.

Proteins [121]

- ❖ Protein is an important component of every cell in the body.
- ❖ Body uses protein to build and repair tissues.
- ❖ Amino acids delay the progressive nature of the diseases and aging process.

A synergistic effect of all these Glycosides, Saponin, Carbohydrate, Phytosterol, Phenol, Triterpenes and Protein increases the potency of the drug against Bronchial asthma.

TLC/HPTLC analysis of chloroform extract

HPTLC analysis

Chloroform extract was applied in TLC aluminium sheet silica gel 60 (E. MERCK) and plate was developed using the solvent system Toluene: Ethyl acetate (9:1). After development, the plate is allowed to dry in air and examined under UV - 254nm, 366 nm and Visible light (Vanillin - Sulphuric acid).

HPTLC Chloroform extracts Photos

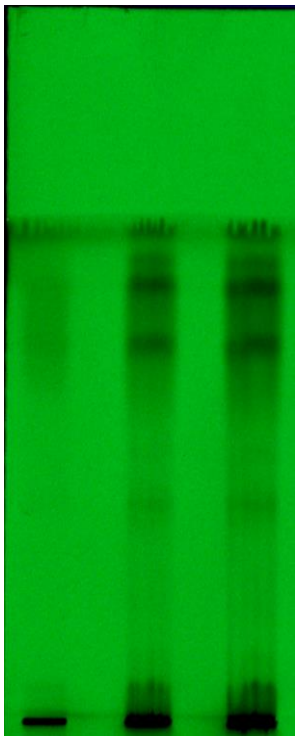
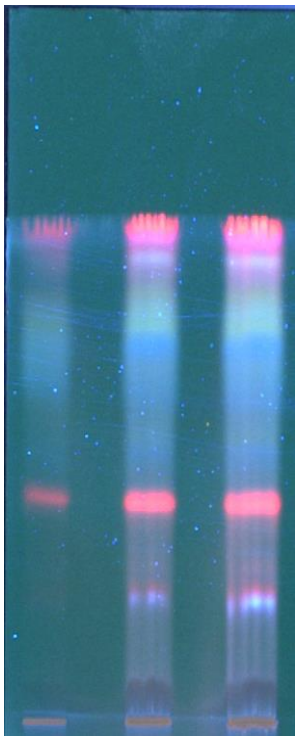
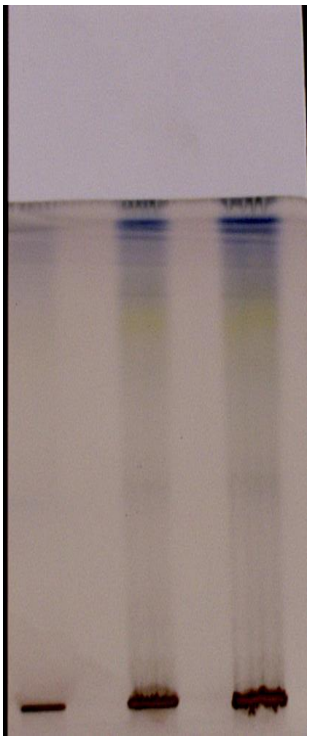
		
UV-254 nm	**UV-366 nm**	**White light after dipping in Vanillin-Sulphuric acid**

Fig.4.1 HPTLC finger print studies

Table: 5. Values for the chloroform extract of *Nagarasingadhi Chooranam*

Color	Rf value(s)	Color	Rf value(s)	Color	Rf value(s)
Green	0.08	Blue	0.07	Pale yellow	0.76
Green	0.75	Pale blue	0.23	Violet blue	0.91
Green	0.87	Rose	0.26	Magenta blue	0.94
Green	0.98	Rose	0.43	Magenta blue	0.95
		Bluish green	0.77		
		Rose	0.90		
		Rose	0.94		
		Rosy red	0.98		

HPTLC finger print analysis for chloroform extract

The finger print chromatogram was recorded at 254 nm. It showed 9 peaks of which peaks at Rf. were the major peaks and others were moderately smaller peaks.

