EVALUATION OF CLINICAL EFFICACY OF URAI MATHIRAI AS IMMUNO MODULATOR

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DECLARATION

I declare that the thesis entitled ***"Evaluation of Clinical Efficacy of Urai Mathirai as Immuno Modulator"*** submitted by me to The Tamil Nadu Dr. M.G.R. Medical University for the award of the degree of Doctor of Philosophy is the record of research work carried out at the study centre Siddha Central Research Institute, Arumbakkam, Chennai, during the period from 1/1/2012 to 1/1/2018 under the guidance of Dr. R. Pattarayan M.D. (Siddha), Professor & HOD (Rtd.), Dept. of Kuzhandhai Maruthuvam, National Institute of Siddha and thesis has not formed previously the basis for the award of any degree, diploma, associateship, fellowship titles in this or any other University or similar Institution of higher learning.

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CERTIFICATE

This to certify that the thesis entitled **"*Evaluation of Clinical Efficacy of Urai Mathirai as Immuno Modulator*"** submitted to The Tamil Nadu Dr. M.G.R. Medical University for the award of the degree of Doctor of Philosophy is the original and independent work of Dr. P. Sathiyarajeswaran, M.D. (Siddha), M.Phil. (Siddha)., carried out at the study centre - Siddha Central Research Institute, Arumbakkam, Chennai, under my supervision and this thesis has not formed previously the basis for the award of any degree, diploma, associateship, fellowship titles in this or any other University or similar Institution of higher learning.

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Place:

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ABBREVATIONS

- % - Percentage
- & - And
- < - Less Than
- µl – Microlitre
- ^{0}C - Degree Of Celsius
- B – Beta
- AAS – Atomic Absorption Spectrophotometer
- ACP – Alkaline Phosphates
- ANOVA – Analysis Of Variance
- ANOVA – Analysis Of Variance
- ATCC – American Type Couture Collection
- AYUSH – Ayurveda Yoga Unani Siddha Homoeopathy
- B.Wt – Body Weight
- BDL – Below Detectable Limit
- CARIF – Canadian Acute Respiratory Illness And Flu scale
- CCRAS – Central Council For Research In Ayurvedic Sciences
- CPCSEA – Committee For The Purpose Of Control And Supervision Of Experiments On Animals
- CPM – Chlorpheninamine
- CRF – Case Report Form
- CTRI – Clinical Trial Registry – India
- DA – Dopaminerigic
- DC – Differential Count
- DNA – Deoxy Ribonucleic Acid
- EDTA – Ethylene Diamine Tetra Acetic Acid
- ESR – Erythrocyte Sedimentation Rate
- FFA – Free Fatty Acids
- FTIR – Fourier Transform Infrared Spectroscopy
- GCP – Good Clinical Practice
- GOT – Glutamate Oxaloacetate

- GPT – Glutamate yruvate Transaminas
- HPTLC – High Performance Thin – Layer Chromatography
- HPTLC – High Performance Thin Layer Chromato Graphy
- Hr – Hour
- HRBC – Human Red Blood Cell
- IAEC – Institutional Animal Ethics Committee
- IC_{50} – Inhibitory Concentration At 50%
- IEC – Institution Ethics Committee
- Ige – Immunoglobulin E
- IPD – In Patient Department
- Kg – Kilogram
- L – Litre
- LPS – Lipopoly Saccharide
- MBC- Minimum Bactericidal Concentration
- MCC – Microcrystalline Cellulose
- MCV – Mean Cell Volume
- MDA – Malondialdehyde
- Mg – Milligram
- Min – Minutes
- Ml / Kg – Millilitre Per Kilogram Body Weight
- MPTP – Mitochondrial Permeability Transition Pare
- Mptp – Mitochondrial Permeability Transition Pore
- NF – Newrofbromatosis
- OECD – Organization For Economic Co-Operation And Development
- P.O – Per Oral
- PCE – Polychromatic Erythrocytes
- PG - Prostaglandin
- PMCAO – Permanent Middle Cerebral Artery Occlusion
- PTZ – Penty Lenetetrazol
- ROS – Reactive oxygen Species
- S.D – Standard Deviation

- S.E.M – Standard Error Of Mean
- SEM – Standard Error Mean
- SRBC – Sheep Red Blood Cell
- TLC – Thin Layar Chromatography
- TNF – Tumor Necrosis Factor
- URT – Upper Respiratory Tract
- V/V – Volume / Volume
- W/V – Weight / Volume
- W/W – Weight / Weight
- WBC –White Blood Cell
- WHO – World Health Organisation

INTRODUCTION

1. INTRODUCTION

From time immemorial, man depends on plants as medicine. From a historic attitude, it is obtrusive that the fascination for plants is also as vintage as mankind itself. The plant kingdom represents a wealthy storehouse of natural compounds, lots of that have been used for medicinal functions and will serve as lead for the improvement of novel retailers having properly efficacy in diverse pathological issues in the coming years.

Herbal medicines are being used by nearly about 80% of the world's population, largely in developing countries for primary health care[1]. Assessing the current status of health care system in adequacies of synthetic drugs is likely to be more glaring in the coming years. It has been reported that there has been an alarming increase in number of diseases and disorders caused by synthetic drugs, prompting a switch over to traditional herbal medicines[2]. Alternative system of medicine is a major component of health care globally and many healthcare providers and organizations are being forced to consider integrating them into their practice and treatment guideline[3].

Siddha system, being the oldest ancient ways in which of maintaining a healthy life vogue remains rife and emphasizes the importance of physical, emotional, psychological, social well-being. Siddha system describes concerning cycles of birth, death and also got to maintain one's harmony among that has been later delineate as motcham or Eternal Bliss.

Healthy seeds yield healthy generation. Siddha Peadiatrics (Balavagadam) being one of the fine focus of Siddhars, they saw the perspective of a healthy seed for an entire life within the child. Hence meticulously brought out steps to nurture it right from the beginning.

The real strength of Siddha system relies on preventive and encouraging health care deliveries and additional stress is given towards malady interference than management. Contribution towards NCD'S and chronic diseases remains thanks to this

strength. Thirumoolar conjointly expressed, one who maintains sensible physical health can pave a path towards life fulfillment.

The global pharma industry put extra effort in designing a drug especially for a child, which should not cause addiction, which should be palatable and will not produce cumulative toxicity. Safe, efficacious, economical drugs are additional demand.

Children with in first decade are commonly affected by respiratory as well as gastro intestinal complaints. Repeated respiratory infections and especially upper respiratory infections is a problem of paediatricians[4].

School age children acquires respiratory infection due to closed room and crowded environment, no proper training on hand wash and prevention of contaminations through touch, droplet etc are the reasons for continuous spread. Environmental cleanliness, awareness and education of personal hygiene decreases respiratory infections[5].

4.5 million Deaths are reported among school age kids every year in developing countries. Pneumonia without measles(70%), Pneumonia with measles(85%), Whooping cough (10%), Bronchiolitis, Croup syndrome stands next to Streptococcal pneumonia, Haemophilus influenza and Staphylococcus aureus stands large in causing recurrent respiratory infections[6].

The WHO report, shows that particular death rate because of intense respiratory tract disease is 10-15 times higher in developing nations than developed nations. Consistently intense respiratory tract diseases in youngsters is responsible of an expected 4.1 million deaths around the world.

In India acute respiratory tract infections make contributions a major public health problem and are the most vital contributory to mortality and morbidity in younger kids mainly schoolers who go to class, representing 15-34 percent of all infantile deaths[7].

INTRODUCTION

School children who go to class are substantial repositories for ARIs and they exchange contamination with other kids and to the individuals who nurture them. They have around 3-8 viral respiratory sicknesses for every year. Common cold is the main reason of morbidity. Risk factors that growth the prevalence and severity of upper breathing infection in growing nations encompass big own family length, lateness within the birth order, crowding, low birth weight, malnutrition, nutrition deficiency, loss of breast feeding, pollution, and young age. Effective interventions for prevention and clinical case management are urgently needed to keep the lives of many youngsters predisposed to extreme ailment.[8].

Acute Respiratory tract contamination most typically arise throughout infancy until school age of lifestyles. The put off in receiving hospital therapy is considered to be an important cause for the high mortality associated with acute respiratory tract infections in the developing nations. Far distance of the medical institution turned into the main motive for now not receiving treatment, followed by using lack of know-how, circle of relatives issues and so on. These reasons may compel the guardians to look for treatment from other exchange sources[5].

The illnesses as a result of an acute respiration contamination consists of tonsillitis, pharyngitis, laryngitis, sinusitis and otitis media. As the incidence of respiratory illness is quite appreciable among school children in Chennai, the investigator felt the need to assess the effectiveness of *Urai Mathirai* with that of Standard drug *Thalisathy chooranam* as a remedy for faculty kids on symptoms of acute upper respiratory infection. Ethno medicinal studies in child health authenticate certain procedures and practices which were prevailing in rural areas account for wellbeing of children. *Urai Mathirai* is a drug used for the past 3 decades indicated in Hospital pharmacopeia of Govt Siddha Medical College, *Palayamkottai*. The children nursed with *Urai Mathirai* are said to be almost free from Pre-School and Schooling age health hazards such as frequent respiratory infections /gastrointestinal infections and anorexia.

Claims suggest even usage of antibiotics were minimal at the period of *Urai Mathirai* administration. Lack of Scientific evidence classifies these claims as tall claims. *Urai Mathirai* as an Immune enhancer is empirical and a thorough study on the drug from standardization up to phase III clinical trials is to be carried out.

Thaaleesaadhi chooranam is a polyherbal formulationprepared from 27 different herbs being used for treatment of various respiratory diseases. The Thaaleesaadhi chooranam is used to treat cough,cold,phlegamatic conditions,pneumonia and a widerange of kapha disease and ear disease[9].

Multiple etiologies of the respiratory diseases and the lack of effective vaccines underline the need for clinical trials and the practical application of Immunomodulatory preparations which may also help in treating viral origins.

There is an increased demand in usage of alternative medicines including Siddha preparation around the world for treating various diseases. There are various types of Siddha preparation which includes *Chooranam, Parpam, Mathirai. Mathirai* is basically a single or compound herb given as such to the patients. However the amount of effectiveness and the safety of the Siddha formulation are under research so this system didn't reach to community wise hence the system lacks.

Aim and Objectives

2. AIM AND OBJECTIVES

The primary and secondary objectives of the study are as follows.

Primary and Secondary objectives:

- To pharmacognostically identify the selected individual plant *Chukku* (*Zingiber officinale*), *Athimathuram* (*Glycyrrhiza glabra*), *Akkarakaram* (*Anacyclus pyrethrum*), *Vasambu* (*Acorus calamus*), *Jathikkai* (*Myristica fragrans*), *Kadukkai* (*Terminalia chebula*), *Masikkai* (*Quercus infectoria*), *Poondu* (*Allium sativum*), *Perunkayam* (*Ferulla asafoetida*), *Thippili (Piper logum*) for authentication.
- To develop the *Urai Mathirai* (Siddha formulation) from the above herbs.
- To investigate the phytochemical potential of individual plant crude powders and *Urai Mathirai* (Siddha formulation) by Chemical tests, HPTLC.
- Evaluation of formulation by standard parameters as per AYUSH guidelines.
- To develop and analyze tablet by Pre - formulation and Post - compression studies.
- To analyze the *Urai Mathirai* (Siddha formulation) for *In - Vitro* studies like Anti-Oxidant, Anti- Inflammatory, and Anti- Microbial activities.
- To assess the safety of the *Urai Mathirai* (Siddha formulation) according to OECD guidelines by animal models.
- To evaluate the *Urai Mathirai* (Siddha formulation) for *In - Vivo* studies like Anti- Inflammatory, Immuno modulatory and Analgesic activities.
- To evaluate the clinical efficacy of the trial drug in controlling repeated Respiratory infections with an active control in parallel to evaluate its efficacy and increasing the time gap between episodes.
- To bring down evidence and scientific support for the *Urai Mathirai* - a traditional Siddha formulation.

Review of literature

3. REVIEW OF LITERATURE

A detailed and thorough literature survey was carried for the individual selected plants and *Urai Mathirai* from well-established institutes (IIT Chennai, CLRI Chennai, The T.N. Dr. M.G.R. Medical University Chennai, Govt. Siddha Medical College, Chennai and Palayamkottai, National Institute of Siddha, Chennai, CCRS Chennai), and various literature sources such as Medline via Pub Med, Science Direct, and Google Scholar.

The collected scientific reports were presented.

3.1 ETHNOBOTANICAL REVIEW

3.1.1 PLANT PROFILE (*Zingiber officinale Roscoe*)

Botanical Source : *Zingiber officinale* Roscoe

Family : Zingiberaceae

Synonyms : *Amomum zingiber* L., *Amomum angustifolium* Salisb.

Parts Used : Rhizome

Taxonomic classification

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Zingiberales

Family : Zingiberaceae

Genus : *Zingiber*

Species : *Zingiber officinale*

Vernacular Names

Sanskrit	: Adraka
Hindi	: Adrak
Kannda	: Alla, Shunthi
Malayalam	: Inchi, Enchi
English	: Ginger
Tamil	: Inji
Telugu	: Allam, Allamu

Distribution

Cultivated in warmer parts of India without any report of its natural occurrence in the wild[10].

Description of drug

Dried drug consists of sympodially branched laterally compressed pieces of horizontal growing rhizome known as 'races' or 'hands', 5 to 12 cm in length, 3 to 5 cm in height and 1 to 2 cm in thickness, the surface is marked with circular closely placed leaf scars and small circular root scars at places, clearly visible on unpeeled or partially peeled pieces of rhizomes, surface of the later one is rough, longitudinally striated and somewhat fibrous, at places attached with the fragments of cork and with a small circular depression of bud scar at the tip of the fingers; fracture short and fibrous, mealy or hard, pale buff to brownish in colour[10].

Habit

A perennial aromatic stout horizontally growing rhizomatous herb having several sympodial lateral tubers and elongated erect leafy shoot up to 60 cm high[10].

Chemical Constituents

Rhizome:

6-Shogaol[11], 6-gingerol[11,12], zingiberol[13], beta-phellandrene[14,16], alpha-zingiberene, ar-curcumene, beta-bisabolene[15,17].

Traditional Medicinal Uses

Indigestion, cough, painful gastrointestinal disorders with indigestion, *vata*, heart burn and headache[18]. **Fig.No. 01**

Zingiber officinale
(சுக்கு)

PHYTOCHEMICAL INVESTIGATIONS

- The volatile oil consists mainly of the mono- and sesquiterpenes; camphene, β-phellandrene, curcumene, cineole, geranyl acetate, terphineol, terpenes, borneol, geraniol, limonene, β-elemene, zingiberol, linalool, α-zingiberene, β-sesquiphellandrene, β-bisabolene, zingiberenol and α- farmesene[19,20].
- Zingiberol is the principal aroma contributing component of ginger rhizome[21].

PHARMACOLOGICAL ACTION

- Antioxidant activity - Ginger can be regarded as the storehouse of antioxidants. It has an extraordinary property of scavenging reactive oxygen species (ROS), free radicals, peroxides, and various other damaging oxidants. The active ingredients like gingerols, shogaols, zingerone, and so forth present in ginger exhibit antioxidant activity. It inhibits an enzyme, namely, xanthine oxidase, which is mainly involved in the generation of reactive oxygen species. Zingerone has been reported to protect in vitro DNA against stannous chloride induced ROS oxidative damage[22].
- The ability of a hexane fraction of dried ginger methanolic extract to suppressproinflammatory gene expression in LPS-activated BV2 microglial cells, thus displaying anti-neuroinflammatory activity[23].

- A Soxhlet extract of ginger in 80% ethanol reduced yeast-induced fever in rats by 38% when administered orally (100 mg/kg). This was comparable to the antipyretic effect of acetylsalicylic acid at the same dose[24].
- Few studies have examined the potential immunomodulatory activity of ginger. Non-specific immunity was increased in rainbow trout eating a diet containing 1% of a dried aqueous ginger extract for three weeks[25].
- STZ treated-type 1 diabetic rat model reported that, oral administration of ethanolic extract of ginger significantly decrease fasting blood glucose level[26].
- In a more recent study, air-dried ginger powder (100 mg/kg orally daily) fed to rabbits with experimentally induced atherosclerosis for 75 days inhibited atherosclerotic changes in the aorta and coronary arteries by about 50%[27].
- The compound aids in restoring renal functions, reducing lipid peroxidation and enhancing the levels of reduced glutathione, superoxide dismutase and catalase activities at doses of 12.5, 25, and 50 mg/kg, respectively[28].
- ginger as neuroprotector suggests that, it exhibit neuroprotective effect by accelerating brain anti-oxidant defence mechanisms and down regulating the MDA levels to the normal levels in the diabetic rats[29].
- Ginger and its constituents show a vital role in ulcer prevention via increasing mucin secretion. Earlier findings have shown anti-ulcerative effects of ginger in experimental gastric ulcer models[30].

MICROBIAL ACTIVITY

- Escherichia coli induced diarrhoea is the leading cause of death in developing countries and recently it was documented that zingerone exerted protective effect on E. coli induced diarrhea[31].

3.1.2 PLANT PROFILE (*Glycyrrhiza glabra L*)

Botanical Source : *Glycyrrhiza glabra* L.

Family : Leguminosae

Synonyms : *Glycyrrhiza glandulifera* Waldst. & Kit., *Glycyrrhiza hirsuta Pall.*

Parts Used : Root

Taxonomic classification

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Fabales

Family : Fabaceae

Genus : *Glycyrrhiza*

Species : *Glycyrrhiza glabra*

Vernacular Names

Sanskrit : Jalayashti

Hindi : Jeti-madh

Kannda : Atimathura

Malayalam : Atimathuram

English : Liquorice

Tamil : Adimathuram

Telugu : Athimathuram

Distribution

Widely distributed throughout Myanmar, Andaman Islands and in India. It is cultivated in Punjab and Sub-Himalayan tracts[32].

Description of drug

Root pieces are cylindrical, 14 – 20 cm in length and 5 – 20 cm in diameter, surface rough, longitudinally wrinkled, at places shows scars left by removal of the lateral roots; fracture outer fibrous and inner splintery, externally dark brown, internally golden yellow, transversely cut surface exhibits wide central xylem, cambium ring, outer narrow phloem and wide radiating medullary rays[32].

Habit

Erect shrub, up to 1.5 m high, bearing pailionaceous purplish blue coloured flowers[32].

Chemical Constituents

Roots

Glycyrrhizin[33], Glycyrrhizic acid[34,35].

Traditional Medicinal Uses

Burning micturition, Bone diseases, cough, Jaundice, Eye diseases, Excessive thirst, Peptic ulcer, Leucoderma, Fever[36].

Fig. No. 02

Glycyrrhiza glabra
(அதிமதுரம்)

PHARMACOLOGICAL ACTION

- The stem decoction showed significant antiarthritic activity against formaldehyde-induced rat paw edema at a dose of 1ml/100 g p.p., when administered for 10 days[37,41].

- The aqueous extract of the roots (1,2,3,4 g/kg/d p.o.) and glycyrrhetic acid (100 mg/kg, p.o.) exhibited anti-arthritic activity in mycobacterial adjuvant-induced arthritis in male albino rats, when administered for 14 days. The activity of the extract was dose-dependent and at highest tested dose, it produced 73 per cent inhibition of thickening of foot while glycyrrhetic acid produced 38 per cent inhibition. However, these were less effective than the standard drug phenylbutazone (100 mg/kg) in inhibiting the secondary lesions. ED50 of the extract was found to be 2.75g/kg[38].

- The total alcoholic extract of the plant (50 mg/100 g p.o. for 15 days) increased the acetylcholine content of the cortex(not of the whole brain) in normal rats, while it prevented the rise in brain acetylcholine level it stressed rats. Similarly it increased (although not significantly) the brain catecholamine levels in normal rats, without affecting the stress-induced increase in brain catecholamine level. It did not cause any significant change in brain 5-HT levels either in normal or stressed rats. In normal and stressed rats, whole brain histamine level was decreased by the drug while it was increased in stressed rats[39].

- The 70 per cent ethanolic extract of both roots and rhizome (10-100mg/kg i.p.) significantly and dose- dependently reduced the onset and incidence of PTZ-induced seizures in mice and intensity of lithium-pilocarpine-induced status epilepticus in rats. However it did not show any protective effect against maximum electroshock induced seizures in mice, even up to 500mg/kg dose[40].

- Glycyrrhetic acid (40 mg/kg i.p.) was devoid of hypothermic effect in albino rats but showed significant antipyretic effect against Brewer's yeast induced pyrexia by reducing the body temperature at 2 and 4 h of

administration in a manner comparable with standard drug sodium salicylate (600 mg/kg)[41].

- The 50 per cent ethanolic, methonolic and aqueous extracts of the roots at 500,500 and 600 µg i.p. doses, respectively reduced the proliferation of Ehrlich ascites tumour (EAT) cells in mice as evidenced by reduction in EAT cell number and ascites volume after 12 days treatment. The peritoneal angiogenesis and (vascular endothelial growth factor) levels were also inhibited in these mice indicating that the extracts decreased VEGF production and neovascularization induced by it. The aqueous extract was most active. The anti-proliferative and anti-angiogenic effects were further confirmed in vitro by inhibition of 3[H] thymidine uptake in EAT cells and neovascularization in chorioallantonic membrane (CAM) assay, respectively[42].

- The ethanolic(70 per cent) extract of both root as well as runner (2,4,6 and 8 mg/ plate) showed concentration-dependent protective effect against ethyl methane sulfonate induced mutagenic toxicity in Salm typhimurium. At the highest tested concentration, 97 percent cells were protected[43].

- The 95 per cent ethanolic root extract when applied topically as 10 per cent ointment (0.5 g) showed a significant reduction in the period of epithelization on the excision wound healing model in wistar albino rats. It also significantly increased the tensile strength of incision wound on oral administration at 1 g/kg p.o. for 10 days in a manner comparable with standard drug, -tocopherol (200mg/kg)[44].

- Glycyrrhetic acid administered at dose 80 mg/kg i.p. did not show significant protection against egg albumin aerosol-induced bronchospasm in sensitized guinea pigs[45].

TOXICITY STUDIES

- The root powder did not manifest any toxic effect upto 6000 to 10000 mg/kg single doses in albino mice and albino rats, respectively[46].

ANTI MICROBIAL ACTIVITY

Anti-tubercular activity

- The ethanolic extract of the roots and its ethyl acetate fraction and glabridin showed anti-mycobacterial activity against two strains namely H37Ra and H37Rv of Mycobacterium tuberculosis with MIC of 500, 100-250 and 29.16 µg/ml, respectively. However, hexane and methanol fractions hispaglabridin B were found inactive fractions and glabridin were no better than standard drugs viz., rifampicin, isoniazid, streptomycin and ethambutol[47].

CLINICAL STUDIES

- In a double blind placebo controlled clinical trial conducted on 40 patients of peptic ulcer at Gastroenterology and Clinical research wards of Osmania General Hospital, Hyderabad administration of one capsule containing 400 mg of deglycyrrhizinated liquorice every six hour for 8 wks to test group(20 patients) resulted in good response in 18 patients (90 per cent) as compared to good response in 11 (55 per cent) out of 20 placebo treated group48.

3.1.3 PLANT PROFILE (*Anacyclus pyrethrum (L.) Lag.*)

Botanical Source : *Anacyclus pyrethrum* (L.) Lag.

Family : Compositae

Synonyms : *Anacyclus depressus* Ball, *Anacyclus freynii* Porta & Rigo ex Willk.

Parts Used : Root

Taxonomic classification

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Asterales

Family : Asteraceae

Genus : *Anacyclus*

Species : *A.pyrethrum*

Vernacular Names

Sanskrit : Agragrahi

Hindi : Akarkara

Kannda : Akalakara

Malayalam : Akkikaruka, Akravu

English : Pellitory

Tamil : Akkirakaram

Telugu : Akarakaram

Distribution

Native to North Africa and imported to India for medicinal purpose[49].

Description of drug

Roots tough, cylindrical 7 – 15 cm in length, tapering slightly at both ends, with a few hair-like rootlets and occasionally topped by bristly remains of leaves; external surface, rough, brown, shriveled bark upto 3 mm, thick not easily separable[50].

Habit

Annual hairy herb with numerous spreading prostate or ascending branched stems[50].

Chemical Constituents

Root

Volatile oil, Anacyclin, Pellitorine, Pyrethrin[50].

Traditional Medicinal Uses

Dystaste, arthritis, dryness of tongue, thirst, tooth ache, hiccough**[50].**

Fig. No. 03

Anacyclus pyrethrum
(அக்கரகாரம்)

PHYTO CHEMICAL INVESTIGATIONS

- The plant has been reported to contain daidzein, genistein, coumestrol, formononetin and biochanin A[51] . The root is reported to contain heavy metals and minerals[52].

PHARMACOLOGICAL ACTION

- The seeds revealed weak abortifacient (15 per cent) activity in rats in the dose of 175 mg/kg administered orally once a day from day 1 to 10 of post mating period[53].

- The chloroform extract of the root at 1 mg/plate produced inhibition of tobacco-induced mutagenesis, using Ames Salmonella/microsome assay. The extract also inhibited the nitrosation of methylurea in a dose dependent manner[54].

TOXICITY STUDIES

- The LD50 for the aqueous extract of the root was found to be 750 mg/kg i.p. in mice[55].

CLINICAL STUDIES

- A decoction of a combination of A. pyrethrum and Helleborus /tiger (1 : 3) was reported to have useful effects in a clinical study involving fifteen patients suffering from diabetes mellitus. The study was, however, of a very preliminary value without any control or determination of the effects of prescribed regimen.

- In a double blind study involving 200 patients presenting for oral surgery, a 2 per cent concentration of root extract was reported to be a safe local anaesthetic and produced a pterygomandibular block with infiltration for the long buccal nerve. The time lapse between administration and onset of paresthesia, establishment of surgical anaesthesia, regression of anaesthesia and complete return of normal secretion. were found to be comparable to xylocaine hydrochloride (2 per cent). The plant extract had a longer duration of action[56].

3.1.4 PLANT PROFILE (*Acorus calamus L.*)

Botanical Source : *Acorus calamus L.*

Family : Acoraceae

Synonyms : *Acorus angustatus* Raf., *Acorus angustifolius* Schott

Parts Used : Rhizome

Taxonomic classification

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Acorales

Family : Acoraceae

Genus : *Acorus*

Species : A.calamus

Vernacular Names

Sanskrit : Bacha

Hindi : Bach

Kannda : Baje

Malayalam : Vaembu

English : Sweet flag

Tamil : Vashambu

Telugu : Vasa

Distribution

Occurs in wild and cultivated throughout India and in neighbouring countries, especially occurs plenty in Sikkim, Manipur and Naga hills and ascending the Himalayas up to 1800 m altitude[57].

Description of plant

Dried rhizome pieces are subcylindrical, occasionally bent at places, rarely straight, dorsiventrally slightly compressed, 5 to 15 cm in length and 1 to 2 cm thickness, covered with thin corky skin and adherent triangular shriveled scaly withered leaf bases visible clearly, encircles the upper surface, lower surface shows irregularly placed elevated circular tubercular root scars; fracture short, fractured surface somewhat spongy, minutely porous and light buff in colour and exhibits a ring of endodermis, central wide stellar tissue and outer cortex, externally pale brown[57].

Habit

Aromatic marshy herb with perennial thick creeping samples[57].

Chemical Constituents

Alpha-asarone[58,59], beta-asarone[58-65], and shyobunone [59,61,62,66].

Traditional Medicinal Uses

Cancerous ulcer, lancinating pain, simultaneous extreme derangement of three humors, filariasis leg, cough, painful gastrointestinal disorders, snake bites, disease of the spleen, hypertension, worm infestation, toxic anasarca.

Fig. No. 04

Acorus calamus
(வசம்பு)

PHYTOCHEMICAL INVESTIGATIONS

- A preliminary examination of the oil from the rhizome of the plants grown in India revealed that it was not identical to the European varieties but resembled the oils of Java varieties. The

Indian oil differed from the commercial varieties of calamus oil in respect of predominance of asarone (82 per cent). The Commercial varieties contained 7 per cent of asarone. The oils obtained from the plants of different parts of India were analyzed by various workers for their chemical constituents. The unpeeled dry root from Coimbatore contained 1.5-3.5 per cent of the oil, while the fresh root, which contained 70-75 per cent of water, yielded 0.8 per cent of the oil[67].

PHARMACOLOGICAL ACTION

- Neuro-pharmacological actions of the oil (from the rhizome) revealed sedative-tranquilizing action in rats, mice, cats, dogs and forced motor activities in mice. In high doses, the oil inhibited monoamine oxidase[70].

- Asarone and 3-asarone possessed many pharmaco dynamical actions quite similar to some of the well-established tranquilizers, 3-asarone appeared to be more potent. Both the compounds (5 mg/kg i.p.) significantly ($p<0.01$) enhanced the anaesthetic activity of pentobarbitone, hexobarbitone and ethanol in mice. Hypnotic potentiating as also the tranquilizing activity of 3-asarone was significantly higher than that of asarone. 3-Asarone also caused generalized convulsions and potentiated the metrazol seizures in rats, while asarone had a definite tendency to protect against metrazol convulsions and modified electroshocks. Both the compounds exerted no effect on the spontaneous motor activity of the animals, caused a fall in rectal temperature in mice, showed effects on spontaneous behaviour patterns in cats and antiacetylcholine action on frog heart[71].

- The water soluble dried powder of the alcoholic extract of the root and rhizome at 10 and 20 mg/kg i.p. did not afford protection to strychnine-induced convulsions. Dosages of 10, 25 and 50 mg/kg i.p. of herbal extract antagonized spontaneous

motor activity and also amphetamine-induced hyperactivity in mice. It was less potent than chlorpromazine, though it exerted sedative and tranquilizing action. The local anaesthetic activity was found to be absent at 0.5 and 1 per cent dose levels[72].

- The anti-inflammatory activity of the boiled coconut oil extract of the rhizome was studied in rats using acute and chronic experimental models. The oral administration of 2 ml of the extract produced 45 per cent inhibition of the carrageenin-induced paw oedema, 13.6 per cent inhibition of cotton pellet granuloma formation and 61 per cent inhibition of croton oil granuloma pouch inflammatory response in rats. In comparison, phenylbutazone 100 mg/kg orally produced 46 per cent, 25 per cent and 45 per cent inhibition of the inflammatory response in the three models, respectively. In adrenalectamized rats the extract produced 28 per cent inhibition of carrageenin-induced oedema as compared to 53 per cent inhibition with that of phenylbutazone[73].

ANTI MICROBIAL ACTIVITY

- The alcoholic, ether, acetate buffer and dilute sulphuric acid extract of the root showed activity against Staphylococcus aureus[74].

- The alcoholic extract of the root showed marked antibacterial activity against Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Staphylococcus citreus, Bacillus megaterium, Salmonella paratyphi A and B, Salrn marcescens, Proteus vulgaris and Shigella dysomei[75].

- The essential oil obtained from the rhizome exhibited *In-vitro* antibacterial activity against Bacillus proteus, Escherichia coli, Staphylococcus pyogenes, Shigella shiga[76] and against Shigella boydii, Salmonella typhi and Salm paratyphi[77].

TOXICITY STUDIES

- Acute toxicity studies with the Indian oil in mice revealed a LD50 of 0.177 g/kg i.p. The elimination of the phenolic and aldehydic fractions from the oil resulted in an increase in toxicity of the oil as well as in the sedative potentiation activity of remaining oil[68,69].

CLINICAL STUDIES

- In a preliminary clinical trial on 15 patients with moderate to severe bronchial asthma, 1 to 2g of the fresh root divided in three doses was administered by chewing method for 2-4wk. The drug was found to have antiasthmatic potential free from side effects[78]. In another preliminary uncontrolled clinical trial, small pieces of the rhizome weighing 1 g were administered to asthmatic patients four times a day for 8d by chewing method. Marked effect in relieving of bronchospasm was observed in some of the cases. No side effects were observed[79].

- In another clinical trial on 147 children (age 5-11 years) having round worms infestation, calamus powder 250 mg t.i.d was given for three days. 122 patients (83 per cent) were completely cured while 25 patients (17 per cent) remained unchanged[80].

- In a clinical study in fifty cases of depression at the O.P.D. of the S.S. Hospital, Banaras Hindu University, Varanasi, A. calamus (500 mg in a dose of 2 tablets three times a day after meal with water) given for six weeks showed reduction in the degree of severity of depression and better rehabilitation. There was also a significant improvement in assessment based on rating of symptoms on Hamilton depressions rating scale. The rate of improvement before and after treatment was statistically significant ($p<0.001$)[81].

3.1.5 PLANT PROFILE (*Myristica fragrans Houtt.*)

Botanical Source : *Myristica fragrans* Houtt.

Family : Myristicaceae

Synonyms : *Aruana silvestris* Burm.f., *Myristica aromatica* Sw.

Parts Used : Seed

Taxonomic classification

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Magnoliales

Family : Myristicaceae

Genus : *Myristica*

Species : *Myristica fragrans*

Vernacular Names

Sanskrit : Jatiphala

Hindi : Jati-phal

Kannda : Jakayi

Malayalam : Jathi

English : Nutmeg

Tamil : Catikkai

Telugu : Jajikaya

Distribution

Indigenous to the eastern Molucca islands and is now found under cultivation in India, Sri Lanka, Philippines, Mauritius, New Guinea and West Indies. In India mainly grown in Kerala, Tamil Nadu and North-Eastern states[82].

Description of drug

Ovoid, 2.2 to 3.2 cm in length and 1.5 to 2.5 cm breadth, the kernel is greyish brown externally and is marked with numerous minute dark reddish-brown points and lines; it is also reticulately marked with small furrows. The outer region is a thin layer of perisperm, which grows inwards to the endosperm at the position of the external furrows. The endosperm, which forms the bulk of nutmeg, is greyish brown; raphe prominent, whitish, extending along the entire length of the seed on one side; testa hard, dark brown, 0.9 to 1.4 mm thick marked by the impressions left by the lobes of the aril. Perisperm is thin, membranous, pale brown; kernel of seed (endosperm) rather soft but firm, whitish and strongly aromatic with a warm slightly bitter taste[82].

Habit

Plant is a dioecious or rarely monoecious medium-sized tree[82].

Chemical Constituents

Seed

Seed is a good source of essential oil (6.88 to 7.78% v/w). The major constituents include eugenol[83], isoeugenol[83], methyl eugenol[84], myristicin[85] and trimyristicin[85].

Traditional Medicinal Uses

Dyspepsia, wheezing, cough, chronic diarrhea, severe diarrhea, oligospermia[86].

Fig. No. 05

Myristica fragrans
(ஜாதிக்காய்)

PHARMACOLOGICAL ACTION

- The ethanolic extract of the kernel administered at 500 mg/kg, p.o. for 30 and 60 days reduced the atherogenic diet induced hyperlipidemia in albino rabbits. It reduced the serum total cholesterol, LDL cholesterol, triglycerides levels in both the treatments as compared to the control group. However, there was no change in HDL cholesterol level at 60 days[87]. Oral administration of the hydroalcoholic extract of the fruits at 150 and .450 mg/kg for 7 days, inhibited the chlorpromazine-induced increase in blood glucose and triglyceride levels by 41 and 53 per cent in male Swiss albino mice. At 450 mg/kg, its activity was comparable to the standard drug, rosiglitazone. Further, administration of the extract for 7 days to rats along with high cholesterol diet feeding, inhibited the increase in plasma triglyceride and cholesterol levels by 47 and 66.7 per cent, respectively and also exhibited a reduction in hepatic triglyceride secretion after tyloxapol administration[88].

- The n-hexane fraction of the acetone insoluble part of petroleum ether extract of the seeds at 10, 30, 100 mg/kg, i.p. protected the Swiss albino mice against maximal electroshock, pentylenetetrazole and lithium-pilocarpine induced seizures in a dose-dependent manner. However, it potentiated the haloperidol induced catalepsy[89].

- The plant extract at 500 mg/kg, p.o. reduced the immobility period of male *Wistar* albino rats in forced swimming test on reserpine-induced immobility and haloperidol-induced catalepsy that was comparable with standard drug, imipramine (1 5 rag/kg), while it did not show any effect on pentobarbitone induced sleeping time in rats[90].

- Intra-peritoneal administration of n-hexane extract of seeds (10 and 30 mg/kg), its acetone insoluble fraction (30, 100 and 300

mg/kg), and isolated compound trimyristin (10, 30 and 100 mg/kg) to male albino mice showed dose-dependent anxiogenic effects in elevated plus maze test. It decreased the number of entries and time spent in open arms. Further the acetone insoluble fraction and trimyristin showed anxiogenic effects in open field test and hole board test and reduced the anxiolytic effects of the standard drugs viz., ondansetron, buspirone and diazepam indicating non-specific anxiogenic activity of the extract[91,92].

- Oral administration of the n-hexane extract of seeds at 5 mg/kg for 3 days, improved the learning and memory of young as well as aged male Swiss albino mice as assessed in elevated plus maze and passive avoidance tests. The higher doses (10 and 20 mg/kg) of the extract were effective in aged rats only. At 5 mg/kg, the extract also reversed the scopolamine and diazepam-induced learning and memory impairment in young mice without affecting their loco-motor activity[93].

- Eugenol, a constituent of the oil at 1-100 mg/kg, i.p. inhibited the acetic acid-induced writhing and paw licking in neurogenic as well as inflammatory phases of formalin induced pain in Swiss albino mice in a manner comparable with the standard drug, indomethacin (20 mg/kg). However, it did not show activity in Eddy's hot plate test and affect motor coordination[94].

- The methanolic and successive aqueous extracts of the fruits at 50 ug/m1 concentration were screened for Rho-kinase 2 (ROCK-II) inhibiting potential of erectile dysfunction. Only the successive aqueous extract showed 19.88 per cent inhibition of ROCK-II[95].

- Eugenol isolated from the fruits at a dose of 10.7 mg/kg/d inhibited the accumulation of lipid peroxidation products in red blood cells and maintained the activities of the antioxidant enzymes viz., superoxide dismutase, catalase, glutathione peroxidase, glutathione-S- transferase, glutathione reductase and

glucose-6-phosphate dehydrogenase in CC14- induced rats. It exhibited a concentration-dependent binding into RBC membranes in vitro and also inhibited RBCs induced by liver S9 fraction[96].

- The seed kernel powder administered at 40 mg/rat, twice daily, p.o. for 7 days inhibited prostaglandin biosynthesis. Further, the petroleum ether fraction of the seed kernels and its sub-fraction obtained through silica column with 90 per cent benzene and 10 per cent chloroform, inhibited the biosynthesis of prostaglandins in vitro in rat kidney tissue[97].

TOXICITY STUDIES

- In an acute toxicity study, the petroleum ether and aqueous extracts of the seeds were found to be safe up to 5 g/kg, p.o. in mice[98].

ANTI MICROBIAL ACTIVITY

- In a preliminary biological screening, the 80 per cent alcoholic extract of the stem was found to be devoid of antibacterial, antifungal, antiprotozoal, anthelminthic, antiviral, anti-implantation, hypoglycemic and diuretic activities, and effect on CVS, CNS and isolated tissues of experimental animals[99].

3.1.6 PLANT PROFILE (*Terminalia chebula Retz*)

Botanical Source : *Terminalia chebula* Retz.

Family : Combretaceae

Synonyms : *Buceras chebula* (Retz.) Lyons, *Myrobalanus chebula* (Retz.) Gaertn.

Parts Used : Dried pericarp

Taxonomic classification

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Myrtales

Family : Combretaceae

Genus : *Terminalia*

Species : *Terminalia chebula*

Vernacular Names

Sanskrit : Kayastha

Hindi : Harra

Kannda : Halle

Malayalam : Kattuka

English : Chebulic myrobalan

Tamil : Katukkai

Telugu : Nallakaraka

Distribution

The plant is found throughout India chiefly in deciduous forests. It occurs abundantly in North India. Its range extends southwards at 300 to 900 m altitude[100].

Description of drug

Fruit is a hard stony drupe, greenish yellow in colour, odourless, ovate, longitudinally wrinkled, 3.5 to 4 cm in length, 1.5 to 2 cm wide and has 5 to 6 ridges (longitudinal ribs). In some, the basal portion is narrower and somewhat elongated on tapering[101].

Habit

Moderate sized or large tree[107].

Chemical Constituents

Fruit

Tannins (20-40%)[102], which on hydrolysis gibe chebulic acid and D-galloyl glucose, chebulagic acid, chebulinic acid, ellagic acid and gallic acid; a tannin terchebin[103], an ellagitannin terchebulin[104], syringic acid[105], gallic acid (1.21%)[106].

Traditional Medicinal Uses

Jaundice, eye diseases, bleeding disorders, laxative, ascites[107].

Fig. No. 06

Terminalia chebula
(கடுக்காய்)

PHYTOCHEMICAL INVESTIGATIONS

- Terminalia chebula contains the triterpenes arjun glucoside 1, arjungenin and the chebulosides 1&2. Other constituents contains tannins up to 30%, chebulic acid 3-5%, chebulinic acid 30%, tannic acid 20-40%, ellagic acid, 2,4-chebulyi–β-D-glucopyranose, gallic acid, ethyl gallate, punicalagin terflavin A , terchebin, some purgative of thenature of anthraquinone , flavonoids like luteolin, rutins, and quercetin etc.

- The water soluble fraction of T. chebula (WFTC) was effective against systemic and local anaphylaxis. Shin et al. showed that the injection of WFTC with doses of 0.01–1.0 g/kg inhibited anaphylactic shock 100%. When WFTC was pretreated at concentrations ranging from 0.005 to 1.0 g/kg, the serum histamine levels were reduced dose-dependently. In addition, this study showed that WFTC increased anti-dinitrophenol and IgE-induced tumor necrosis factor- (TNF-) α production[108].

- Reactive oxygen species and oxidative stress increase the formation of amyloid-β and senile plaques in the brain are the hallmark of AD[109].

3.1.7 PLANT PROFILE (*Quercus infectoria G.Olivier*)

Botanical Source : *Quercus infectoria* G.Olivier

Family : Fagaceae

Synonyms : *Quercus carpinea* Kotschy ex A.DC., *Quercus grosseserrata* Kotschy ex Wenz.

Parts Used : Leaf gall

Taxonomic classification

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Fagales

Family : Fagaceae

Genus : *Quercus*

Species : *Quercus infectoria*

Vernacular Names

Sanskrit : Machika

Hindi : Machika

Kannda : Machikai

Malayalam : Manja-kani

English : Oak gall

Tamil : Masikkai

Telugu : Mashikaya

Distribution

Native of Greece, Asia Minor, Syria and Iran[110].

Description of drug

Gall spherical or pear-shaped, hard and brittle, 1.2 – 2.5 cm in diameter having a short basal stalk and numerous rounded projections on the upper part of the gall; galls usually sink in water, surface smooth, rather shining, bluish-green, olive green or white-brown; a few galls show the escape route of insect, in the form of small rounded hole leading to a cylindrical canal which passes to the center of the gall[110].

Habit

A small tree or shrub 2 – 5m high[110].

Chemical Constituents

Galls

The galls contain gallotannic cid, gallic acid, ellagic acid, gum, starch, sugar and essential oil[111].

Traditional Medicinal Uses

Dysentry, diarrhoea, gargle[112], mouth ulcers, genital discharging diseases, ricketic heat in children, general debility[110].

Fig. No. 07

Quercus infectoria
(மாசிக்காய்)

PHYTOCHEMICAL INVESTIGATIONS

- The galls contain 50-70% of the tannin known as gallotannic acid. This is a complex mixture of phenolic acid glycosides varying greatly in composition. It is prepared by fermenting the galls and extracting with water-saturated ether[113].

- An Aleppo gall contains 50-60% of tannin (tannic acid). A Chinese gall contains 70% of tannic acid. Oak bark contains upto 16% tannic acid to which it owes its effects. Pure gallic acid is in the form of white or colourless feathery crystals of a beautiful silky luster; it is a commercial acid. However, it is pale yellow in color, soluble in alcohol and also sparingly in ether. Its solution in water undergoes decomposition when exposed to air. Gallic acid is converted into metagallic acid when strongly heated[114.]

PHARMACOLOGICAL ACTION

- The local anaesthetic action of a sub fraction prepared by chloroform-methanol extraction of Quercus infectorius galls was found due to the complete blockade of the isolated frog sciatic nerve conduction. The data obtained indicates that it is a potent local anaesthetic. The action potential was completely abolished within 7 minute when an isolated nerve was placed in a 4% solution of subfraction[115,116,117.]

- A dried acetone-treated methanol extract of Quercus infectori dissolved in water was studied for its analgesic effect in an experimental model using the rat tail-flick test. The result showed analgesic effect in rats[115,116,117]

- The study was carried out to determine the potential of galls of Quercus infectoria as an anti-proliferative agent towards the cervical cancer cells and ovarian cancer cells. The toxicity in-*vitro* was evaluated on non-malignant cell line. The results suggested that Quercus infectoria galls extracts have significant anticancer effect[118.]

- A dried acetone-treated methanol extract of Quercus infectoria dissolved in water was studied for its hypoglycemic effect in an experimental model. The result revealed that it significantly reduced blood sugar level in rabbits[119].

- Quercus infectoria galls have been reported to cause a significant reduction in the blood pressure in rabbits[117].

- A study was designed to evaluate anti-inflammatory effect of alcoholic extract of Quercus infectoria galls on various experimental models of inflammation. Oral administration of gall extract significantly inhibited carrageenan, histamine, serotonin and prostaglandin E2 (PGE2) induced paw edemas, while topical application of gall extract inhibited phorbol-12-myristate-13-acetate (PMA) induced ear inflammation. The extract also inhibited various functions of macrophages and neutrophils relevant to the inflammatory response[120].