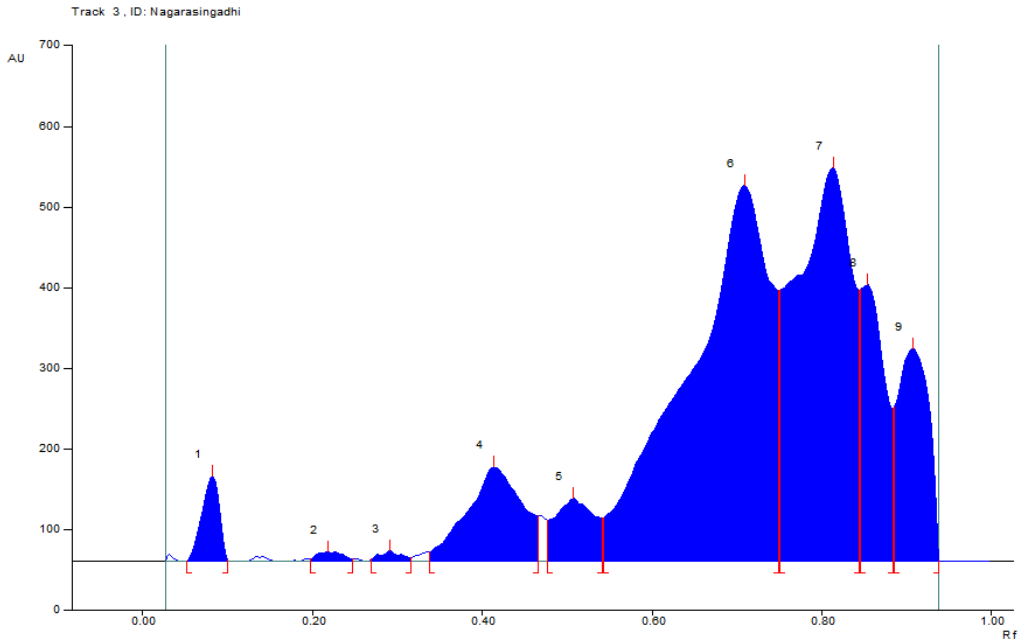


Fig: 4.2. HPTLC finger print for chloroform extract

Table: 6. Chloroform extracts - Rf values in HPTLC finger print

Track 3, ID: Nagarasingadhi

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	0.4 AU	0.08 Rf	105.0 AU	5.58 %	0.10 Rf	1.6 AU	1827.4 AU	1.98 %
2	0.20 Rf	3.4 AU	0.22 Rf	12.0 AU	0.64 %	0.25 Rf	2.3 AU	305.3 AU	0.33 %
3	0.27 Rf	2.2 AU	0.29 Rf	13.6 AU	0.72 %	0.32 Rf	4.5 AU	268.0 AU	0.29 %
4	0.34 Rf	11.1 AU	0.42 Rf	116.5 AU	6.19 %	0.47 Rf	56.4 AU	6235.7 AU	6.77 %
5	0.48 Rf	51.1 AU	0.51 Rf	77.8 AU	4.13 %	0.54 Rf	53.8 AU	3040.9 AU	3.30 %
6	0.54 Rf	54.2 AU	0.71 Rf	465.3 AU	24.71 %	0.75 Rf	35.9 AU	37262.5 AU	40.43 %
7	0.75 Rf	336.0 AU	0.82 Rf	487.1 AU	25.87 %	0.85 Rf	36.1 AU	27207.2 AU	29.52 %
8	0.85 Rf	336.6 AU	0.86 Rf	342.3 AU	18.18 %	0.89 Rf	89.4 AU	8191.0 AU	8.89 %
9	0.89 Rf	191.0 AU	0.91 Rf	263.3 AU	13.98 %	0.94 Rf	0.0 AU	7818.2 AU	8.48 %

DISCUSSION:

A qualitative fingerprinting of *Nagarasingadhi Chooranam* 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 *Nagarasingadhi Chooranam*. The method may be applied to identify the *Nagarasingadhi Chooranam* from other manufacturing process.

The present study revealed that *Nagarasingadhi Chooranam* showed best results in Toluene: Ethyl Acetate: 9:1 solvent system. After scanning and visualizing the plates in absorbance mode at both 254 nm, 366 nm and visible light range, best results were shown at 254 nm.

TLC plate showed different colour phyto constituents of chloroform extract of *Nagarasingadhi Chooranam.* The bands revealed presence of four greenish, one blue, one pale blue, four rose, one bluish green, one rosy red, one pale yellow, one violet blue and two magenta blue bands showing the presence of glycosides, carbohydrate, phytosterol, alkaloids, phenol,triterpene and saponins.

The results from HPTLC finger print scanned at wave length 254 nm for chloroform extract of *Nagarasingadhi Chooranam*. There are ten polyvalent phyto constituents and corresponding ascending order of Rf values start from 0.05 to 0.89 in which highest concentrations of the phyto constituents was found to be 25.87 % and 24.71 % with its corresponding Rf value were found to be 0.75 and 0.54 respectively.

BIO CHEMICAL ANALYSIS:

Table: 7. Results of basic radicals studies

S.NO	Parameter	Observation	Result
1	Test for Potassium	Formation of Yellow colour precipitate	Positive
2	Test for Calcium	Formation of White colour precipitate	Positive
3	Test For Magnesium	Formation of White colour precipitate	Positive
4	Test For Ammonium	-	Negative
5	Test For Sodium	-	Negative
6	Test for Iron (Ferrous)	Appearance of Blood red colour	Positive
7	Test For Zinc	-	Negative
8	Test For Aluminium	-	Negative
9	Test For Lead	-	Negative
10	Test for Copper	-	Negative
11	Test For Mercury	-	Negative
12	Test for Arsenic	-	Negative

Interpretation

The basic radicals studies of *NSC* shows the presence of **Potassium, Calcium, Magnesium and Iron** and absence of heavy metals such as lead, arsenic and mercury.

- Presence of iron in the drug has increased haemoglobin concentration in the blood. It enhances the arterial oxygen level. The drug enhances oxygen supply, promotes the normal ventilation of the lungs and reduces the dyspnea. It also reduces airway hyperactivity and eosinophilia.
- Calcium is important for normal muscle contraction and blood vessel structure. Calcium is a cell signaling mineral, which means it plays a vital role in cell-to-cell communication. Contraction and expansion of respiratory muscles allows the lungs to breathe in and out. Calcium is crucial for muscle movement, and it helps an individual's to maintain normal breathing rhythm.
- Calcium and potassium cell-signaling channel plays almost important role in regulatory part of the respiratory system, breathing rhythm, and the body's response to insufficient oxygen levels. So this drug stimulates normal respiratory mechanism [(122)].
- The Mg ions are responsible for bronchodilator and anticholinergic action which helps in acute asthma [(123)].

So Calcium, Potassium, Magnesium and Iron of this drug help to achieve its activity on bronchial muscles.

Table: 8. Results of acid radical studies

S.NO	Parameter	Observation	Result
1.	Test for Sulphate	-	Absent
2.	Test for Chloride	Formation of white precipitate	Present
3	Test for Phosphate	Formation of yellow precipitate	Present

S.NO	Parameter	Observation	Result
4	Test for Carbonate	-	Absent
5	Test for fluoride & oxalate	-	Absent
6	Test For Nitrate	-	Absent

Interpretation:

The acid radical study of *NSC* shows the presence of **Chloride, Phosphate**.