- In vitro antibacterial activity of methanol & aqueous extract of Quercus infectoria galls against several bacterial pathogens of the urinary tract infection was evaluated using disc diffusion method at the concentration of 5 mg/disc. Both the extracts showed similar inhibitory effects against 4 Gram-positive bacteria (Staphylococcus saprophyticus, Streptococcus agalactiae, Streptococcus pneumonia and Enterococcus faecalis) and a Gram-negative bacteria Proteus mirabilis. It has also been reported to be effective against Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Pseudomonas aeruginosa and Bacillus subtilis in another similar study [121].

3.1.8 PLANT PROFILE (*Allium sativum L.*)

Botanical Source : *Allium sativum* L.

Family : Amaryllidaceae

Synonyms : *Allium controversum* Schrad. ex Willd., *Allium longicuspis* Regel

Parts Used : Bulb

Taxonomic classification

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Asparagales

Family : Amaryllidaceae

Genus : *Allium*

Species : *Allium sativum*

Vernacular Names

Sanskrit : Lasuna

Hindi : Lahsun

Kannda : Belluli

Malayalam : Velluli

English : Garlic

Tamil : Acanam

Telugu : Velluli

Distribution

Cultivated throughout India upto an altitude of 2000 m in the hills[122.]

Description of drug

A tunicated, globular bulb, 3.5 – 6.5 cm in diameter, gradually tapering towards the apex, bearing sterile, sheathing leaves, placed on a woody disc like stem, attached with 20 – 40 stout fibrous root at the base, enclosing 10 – 25 fleshy bulblets called 'clove' arranged in whorled fashion around the central, erect, pithy, cylindrical scape; each clove asymmetrical in shape except those lying near the centre, plano-convex with 2 or 3 ventral flat surfaces separated by longitudinal ridges meeting at the base forming an irregular margin around the 5 to 10 mm wide depression[122.]

Habit

The plant is a bulbous, perennial herb[122.]

Chemical Constituents

Bulb

Allyl alcohol[123], Sulphur compounds such as allicin (diallyl thiosulphinate[123-126], alliin ((+)-S-allyl-L-cysteine sulphoxide) [123,126,127], diallyl disulphide[123,124] and allyl propyl sulphide[123,124] are the major constituents of the essential oil of the bulb. Its also rich in nicotinic acid, vitamin A, Thiamine, riboflavin, niacin, biotin, folic acid, etc[123].

Traditional Medicinal Uses

Simultaneous extreme derangement of three humours, anorectal disorders, sinusitis, headache[128].

Fig. No. 08

Allium sativum
(பூண்டு)

PHYTOCHEMICAL INVESTIGATIONS

- The lipids of garlic amounted to 0.6 per cent on the dry weight basis. Fractionation on silicic acid column chromatography showed that garlic lipids comprised 62.6 per cent neutral lipids, 14.0 per cent glycolipids and 23.4 per cent phospholipids. The fatty acid composition of the total Lipids and component fractions showed that pal mi tic, oleic, linoleic and linolenic acids constituted the major fatty acids; capric, lauric, myristic and stearic acids amounted to about 6 per cent in all lipid fractions. The unsaturated fatty acids together amounted to 72-80 per cent and among these linoleate was predominant in the total lipids as well as in the neutral and phospholipid fractions[129].

- The nutrient composition, consumption and contribution of garlic to dietary value were analyzed. The proximate principles quantitatively analyzed were dry matter, nitrogen, proteins, fats, carbohydrates and minerals such as calcium, phosphorus, iron, manganese, magnesium and zinc. The composition of other edible components was starch, sugars, total fibre, uronic acid, tannins and phytic acid. In addition, eighteen amino acids were found, the major ones being arginine, glutamic acid and aspartic acid[130].

PHARMACOLOGICAL ACTION

- Oral administration of garlic extract (1 ml per 100 g bw for 15d) produced a decrease of 42 per cent in phospholipids and an increase of 40 per cent in the total cholesterol contents in brain of rats fed on low dietary protein. The esterified cholesterol values decreased by 57 per cent in the high protein fed rat brain. The free cholesterol levels were increased more than 20-fold both in the high as well as low protein fed rat brain. Garlic induced changes in rat brain possibly by influencing the susceptibility of brain to allicin and that the effects of garlic on cholesterol levels of rat brain are tissue specific[131].

- The ethyl ether extract of dried cloves of garlic at a dose of 0.5 g/kg was found to exhibit sufficient hypoglycemic activity on oral administration to fasting rabbits as compared to the standard dose of tolbutamide. The extract was effective in controlling the hyperglycemic response of glucose feeding (1 g/kg) in glucose tolerance experiment in the fasting rabbits[132].

- The effect of various extracts of garlic viz., ethyl alcohol, petroleum ether and ethyl ether in a dose of 0.25 g/kg was studied on blood glucose levels in alloxan-induced diabetic rabbits. The extracts revealed hypoglycemic activity, the ethyl ether extract being the most potent. The activity of the various extracts of garlic might be due to increased insulin-like activity of plasma either due to increased pancreatic secretion of insulin from the /3-cells or its release from bound form. Such a process of liberating fixed insulin or the direct effect of a metabolite of organic sulphides chiefly present in the hypoglycemic principles explained the mechanism of action of garlic as a hypoglycemic agent[133].

- The juice obtained from the bulbs of garlic at a dose of 150 mg/100 g bw (1 ml) for 5d revealed good estrogenic activity in albino rats[134]. The petroleum ether, alcoholic and aqueous extracts of the seeds of garlic at a dose of 100-200 mg/kg bw orally did not reveal any anti-implantation activity in female albino rats[135,136].

- The Genotoxic effects of orally administered garlic were evaluated in bone marrow cells of mice by performing the micronucleus test. Acute administration of garlic (7.5, 5 and 2.5 g/kg bw) failed to induce a significant increase in the yield of micronuclei in polychromatic erythrocytes (PCEs) which was not significantly different from the values of the positive controls cyclophosphamide[137].

- The chemo-preventive action of garlic extract on 7, 12-dimethylbenz[a] anthracene (DMBA)induced complete skin carcinogenesis system was studied in random bred 6-7wk old, male Swiss albino mice. Topical weekly application of DMBA for 25wk at two dose levels i.e. 200 nmol during the first week followed by 100 nmol during subsequent weeks or 400 nmol during the first week followed by 200 nmol during subsequent weeks, resulted in 73.9 per cent and 100 per cent tumour incidences, respectively. When garlic extract was topically applied twice daily for 3d every week prior to above stated dose schedules of DMBA, the incidences of tumours were reduced to 31.8 per cent ($p<0.01$) and 43.4 per cent ($p<0.01$), respectively[138].

- The decoction of the bulbs of garlic did not show any effect against formaldehyde-induced rat paw oedema[139], whereas in a preliminary biological study, the ethanolic extract of the bulbs of garlic revealed anti-inflammatory activity in rat[140].

- The pharmaceutical preparation allisatin (concentrated preparation of fresh *A. sativum*) in a dose of 200 mg/100 g bw/day showed slight anti-inflammatory activity against formalin arthritis and not against granuloma pouch in albino rats. Allisatin also appeared to suppress the delayed periarticular changes more than the acute inflammatory reactions. It had no action on the adrenal gland. None of the rats developed gastric ulcer or hemorrhage as compared to the 3-methasone control incidence of 25 per cent. In another study, the preparation did not show any anti-inflammatory activity against carrageenan-induced edema in rat hind paw[141].

- The effect of oral feeding of garlic (1 g capsule containing whole dehydrated powder) was studied on some haematological parameters in rabbits when administered daily for 6wk. There was no appreciable changes in the haemogram. However, it slightly

depressed the WBC count and bleeding and clotting time in rabbits[142].

- Studies on the protective properties of garlic oil against acetaminophen-induced hepatotoxicity in rats were also carried out. Pretreatment of rats with garlic oil (5 per cent and 10 per cent) for 6d followed by a single intraperitoneal dose of acetaminophen (125 mg/kg) protected against acetaminophen-induced rise in liver alkaline phosphatase, glutamate oxaloacetate (GOT) and glutamate pyruvate transaminase (GPT) activities. The oil (10 per cent) also prevented the formation of thiobarbituric acid reactive substance and depletion of reduced glutathione (GSH) in the livers of acetaminophen- treated rats[143].

ANTI MICROBIAL ACTIVITY

- The antimicrobial action of crushed garlic is mainly due to the formation of allicin which is broad spectral in nature. The other antimicrobial factors known to be present in garlic are allistatin-I, allistatin-II[144]; scrodinine and heat stable factors developed during storage of garlic extract at elevated temperature.

- The alcoholic extracts of the bulbs at a concentration of 200 mg/ml using agar diffusion method showed *In-vitro* antibacterial activity against Bacillus subtilis, Escherichia coli, Staphylococcus aureus (10-19 mm of inhibition), Salmonella typhimurium, Pseudomonas aeruginosa (5-9 mm of inhibition), while it had no inhibition against Proteus vulgaris. The hexane and aqueous extracts were devoid of any antibacterial activity against all the organisms (Ahmad et al., 1998). The extract of garlic showed inhibition in *in-vitro* studies against Staphylococcus citreus and different strains of Escherichia coil[145].

- The effect of garlic cloves extract was studied on the growth of yeasts and moulds viz., Aspergillus niger, Penicillium notatum and Torula utilis. At lower concentration garlic acted as fungistatic agent while, at higher concentrations it had fungicidal effect[146].

3.1.9 PLANT PROFILE (*Ferula foetida*)

Botanical Source : *Ferula foetida* (Bunge) Regel

Family : Apiaceae

Synonyms : *Ferula scorodosma* Bentley & Trimen, *Scorodosma foetidum* Bunge

Parts Used : oleo-gum resin

Taxonomic classification

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Apiales

Family : Apiaceae

Genus : *Ferula*

Species : *Ferula foetida*

Vernacular Names

Sanskrit : Hingu, Ramatha

Hindi : Hingra, Hing

Kannda : Hingu, Ingu

Malayalam : Kayam

English : Asafoetida

Tamil : Perunkayam

Telugu : Inguva

Distribution

Its found over the Southern Turkistan as far north as river Syrdarja, sandy deserts and arid hills of Eastern Perisa, in Khorassan and neighbouring parts of Afghanistan near

Herat. It has also been collected further north in Central Asia between Caspian and sea of the Aral, deserts of Rajasthan and Punjab in India[147].

Description of drug

Tears when fractured shows conchoidal surface which changes from milky white to purplish pink, has a powerful persistent alliaceous odour and a bitter acrid alliaceous taste[147].

Habit

A herb with a circular mass of foliage, springing annually from the perennial root stock. The flower plan shoots up a stem peculiarly massive and pillar-like[147].

Chemical constituents

Dimethyl trisulphide, 2-butyl methyl disulphide, 2-butyl methyl trisulphide, di-2-butyl disulphide, di-2-butyl trisulpide, di-2-butyl tetrasulphide, asadisulphide, azacoumarin A and B, R-2-butyl-1-prepenyl disulphide, 1-(1-methyl thio propenyl)-1-propenyl disulphide, ferulic acid, asaresinol ferulate, fenchone, linalool, foetidin, asafoetidin, beta-caryophyllene, beta-selinene, ferocolicin[148].

Traditional Medicinal Uses

Dysmennorhoea, eructation, painful gastrointestinal disorders with indigestion, gum diseases, ascites, diseases due to vata humor[148].

Fig. No. 09

Ferulla asafoetida
(பெருங்காயம்)

PHYTOCHEMICAL INVESTIGATIONS

- Several sesquiterpenoid coumarins have been isolated from the ether extract of the gum resin. Among these the coumarins identified were assafoetidin, ferocolicin[149]; saradeferin[150]; and asimafoetida besides ferulic acid, famesiferol A and C[151] and asimafoetidnol[152]. In addition, umbel liferone was isolated from the ethanol extract fraction of the dried latex powder[153].

- Seven phenolic acids viz., tannic, gallic, caffeic, cinnamic, chlorogenic, ferulic and vanillic were found to be present in the rhizome[154].

PHARMACOLOGICAL ACTION

- Oral administration of the methanolic extract of the resin (400 mg/kg/ d) on days 1-10 post-coitum *vented pregnancy ($p<0.01$) in 80 per cent of rats. When administered as a polyvinyl pyrrolidone (1:2) complex, 100 per cent pregnancy inhibition was observed by the extract at the same dose. Lower doses of the extract caused a marked reduction in the mean number of implantations. On column chromatography, significant antifertility activity was also observed in the hexane and chloroform eluents of this extract. The methanolic extract was devoid of any estrogenic activity in immature rat bioassay[155].

- Asafoetida significantly restored the level of antioxidant system, depleted by N-methyl- N-nitrosourea (MNU) treatment at two different doses (1.25 and 2.5 per cent w/w in diet) in female Sprague-Dawley rats. In long term studies, where MNU was used to induce mammary carcinoma, asafoetida treatment resulted in a significant reduction in the multiplicity and size of palpable mammary tumours and a delay in mean latency period of tumour appearance[156].

- The 70 per cent ethanolic extract (25 and 50 mg/plate) of resin gum did not show any anti-mutageniei iy activity in *Salm.*

typhimurium strains TA100 and TA1535. Asafoetida extract was Found to inhibit the microsomal activation-dependent mutagenicity of 2-acetamidofitiorene[157].

- The oleo-gum resin, at doses of 34, 45 and 55 mg/kg orally, showed 63, 80 and 100 per cent protection against pentylenetetrazol (PTZ)-induced convulsions respectively, in albino mice. A significant dose-dependent anticonvulsant activity without sedative effect was observed. Also, it had a large therapeutic index which indicated the safety margin of the test drug. on autopsy, except visceral congestion, no histopathological changes were seen[158].

- The aqueous extract of the gum (200 and 400 mg/kg p.o. for 15 days) showed dose-dependent memory and learning enhancing effect in male wistar albino rats as evidenced by decrease in transfer latency, in elevated plus maze test and increase in step through latency in passive avoidance test in comparison to control group. It also inhibited the brain acetylcholinesterase activity and increased serum protein thiols. However, it was less effective than the standard drug, rivastigmine (5 mg/kg p.o.) [159].

- The aqueous extract of oleo-resin (containing 60 per cent ferulic acid) did not show any hypoglycemic and hyperglycemic activities in STZ-induced type I diabetic rats. At the doses of 100 and 200 mg/kg i.p. once daily, the extract showed significant increase in the level of fasting blood glucose after 3 wks. The hypoglycemic effects were comparable with insulin at a dose of 5 µ/kg.

- The effect of asafoetida (1.25 and 2.5 per cent w/w in diet) was studied on drug metabolizing enzymes in the livers of N-methyl-N-nitrosourea treated rats. Asafoetida treatment significantly reduced ($p<0.05$) the levels of cytochrome P450 and b5. There was enhancement in activities of glutathione-S-transferase, DT-diaphorase, superoxide dismutase and catalase and in the level of

reduced glutathione. The treatment restored the levels of antioxidant system. Lipid peroxidation was also found to be significantly reduced ($p<0.005$) [156].

- The oleo-gum resin incorporated at 50 and 250 mg per cent in basal diet of rats did not affect the hepatic mixed function oxygenase system. However, at higher dose asafoetida reduced the cytochrome b5 level (Sambaiah and Srinivasan, 1989). In another study, the incorporation of oleo gum resin (250 mg per cent) in basal diet of rats caused increased biliary secretion of cholesterol and phospholipids without affecting bile acid content[160].

- The gum did not show any beneficial antiarthritic effect against formalin-induced arthritis in rats[161].

- Asafoetida, when studied for its action on digestive enzymes showed activation of pancreatic amylase, but had an inhibitory influence on lipase activity. Proteolytic enzymes were not affected by asafoetida[162].

TOXICITY STUDIES

- The aqueous extract of the gum, in acute toxicity study, did not cause any mortality up to 2000 mg/kg, p.o. dose in rats[159].

ANTI MICROBIAL ACTIVITY

- The alcoholic and aqueous extracts of the gum resin did not show antibacterial activity against Staph. aureus and Esch. coli. In another study, the aqueous extract of asafoetida showed slight inhibition of growth of Staph. aureus and Sh sonnei while being inactive against Esch coil, Aerobacter aerogenes, Lactobacillus came and Staph faecalis[163].

CLINICAL STUDIES

A study was conducted on fat (butter)-induced hyperlipaemia on10 healthy subjects. Asafoetida-resin (3 g) or an equivalent amount of its ether

extracted essential oil showed significant ($p<0.001$) protective action, by increasing the plasma fibrinogen and decreasing the coagulation time and fibrinolytic activity. There was an insignificant decrease in serum cholesterol. The essential oil fraction (mainly allyl persulphide) produced all the beneficial effects of whole asafoetida[164].

2.1.10 PLANT PROFILE (*Piper longum L*)

Botanical Source : *Piper longum* L.

Family : Piperaceae

Synonyms : *Chavica longa* H.Karst.. *Chavica roxburghii* Miq

Parts Used : Dried fruit

Taxonomic classification

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Piperales

Family : Piperaceae

Genus : Piper

Species : *Piper longum*

Vernacular Names

Sanskrit : Magadhi

Hindi : Pipli

Kannda : Lippili

Malayalam : Tippali

English : Long pepper

Tamil : Tippili

Telugu : Pipallu

Distribution

Widely distributed in Western ghats, Central Himalayas to Assam, Khasi and Miker hills and lower hills of Bengal[165].

Description of drug

Infloresecence is a spike, fruits small, ovoid sunken structures embedded in a fleshy spike, which is 2.5-4 cm long, ovoid, oblong, light green when immature and blackish-green and shining on ripening. Samples of commerce are dull dark brown to black[166].

Habit The plant is a slender climber[165].

Chemical Constituents

Fruit: Alkaloids piperine, piperlongumine (piperlatine[167].), piperlonguminine [167,168] and also methyl-3,4,5-trimethoxycinnamate[167-169].

Traditional Medicinal Uses

Fruits are used for diseases of respiratory tract, ie, cough, bronchitis, asthma, as a counter-irritant for inflammation, emmenagogue, abortifacient, anthelminthic, dysentery and leprosy.

Fig. No. 10

Piper Logum
(திப்பிலி)

PHYTOCHEMICAL INVESTIGATIONS

- The alkaloids piperine, piperlongumine (piplartine)2, piperlonguminine" and also methy1-3,4,5- trimethoxycinnamate2-4.

Sesamin, a lignan5, dihydrostigmasterol and two low melting unstable compounds, one of which appeared to be isobutylamide of an unsaturated acid, n-isobutyl-deca-trans-2-trans-4-dieneamide, essential oil consisting of n-hexadecane, n-heptadecane, n-octadecane, n-nonadecane, n-cicosane, n-hencosane, a-thujene, terpinolene, zingiberine, p-cymene, p-methoxyacetophenone, dehydrocarveol and two monocyclic sesquiterpenes. The presence of L-tyrosine, L-cysteine hydrochloride, DL-serine and L-aspartic acid as free amino acids also has been reported in the fruits9. The seeds contain sylvatine, dieudesminw. In addition to palmitic, hexadecenoic, stearic, linoleic, oleic, linolenic, higher saturated acids, arachidic and behenic acids are also reported[170].

PHARMACOLOGICAL ACTION

- Our present study investigated effects of these herbs on inflammation in rat model with cerebral ischemia. After subjecting the rats to permanent middle cerebral artery occlusion (pMCAO) for 6 h, at doses of 100 and 200 mg/kg, dichloromethane fraction from white pepper and long pepper, respectively, was intragastrically administered once a day for seven consecutive days. Cerebral cortical and hippocampal tissues were collected after seven days. Superoxide dismutase, malonaldehyde, tumor necrosis factor-alpha (TNF-alpha), interleukin-1 beta (IL-1 beta), and IL-6 were measured by spectrophotometer. These results show that dichloromethane fraction provides protection against cerebral ischemia. The possible mechanism is related to anti-inflammatory activity and reduction in oxygen-free radicals. (C) 2017 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda[171].

- Present study evaluated in vitro antioxidant activity and cytotoxicity of four important Piper species (P. nigrum L., P. chaba Hunter, P. longum L. and P. colubrinum Link.) and six black pepper varieties (Sreekara, Subhakara, IISR Malabar Excel, Panniyur-1, Panchami and IISR

Thevam). It was performed with sequential extracts of the dried berries/fruits using n-hexane, chloroform, methanol and water in the order of increasing polarity. Concentrated extracts were tested for total phenolic content, in vitro antioxidant activity and cytotoxicity. Methanol and chloroform extracts showed high antioxidant activity than hexane and water extracts. In vitro cytotoxicity of the extracts was tested on cervical cancer cell line CaSki by MTT assay and compared with that of synthetic anticancer drug Doxorubicin. Results showed more cytotoxicity with more extract and increased time of exposure with CaSki. Chloroform extract of P. longum and P. colubrinum were found to be highly toxic to CaSki than other extracts[172].

ANTI MICROBIAL ACTIVITY

- Sixty-three amide alkaloids, including three new, piperflaviflorine A (1), piperflaviflorine B (2), and sarmentamide D (4), and two previously synthesized ones, (1E,3S)-1-cinnamoyl-3-hydroxypyrrolidine (3) and N-[7'-(4'-methoxyphenyl) ethyl]-2-methoxybenzamide (5), were isolated from the aerial parts of Piper flaviflorum and Piper sarmentosum. Their structures were elucidated by detailed spectroscopic analysis and, in case of 3, by single-crystal X-ray diffraction. Most of the isolates were tested for their antifungal and antibacterial activities. Ten amides (6-15) showed antifungal activity against Cryptococcus neoformans ATCC 90113 with IC50 values in the range between 4.7 and 20.0 mu g/mL[173].

- P. longum possesses bioavailability enhancing properties. Piperine was shown to enhance the bioavailability of antitubercular drugs rifampicin'3, pyrazinamide, isoniazid and ethambuto1R14 and also the antileprotic drug dapsone'5. The essential oil of fruit showed antibacterial, anti fungal'" and anthelmintic18 activities[174].

- Anti-allergic activity using immunoglobulin E (IgE)-sensitized beta-hexosaminidase in the rat basophilic leukemia-2H3 (RBL-2H3) cells and anti-inflammatory activity using lipopolysaccharide (LPS)-induced

nitric oxide (NO) and tumor necrosis factor-alpha (TNF-alpha) in the murine macrophage (RAW 264.7) cells. The ethanolic extracts of BJK showed anti-allergic activity (IC50 = 12.69 mu g/ml) and exhibited potent NO inhibitory effect (IC50 = 16.60 mu g/ml), but inactive on TNF-alpha release. Moreover, 6-shogaol and plumbagin, two pure compounds from BJK, showed higher anti-allergic activity than the ethanolic BJK extract with IC50 values of 0.28 and 4.03 mu g/ml, respectively. These compounds were significantly higher than chlorpheniramine (CPM), standard drug, with IC50 value of 17.98 mu g/ml. Determination of the anti-inflammatory activity by measuring the inhibition of NO production presented that plumbagin and 6-shogaol exhibited higher than crude BJK extract with IC50 values of 0.002 and 0.92 mu g/ml, respectively[175].

- Arthritis was induced by intradermal injection of complete Freund's adjuvant (0.1 ml) into the left hind paw of the *Wistar* albino rats. PHF (100 & 200 mg/kg b.wt) and prednisolone (PDL) (5 mg/kg b.wt) were administered orally from 1st day to 28th day after adjuvant induction. Induction of arthritis significantly increased hind paw volume (HPV), levels of reactive oxygen species (LPO and NO), and inflammatory cytokines (TNF-alpha, IL-1 beta and IL-6) with subsequent decrease in the anti-oxidant status (GSH, SOD and CAT) in arthritic rats compared to controls. Furthermore, the mRNA expression of inflammatory enzymes (iNOS and COX-2), and transcription factor (NF-kappa B) was found up regulated in the joint tissues of arthritic rats in RT-PCR analysis. In conclusion, these findings showed that PHF exerted beneficial effects on rheumatoid arthritis in rats[176].

- The present study investigated the mechanistic basis for the observed protective effects of PLL. Rats treated with PLL-derived alkaloids showed improvement in rotenone-induced motor deficits, while reactive oxygen species (ROS) production was decreased, mitochondrial membrane potential was stabilized, and the opening of the mitochondrial permeability transition pore (mPTP) which is

involved in ROS production was inhibited. In addition, rotenone-induced apoptosis was abrogated in the presence of these alkaloids, while a pretreatment stimulated autophagy, likely mitigating neuronal injury by the removal of damaged mitochondria. These findings provide novel insight into the neuro-protective function of PLL as well as evidence in favor of its use in PD treatment[177].

- The current study was done to evaluate anti-stress activity in rats subjected to forced swim stress one hour after daily treatment of P. longum extract. Urinary vanillylmandellic acid, 5-hydroxyindoleacetic acid, homo vanillic acid and ascorbic acid, estimated by HPLC and spectrophotometric methods in all groups, were selected as non-invasive biomarkers. Anti-stress and nootropic activity activities of aqueous extract of P. longum fruit extract were estimated as locomotor and working memory in rats in a Y-maze apparatus. The in vitro antioxidant activity was determined based on the ability of the P. longum to scavenge free radicals. Daily administration of aqueous extract of P. longum at doses of 100, 200 and 300 mg per kg body weight one hour prior to induction of stress increased the stress-induced urinary biomarker levels in a dose-dependent manner. P. longum treatment showed significant dose-dependent variation in non-invasive biomarker levels in urine samples of rats taken after 24 h. Cognition, determined by working memory and locomotor activity results, were shown to be dose-dependent. The results of this study suggest anti-stress and nootropic activity effect of P. longum in rodents[178].

- The two pure phytochemicals characterized as cis-piperine and trans-piperine isolated from the dried fruits of Piper longum were tested for anti-hepatotoxic activity at the dose of 50 mg/kg orally respectively, against silymarin as a standard reference. The toxicity was induced by CCl4, and then various biological parameters such as AST, ALT, ALP, SOD, GPx, GSH, total protein, total albumin and total bilirubin were measured at 134.24 IU/L 71.96 IU/L, 375.02 IU/L, 37.02 U/g, 17.02

U/g, 29.12 U/g, 1.95 g/dl, 1.09 g/dl, 4.16 g/dl respectively. The phytochemicals cis-piperine and trans-piperine showed significant anti-hepatotoxic activity[179].

- Effect of piperine on haematological parameters of mice in acetic acid induced IBD was also determined which involves the estimation of FFA using a commercial free fatty acid fluorometric assay kit. Result Piperine significantly attenuated acetic acid induced DAI score which implies that it suppresses weight loss, diarrhoea, gross bleeding and infiltration of immune cells. Piperine administration also effectively and dose dependently prevented shortening of colon length and enlargement of spleen size. Histological examination indicated that piperine reduces oedema in sub-mucosa, cellular infiltration, reduced haemorrhages and ulceration as compare to acetic acid induced colitis in mice. Furthermore piperine inhibited abnormal secretion of pro-inflammatory mediators namely NO, cytoldnes TNF-a and reduces FFA induced TLR4 mediated inflammation. These results suggest that piperine has an anti-inflammatory effect at colorectal sites that is due to down- regulations of the productions and expression of inflammatory mediators and it also reduces FFA induced TLR4 mediated inflammation[180].

- PLA was prepared by extracting the dry seed of P. longum using 85% ethanol. Adult male C57BL/6 mice were divided into eight groups of 12 rats each. Experimental and control groups received an equivalent volume of saline, 0.5% CMC-Na, and 0.1% Tween 80, treated groups received oral PLA (30, 60, and 120 mg/kg), other groups treated with piperine (60 mg/kg) or Madopar (50 mg/kg). The PLA prevention group (PLA-Pr) administrated PLA (120 mg/kg) for 1 week before MPTP challenged. Except for the PLA-Pr group, others were treated for seven consecutive weeks. Parkinson's disease was induced by injecting MPTP intra-peritoneally (25 mg/kg) twice weekly for five consecutive weeks. Dopaminerigic (DA) neurons and their metabolism were detected by UFLC-MS/MS. Tyrosine hydroxylase (TH)-

immunohistochemistry assay and Western blotting were performed. The antioxidant enzymatic levels were determined by kit-based assays[181].

CLINICAL STUDIES

- A double-blind, within person-randomized controlled study of 30 healthy volunteers to determine efficacy and safety of topical *Trikatu* on mosquito bite reactions. Methods: All subjects were bitten by Aedes aegypti laboratory mosquitoes on their forearms and they were randomly assigned arms to apply either *Trikatu* or reference product on the mosquito bite papule. The main outcome was the difference of papule size reduction at 30 min, measured by a caliper, between the *Trikatu* and reference arms. Pruritis, redness, pain, and patient satisfaction were assessed at 15, 30, 60, 180, and 360 mm as secondary outcomes. Results: There were no significant differences between treatment and reference arms on any outcome at any time of measurement. Conclusion: *Trikatu* did not show additional effects for relieving mosquito bite reaction as compared with the reference product containing camphor, menthol, and eucalyptus. For further study, it is very important to consider a proper selection of subjects, comparator product, and concentration of extract.

Urai Mathirai

The earlier Anti - microbial studies conducted supports the usefulness of the drug in group of organisms of Enterobacteriaceae. The Minimal inhibitory Zones in compared with Antibiotics of Biomedicine are Encouraging as Urai mathirai showed Higher Inhibition in organisms causing Nosocomial infections[182].

Immuno modulatory review

In 2004 and 2005, a multi-centre pediatric study was carried out in the Czech Republic and Slovakia, to determine the effects on the frequency of upper respiratory tract (URT) infections in children. The study involved 215 children, aged three to seven years, with a history of at least five URT infections during the year prior to

treatment. Beta-(1,3/1,6)-D-glucan (as syrup) was administered to patients for a period of at least three months. Astatistically significant positive effect of syrup administration was demonstrated in 153 (71.2%) children. In these children, a reduction of 60% in the occurrence of URT infections was recorded during the following year.

An improvement in health was observed in 17 out of 20 patients with recurrent viral and mycotic infections, after a three-month course of beta-(1,3/1,6)-D-glucan. In five patients, in whom infection re-occurred during the treatment, the infection was mild and did not need anti-infection therapy. Beta-(1,3/1,6)-D-glucan appears to be a suitable nonspecific natural immune-modulator for the prevention of recurrent infections, mainly of the respiratory tract, but also of urinary or gynaecological infections and herpes infections in patients with severe allergic or immune-pathological diseases, such as bronchial asthma, polyvalent allergy, rheumatic diseases, multiple sclerosis, etc[183].

Beta-(1,3/1,6)-D-glucan syrup was administered to 30 children, aged three to 10 years with recurrent infection of upper and lower respiratory tracts, for a period of two to four months (three months on average). The occurrence of the respiratory tract infections was reduced, six children were not sick at all and children with immunity disorders and polyvalent allergies experienced a decrease of 50-60% in infection frequency. No side effects were recorded. Beta-(1,3/1,6)-D-glucan syrup was rated positively as a nutrition supplement supporting the immune system[183].

Infections which caused ravages in the past centuries are again resurgent and newly emerging pathogens capable of human diseases continue to surface. Multidrug antibiotic resistance has turned into a major medical problem. Judicious concepts for combating infections in the 21st century have acquired a new poignancy. Immunomodulators of natural, synthetic, and recombinant origin can stimulate host defense mechanisms for the prophylaxis and treatment of diverse viral, bacterial, parasitic and fungal diseases. Some immunomodulator preparations are already licensed for use in patients and numerous others are being extensively investigated in preclinical and clinical studies. Immunomodulators offer a novel adjunct to to established antimicrobial therapies[184].

Plan of work

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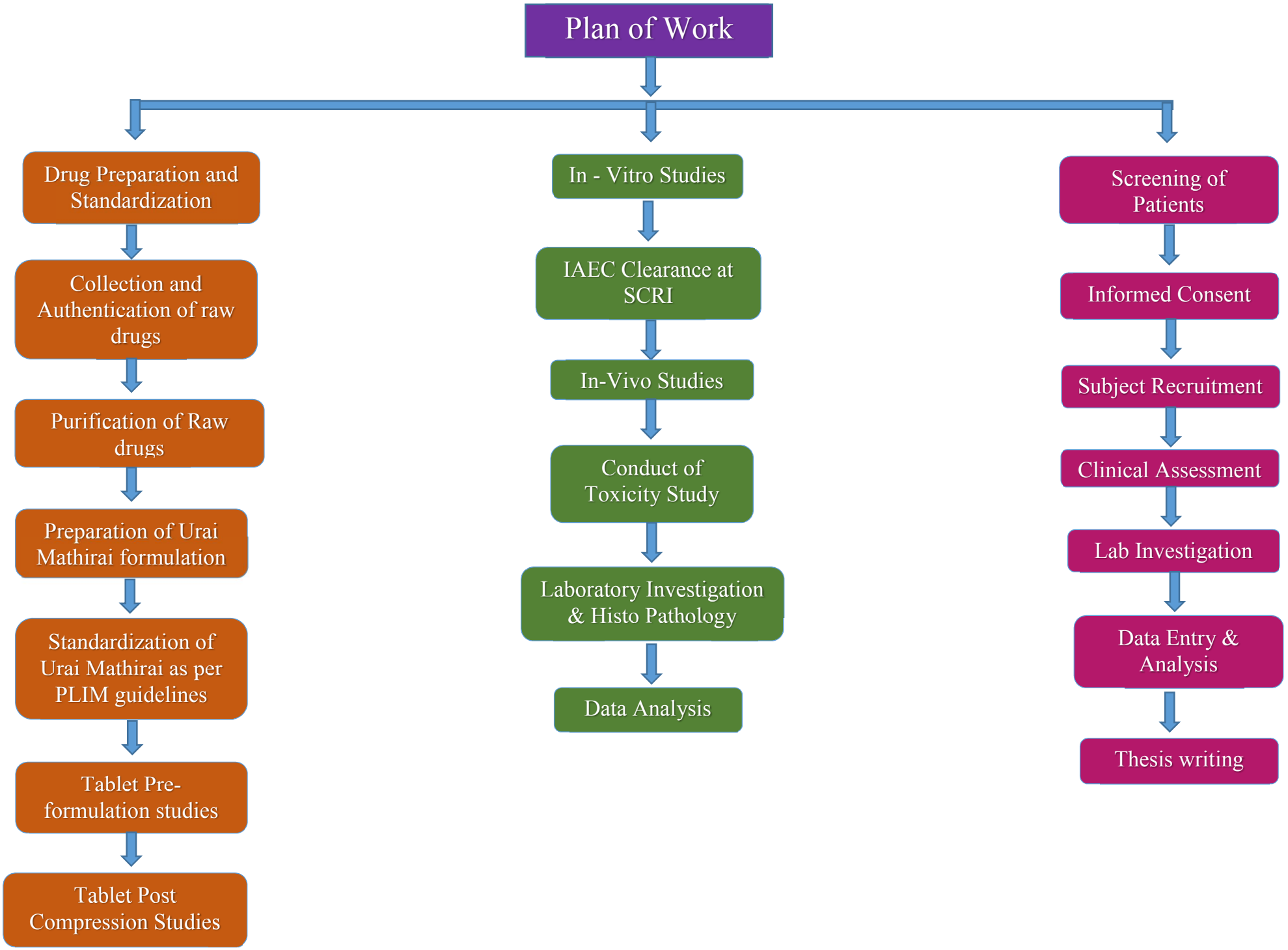

Plan of Work
Drug Preparation and Standardization
Collection and Authentication of raw drugs
Purification of Raw drugs
Preparation of Urai Mathirai formulation
Standardization of Urai Mathirai as per PLIM guidelines
Tablet Pre-formulation studies
Tablet Post Compression Studies
In - Vitro Studies
IAEC Clearance at SCRI
In-Vivo Studies
Conduct of Toxicity Study
Laboratory Investigation & Histo Pathology
Data Analysis
Screening of Patients
Informed Consent
Subject Recruitment
Clinical Assessment
Lab Investigation
Data Entry & Analysis
Thesis writing

Materials and Methods

5. MATERIALS AND METHODS

5.1. COLLECTION AND AUTHENTICATION OF RAW DRUGS

The selected raw drugs for the proposed study were collected from Siddha Central Research Institute, Pharmacy RDS. It was identified and authenticated by Research Officer (Botany), SCRI, Chennai. A voucher specimen has been deposited for further reference.

5.1.1. MACROSCOPIC CHARACTERS

The Organoleptic evaluation such as appearance, colour, odour, taste and texture were observed.

5.1.2. POWDER MICROSCOPY

The specified organs were cut and removed from the selected plants, shade dried and powdered individually. The shade dried powdered sample was passed through sieve no 60 and then subjected to powder microscopy study. A small quantity of powder was taken and a few drops of Dilute NaOH [185] was added and heated for one to two minutes. Dilute NaOH was used as a clearing agent to the tissues. To the cleared powder phloroglucinol and concentrated Hydrochloric acid in the ratio of 1:1 was added and kept as such for 3 to 4 minutes. Then 2 to 3 drops of dilute Hydrochloric acid was added and finally mounted in glycerine. The lignified tissues will acquire a pink colour. To detect the presence of starch grains, the powder was mounted in 1 to 2 drops of dilute iodine and mounted in glycerin. Starch grains appear as blue to purple colour.

PHOTOMICROGRAPHS

Microscopic description of tissues is supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars.

Descriptive terms of the anatomical features are as given in the standard Anatomy books[186].

The observations of powder microscopy were exhibited in result.

5.2. PURIFICATION OF RAW DRUGS

All the raw drugs were purified as per the methods mentioned in Siddha literature.

5.2.1 PREPARATION OF *URAI MATHIRAI* FORMULATION

The following selected purified raw drugs were used to prepare *Urai Mathirai* formulation: **Table No. 01**

S.No	Tamil Name	Botanical name	weight
1.	Chukku-	*Zingiber officinale*	80 gms
2.	Athimathuram	*Glycyrrhiza glabra*	50 gms
3.	Akkarakaram	*Anacyclus pyrethrum*	30 gms
4.	Vasambu	*Acorus calamus*	30 gms
5.	Jathikkai	*Myristica fragrans*	30 gms
6.	Kadukkai	*Terminalia chebula*	30 gms
7.	Masikkai	*Quercus infectoria*	30 gms
8.	Poondu	*Allium sativum*	30 gms
9.	Perunkayam	*Ferulla asafoetida*	30 gms
10.	Thippili	*Piper logum*	25 gms

The identified above raw drugs are ground with water and made into tablets[187].

5.3. STANDARDIZATION OF FORMULATION AS PER AYUSH GUIDELINES

5.3.1. DESCRIPTION

5.3.1.1. MACROSCOPIC

The Organoleptic evaluation such as Appearance, colour, odour, taste and texture were observed[188]

5.3.1.2. POWDER MICROSCOPIC STUDY

A small quantity of *Urai Mathirai* powder was taken and a few drops of Dilute NaOH[185] was added and heated for one to two minutes. To the cleared formulation phloroglucinol and concentrated Hydrochloric acid in the ratio of 1:1 was added and kept as such for 3 to 4 minutes. Then 2 to 3 drops of dilute Hydrochloric acid was added and finally mounted in glycerin. The lignified tissues showed a pink colour. To detect the presence of starch grains, the Chooranam was mounted in 1 to 2 drops of dilute iodine and mounted in glycerine. Starch grains appeared as blue to purple colour.

The observation of macroscopic and powder microscopy were exhibited in figure No 11.

5.3.2. PHYSICO-CHEMICAL ANALYSIS

5.3.2.1. DETERMINATION OF LOSS ON DRYING

This parameter is used for determination of moisture content. Loss on drying is the loss in weight in % w/w determined as per the standard procedure. Weighed stopper glass bottle that has been dried for 30 minutes under the same conditions to be employed in the determination was weighed. The individual herbal powders and *Urai Mathirai* formulation were placed separately in to the bottle and the contents were accurately weighed. The sample was distributed evenly to depth not exceeding 10 mm. The loaded bottle was placed in a drying chamber (oven) and the stopper was removed. The sample was dried to constant weight at a temperature of 105^{o} C in hot hair oven for 5 hours. The percentage of loss on drying was calculated with reference to the air-dried drug. This procedure was continued until difference between two successive weighing was not more than 0.25% of constant weight. The value was then calculated[188,189].

5.3.2.2. ASH VALUE

This parameter can be used for the determination of inorganic materials such as carbonates, silicates, oxalates and phosphates. Heating causes the loss of organic material in the form of carbon dioxide leaving behind the inorganic components. Ash value is an important characteristic of a drug to detect the extent of adulteration as well as to establish the quality and purity of the drug. There is a considerable difference in the ash values of different drugs but mostly the difference varies within narrow limits in case of the same drug. The acid insoluble ash consists mainly of silica and high acid insoluble ash thereby indicating the contamination with earthy materials. The water-soluble ash is used to estimate the amount of inorganic elements[188,189].

DETERMINATION OF TOTAL ASH

About 2 gm of air-dried individual herbal powder and its *Urai Mathirai* formulation were weighed accurately in a tarred platinum or silica dish and was incinerated at a temperature not exceeding 450^{o}C until free from carbon. It was then cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug. This procedure was continued until difference between two successive weighing was not more than 0.25% of constant weight. The value was then calculated[188,189].

5.3.2.3. DETERMINATION OF WATER SOLUBLE ASH

The ash obtained in the previous method was boiled with addition of 15ml of water for 10 minutes. It was filtered in an ash less filter paper (Whatman No. 41) and the residue was ignited in the furnace to get a constant weight. The water soluble ash value was calculated with reference to the weight of powdered plant material and its *Urai Mathirai* formulation[188,189].

5.3.2.4. DETERMINATION OF ACID INSOLUBLE ASH

The ash obtained above was boiled with 25 ml of 2M hydrochloric acid for 15 min. The insoluble matter was collected in a Gooch crucible or an ash less filter paper. It was washed with hot water and ignited. It was then cooled in desiccators and weighed. The percentage of acid insoluble ash was calculated with reference to air-dried drug. This procedure was continued until difference between two successive weighing was not more than 0.25% of constant weight. The value was then calculated[188,189].

5.3.2.5. EXTRACTIVE VALUES

The amount of an extract that a drug yields in a particular solvent is often an approximate measure of the amount of certain constituents that the drug contains The drug should be extracted with different solvents in the order of increasing polarity to get the correct and dependable values. Generally alcohol and water extractives are taken into consideration for fixing the standard of a drug. Alcohol can dissolve almost all the substances but is generally used for determining the extractive index for those drugs which contain glycosides, resins, alkaloids etc. Water is used for the drugs containing water- soluble substances as chief constituents[185].

DETERMINATION OF WATER SOLUBLE EXTRACTIVE

5 grams of the individual herbal powders and its *Urai Mathirai* formulation were macerated separately with 100 ml distilled water in a closed flask for 24 hours, shaking frequently for 6 hours and allowed to standing for 18 hours. It was filtered rapidly and 25ml of the filtrate was evaporated to dryness at 105^{o}C and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug. The value was then calculated.

5.3.2.6. DETERMINATION OF ALCOHOL SOLUBLE EXTRACTIVE

5 grams of the individual herbal powders and its *Urai Mathirai* formulation were macerated separately with 100 ml of 95 % alcohol in a closed flask for 24 hours, shaking frequently for 6 hours and allowed standing for 18 hours. It was filtered rapidly taking precautions against loss of alcohol and 25 ml of the filtrate was evaporated to

dryness at 105^{o}C and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug. The value was then calculated.

5.3.2.7. pH VALUE

Five gram of powdered plant materials and its *Urai Mathirai* formulation was added to 100 ml of distilled water in a stoppered flask. It was stirred well and allowed to stand for 1 hours. Then, it was filtered and the clear solution was used for the measurement for pH.

The results of Physio-chemical parameters such as Ash values, Extractive values, Loss on drying and pH value were tabulated in Table. No.1

5.3.3. PHYTOCHEMICAL TEST FOR IDENTIFICATION OF COMPOUNDS

The *Urai Mathirai* formulation were subjected to preliminary phytochemical screening for identification of phytochemical constituents[190,191,192].

1. **Tests for Alkaloids**

Mayer's test: A pinch of Urai mathirai formulation were taken separately and 4 mL of dilute hydrochloric acid was added, mixed and filtered. To the filtrate, one or two drops of Mayer's reagent were added.

Dragendorff's test: A pinch of Urai mathirai formulation was treated separately with 4 mL of 2% acetic acid, mixed thoroughly and filtered. To the filtrate 2 drops of Dragendorff's reagent was added.

Hager's Test: A pinch of Urai mathirai formulation were taken separately and 4 mL of dilute hydrochloric acid was added, mixed and filtered. To the filtrate, one or two drops of Hager's reagent were added.

Wagner's Test: A pinch of Urai mathirai formulation were taken separately and 4 mL of dilute hydrochloric acid was added, mixed and filtered. To the filtrate, one or two drops of Wagner's reagent were added.

2. **Tests for Sugars and Carbohydrates**

Molish's test: A small quantity of Urai mathirai formulation was dissolved separately in 4 mL of distilled water and filtered. Filtrate was treated with 2-3 drop of 1% alcoholic α-napthal solution and 2 mL of concentrated sulphuric acid was added from the sides of the test tube.

Fehling's test : A small quantity of Urai mathirai formulation were dissolved separately in 4 mL of distilled water and filtered. Filtrate was boiled on water bath with 1 mL each of Fehling solutions A and B.