Chloride

- Chloride is needed to keep the proper balancing of body fluids [(124)].
- It maintains proper blood volume and pressure [(125)].
- It plays critical roles in inflammatory airway diseases such as Bronchial asthma, Allergic rhinitis [(126)].

Phosphate

- Phosphate is a charged particle that contains the mineral phosphorus [(127)].
- The mineral Phosphorus is primarily used for growth and repair of body cells and tissues [(128)].
- It reduces the histamine release by activated mast cells [(129)].

AVAILABILITY OF MICROBIAL LOAD IN *NAGARASINGADHI CHOORANAM*

Bacterial load

Fig: 5.1. Bacterial load 10^{-4}

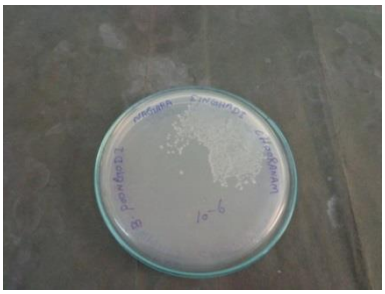


Fig: 5.2. Bacterial load 10^{-6}

Fungal load

Fig: 5.3. Fungal load 10^{-2}

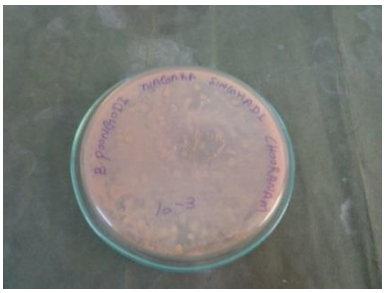


Fig: 5.4. Fungal load 10^{-3}

Total bacterial load in 10^{-4} dilution is 15 and in 10^{-6} dilution is 9

Total fungal load in 10^{-2} dilution is 9 and 10^{-3} dilution is 4 .

DISCUSSION:

The Herbo-mineral drug are 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 [(131)].

Here the contamination of *NSC* have been examined by bacterial and fungal load.

- Total bacterial load in 10^{-4} dilution is 15 and in 10^{-6} dilution is 9.
- Total fungal load in 10^{-2} dilution is 9 and in 10^{-3} dilution is 4.

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

INSTRUMENTAL ANALYSIS

FT-IR (Fourier Transform Infra-Red Spectroscopy)

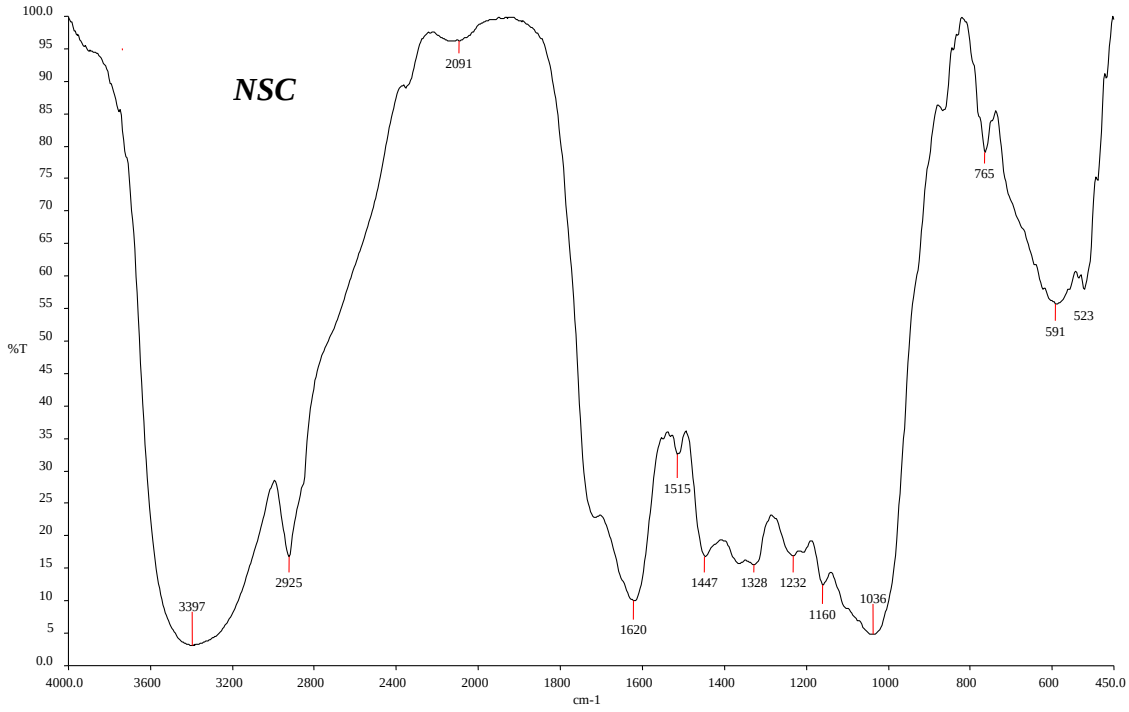


Fig: 6. FT-IR Graph of *Nagarasingadhi Chooranam*

Table 9: FT-IR Interpretation of *Nagarasingadhi Chooranam*

Absorption peak cm^{-1}	**Stretch**	**Functional group**
3393	O-H(stretch, H- Bonded), N-H stretch, N-H stretch	Alcohols, Amide and Amine
2925	C-H stretch, O-H stretch	Alkane and Acid
1620	C=C stretch	Alkene
1515	C=C stretch	Aromatic
1447	-C-H stretch, C=C stretch	Alkane, Aromatic
1328	C-F stretch, C-N stretch	Alkyl Halide, Amine
1232	C-F stretch, C-N stretch, C-O stretch, C-O stretch, C-O stretch	Alkyl Halide, Amine, Ether, Ester and Acid
1160	C-N stretch, C-F stretch, C-O stretch, C-O stretch	Amine, Alkyl Halide, Ether, Ester
1036	C-F stretch, C-O stretch, C-O stretch	Alkyl Halide, Ether, Ester
765	C-Cl stretch, =C-H bending	Alkyl Halide, Alkene
591	C-Br stretch	Alkyl Halide
523	C-Br stretch	Alkyl Halide

DISCUSSION:

FTIR instrumental analysis was done. The test drug was identified to have 12 peaks. They are the functional groups present in the trial drug Nagarasingadhi *Chooranam*.