Benedict's test : A small quantity of Urai mathirai formulation was dissolved separately in 4 mL of distilled water and filtered. To the filtrate, 0.5 mL of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min.

3. Tests for Proteins

Small quantity of Urai mathirai formulation was dissolved separately in a few mL of water, filtered and subjected to the following test:

Biuret Test: To the filtrate few drops of Biuret reagent solution was added

Xanthoproteic test:A little quantity of the test filtrate was taken with 2 mL of water and 0.5mL of concentrated nitric acid was added to it.

4. Test for Amino Acids

Ninhydrin Test: To the aqueous extract of Urai mathirai formulation, few drops of Ninhydrin reagent were added.

5. **Test for Flavonoids**

Shinoda test: A pinch of Urai mathirai formulation was dissolved separately in ethanol, mixed thoroughly and filtered. To the filtrate a piece of Magnesium metal and con. Hydrochloric acid were added and heated.

6. Test for Terpenoids

Noller's Test (or) Salkowski Test: To the alcoholic extract of Urai mathirai formulation in a test tube, a bit of Tinfoil and 0.5 mL of thionyl chloride was added. It was heated gently.

7. Tests for Phenolic Compounds

Ferric Chloride Solution Test: The Urai mathirai formulation was taken, placed separately in water and warmed. To this 2 mL of Ferric chloride solution was added. ***Lead Acetate Solution Test:*** To the aqueous extract Urai mathirai formulation, lead acetate solution was added separately.

8. Tests for Tannins

A pinch of Urai mathirai formulation was dissolved separately in ethanol, mixed thoroughly and filtered. The filtrate is tested for the presence of tannins by the following test:

***Ferric chloride*:** To the filtrate dilute ferric chloride solution was added.

Lead acetate: To the filtrate Lead acetate solution was added (10%).

9. Tests for Steroids

Liebermann's Burchard test: The Urai mathirai was dissolved in 4 mL of chloroform and 10 drops of acetic anhydride, 2 - 4 drops of concentrated sulphuric acid were added. ***Salkowski Test:*** A pinch of Urai mathirai was dissolved in 4 mL of chloroform .Then treated with few drops of concentrated sulphuric acid. The lower layer of the chloroform showed red colour due to the presence of steroids.

10. Tests for Glycosides

Anthrone test: A pinch of Urai mathirai formulation was taken separately in a watch glass and 2 drops of alcohol was added. An equal quantity of anthrone was added and mixed thoroughly and dried. Then one drop of concentrated sulphuric acid was added, spreaded in a thin film with a glass rod in a watch glass and heated over the water bath.

11. Detection of Fixed Oils and Fats

Spot test: A small quantity of Uraimathirai was pressed separately between two filter papers.

12. **Test for Saponin**

Foam test: The Urai mathirai formulation was diluted separately with distilled water to 10 mL, filtered and shaked in a graduated cylinder for 15 minutes.

5.3.4. IDENTIFICATION BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

HPTLC studies were carried out as per following method[193].

HPTLC experiments were performed for hydroalcoholic extract of powdered plant materials and *Urai Mathirai* formulation on aluminum packed silica gel $60F_{254}$ HPTLC plates (Merck). The mobile phase was Toluene : Ethyl Acetate : Formic Acid (5 : 2.5 : 0.5) and it was poured into the Camag twin trough glass chamber and allowed to equilibrate for 30 min. The Samples (5 -15 uL) were applied to the plates as sharp bands by using of CAMAG Automatic TLC Sampler 4 (ATS4) applicator. After drying the spots in a current of air the plats were placed in one trough of CAMAG twin trough glass chamber and then developed until the solvent front had travelled a distance of 8 cm above the position of sample application. The developed plate was air dried, visualized under UV 254, 366 nm for documenting the TLC chromatograms; Then scanned in both wavelengths for generating the finger print profiles. The photo documentation and finger printing was also done at 575 nm after dipping the plate in vanillin-sulphuric acid reagent, followed by heating in an oven till the appearance of color of the spots.

The results of HPTLC of *Urai Mathirai* formulation and its individual herbal ingredients were expressed in chromatogram, finger print (TLC Pattern) in Figure.No.12 to 22 and table 3 to 13

5.3.5. HEAVY METAL ANALYSIS:

Preparation of samples by acid digestion method

Accurately weighed 2g of sample of *Urai Mathirai* were taken in Kjeldahl flask. Acid mixture of HNO_3: $HClO_4$ (4:1) were added in the flask and heated continuously till the solution is colourless. The sample was then transferred in a 25 ml volumetric flask and the volume was made-up with distilled water. Reagent blank was synchronously prepared according to the above procedure. The standards of Lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) were prepared as per the standard procedure and the calibration curve was developed for each of them.

Detection

Then samples were analyzed for the presence of Pb, Cd, As and Hg using Atomic Absorbance Spectrophotometer (AAS) 6300 (by SHIMADZU) [188].

The results of Heavy metal analysis were tabulated in Table No.14

5.3.6. MICROBIAL CONTAMINATION:

Microbial contamination was carried out as per procedure of and WHO Guidelines. It included the test of Total Bacterial Count, Total Fungal Count, and presence of specific pathogens like *Escherichia coli*, *Salmonella spp*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The media used for the microbial limit test were of HiMedia Pvt. Ltd[188].

ENUMERATION OF MICROBIAL LOAD

THE TOTAL AEROBIC BACTERIA

The total aerobic bacteria in *Urai Mathirai* formulation were determined by the surface plate agar with a medium nutrient agar. Two gram of each plant sample was added to 20 ml of 0.01% Tween- 20 sterile water and mixed thoroughly. Each suspension was properly diluted with the same sterile water, as necessary depending on the expected bacterial load of the material being examined. Aliquots of 0.2 ml from each dilution were then spread on the surface of the agar plates. The bacteria were counted after 3 days incubation at 30ºC. The total aerobic mesophilic counts were calculated by multiplying the average number of colonies with dilution factor and reported as a colony-forming unit per gram (cfu/g) of sample.

***Coliform spp*.**

The *coliform spp* bacteria were determined using MacConkey agar media for 24 hours incubation at 37°C. Pink colonies were counted and calculations were made. Presumptive *E. coli,* colonies appearing on MacConkey agar media were then sub cultured on Eosin Methylene Blue agar (EMB) and incubated at 37°C for 24- 48 hours. Colonies with characteristic greenish metallic colour were subjected.

Salmonella spp

For *Salmonella spp*. 25 g of sample was aseptically weighted and added into 225 ml of sterile lactose broth and incubated at 37°C for 18-20 h. An inoculum of 0.2 ml was transferred into a tube containing 9.8 ml of Selenite Cystine Broth by subsequent incubation at 40°C for 24 hours and afterward plated onto bismuth sulphite agar and

xylose lysine desoxycholate agar plates for 24-48 hours at 37°C and then examined for typical colonies.

YEAST AND MOULDS

Total fungal count of the *Urai Mathirai* formulation was determined by plate count method using Sabouraud Dextrose Agar. Plates were incubated at a temperature of 28 ° C for 3-5 days and the number of colony-forming units (cfu) was counted

Pseudomonas aeruginosa

1.0g inoculate 100 ml of fluid soyabean-casein digest medium with a quantity of the solution, suspension or emulsion thus obtained containing 1 g or 1 ml of the preparation being examined. Mix and incubate at 35º to 37º for 24 to 48 hours. Examine the medium for growth and if growth is present, streak a portion of the medium on the surface of cetrimide agar medium, each plated on Petri dishes. Cover and incubate at 35º to 37º for 18 to 24 hours.

Staphylococcus aureus

Proceed as described under *Pseudomonas aeruginosa*. If, upon examination of the incubated plates, none of them contains colonies having the characteristics the sample meets the requirements Vogel-Johnson agar, Mannitol-salt agar, Baird-Parker agar media used for the absence of *Staphylococcus aureus.*

MICROBIAL GROWTH MEDIUM

Nutritional media used for evaluation of microbial limits were procured from Hi-Media Laboratories Ltd. and were ready to use dehydrated media. Soybean casein digest agar,

Mannitol salt agar, Cetrimide agar, Brilliant green agar, Potato dextrose agar, MacConkey's agar were utilized during microbial evaluation studies.

The results of Microbial contamination were tabulated in Table No.15

ANTI-MICROBIAL STUDIES

The antimicrobial activity of the extracts was determined by Kirby Bauer method in agar well diffusion. In this method, initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 h in an incubator. Then agar plates of the Muller Hilton's Agar media were prepared. Each plate was inoculated by swabbing with bacterial suspension or with 18 h fresh culture (10^5 10^6 colony forming unit "CFU"/ml), which was swabbed evenly on the surface of solid agar media by the help of cotton swab. After 20 min, wells of 6 mm diameter were made in solid agar medium with the help of gel puncher and filled with 100 µl of test sample. The positive control wells were filled with Chloramphenicol (Standard drug). All the plates were incubated at 37°C for 24 h and then during observation after 24 h, the diameter of the zone of inhibition was measured in millimeter. As well as proper labeling of the petri plates must be done before performing.

The results are represented as average zone of inhibition for test sample.

The results of Microbial contamination were shown in Figure No.23,24,25,26 and Table No. 16

5.3.7. DETERMINATION OF PESTICIDAL RESIDUE BY TLC

Extraction of common pesticide from material

10 g of *Urai Mathirai* formulation was taken in a round bottom flask and added Sodium sulphide with 100 ml n-Hexane. It was refluxed for 1 hour and filtered. The filtrate extracted with 50 ml and 25 ml of Acetonitrile. The Acetonitrile layer was mixed with 500 ml demineralised water with 2.5 ml saturated sodium sulphide and then extracted with an n-Hexane layer and evaporated on a water bath. This residue was used for the analysis of Organochloro, Organophosphate and Pyrethroid pesticides by thin layer chromatography using reference standards Chlorpyriphos, DDT, Endosulfan, Malathion, Parathion (Accu standards, USA)[194].

TLC details

Sample solution	:	Residue in methanol
Development system	:	Benzene: Methanol (60: 40)
Stationary Phase	:	Silica gel 60 F_{254} TLC plate of 0.2mm thickness.
Detection	:	By UV Absorption Range from 200 to 300nm.

The Extracts were spotted along with reference sample and chromatogram was developed and analyzed under UV from 200 to 300 nm.

The results of Pesticidal residue parameters were tabulated in Table No.17

5.3.8. AFLATOXIN

HPLC analysis

A 5gm *Urai Mathirai* formulation was ground with a mortar and pestle with 3gm of ASE Prep DE, a diatomaceous earth used to remove moisture from the sample and to fill up any empty space in the cell to reduce solvent usage. The ground sample was added to a 22 mL extraction cell and was extracted using 50/50 v/v% methanol/acetonitrile with two 5 min extraction cycles at high pressure and 80 °C. A total solvent volume of 16.5 mL was used in the two cycles to extract the sample. After the complete extraction procedure, the cell was flushed with nitrogen 120 s to remove any solvent retained in the sample.

The extract was analyzed by on-line solid-phase extraction coupled to a high-performance liquid chromatographic system (online SPE-LC). The Dionex UltiMate® ×2 Dual-Gradient HPLC system was used, comprising a six channel on-line degasser; two integrated gradient pumps used for sample loading, sample cleanup, and separation on the analytical column; a cooled well-plate autosampler with split loop injection; a thermostatted column compartment equipped with a 6-port switching valve; a photochemical derivatizer; and a fluorescence detector. The stationary phase of the Venture™ AF SPE immunoaffinity 15–20 µm 50 × 2.1 mm column selectively retained the target analytes (aflatoxins) from the sample matrix. The enriched analytes were transferred in a back-flush mode to an Acclaim® 120 C18 3 µm, 4.6 × 150 mm column for the reversed-phase separation of the B1, B2, G1, and G2 aflatoxins. After separation,

the aflatoxins B1 and G1 are photo chemically derivatized by irradiation with UV light at 254 nm, allowing detection of these aflatoxins with a fluorescence detector[188].

The results of Aflatoxin parameters were tabulated in Table No.18

5.4. FORMULATION ASPECT OF TABLETS

5.4.1. GRANULATION TECHNOLOGY

5.4.1.1. Direct compression: Processing steps are:

Raw material → Weighing → Screening → Mixing → Compression.

Direct compression consists of compressing tablets directly from powdered materials without modifying physical nature of materials. This method is applicable for crystalline chemicals having good compressible characteristic and flow properties such as: Potassium salt (chlorate, chloride, bromide), Sodium chloride, Ammonium chloride, Methenamine etc.

If necessary, direct compression vehicles can be used which are having good flow and compressible characteristics. Commonly used directly compression diluents are: MCC (Microcrystalline cellulose (Avicel), Spray dried lactose, Starch - (Sta Rx 1500, Embdex, Celutab), Sugar (Sugartab, Nutab), Dicalcium phosphate dihydrate (Di-Tab), Mannitol for chewable tablet[195].

Advantages:

1. Low labour input

2. A dry process

3. Fewest processing steps

Disadvantages:

1. Stratification may occur due to differences in particle size and bulk density which results poor content uniformity.

2. A large dose drug may cause problem in direct compression. It requires diluents. The tablet becomes large in size which is difficult to swallow and also costly.

3. During handling of dry materials static charge may form which may present uniform distribution of drug.

4. Direct compression diluent may interact with the drug. For example, amine drug with Lactose produce discoloration of tablet.

5.4.1.2. Dry granulation: Processing steps are:

Raw material → weighing → Screen → Mixing → Slugging → Milling → Screening → Mixing → Compression

When tablet ingredients are sensitive to moisture and/or unable to withstand elevated temperature during drying and when the tablet ingredient have insufficient cohesive properties, slugging may be used to form granules. This method is referred to as dry granulation. This technique is used in preparation of aspirin, aspirin combination, acetophenetidin, thiamine hydrochloride, ascorbic acid, magnesium hydroxide.

Compression granulation involves the compaction of the components of a tablet formulation by means of flat punch. These compact masses are called slug and the process is called slugging. Slugs are then milled and screened to produce a granular form. On large scale operation compression granulation can be performed on specially designed machine called Roller compactor (Chilsonator roller compactor)[196].

5.4.1.3. Wet granulation: Processing steps are:

Raw materials → Weighing → Screening → Wet massing → Sieving/Milling → Drying → Screening → Mixing → Compression

The most widely used and most general method of tablet preparation is the wet granulation method. The active ingredient, diluent and disintegrates are mixed or blended well. For large-scale production twin shell blender, double cone blender, planetary mixer, sigma blade mixer, ribbon mixer etc. are commonly used. Solutions of the binding agent are added to the mixed powder with stirring. The powder mass is wetted with the binding solution until the mass has the consistency of damp snow. If the granulation is over wetted the granules will be hard, if not wetted sufficiently, the resulting granules will be too soft, breaking down during lubrication. The wet mass is forced through a 6 or 8 mesh (Mesh no. is the number of wires passing through an inch) screen or several mills can be used. Moist materials from wet milling steps is placed on large trays and placed in drying chambers with a circulating air current and thermostable heat controller[197]. Commonly used dryers are tray dryer, fluidized bed dryer. After drying, the granulation is reduced in particle size by passing smaller mesh screen. The screen size depends on the diameter of the punch as follows:

- Tablet upto 3/16 in diameter ---------- 20 mesh
- Tablet upto 7/32 into 5/16 in diameter ----------16 mesh
- Tablet upto11/32 into13/32 in diameter ----------14 mesh
- Tablet upto 7/16 in and more ----------16 mesh

After drying granulation, the lubricant or glidants is added as fine powder to promote flow of granules. These granules then compressed to get tablet.

EVALUATION OF TABLETS

The delayed release tablets were undergoes various evaluation such as

- General appearance (like size & shape, organoleptic properties)
- Weight variation test.
- Thickness of the tablets.
- Hardness and friability test.
- Disintegration test

MATERIALS & METHODS

Urai Mathirai Preprocess coarse powder, polyvinyl pyrrolidone (sigma), Talc (sigma), all other reagents were obtained from Siddha Central Research Institute, Arumbakkam, Chennai. Micro crystalline cellulose obtained from Signet Chemical Corporation Pvt Ltd. Pre-formulation and post compression parameter testing is an investigation of physical and chemical properties of a drug substance and its formulation. It involves physical characterization, bulk characterization, drug excipient compatibility study, weight variation, thickness, hardness and friability.

LIST OF EQUIPMENTS

Table No. 02

S.NO	EQUIPMENT	MANUFACTURE NAME
1.	Moisture Analyzer	Sartorius
2.	Tapped Volumeter	Erweka
3.	Hot air oven	Remi
4.	Compression Machine	Erweka
5.	Hardness tester	Pfizer

6.	Thickness Apparatus	Vernier caliper
7.	Friabilator	Electrolab
8.	Electronic Weighing Balance	Shimadzu
9.	Disintegator	Electrolab
10.	Bruker	FTIR spectrophotometer

UG PROFILE

- **Name of the drug substance:** *Urai Mathirai*
- **Official status :** Siddha Hospital
- **Category :** Immuno-modulatory drug
- **Description:** A deep green colour powder

SOLUBILITY:

Water	Slightly Soluble
Alkaline Solution	Soluble
Alcohol	Very Slightly Soluble

THERAPEUTIC APPLICATION:

Urai Mathirai is indicated in repeated respiratory infections and for immunomodulation in children.

5.4.2. EXCIPIENT PROFILE

1. MICROCRYSTALLINE CELLULOSE

Nonproprietary Names	**:** BP: Microcrystalline Cellulose JP:Microcrystalline Cellulose PhEur: Cellulose, Microcrystalline USP-NF**:** Microcrystalline Cellulose
Synonyms	**:** AvicelPH, microcristallinum, Celphere, Ceolus KG; Crystalline cellulose, Emcocel, Ethispheres, Fibrocel, Pharmacel,Tabulose, Vivapur.
Functional Category	**:** Adsorbent**,** Suspending agent**,** Tablet and capsule diluents**,**Tablet disintegrant
Description	**:** Microcrystalline cellulose is a purified, partially depolymerized cellulose that occurs as a white, odorless, tasteless, crystalline powder composed of porous particles. It is commercially available in different particle sizes and moisture grades that have different properties and applications.
Moisture content	**:** Typically less than 5% w/w
Solubility	**:** Slightly soluble in 5% w/v sodium hydroxide solution practically insoluble in water, dilute acids, and organic solvents.
Storage Conditions	**:** Stored in a well-closedcontainer in a cool,dry place.
Incompatibilities	**:** Incompatible with strongoxidizingagents.
Application	: It is used as binder/diluent in oral tablet and capsule formulations .in both wet-granulation and direct-granulation.

USE	CONCENTRATION (%)
Adsorbent	20–90
Antiadherent	5–20
Tablet &Capsule binder/diluents	20–90
Tablet disintegrant	5–15

2. POVIDONE

Nonproprietary Names : BP: Povidone
PhEur: Povidone
USP: Povidone

Synonyms : E1201; Kollidon; Plasdone; poly[1-(2-oxo-1-pyrrolidinyl) ethylene]; polyvidone; polyvinyl pyrrolidone; PVP; 1-vinyl-2-pyrrolidinone polymer

Functional Category : Disintegrant; dissolution aid; suspending agent; tablet binder.

Description : Povidone occurs as a fine, white to creamy-white colored, odorless or almost odorless, hygroscopic powder.

Moisture content : It is very hygroscopic, significant amounts of moisture being absorbed at low relative humidities.

Solubility : Freely soluble in acids, chloroform, ethanol (95%), ketones, methanol, and water; practically insoluble in ether, hydrocarbons, and mineral oil.

Storage Conditions : Stored in a well-closed container in a cool,dry place.

Incompatibilities : Incompatible in solution with a wide range of inorganic salts, natural and synthetic resins, and other chemicals. It forms molecular adducts in solution with sulfathiazole, sodium salicylate, salicylic acid, phenobarbital, tannin, and other compounds; see Section 18. The efficacy of some preservatives, e.g. thimerosal, may be adversely affected by the formation of complex.

Application **:** Although povidone is used in a variety of pharmaceutical formulations, it is primarily used in solid-dosage forms. In tableting, povidone solutions are used as binders in wetgranulation processes. Povidone is also added to powder blends in the dry form and granulated in situ by the addition of water, alcohol, or hydroalcoholic solutions. Povidone is used as a solubilizer in oral and parenteral formulations and has been shown to enhance dissolution of poorly soluble drugs from solid-dosage forms. Povidone solutions may also be used as coating agents. Povidone is additionally used as a suspending, stabilizing, or viscosity-increasing agent in a number of topical and oral suspensions and solutions. The solubility of a number of poorly soluble active drugs may be increased by mixing with povidone.

USE	CONCENTRATION (%)
Carrier for drugs	10–25
Tablet binder, tablet diluent, or coating agent	0.5-5
Suspending agent	Upto 5
Dispersing agent	Upto 5

3. TALC

Nonproprietary Names **:** BP: Purified talc

JP: Talc

PhEur: Talcum

USP: Talc

Synonyms **:** Altalc, E553b, Hydrous magnesium calcium silicate,

Hydrous Magnesium silicate, Luzenac Pharma, Magnesium hydrogen Metasilicate, Powdered talc, Purified French chalk, Purtalc, Soapstone, Steatite, Superiore;

Functional Category **:** Anticaking agent, glidant, tablet and capsule diluents, lubricant.

Description **:** Talc is a very fine, white to grayish-white, odorless, impalpable, unctuous, crystalline powder. It adheres readily to the skin and is soft to the touch and free from grittiness.

Moisture content **:**Maximum moisture absorption is approximately 90%

Solubility **:**Practically insoluble in dilute acids, alkalis, water and organic solvents.

Storage Conditions **:**Stored in a well-closed container in a cool, dry place

Incompatibilities **:**Incompatible with Quaternary ammonium compounds

Application **:** It is widely used as a dissolution retardant in the development of controlled-release products. Talc is also used as a lubricant in tablet formulations, in a novel powder coating for extended-release pellets and as an adsorbant. In topical preparations, talc is used as a dusting powder, although it should not be used to dust surgical gloves. It is used to clarify liquids and is also used in cosmetics and food products[198].

USE	CONCENTRATION (%)
Dusting powder	90.0–99.0
Glidant and tablet lubricant	1.0–10.0
Tablet and capsule diluents	5.0–30.0

5.4.3. METHODS

PRE-FORMULATION STUDIES

Pre-formulation activities range from supporting discovery's identification of new active agents to characterizing physical properties necessary for the design of dosage form. Critical information provided during pre-formulation can enhance the rapid and successful introduction of new therapeutics entities for humans. Pre formulation testing is an investigation of physical and chemical properties of a drug substance.

5.4.3.1. Physical Characterization of drug

a). Organoleptic properties

- **Colour:** A small quantity of drug powder was taken in butter paper and viewed in well-illuminated place.

- **Taste and odour:** Very less quantity of drug was used to get taste with the help of tongue as well as smelled to get the odour.