The above table shows the presence of Alcohol, Amides, Amines, Acid, Aromatics, Alkyl halides, Alkene, Ether and alkanes which are represents the peak value.

- OH group has anti asthmatic effect. It has higher potential towards inhibitory activity against airway inflammation [(131)].
- Amide has mucolytic activity. It makes the mucus less thick and sticky and easier to cough up [(132)].

SEM: (SCANNING ELECTRON MICROSCOPE)

SEM images of *Nagarasingadhi Chooranam*

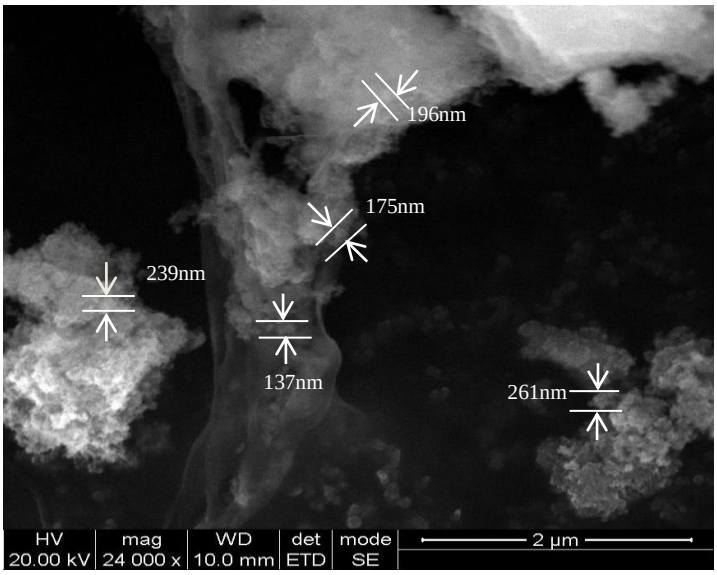


Fig: 7.1. SEM image of 2 µm of *Nagarasingadhi Chooranam*

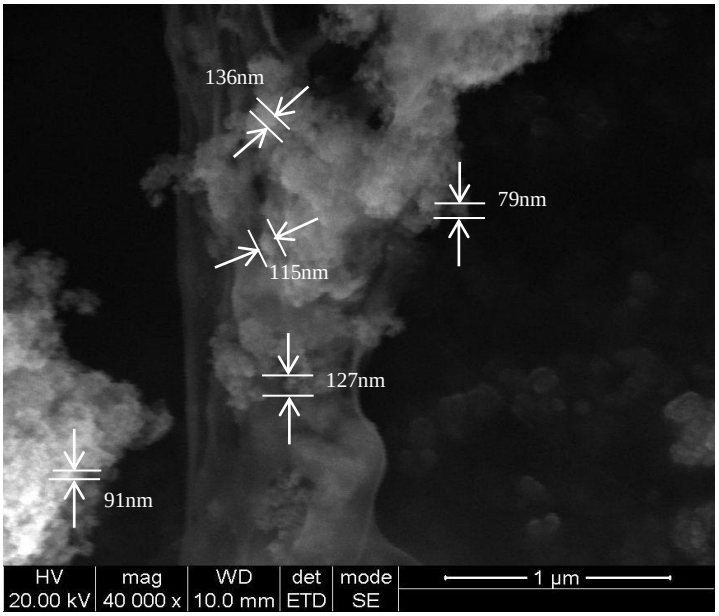


Fig: 7.2. SEM images of 1 µm of *Nagarasingadhi Chooranam*

Interpretation for SEM

- SEM analysis of the test drug *NSC* revealed the presence of Nano and Micro particles of size 79nm, 91nm, 136nm, 196nm and 261nm.
- Micro particles are defined as particulate dispersion or solid particles with a size in the range of 100 to 1000nm in diameter and Particles ranging from 1 to 100nm are called Nano particles
- Size and surface of micro particles can be easily manipulated to achieve both passive and active drug targeting. Nano medicine has its benefit in the treatment for many chronic diseases. Nano particles are smaller in size which enhances the solubility and bioavailability of the drug [133].
- The particles of Nano and micro particles control and sustain the release of drug during the transportation and at the site of localization, alters drug distribution in the body and subsequent clearance of the drug so as to achieve increased drug therapeutic efficacy thereby bio-availability and reduced side effects.

ICP-OES RESULTS OF *NAGARASINGADHI CHOORANAM*

Table 10: ICP –OES Results of *Nagarasingadhi Chooranam*

S. no	Elements	Detected levels
1.	Potassium	210.881mg/L
2.	Calcium	12.150 mg/L
3.	Phosphorus	88.571 mg/L
4.	Iron	18.370 mg/L
5.	Sodium	13.180 mg/L
6.	Arsenic	BDL
7.	Mercury	BDL
8.	Nickel	BDL
9.	Lead	BDL
10.	Cadmium	BDL

BDL:Below Detectable Limit

1% = 10000ppm,

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

The toxic metals and the permissible limits

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

DISCUSSION

ICP-OES reveals the concentration of many physiologically important minerals like Ca, Na, Fe, K & P in the drug *Nagarasingadhi Chooranam.* The heavy metals like As, Cd, Hg, Pb and trace element like Ni were below detectable level. This reveals the safety of the drug. Hence the formulation *Nagarasingadhi Chooranam* is extremely safe.

Phosphorus

- Phosphorus is an essential mineral primarily used for growth & repair of body cells and tissues [(128)].
- Phosphorus is best suited for cough that occurs with asthma.
- It is indicated in the treatment of bronchial asthma [(134)].

Iron

- Presence of iron in the drug has increased haemoglobin concentration in the blood and enhances the arterial oxygen level. The drug enhances oxygen supply, promotes the normal ventilation of the lungs and reduces the dyspnea.
- It also reduces airway hyperactivity and eosinophilia.

Calcium and Pottasium

- A potassium and calcium cell-signaling channel plays a most important role in regulatory part of the respiratory system, breathing rhythm and the body's response to insufficient oxygen levels [(121)].
- So this drug *NSC* stimulates normal respiratory mechanism.

So Calcium, Potassium, Iron, Sodium and phosphorus of this *NSC* drug help to achieve its activity on bronchial muscles.

TOXICOLOGICAL STUDIES RESULTS

Acute oral toxicity in rats:

Dose finding experiment and its behavioral Signs of Toxicity for *Nagarasingadhi choornam.*

Table: 11 Observation of acute toxicity studies

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: 12 Dose finding experiment and its behavioural Signs of Toxicity for *Nagarasingadhi Chooranam*

Dose mg/kg	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
2000	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

1. Alertness 2.Aggressiveness 3. Pile erection 4. Grooming 5.Gripping 6. Touch Response 7. Decreased Motor Activity 8.Tremors 9 Convulsions 10. Muscle Spasm 11.Catatonia 12.Musclerelaxant 13.Hypnosis 14.Analgesia 15.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 *Nagarasingadhi Chooranam* from the range of 5mg/kg to 2000mg/kg which did not produce signs of toxicity, behavioural 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 *Nagarasingadhi Chooranam* was found to be non-toxic at the dose level of 2000mg/ kg body weight.

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

Table: 13. Body weight(g) changes of rats exposed to *Nagarasingadhi Chooranam*

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
NSC 100	121.26 ± 0.49	122.91 ± 0.57	123.78 ± 0.51	127.65 ± 0.61	131.72 ± 0.52
NSC 200	121.97 ± 0.51	122.98 ± 0.52	123.97 ± 0.50	128.36 ± 0.61	132.05 ± 0.65

Values are expressed as mean ± S.D. N=3

Table: 14. Effect of *Nagarasingadhi Chooranam* on Organ weight in rats

Organ	**Control**	**100 mg/kg**	**200 mg/kg**
Liver (g)	3.07±0.20	3.26±0.15	3.37 ± 0.19
Heart (g)	0.32 ± 0.02	0.32 ± 0.06	0.33 ± 0.01
Lung (g)	0.28±0.05	0.29±0.04	0.31±0.06
Spleen (g)	0.25 ± 0.06	0.25 ± 0.05	0.25 ± 0.07
Brain (g)	0.37± 0.05	0.38 ± 0.01	0.38 ± 0.03
Kidney (g)	0.76 ± 0.05	0.76 ± 0.06	0.76± 0.07

*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: 15. Effect of *Nagarasingadhi Chooranam* on Haematological parameters in rats

Parameter	Control	100mg/kg	200 mg/kg
RBC(x 10^6/mm^3)	8.29±0.43	9.59±1.52	10.71±0.58
PCV (%)	49.66±0.77	50.79±0.70	52.46±0.65
WBC(x 10^3/mm^3)	11.75±0.85	12.97±0.89	13.75±1.14
Neutrophils (%)	23.29±0.73	23.79±0.56	24.41±0.61
Eosinophils 10(%)	4.10±0.23	4.03±0.23	3.15±0.33
Hb (%)	15.13±0.39	15.86±0.49	16.42±0.65
Platelets(x 10^3/mm^3)	425.73±1.35	427.9±1.36	430.56±1.37
Lymphocytes (%)	85.5±0.46	86.53±0.75	87.43±0.91

*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:16. Effect of Nagarasingadhi Chooranam on Biochemical parameters in rats

Parameters	Control	100 mg/kg	200 mg/kg
Glucose (mg/dl)	108.63±0.81	105.78±0.79	104.56±1.32
BUN(mg/dl)	22.06±1.55	22.37±1.48	21.64±1.45
Creatinine (mg/dl)	0.85±0.07	0.92±0.45	0.92±0.25
SGOT (U/L)	74.35±1.23	74.55±1.21	73.82±0.91
SGPT(U/L)	27.07±0.84	27.35±0.90	26.77±1.01
ALT (U/L)	104.63±1.14	104.87±1.11	103.98±1.09
Protein (g/dl)	8.58±0.68	9.47±0.59	11.51±0.56
Albumin (g/dl)	5.34±0.40	5.87±0.47	5.77±0.38
Total Cholesterol (mg/dl)	93.21±1.16	91.52±1.13	88.96±1.25
Triglycerides (mg/dl)	52.58±1.56	51.04±1.58	49.42±1.58

*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: 17 Effect of Nagarasingadhi Chooranam on Urine parameters in rats

Parameters	**Control**	**100 mg/kg**	**200 mg/kg**
Colour	Yellow	Yellow	Yellow
Transparency	Clear	Clear	Turbid
Specific gravity	1.01	1.02	1.04
Ph	7.2	7.4	6.9
Protein	Nil	Nil	Nil
Glucose	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve
Ketones	-ve	-ve	-ve
Blood	Absent	Absent	Absent
RBCs	Nil	Nil	Nil
Epithelial cells	Nil	Nil	Nil
Casts	Nil	Nil	Nil

DISCUSSION:

The dose selected for the Sub acute toxicity study was 100mg, 200mg/kg of Nagarasingadhi *Chooranam*

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 and body weight recorded did not show any significant differences in the treatment and the control group indicating that *Nagarasingadhi Chooranam* was not toxic to kidney, liver and spleen.