The results were shown in the Table No. 19

5.4.3.2. BULK CHARACTERIZATION LUBRICATED BLEND

5.4.3.2.1.*Bulk Density***:** It refers to a measurement to describe packing of particles. Bulk density is used to determine the amount of drug that occupies the volume in mg/mL. It is the ratio of total mass of powder to the bulk volume of powder. It was measured by pouring the weighed powder in to a measuring cylinder and the initial volume was noted. This initial volume is called bulk volume. From this, the bulk density is calculated according to the formula mentioned below. It is expressed in g/mL and is given by

$$\text{Bulk density (g/mL)} = \frac{\text{Weight of sample (g)}}{\text{Volume occupied by the sample (mL)}}$$

5.4.3.2.2. *Tapped Density:* Weighed quantity of drug was taken in a graduated cylinder. Volume occupied by drug was noted down. Then the cylinder was subjected to 500, 750

& 1250 taps in tap density tester According to USP, the blend was subjected for 500 taps. The percentage volume variation was calculated and subjected for additional 750 taps. The percentage variation was calculated and recorded.

$$\text{Tapped bulk density (g/cc)} = \frac{\text{Mass of powder (g)}}{\text{Tapped volume of the powder (cc)}}$$

5.4.3.2.3. *Compressibility Index:* Weighed amount of drug was transferred to 100ml graduated cylinder and subjected to 500,750 & 1250 taps in tap density tester. The difference between two taps should be less than 2%. The percentage of compressibility index was calculated using formula

$$\text{Compressibility Index (CI)} = \frac{V_i - V_t}{V_i} \times 100$$

Where, V_t = Tapped volume; V_i = Untapped volume

5.4.3.2.4. *Hausner's ratio:* It provides an indication of the degree of densification which could result from vibration of the feed hopper.

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Table No 03 Compressibility index& Hausner's ratio

S.No.	Compressibility Index (%)	Flow Character	Hausner Ratio
1.	≤10	Excellent	1.00–1.11
2.	11–15	Good	1.12–1.18
3.	16–20	Fair	1.19–1.25
4.	21–25	Passable	1.26–1.34
5.	26–31	Poor	1.35–1.45
6.	32–37	Very poor	1.46–1.59
7.	>38	Very, very poor	>1.60

5.4.3.2.5. *Angle of repose:* Angle that can be obtained between the free surface of a powder heap and horizontal plane. The angle of repose was measured by allowing the powders to fall over a graph sheet placed on horizontal surface through a funnel kept at a certain convenient height.

The height of the heap was measured and then circumference of the base of heap was drawn on a graph sheet with the help of a pencil. The radius of the circle obtained was measured. The angle of repose is given as,

$$\theta = \tan^{-1}\left(h/r\right)$$

θ = angle of repose;
h = height of the heap;
r =radius of the base of the heap.

Table No. 04

SNO	Angle of Repose (θ)	Type of Flow
1	<20	Excellent
2	20-30	Good
3	30-34	Passable
4	>40	Very Poor

5.4.4. DRUG –EXCIPIENT COMPATIBILITY STUDIES

FT- IR Spectroscopy

FTIR spectroscopy method was used to find out the interaction between the drug and excipients used in the formulation. In this method, a physical mixture of URAI and each excipienst in the ratio of 1:1 were prepared and analyzed the interference by the excipients using KBR pellet method. Spectra were obtained for pure drug, excipients, drug and excipients, using Bruker ALPHA II FT-IR spectrometer and compared[199].

FORMULATION

The *Urai Mathirai* 50mg were prepared by wet granulation method using microcrystalline cellulose (diluents), polyvinyl pyrollidone k30 (binder), and talc (glidant & lubricant). The active ingredient and diluent were weighed accurately, passed through sieve 40 mesh and mixed thoroughly with help of poly bag for 15mins. . Solutions of the binding agent were prepared by adding polyvinyl pyrollidone k30 into water with stirring. The powder mass was wetted with the binding solution until the mass has the consistency of damp. The wet mass was passed through a 20 mesh screen. Collected wet granules was placed on large trays and allowed to drying with use of tray dryer, fluidized bed dryer. After drying, the granulation was reduced in particle size by passing smaller mesh screen 50 % of talc was added finally and mixed for 5 mins. The powder blends were evaluated for pre compression parameters like bulk density, tapped density, angle of repose, compressibility index and hausner's ratio. The blends were compressed into tablets on a 16 station tableting machine and evaluated for post compression parameters[200].

- In formulation F1 & F2, it was observed that tablet showed poor hardness, and the friability was not complied with the USP limit. The reason may be due to the lack of binding.

- In formulation F2, the total tablet weight was increased to 60mg. Slight improvement in the hardness.

- In formulation F3 the MCC112 was replaced with MCC 101 then the tablet gave good hardness and friability. The reason may be MCC 101 gives binding effect thereby improving the hardness[201].

- Finally F3 formulation was optimized and the values shown below

Table No. 05: Formulation of *Urai Mathirai* tablets

Materials	F1	F2	F3
Urai	50 mg	50mg	50mg
MCC (112) (%)	1.85	-	-
MCC (102) (%)	-	11.67	11.67
PVP k30 (%)	5.56	4.17	4.17
Talc (%)	2.78	0.83	0.83
Total	54mg	60 mg	60mg

MCC- Microcrystalline cellulose PVP-Polyvinyl pyrrolidone

Fig. No 11

Urai Mathirai

5.4.5. EVALUATION

5.4.5.1. Pre compression parameters of lubricated blends

The powder blends were evaluated for pre compression parameters like Bulk density, Tapped density, Angle of Repose, Compressibility Index and Hausner's Ratio as per the method described earlier[202].

5.4.5.1. Post compression parameters

Hardness:

Hardness is required to withstand mechanical shocks of handling in manufacturing, packing, handling & shipment. For all formulations, the hardness was checked using the hardness tester like Monsanto Tester, Pfizer Tester, Erweka Tester[203].

Thickness:

The thickness of formulations was determined using a Vernier caliper in mm.

Weight Variation test (U.S.P.): Weight variation test to evaluate volumetric fill of die cavity. 20 tablets were weighed individually. Calculate average weight and compared the individual tablet weight to the average. The tablet passed the U.S.P. test if no more that 2 tablets are outside the percentage limit and if no tablet differs by more than 2 times the percentage limit.

Table No 06: Weight variation limits

SNO	AVERAGE WEIGHT OF TABLET	PERCENTAGE
1	80 mg or less	± 10%
2	More than 80 mg & less than 250 mg	± 7.5%
3	250 mg or more	± 5%

Friability:

Friability test to evaluate the ability of tablet to withstand abrasion in packing, handling & shipment. For tablets with unit mass equal or less than 650 mg, sample of whole tablets corresponding to 6.5g was taken. For tablets with unit mass more than 650mg, a sample of 10 whole tablets was taken and weighed. Tablets were then placed in friability testing apparatus and rotated at a speed of 25 rpm for 4 mins[204]. Tablets were then reweighed. The difference in weight was noted and percentage friability was calculated. The percentage friability was not more than 1% as per the official limits

$$\% \text{ Friability} = (W1 - W2)/W1 * 100$$

Disintegration Test (U.S.P.):

Disintegration time is time taken by the tablet to break into fine particles. The test for disintegration time, one tablet was placed in each tube and the basket rack was positioned in a 900ml of water, simulated gastric fluid or simulated intestinal fluid at 37 ± 1° C at a frequency of 28 to 32 cycles per minute. According to the test the tablet must disintegrate and all particles must pass through the 10 mesh screen in the time specified. If any residue remains, it must have a soft mass. In case of delayed release time taken by the tablet to break into fine particles was called as lag time period[205].

5.5. *IN-VITRO* EVALUATION

5.5.1. ANTIOXIDANT STUDIES (DPPH radical scavenging activity)

The DPPH (1,1¬Diphenyl¬2¬Picrilhydrazyl) radical scavenging activity for the extracts was determined using the method proposed by Von Gadow et al. 1997[206]. In this, 50 µl of samples of each plant extracts was placed in separate cuvettes, then into it, 2 ml of 6 × 10 −5 M methanolic solution of DPPH radical was added immediately and absorbance was measured immediately at 517 nm. Then after 16 min of incubation

decrease in absorbance was again measured for the entire samples. Methanolic solution of Ascorbic acid of 1 µl/mg concentration was used as a standard. All the determination was performed in triplicates. The DPPH radical scavenging activity was measured in percentage of reduction of the color. The percentage inhibition of the DPPH radical by the sample extracts was calculated by the formula suggested by (Yen and Duh 1994)[207].Control was taken at time zero that mean before the antioxidant present in plant extracts starts its activities.

$$\%\text{Inhibition} = \{(A\ C\ (0) - A\ A\ (t))\ )/A\ C\ (0)\} \times 100$$

Where, A C (0) = Absorbance of control at time, t = 0 min A A (t) = Absorbance of antioxidant at time, t = 16 min

5.5.2. ANTI-INFLAMMATORY STUDIES (Cell membrane stabilization method)

Anti-inflammatory assay was performed using an O^{+ve} human blood and mixed with equal volume of Alsevar solution (dextrose 20%, sodium citrate 8%, citric acid 0.5%, sodium chloride 4.2% and 1L of distilled water). The blood was centrifuged at 3000rpm for 10mins and HRBC were washed (0.9% of NaCl, PH 7.2) and packed with isosaline (10%). Various concentration of crude extract with distilled water (125µg/µl, 250µg/µl, 500µg/µl and 1000µg/µl) was prepared in each test tubes and mixed with 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of HRBC. *Serratia peptidase* tablets (5mg) were used as positive control and distilled water as negative control. The entire sample was incubated at 37°C for 30mins and centrifuged at 3000rpm for 10mins. The

suspension of hemoglobin was collected and the O.D was recorded at 560nm using UV-Vis spectrophotometer. The percentage of inhibition was calculated and noted[208].

Percentage of Hemolysis = O.D of test / O.D of control X 100

Percentage of Protection = 100 – O.D of test / O.D of control X 100

5.5.3 IMMUNO MODULATORY STUDIES

Hemolytic assay for human complement activity

The stock solution of CPW buffer is a 5 concentrated saline buffer (VBS, pH 7.3) containing 0.9 M NaCl, 10 mM diethylbarbituric acid, 9 mM sodium barbiturate, 250 mM Ca^{2+} and 250 mM Mg^{2+}. The stock solution of APW buffer has the same composition as the CPW buffer, without Ca2+ and with 8 mM ethylenglycol-bis (2 aminoethyl) tetra-acetic acid. Human pooled serum (HPS) was used as complement source. In the CPW assay, sheep erythrocytes (SSRBC) were sensitized by incubating 4 10^8 cells/ml with an equal volume of VBS-EDTA(13 mM) containing a 1:2000 dilution of rabbit anti-sheep serum for 20 min at 37C and for 20 min at 4◦C. The excess of antibodies was removed by subsequent washings of the sensitized SSRBC with phosphatebuffered saline. In the APW assay, 1% of uncoated washed rabbit erythrocytes (RRBC) were diluted in APW buffer. Ethanolic plant extracts were dissolved in a CPWethanol (1%) or APW-ethanol (1%) solution.

The initial crude formulation extract concentration was 5 mg/ml in respective buffers. Extracts were further vortexed, sonicated (for maximum 5 min) and heated (45◦C), until complete dissolution. The complement assay was based on the technique

described by and modified as follows. All vegetal samples were diluted in a F-96 well microtiter plate (seven consecutive logarithmic dilutions, from 250 to 3.9 mg/ml with the appropriate CPW or APW buffer). The final volume in each well was 100 ml. Fifty ml of CPW-HPS (95:5) or 30 ml of APW-HPS (75:25) solution were then added to each well[209].

After a preincubation of 30 min at 37C, 50 ml of a 1% SSRBCCPW or RRBC-APW suspension were added. All plates were furthermore incubated at 37C for 60 min, slowly shaken and read at 690 nm with a micro-ELISAreader. As a positive control for both CPW and APW, a serial dilution (from 1000 to 3.9 mg/ml) of heparin, a sulfated polysaccharide with known anti-complement activity, was used[210].

The buffer without Formulation extract served as a negative control. No influence of a 1% ethanol solution could be detected. Because Ca2+ and Mg2+ chelating can impair the complement cascade, the ratio between the results obtained under standard conditions and the results obtained by increasing 4 times the concentrations of Mg2+ (for APW) and Ca2+ and Mg2+ (for CPW) was calculated for each positive extract. All extracts were also subjected to a preincubation with the target cells, in order to detect a spontaneous lysis of erythrocytes. IC_{50} values were calculated with the help of Excel 98 software tendance function[211].

Blood platelet aggregation assay

Nine volumes of blood were mixed gently with one volume of trisodium citrate (3.8% g/v). The samples were centrifuged (200g for 10 min) and platelet rich plasma

(PRP) was collected. Platelet poor plasma (PPP) was prepared after further centrifugation of blood samples at 1000g for 10 min. PRP was then diluted with PPP up to a final concentration of 250.000 thrombo-cytes/ml. Platelets were used within 3 h. Each test was repeated in triplicate with two different samples of PRP, prepared on different days.

A modified smear method was used for platelet aggregation screening. Ethanolic extracts of formulation were dissolved in an 8% saline –ethanolic solution, up to a concentration of 25 mg/ml[212].

5.6. *IN-VIVO* EVALUATION

5.6.1. Safety profile study

Principle

The principle of the test study is that based on a stepwise procedure with the use of a minimum number of animals per step. Sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is 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 is 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.

Test animals and Test conditions:

Sexually mature either sex *albino rats* (120-150g) were obtained from the animal laboratory of the Siddha Central Research Institute, Chennai. All the animals were kept under standard environmental condition (27±2°C). The animals had free access to water and standard pellet diet. Rats were deprived of food but not water (16-18 h) prior to administration of the formulation. The principles of laboratory animal care were followed and the Department's ethical committee approved the use of the animals and the study design IAEC PROTOCOL NO:162/Pharma/SCRI/2017.

Preparation of animals:

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Preparation of doses:

The clinical dose employed in children is 50 mg (HED = 2.5mg/kg body weight). The Rat doses are calculated based on the FDA guidelines[213].

5.6.1.1. Acute toxicity study:

Acute toxicity study is carried out as per guidelines for toxicity / safety profile evaluation of Ayurveda & Siddha plant drugs which were designed by pre-clinical toxicology unit of NIN in association with CCRAS as per international guidelines.

The study will be carried out in albino rats of either sex with a single exposure of recommended therapeutic dose, 5 times of the recommended therapeutic dose and 10 times of the recommended therapeutic dose of test compound. Healthy young adult

animals are randomly assigned to the vehicle control and treatment groups. The experimental grouping of the study is as follows **Table No. 07**:

S. No.	Test Groups	No. of Animals
1	Vehicle control	10 (5M+5F)
2	*Urai Mathirai* Therapeutic Dose (TD) 10 mg/kg	10 (5M+5F)
3	*Urai Mathirai* Average Dose (TDX5) 50 mg/kg	10 (5M+5F)
4	*Urai Mathirai* Highest Dose (TDX10) 100 mg/kg	10 (5M+5F)

Before dosing, animals were fasted overnight and test drug was orally administered in a single dose. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. The volume should not exceed 1ml/100g body weight, except in the case of aqueous solutions where 2ml/100g body weight may be used. Following the period of fasting, the fasted body weight of each animal was determined, and the dose was calculated according to the body weight. After administration of test drug, food was withheld for further 3-4 hrs. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hrs, with special attention given during the first 4 hrs, and daily thereafter, for a total of 14 days. All the rats were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioral changes.

The observations recorded are physical observations, behavioral observations, autonomic effects, sensory responses and reflexes, respiratory effects, and somatomotor

activity. The time of death, if any, was recorded. Necropsy has to be carried out if incase of death of animal. All the data related to the study should be summarized in tabular form and presented as per the guidelines[214].

5.6.1.2. Sub-acute toxicity study:

Sub-acute toxicity study is carried out as per guidelines for toxicity / safety profile evaluation of Ayurveda & Siddha plant drugs which were designed by pre-clinical toxicology unit of NIN in association with CCRAS as per international guidelines[215].

The study will be carried out in albino rats of either sex with a single exposure of recommended therapeutic dose, 5 times of the recommended therapeutic dose and 10 times of the recommended therapeutic dose of test compound. Healthy young adult animals are randomly assigned to the vehicle control and treatment groups. The experimental grouping of the study is as follows **Table No. 08**:

Sr. No.	Test Groups	No. of Animals
1	Vehicle control	12 (6M+6F)
2	*Urai Mathirai* Therapeutic Dose (TD) 10 mg/kg	12 (6M+6F)
3	*Urai Mathirai* Average Dose (TDX5) 50 mg/kg	12 (6M+6F)
4	*Urai Mathirai* Highest Dose (TDX10) 100 mg/kg	12 (6M+6F)

Pre-experimentation phase

Acclimatization of animals

i) Period – 7 days (Recording of body weight and food intake twice in a week)
ii) Urine qualitative test (Ames multiple sticks)
iii) Fecal consistency (Filter paper technique)

II. Experimentation phase

The animals are dosed with the test substance orally, daily seven days each week for a period of 28 days. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. The volume should not exceed 1ml/100g body weight, except in the case of aqueous solutions where 2ml/100g body weight may be used. Animals were observed individually preferably at the same time(s) each day and considering the peak period of anticipated effects after dosing. Once before the first exposure (to allow for within-subject comparisons), and at least once a week thereafter, detailed clinical observations should be made in all animals. Body weight and food/water consumption measurement should be made weekly. Hematological, clinical biochemistry and urine qualitative tests should be made once before the first exposure of the test drug and after the end of test period. All animals in the study shall be subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. Organ weights should be collected from all the animals and necropsy has to be carried out if incase of death of animal during the study also. Histopathological studies of the organs are carried out as per the guidelines. Any other tests or procedures if necessary

would be carried out as per the guidelines. All the data related to the study should be summarized in tabular form and presented as per the guidelines.

5.7. PHARMACOLOGICAL STUDIES

5.7.1. Anti-Inflammatory activity: Carrageenan induced hind paw oedema

Healthy Wister albino rats of either sex (120–150 g) were employed in the study. Carrageenan (1% w/v) was prepared 24 hrs before administration. In short, 0.1 mL of 1 % w/v carrageenan was injected into the sub plantar tissue of left hind paw of each rat. Swelling or paw edema was measured at 0, 1, 2, 3 h using Plethysmometer. Animals were treated with test extract 1hour before the carrageenan injection. Measurement was carried out immediately before and 3hrs following carrageenan injection. Percent inhibition of test drugs was calculated in comparison with vehicle control (100%). The standard drug indomethacine (10mg/kg) was used.

After the experiment animals were sacrificed under anesthesia and the blood was collected. The serum was separated and used for the hematological and biochemical estimations[216].

The treatment was scheduled as follows **Table No. 09**:

S.No	Test Groups	No. of Animals
1	Normal control, normal rats received 0.5 ml normal saline	6
2	0.1 mL of 1 % w/v carrageenan was injected into the sub plantar	6
3	Standard control (Rats receive indomethacin (10 mg/kg b.w.)	6
4	Test group – I, (Rats receive *Urai Mathirai* + 0.1 mL of 1 % w/v Carrageenan)	6

5	Test group – II, (Rats receive *Urai Mathirai* + 0.1 mL of 1 % w/v Carrageenan)	6
6	Test group – III, (Rats receive *Urai Mathirai* + 0.1 mL of 1 % w/v Carrageenan)	6

Parameters monitored:

1. Paw volume
2. Hematological parameters
3. TNF-α

5.7.2. Analgesic activity: Tail-flick method

Analgesiometer an instrument used to measure analgesia by a modified method of D Amour and Smith called as tail flick method. Reaction time in seconds was used as the unit for measurement of pain and an increase in reaction time was indicative of analgesia. Time between placing the tail of the rat on the radiant heat source and sharp withdrawal of the tail was recorded as "reaction time". Cut off time of ten seconds was imposed in all sets of experiments taken as maximum latency so as to rule out thermal injury while noting down the reaction time. Animals that showed a mean reaction time outside the range of five-six seconds, were discarded. In all the groups, tail-flick test was performed prior to drug administration, and at 30, 60, 90 and 120 minutes after drug administration, and the reaction time at each time interval (test latency) was calculated. Percentage analgesia was calculated using the following[217].

Formula:

% Analgesia = MPE=TL-BL / ML-BL × 100

Where, M.P.E. = Maximum possible effect,

M.L. = Maximum latency or cut off time

T.L. = Test latency

B.L. = Basal latency or control latency

5.7.3. Immunomodulatory activity

Hypersensitivity reaction to SRBC was induced in rats, following the prescribed method. The UM (in doses of 10, 50, and 100 mg/kg, body weight) was administered to the animals (test group) orally for five days and the vehicle was administered to the control animals. Each group consisted of six rats – three male and three females. The UM was administered orally on each of the two days prior to the immunisation, on the day of the immunisation and on each of the two days after the immunisation (i.e., Days –2, -1, 0, +1. +2). The rats were immunised by injecting 0.1 ml of SRBC subcutaneously into the right hind footpad on day 0. The animals were challenged seven days later by injecting the same amount of SRBC into the left hind footpad. The thickness of the left hind footpad was measured with a Plethysmograph at 0, 1, 2, 4h and 24 h after the challenge[218].

5.8. CLINICAL STUDY

To evaluate the clinical efficacy of *Urai Mathirai* by an Open Non- randomized clinical trial as one arm and other arm will receive official formulation (Thaleesathy chooranam)[219].

Protocol approval from ethics committee:

The detailed study trial protocol was submitted to the Institution Ethics Committee of Siddha Central Research Institute, Chennai-106. The protocol was approved by the ethics committee and clearance to conduct the trial was given. IEC NO: CCRS/SCRI-1/2014-15/04.

Clinical trial registration:

The clinical trial was registered in Clinical Trials Registry - India (CTRI) on 01.06.2017. The registration number of the trial was given as: CTRI/2017/06/008723.The clinical study was also registered in Clinical Trials gov. The registered protocol is available in the public portal of respective websites.

Case report form:

A detailed clinical case recording form including Assessment questionnaire was prepared prior to the start of the study. The complaints, past history, signs and symptoms before and after treatment, drug compliance, informed consent, laboratory investigations, adverse reactions, reasons for withdrawal were duly recorded in the CRF. Clinical study was conducted as per the guidelines for GCP accepted by AYUSH.

Subject selection:

The study subjects were selected from patients reporting at OPD of RCH Reproductive and Child Health Care (Kuzhandhai Maruthuvam) department of Siddha Central Research Institute. The patients referred were subjected to screening. The screening proforma was filled and patients who satisfied the following inclusion and exclusion criteria were taken up for the trial. A total of 90 patients were screened for Upper Respiratory Tract Infections. Among them 76 patients entered the trial, 64 patients successfully completed the trial.