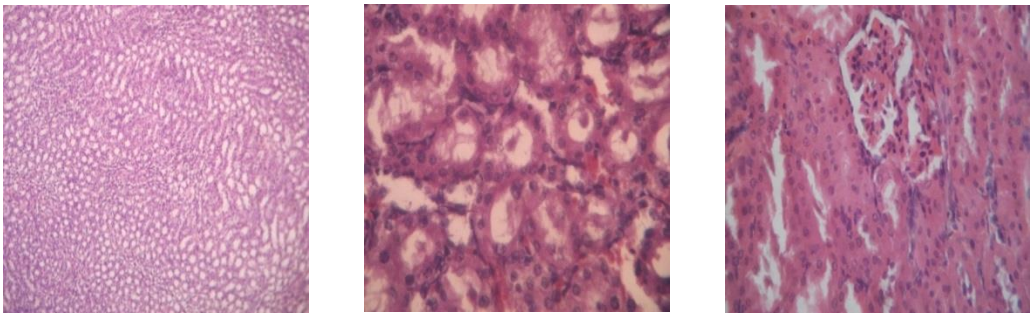
There was no significant changes were observed in haemoglobin (Hb), Red blood cell (RBC), White blood cell (WBC), Packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups.

HISTOPATHOLOGY SLIDES:

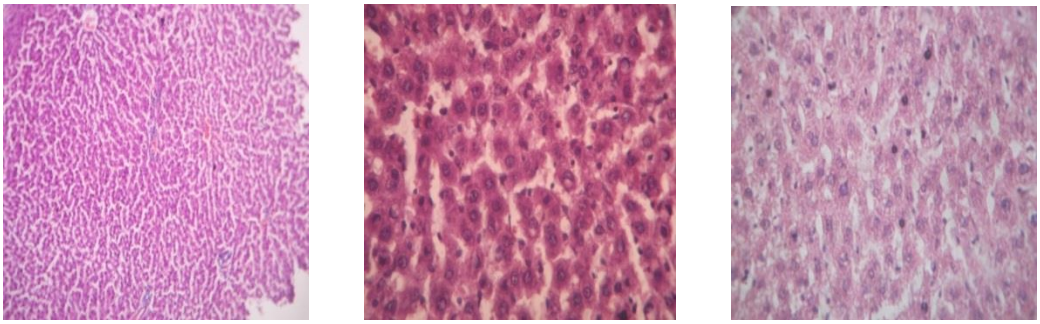
Fig.no.8. Histopathology slides

Control	*NSC* 100mg	*NSC* 200mg

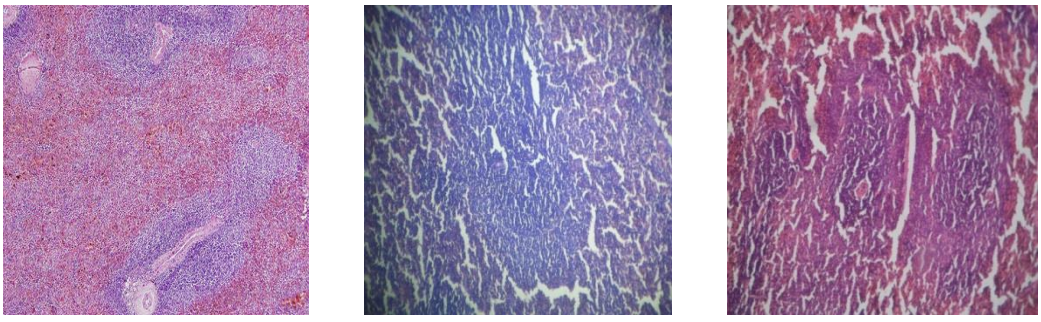
KIDNEY



LIVER



SPLEEN



DISCUSSION:

Histopathology studies were carried out on liver, kidney and spleen and recorded. Blood samples for haematological 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.

The acute and sub-acute toxicity studies of *NSC* drug produced some significant changes but the values were found within normal limits. So the drug was nontoxic and safe. It did not produced any adverse effect. So hopefully it could be used for human beings.

The histopathology studies of acute and sub-acute toxicity shows that there is no toxicological abnormality seen in the vital organs after administration of the test drug *Nagarasingadhi Chooranam*. Thus the safety of the drug is revealed so that it can be administered for long time without any side effects.

PHARMACOLOGICAL STUDY

BRONCHODILATOR ACTIVITY

Table: 18 Bronchodilator activity of *Nagarasingadhi Chooranam*

Serial No	Group	Onset of Convulsion in sec.	% protection
1	Control	96.93±1.31	--
2	Standard (Chlorpheniramine maleate)	989.77±2.03**	
3	Nagarasingadhi Chooranam (200mg/kg)	416.74.±1.80	
4	Nagarasingadhi Chooranam (400mg/kg)	566.92±.2.01*	

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control, N = 6*

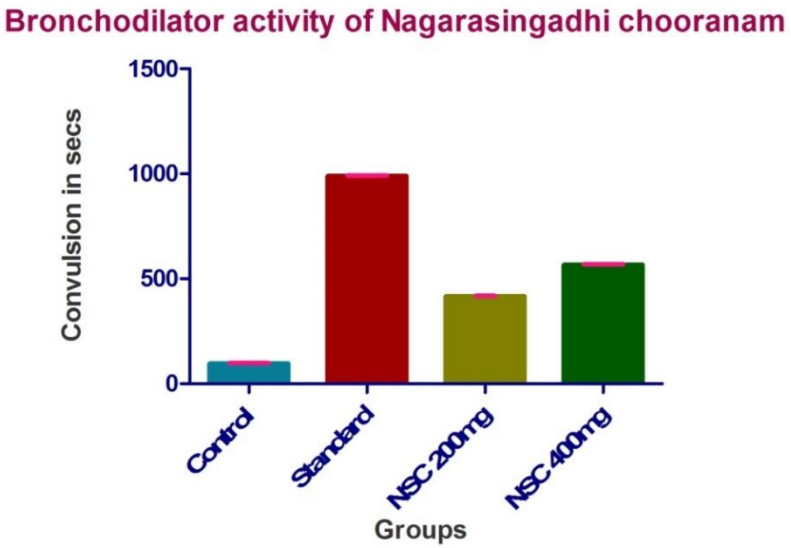


Chart No.1 Bronchodilator activity of Nagarasingadhi Chooranam

DISCUSSION:

From the above results, we can confirm that *NSC* possesses bronchodilator activity nearer to fifty percentage when compared with Chlorpheniramine maleate as a standard drug.

ANTI-HISTAMINE ACTIVITY:

Table: 19 Anti histamine effect of *Nagarasingadhi Chooranam*

S.no	Grouping	Area of protection from exudation of Dye in mm
1	Control	445.94±1.66
2	Cetirizine(STD)	127.11±0.99**
3	Nagarasingadhi Chooranam 100mg	182.02±3.87
4	Nagarasingadhi Chooranam 200mg	160.61±0.93*

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control, N = 6*

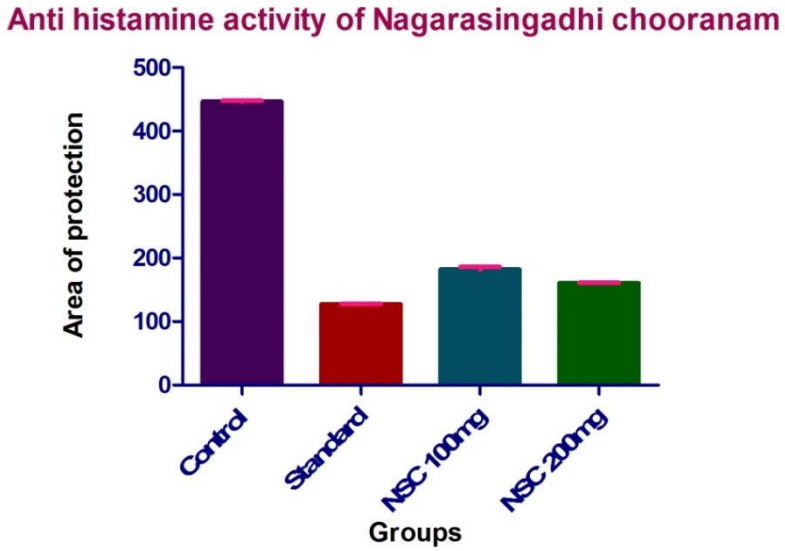


Chart No.2 Anti histamine activity of Nagarasingadhi Chooranam

DISCUSSION

Mediators like histamine, serotonin, and acetylcholine are implicated in various ways in the pathogenesis of Asthma. Histamine is the most implicates mediator in broncho constriction that accompany asthma although the role of serotonin in asthma is uncertain. *NSC* inhibited the histamine induced bronchospasm (vascular permeability) in rats, when compare with cetrizine as standard. Here, *NSC* possesses the Anti-histamine activity.

ANTI OXIDANT ACTIVITY:

Table: 20. DPPH Assay of *Nagarasingadhi Chooranam*

Sample concentration (µg/ml)	Absorbance		Percentage of Inhibition	
	Drug	Standard	Drug	Standard
Control	0.5271	0.312	-	-
1.25	0.1839	0.278	28.11	40.89
2.50	0.1659	0.202	36.52	51.25
5	0.1423	0.084	65.00	74.07
10	0.1286	0.052	75.60	83.33
20	0.0964	0.034	81.71	89.62

***µg/ml: microgram per millilitre. Drug: NSC (1.25-20µg/µl). Standard: Ascorbic acid (10mg/ml DMSO)**

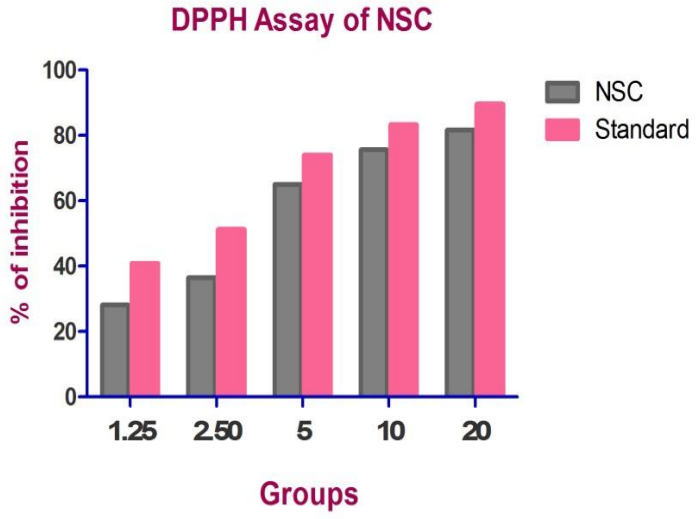


Chart No.3 Anti-oxidant activity of *Nagarasingadhi Chooranam*

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

In the present study, the extract of *NSC* was found to possess concentration dependent scavenging activity on DPPH radicals. The values of DPPH free radical scavenging activity of the *NSC* extract was given in (Table No.20). The extract of *NSC* showed the highest DPPH scavenging activity (81.71%) at 20µg/ml and the lowest percentage of inhibition (28.11%) at 1.25µg/ml. Ascorbic acid (Standard) showed highest percentage of inhibition (89.62%) at 20µg/ml and the lowest percentage of inhibition (40.89%) at 1.25µg/ml. This indicated that % of inhibition increased with increase in concentration of both the standard and *NSC* extract. The *NSC* extract has more or less equal DPPH scavenging activity when compared to the standard. From the present study, it was concluded that the *NSC* extract has a marked antioxidant activity at higher concentrations.

6. CONCLUSION

The trial drug *Nagarasingadhi chooranam* was selected from the classical Siddha literature, "*Anuboga Vaidhiya Navaneedham* (Part 8)" for the evaluation of safety and efficacy of the drug in *Swasakasam* (Bronchial asthma).

The trial drug was identified and authenticated by the botanist and experts of Gunapadam. Since the trial drug, *Nagarasingadhi chooranam* purification processes and the drug was prepared according to the classical methods. The purification process of this drug possible to eliminates their toxins and increases its efficacy and the grinding process of this drug helps to change the particle size of the drug for its better bio availability.

Phytochemical analysis of the drug shows presences of glycosides, saponins, carbohydrates, phytosterol, phenols, triterpene and Protein. Plants steroids are important to cure the chronic inflammatory diseases like Bronchial Asthma. Glycosides inhibit eosinophil accumulation in tissue and allergic inflammation. Repair of epithelial tissue injury in asthma was made by carbohydrates. Saponins quickens the expulsion of mucus from the lungs.

Biochemical analysis of basic radicals confirms the presence of Iron, Calcium, Potassium and Magnesium. Iron enhances enhances oxygen supply and promotes the normal ventilation of the lungs and reduces the dyspnoea. Magnesium ions are responsible for bronchodilator and anticholinergic action which helps in acute asthma.

Calcium and potassium cell-signalling channel plays almost important role in regulatory part of the respiratory system. So this drug stimulates normal respiratory mechanism.

Biochemical analysis of acid radicals shows the presence of Chloride, Phosphate. Chloride plays critical roles in inflammatory airway diseases such as Bronchial asthma. Phosphate reduces the histamine release by activated mast cells.

Instrumental analysis FT-IR results showed presence of Alcohol, Amide, Amine, Acid, Aromatic, Alkyl halides, Alkene, Ether and Alkane groups. Alcohol group has anti asthmatic effect. It has higher potential towards inhibitory activity

against airway inflammation. Amide has mucolytic activity. It makes the mucus less thick and sticky and easier to cough up.

SEM picture represents shows nano and micro particle varying sizes from 79nm to 261nm. It represents the drug is more absorbable and easily to reach the cell. The micro and nano particles present in the drug results in increased drug therapeutic efficacy thereby bio-availability and reduced side effects.

ICP-OES results show the presence of Phosphorus, Iron, Sodium, Calcium and Potassium. Phosphorus is best suited for cough that occurs with asthma. So it is indicated in the treatment of bronchial asthma.

Nagarasingadhi chooranam did not produce any oral acute or sub-acute toxicity in rats. So the drug was non-toxic and safe. The histopathology studies of acute and sub-acute toxicity shows that there is no toxicological abnormality seen in the vital organs after administration of the test drug *Nagarasingadhi chooranam*.

Nagarasingadhi chooranam could be conformed as No-Observed-Adverse Effect Level (NOAEL) drug as it acts harmless under normal usage and to be of no toxicological concern.

After evaluate the safety the drug, the Bronchodilator and anti-histaminic property of *Nagarasingadhi chooranam* is elaborated. So it can be concluded that this drug inhibits the tone of tracheal and bronchial muscles and thus has a bronchodilator action. It is possible that anti-histamine activity of the *Nagarasingadhi chooranam* mainly involves inhibiting the histamine induced bronchospasm. The NSC extract has more or less equal DPPH scavenging activity when compared to the standard. The NSC extract has a marked antioxidant activity at higher concentrations.

From the above scientific evaluation, the author concludes that the drug *Nagarasingadhi chooranam* is proficient with the new hope in the treatment of Bronchial asthma which is cost effective and has fair preparation method.

7. FUTURE SCOPE

The trial drug ***Nagarasingadhi Chooranam*** has its own potency in treating Bronchial asthma in animal model which has been established in this study. An incredible action of this drug value against the disease of Bronchial asthma has been revealed from this study of ***Nagarasingadhi chooranam***. This must be implicated in the future clinical studies and preclinical chronic toxicity studies.

8. SUMMARY

- Siddhars improve their long life and virtual power through the modification of *Pranan*. Imbalance in *Pranan* can cause the vitiation of three humours, it can cause bronchoconstriction. Siddhar describes this ethiology of symptoms in literature named as a disease *Swasakasam*.
- Hence the author conducts the detailed scientific validation of *Nagarasingadhi chooranam* for Bronchodilator activity, Anti-histamine activity and Anti-oxidant activity.
- To collect the information about the drug in various classical siddha and modern text books, literature were referred. From them, the author came to an idea about the drug and its efficacy on bronchial asthma.
- The Phytochemical analysis of the drug evaluates that it contains glycosides, saponins, carbohydrates, phytosterols, phenols, triterpene and protein which contributes much in relieving the symptoms of bronchial asthma
- Bio-Chemical analysis of the drug contains Iron, Magnesium, Pottasium, Calcium, Chloride and Phosphate which involves improving normal respiratory function in bronchial asthma.
- ICP-OES result shows the presence of Phosphorus, Iron, Sodium, Calcium and Potassium which involves in treating the symptoms of *Nagarasingadhi chooranam.*
- SEM analysis represents the drug contains nano and micro particles.
- The preclinical study showed that the drug has got safety and significant Bronchodilator, Anti-histamine activity and Anti-oxidant activities.
- An incredible action of this drug value against the disease of Bronchial asthma has been revealed from this study of *Nagarasingadhi chooranam.* This must be implicated in the future clinical studies and preclinical chronic toxicity studies.

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