Inclusion criteria:

- ✓ Patients of both sexes. Patients between 6 months to 6years of age.
- ✓ Parental consent is important as your children will not be autonomous to take decisions.
- ✓ Patients clinically diagnosed with Upper Respiratory Tract Infections and presenting with URTI at hospital treatment. The definition used for recurrent URTIs was three or more such episodes during the last 12 months.
- ✓ The current episode required for study eligibility was defined by the presence of at least two of the following

- Rhinitis
- Pharyngitis
- Cough
- Hoarseness,
- Temperature More than 38.5°C,
- Prescription of an antibiotic for a URTI, occurring after an asymptomatic Period of at least 1 week without antibiotics.

Exclusion criteria:

- ✓ Occurrence of otitis media and/or sinusitis and/or infection of the lower respiratory tract (ie, bronchitis, pneumonia) and/or proven group A streptococcal angina at the enrolment.
- ✓ Further main exclusion criteria were allergic asthma, significant systemic disease (eg, hepatic and/or renal disease, malignancy), immune system disorders, suspected Malabsorption, major surgical procedure within 3 months of commencement of the study
- ✓ Recent immunosuppressive or immunostimulant therapy or corticosteroids.

Withdrawal criteria:

- ✓ If any untoward side effects / adverse effects reported.
- ✓ If the patient fails to strictly adhere to the study protocol.

- ✓ If the patient turns unwilling to continue in the course of clinical trial.

Study design:

Study Population : Patients between 6 Months to 6Years of age with URTI

Study Centre : Single centre. Siddha Central Research Institute, Chennai- 106.

Trial Design : Non-randomized, Active Controlled Trial (Phase 3)

An open trial was planned as there are no published or documented clinical data available for this drug and moreover there were difficulties in conducting blinded trial.

Allocation : Non-randomized

Sampling method : Purposive sampling.

Primary Purpose : Treatment

Study Period : 2 years.

Sample Size : 60 cases.

Informed consent (Assent Form):

The details about the study were explained to the patients/parents orally. A written information sheet containing the entire details of the study was given to the study participants. The details were given in language understandable to the patients (Form IV A). They were given

ample time to take decision about their participation. The information sheet was prepared in two languages (Tamil and English). The parental consent form was signed by the patient/parent and a witness. The illiterate patients were explained about the study and a left thumb impression was obtained. In that case, a witness who is not related to the study was asked to sign the consent form (Form IV B).

Treatment:

Name of the trial drug : *Urai Mathirai*

Reference : Hospital pharmacopeia of Govt siddha college, Palayamkottai.

Dosage : 50 mg once a day. After food.

Route of administration : Oral

Treatment period : Six Months continuous

Name of the comparator drug : Thaleesathy Chooranam

Dosage : One gram Twice a day. After food.

Route of administration : Oral

Treatment period : Six Months continuous

Drug storage:

The trial drug was stored in clean and dry narrow mouthed container. It was kept in room temperature protected from sunlight.

Dispensing:

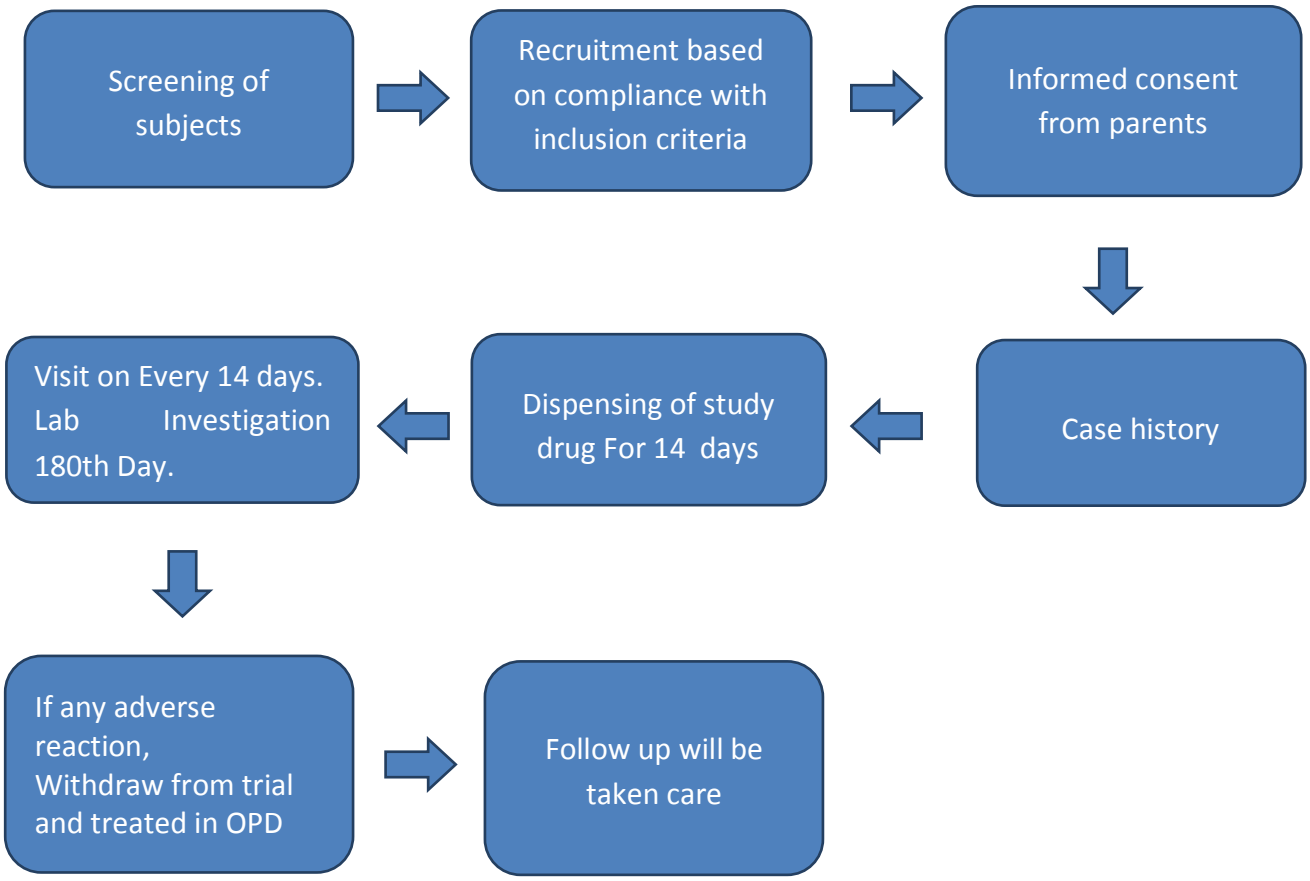
Urai Mathirai (50 mg) was dispensed to patients in airtight container consists of 30 tabs. The patients were instructed to bring unused drug on next visit. Thaleesathy chooranam will be given in butter paper packing of 500 mg twice daily for 6 months. The details were recorded in drug compliance form (Form 1V C).

Study enrolment:

The patients who were to be enrolled were informed about the study, trial drug, possible outcomes and the objectives of the study in the language and terms understandable to them. After ascertaining the patient's willingness, informed consent was obtained in writing in the consent form (Form IV A). All these patients were given unique registration card in which patients' registration number of the study, address, phone number and doctor's phone number etc., were entered so as to report easily if any complication arises.

Filled in screening form (Form 1) was filed separately. Form IA,IIA and Form III was used for recording the patient's history, clinical examination, laboratory investigations respectively.

FLOW CHART OF SUBJECT RECRUITMENT



Data collection:

Details of the complaints, duration of illness, the course of the disease, data on past medical history to reveal the medications taken, family history to get details about the spread of infection from close contacts.

Clinical assessment details at the time of enrolment were entered in Form IIA,B.

The clinical symptoms were assessed and recorded on each visit i.e, $0^{th}/1^{st}$ day, 3^{rd} day,30^{th} day, 60^{th} day,90^{th} day, 120^{th} day, 150^{th} day, 180^{th} day (after treatment period). It was recorded before and after treatment.

The laboratory investigations were done before and after treatment in the biochemistry laboratory of Siddha Central Research Institute, Chennai - 106 and recorded in Form III. It includes blood and X-ray investigation.

Outcome measures:

- Reduction in Recurrence of Respiratory Infections
- Clinical Improvement
- No Antibiotic treatment
- Increase in Anti bodies IgA/IgG
- Decrease in Phagocytosis

Conduct of the study:

The trial drug *Urai Mathirai* was given continuously for six months in OPD of RCH Reproductive and Child Health Care (Kuzhandhai Maruthuvam) department of Siddha Central Research Institute. The patients visited the hospital once in 14 days. At each clinical visit clinical assessment was done and prognosis was noted.

Laboratory investigations and X-ray tests were done on 0^{th} day and 180^{th} day of the trial. The patients were advised to have regular follow-up for next 2 months. No laboratory investigations were carried out at the end of follow up. The patients were watched for any recurrence of symptoms.

Adverse effects/serious effects management:

During the course of treatment if there was any reporting of adverse drug reaction, the trial drug was stopped and withdrawal form was filled up and the patient was treated further in OPD of RCH Reproductive and Child Health Care (Kuzhandhai Maruthuvam) department of Siddha Central Research Institute. In case of emergency, the patients were referred to nearby Government hospital for emergency management. Survival data of the withdrawn subjects were recorded and maintained for 2 months. The contact number was given to the patients with alternate phone number was given to the participants.

Follow up period:

The patients were followed for 2 months after the treatment period. Those patients, who developed symptoms like cough, hoarseness, rhinitis etc., were treated with OPD medicines.

Data management:

After enrolling the patient in the study, a separate file for each patient was opened and all forms were be kept in that file. Study number and Patient number were entered on

the top of file for easy identification. Whenever study patient visited OPD during the study period, the respective patient's file was taken and necessary entries were made at the assessment form or other suitable forms. The screening forms were filed separately. The Data entries were monitored by guide and adverse events if any were intimated to the Pharmacovigilance committee of SCRI. All forms were further scrutinized in presence of Investigators by statistician for logical errors and incompleteness of data to avoid any bias. No modification in the results was made for unbiased reports. The forms were preserved for future references. The cumulative data sheet was electronically maintained. This was done to facilitate the data analysis using GraphPad Prism Software (Version 7.0.3).

Data cleaning:

The data were checked for coding, correctness of the entries, missing data etc.

Data analysis:

Data are presented as mean ± S.E.M. Statistical analysis was performed using GraphPad Prism Software (Version 7.0.3). Each treatment groups vs vehicle group was analysed using Two-way analysis of variance (ANOVA). The level of significance was set at $P< 0.05$.

Results

The present study was aimed at establishing the scientific validation with supporting data on evaluation of the formulated Mathirai (Tablet) of commonly available immunomodulator herbs on its phytochemistry and pharmacological activities with reference to antioxidant, immunomodulator, anti inflammatory and anti microbial activity which has not been reported earlier. The study design also focused on the Standardization of tablet formulation employing various techniques. Plants are gaining more importance as because of their phytoconstituents, with diverse pharmacological property and therapeutic application. Identifying such plants is of significance and as a prelude to this it is mandatory to examine various characters before commencing investigation. Thus various parameters were examined for the individual herbal powders and its formulation was listed.

MACROSCOPY AND POWDER MICROSCOPY OF INDIVIDUAL DRUGS

Drug identified as: *Zingiber officinale*-rhizome

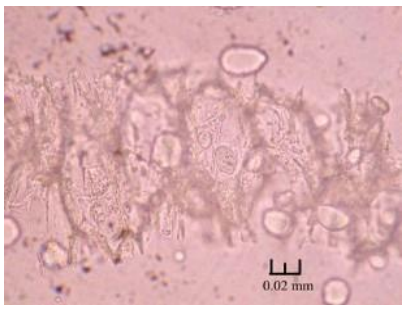
MACROSCOPY:

Fig. No 12

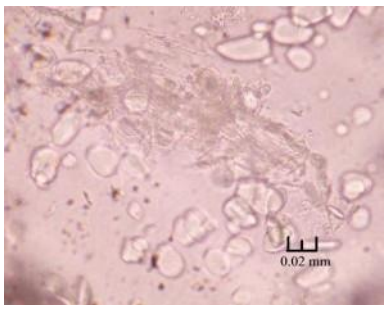
a) Rhizome laterally compressed bearing short, flattish, ovate, oblique branches on upper side, each having at its apex, depressed scar, pieces about 5-15 cm long, 1.5-6.5 cm wide, usually 3-4 cm and 1-1.5 cm thick, externally buff coloured showing longitudinal striations , fracture short, smooth, transverse surface exhibiting narrow cortex, well-marked endodermis, a wide stele showing numerous scattered fibro-vascular bundles and yellow secreting cells when examined under lens

b) Odour: aggreable and pungent

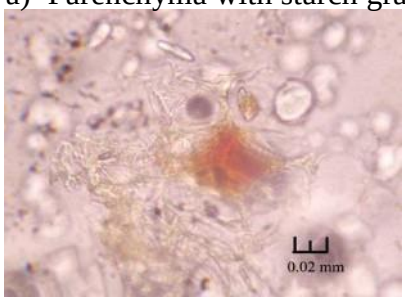
POWDER MICROSCOPY: Fig 12a,b,c,d



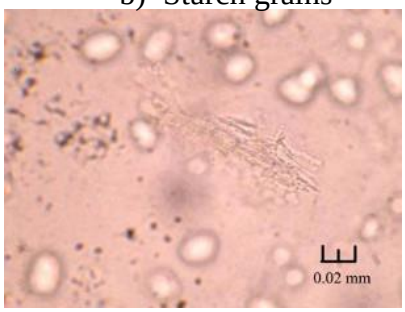
a) Parenchyma with starch grains



b) Starch grains



c) Cell with pigment



d) Starch grains

a) Creamy

b) Shows groups of polygonal thin walled parenchyma cells

c) Yellowish to reddish brown oleo-resin cells

d) Unlignified fibres

e) Vessels with annular reticulate or spiral thickening

f) Numerous round to oval starch grains upto 60 um long,25 um wide and 7 umthick, marked by fine concentric striations

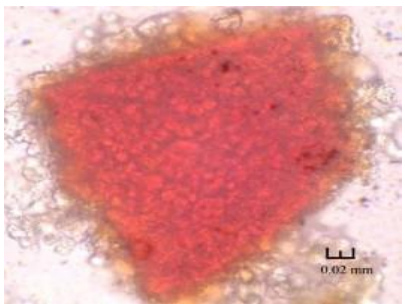
Drug identified as: *Glycyrrhiza glabra*-stolon

MACROSCOPY:

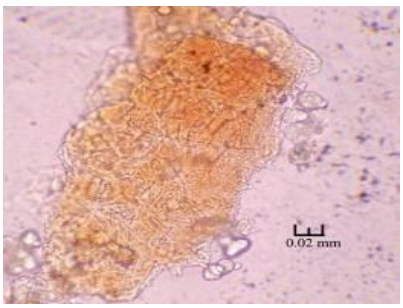
Fig. No 13

a) Stolon consisted of yellowish brown outer layer, externally longitudinally wrinkled, transeversely cut and a smoothed surface showing a cambium ring at about one-third distance from the periphery and a small central pith, fracture coarsely fibrous in bark and splintery in wood
b) Odour: faint and characteristic
c) Taste: sweet

POWDER MICROSCOPY: Fig. No 13a,b,c,d

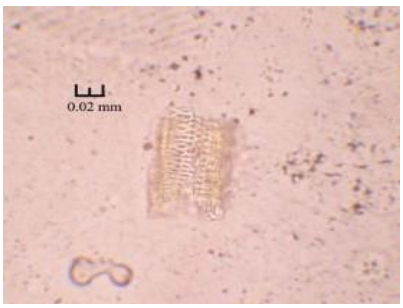


a) Cells with pigment



b) Parenchyma with contents

c) Medullary ray cells attached with fibres



d) Pitted vessel

a) Yellowish cream
b) Parenchyma cells containing a small prism of Calcium oxalate crystals
c) Fragments of fibres
d) Simple starch grains

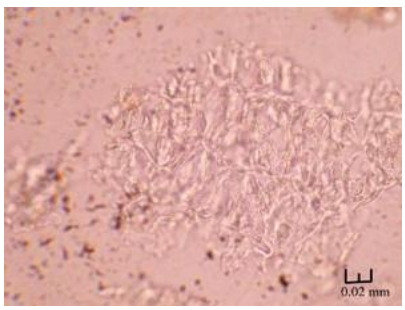
Drug identified as: *Anacyclus pyrethrum*-root

MACROSCOPY:

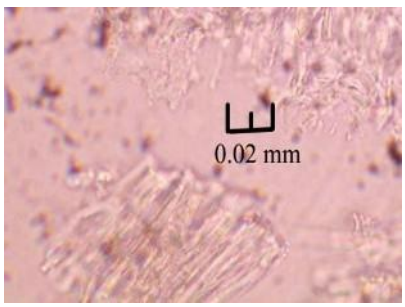
Fig. No 14

a) Roots-tough, cylindrical, 7-15 cm in length, tapering slightly at both ends with a few hair-like rootlets, external surface rough, brown, shriveled, bark upto 3 mm thick, not easily separable, odour slightly aromatic
b) Taste characteristically astringent and pungent on chewing gives a tingling sensation to the tongue and lips and cause excessive flow of salaiva

POWDER MICROSCOPY: Fig. No 14a,b,c,d



a) Parenchyma



b) Fibre bundle

c) Scalariform vessel

d) Fragment of fibre

a) Ash coloured
b) Vessels have scalariform thickening
c) Rosette crystals of Calcium oxalate and fragments of sclerenchyma

Drug identified as: *Acorus calamus*-rhizome

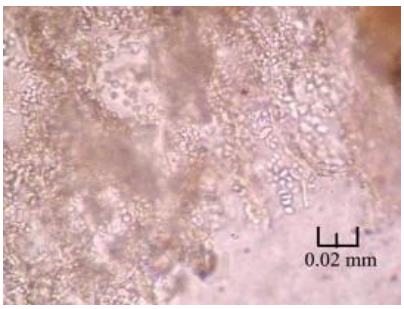
MACROSCOPY:

Fig. No 15

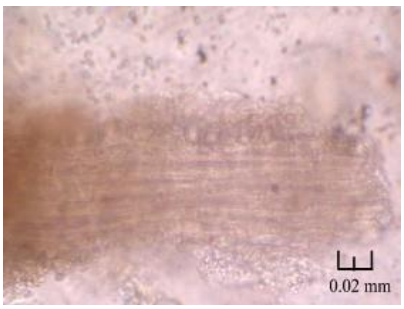
a) Drug was a simple rhizome, sub-cylindrical, slightly flattened usually somewhat tortuous, cut-pieces are 1-1.5 cm long and 0.5-1.5 cm thick upper side marked with remnants of bud scales and almost encircling the rhizome, lower side shows elevated tubercular spots of root scars, light-brown colored externally and buff colored internally

b) Odour: characteristically aromatic, taste: pungent and bitter

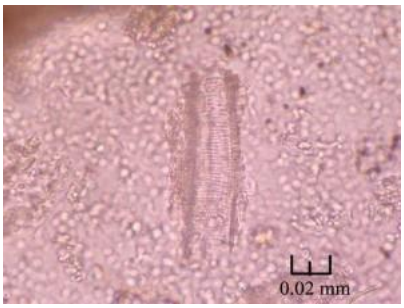
POWDER MICROSCOPY: Fig. 15a,b,c,d



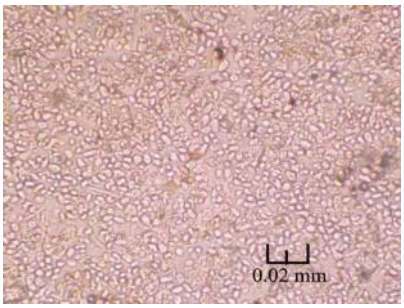
a) Parenchyma cells with starch grains



b) Fibre bundle with crystals



c) Fragment of vessel



d) Starch grains

a) Buff coloured

b) Shows abundant parenchymatous cells with starch grains measuring about 3-6 um in diameter

c) Fibre bundle with crystals

d) Reticulate, annular vessels

Drug identified as: *Myristica fragrans*-kernel

MACROSCOPY:

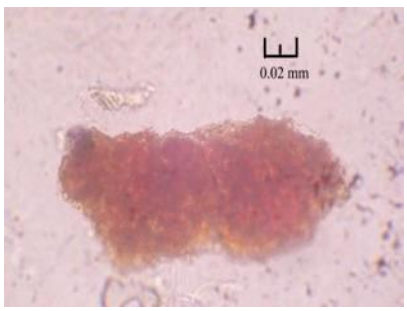
Fig. No 16

a) Kernel ellipsoid 20-30 mm long and about 20 mm broad, externally greenish brown, marked with irregular dark brown patches and lines slightly furrowed reticulately with a small light coloured area at one end indicated the position of the radical, a groove runs along the line of the raphe to the darker chalaza at the opposite end, a thin layer of perisperm with infoldings appearing as dark ruminations surrounding the abundant greyish-brown endosperm

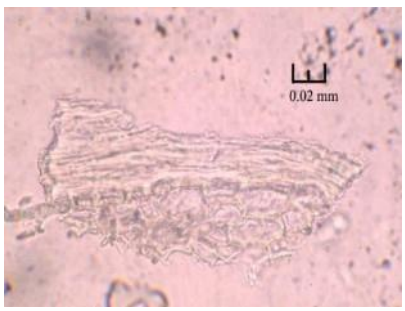
b) Odour: strong and aromatic

c) Taste: Pungent and aromatic

POWDER MICROSCOPY Fig. No 16a,b,c,d

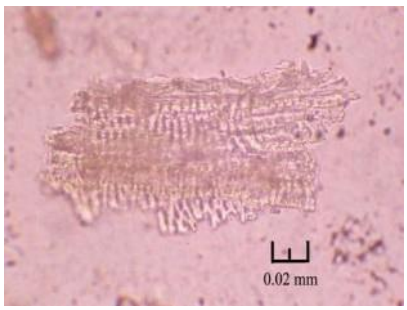


a) Cells of the testa in surface view



b) Perisperm cells

c) Groups of sclereids



d) Pitted vessel

a) Brown coloured, oily

b) Fragments of endosperm cells containing prismatic crystals and starch grains

c) Few cells of endosperm containing brown contents

d) Starch grains oval to rounded mearing upto 20 um in diameter

Drug identified as: *Terminalia chebula*-Dried fruit

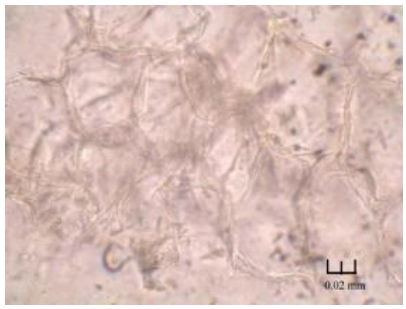
MACROSCOPY:

Fig. No. 17

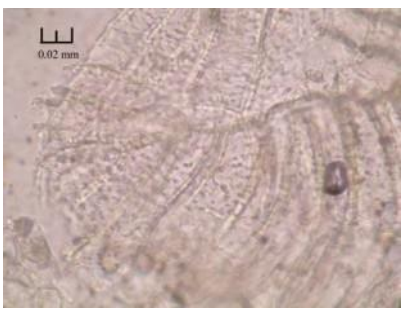
a) Fruit dark brown, ovoid, 20-35 mm long, 13-25 mm wide, wrinkled and ribbed longitudinally, pericap fibrous 3-4 mm thick, non-adherent to the seed
b) Taste: astringent

POWDER MICROSCOPY: Fig. No 17a,b,c,d

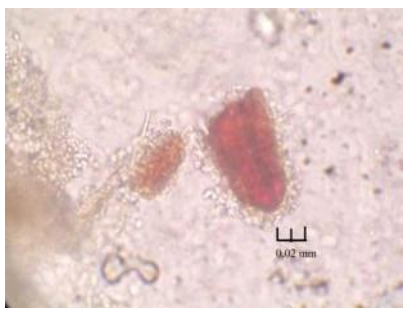
a) Epicarp in surface view



b) Parenchyma of mesocarp



c) Groups of sclereids



d) Tannin cell

a) Brown coloured epicarp cells
b) Polygonal mesocarp cells
c) Sclereids

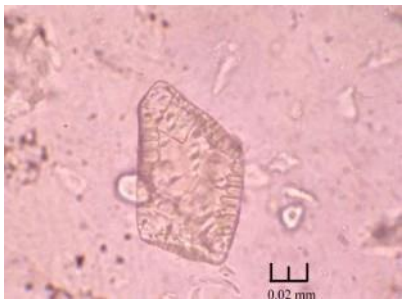
Drug identified as: *Quercus infectoria* - gall

MACROSCOPY:

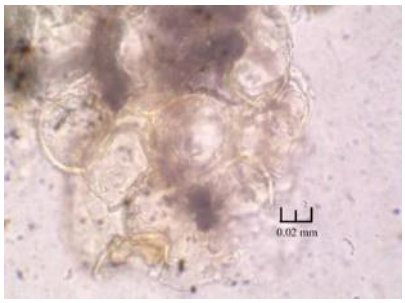
Fig. No. 18

a) Gall spherical, pear shaped, hard and brittle, 1.2-2.5 cm in diameter having a short basal stalk and numerous rounded projections on the upper part of the gall, with smooth shining outer surface, bluish green to olive green
b) Taste: astringent followed by sweetness

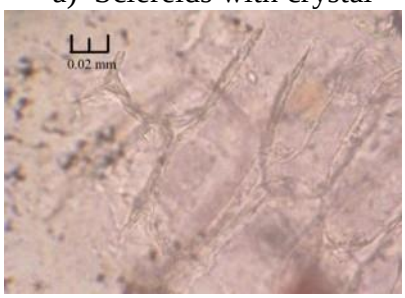
POWDER MICROSCOPY: Fig. No 18a,b,c,d



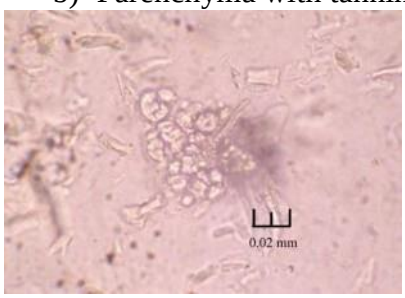
a) Sclereids with crystal



b) Parenchyma with tannin



c) Parenchyma with pitted wall



d) Starch grains

a) Cream colored
b) Fragments of palisade like thin walled cells
c) Oval to polygonal thin walled parenchymatous cells
d) Sclereids with thickened and striated walls with spiral thickening
e) Round to oval starch grains measuring 25 um in diameter

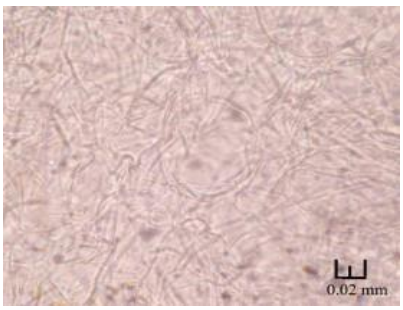
Drug identified as*: Allim sativum*-bulb

MACROSCOPY:

Fig. No. 19

a) Drug occurred as entire bulb or isolated cloves (bulblets), sub-globular, 4-6 cm in diameter, consisting of 8-20 cloves, surrounded by 3-5 whitish papery membranous scales attached to a short, disc-like woody stem having numerous wiry rootlets on the underside, each clove is irregularly ovoid, tapering at upper end with dorsal convex surface, 2 or 3 cm long, 0.5-0.8 cm wide each surrounded by two very thin papery whitish and brittle scales
b) Odour: peculiarly pungent and disaggreable
c) Taste: acrid giving warmth to the tongue

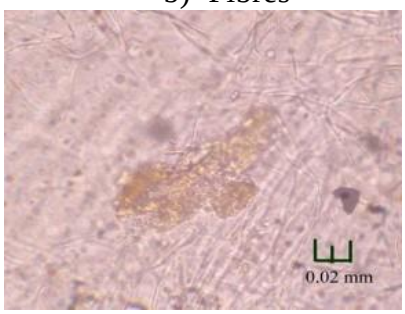
POWDER MICROSCOPY: Fig. No 19a,b,c,d



a) Parenchyma

b) Fibres

c) Annular and spiral vessels



d) Oil cells

a) Yellowish cream coloured
b) Shows fragments of parenchymatous cells
c) Lignified pitted epidermal cells with underlying hexagonal hypodermal cells indicates admixture of fragments of dried outer and inner scales respectively

Drug identified as: *Ferula foetida*-oleo gum resin

Fig No. 20

ORGANOLEPTIC CHARACTERS

a) Colour: Yellowish brown

b) Odour: Strong and characteristic and persistent

c) Taste: Bitter and acrid

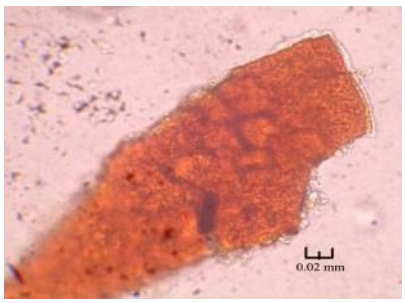
Drug identified as: *Piper longum*-Fruits with bracts

MACROSCOPY:

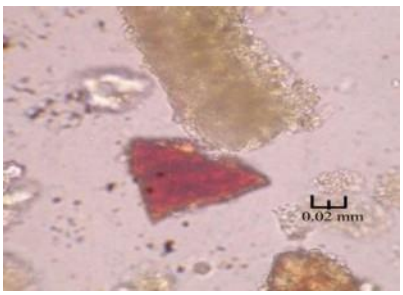
Fig. No 21

a) Fruits, greenish black to black, cylindrical 2.5-5 cm long and 0.4-1 cm thick consisting of minute sessile fruits arranged around an axis, surface rough and composite, broken surface shows a central axis and 6-12 fruitlets arranged around an axis
b) Taste: pungent producing numbness on the tongue
c) Odour: aromatic

POWDER MICROSCOPY: Fig No. 21a,b,c,d

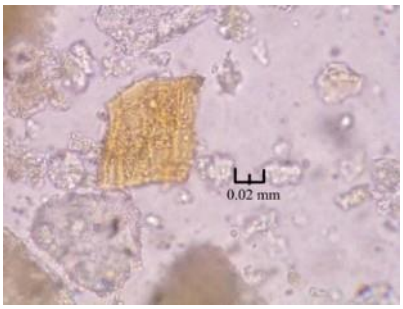


a) Cells of the epicarp



b) Epicarp in surface view

c) Endosperm cells



d) Endocarp cells

a) Deep moss green
b) Shows fragments of parenchyma cells
c) Oval to elongated stone cells
d) Oil globules
e) Round to oval starch grains measuring 3-8 um in diameter

STANDARDIZATION OF FORMULATION – AYUSH GUIDELINES

Macroscopy and Powder Microscopy Of Urai Mathirai

1. Macroscopy of *Urai mathirai-powder*

Fig. No 22

Colour: Brownish black

Odour: Characteristic

Taste: Astringent

2. Powder microscopy of *Urai mathirai*

The following characters were observed under the microscope:

i. Fragments of testa

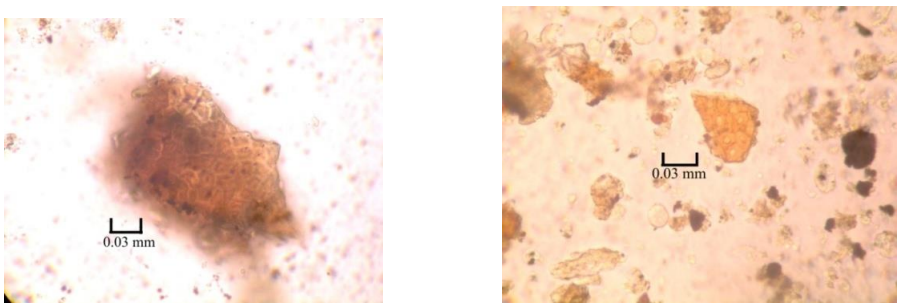


Fig. No 23

ii. Fragments of cells with contents

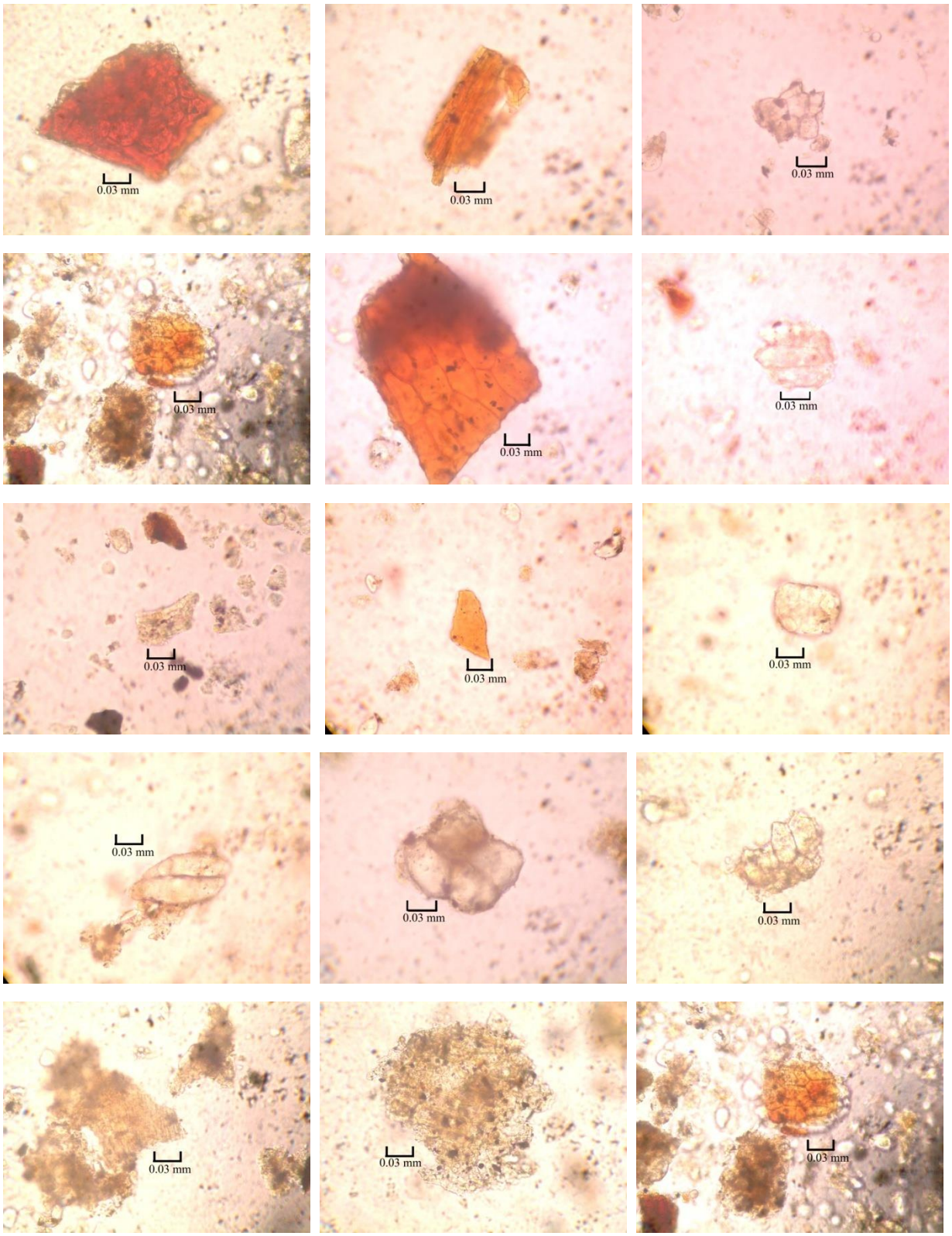


Fig. No 24

iii. Sclereids

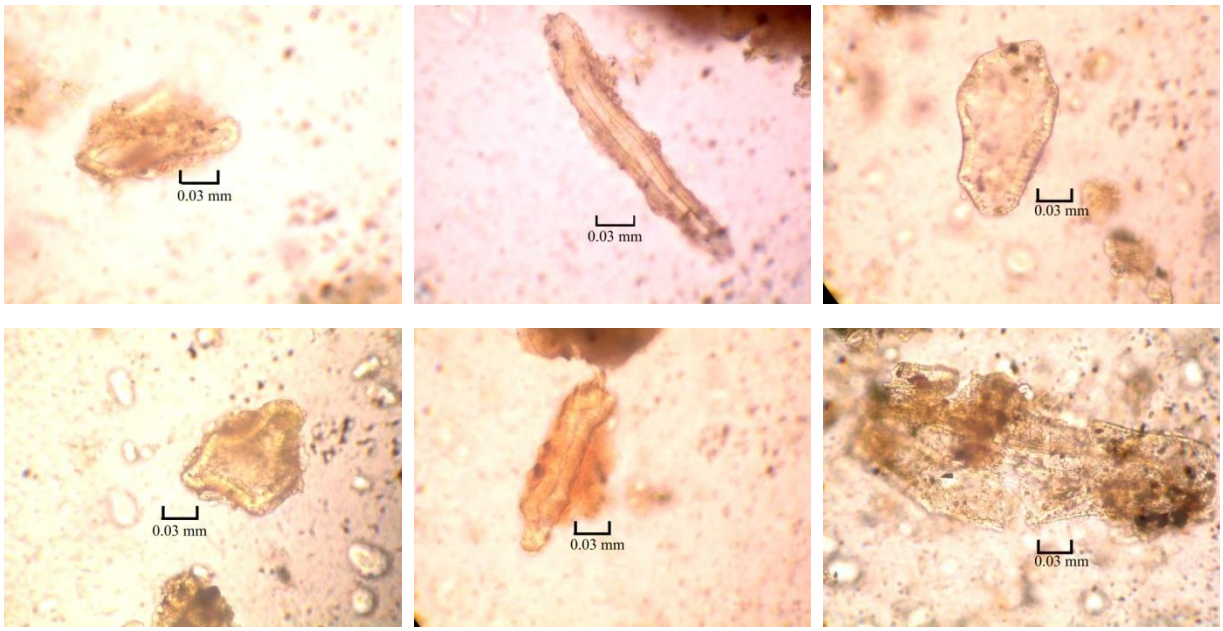
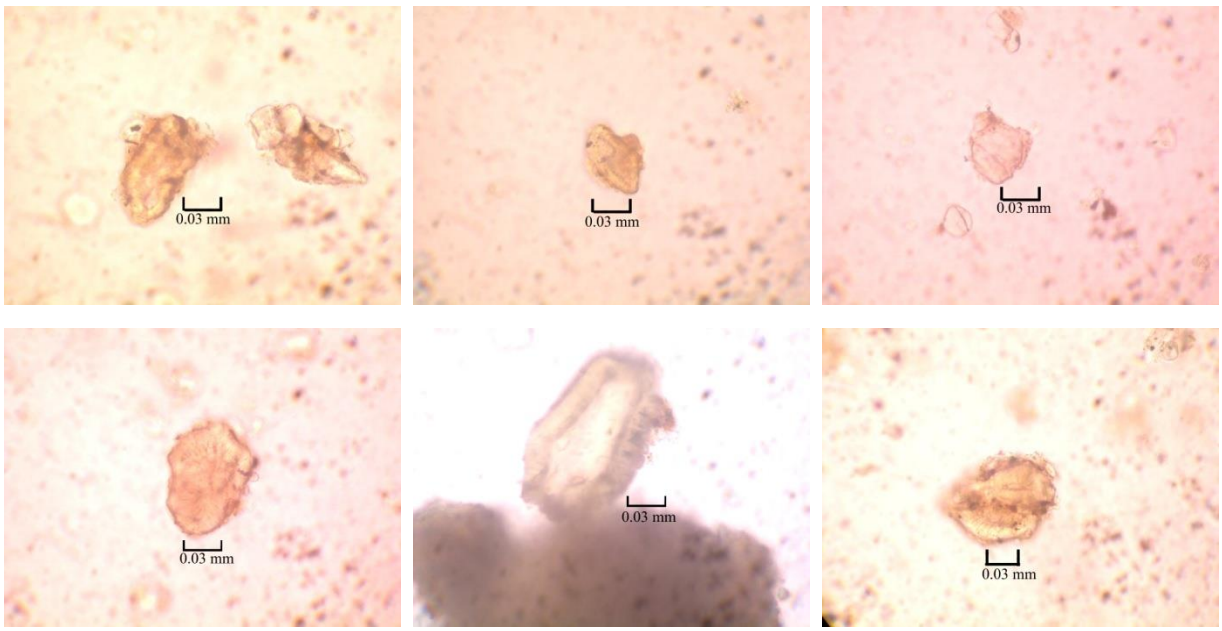


Fig.No 25

iv. Stone cells



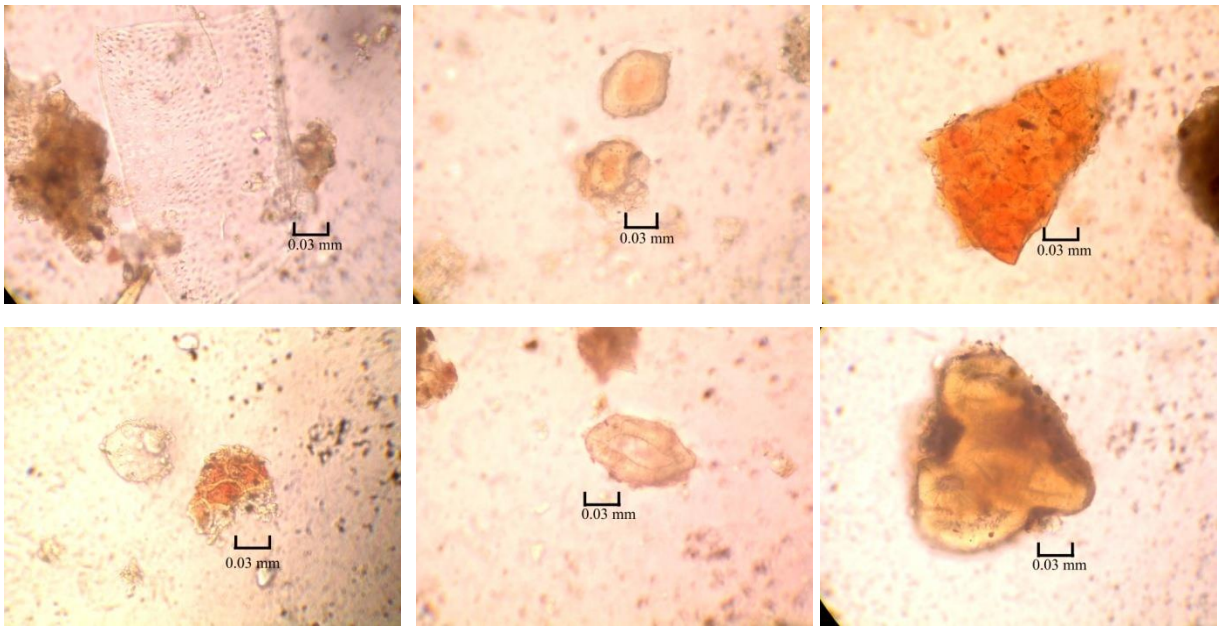


Fig. No 26

v. Fibres

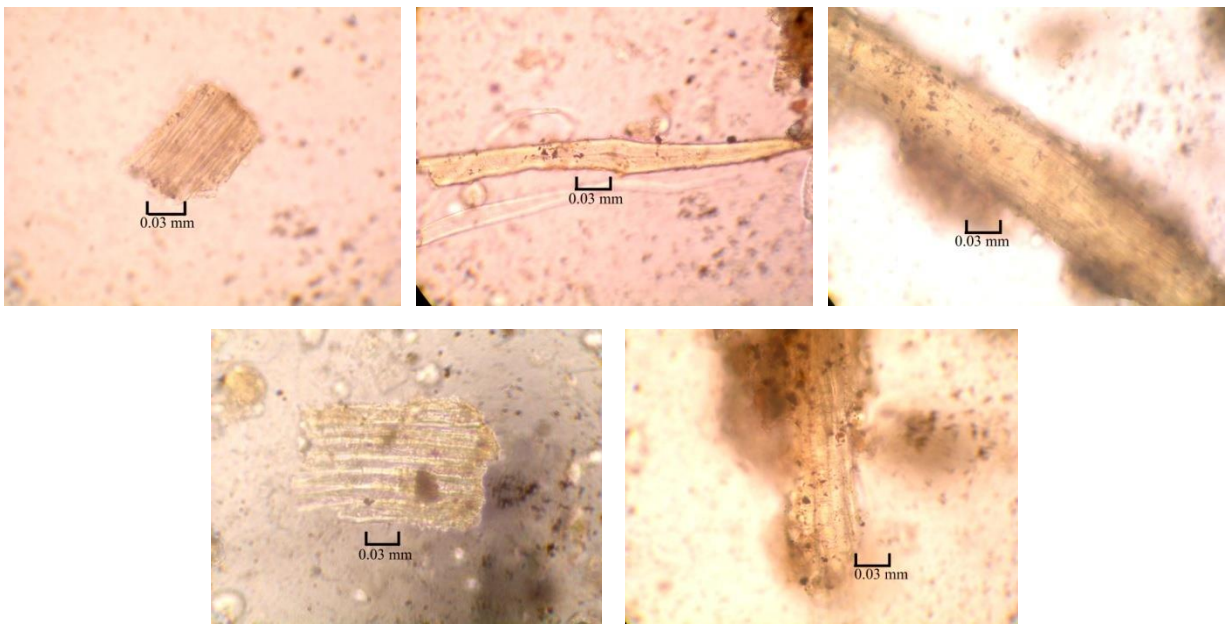


Fig. No 27

vi. Vessels

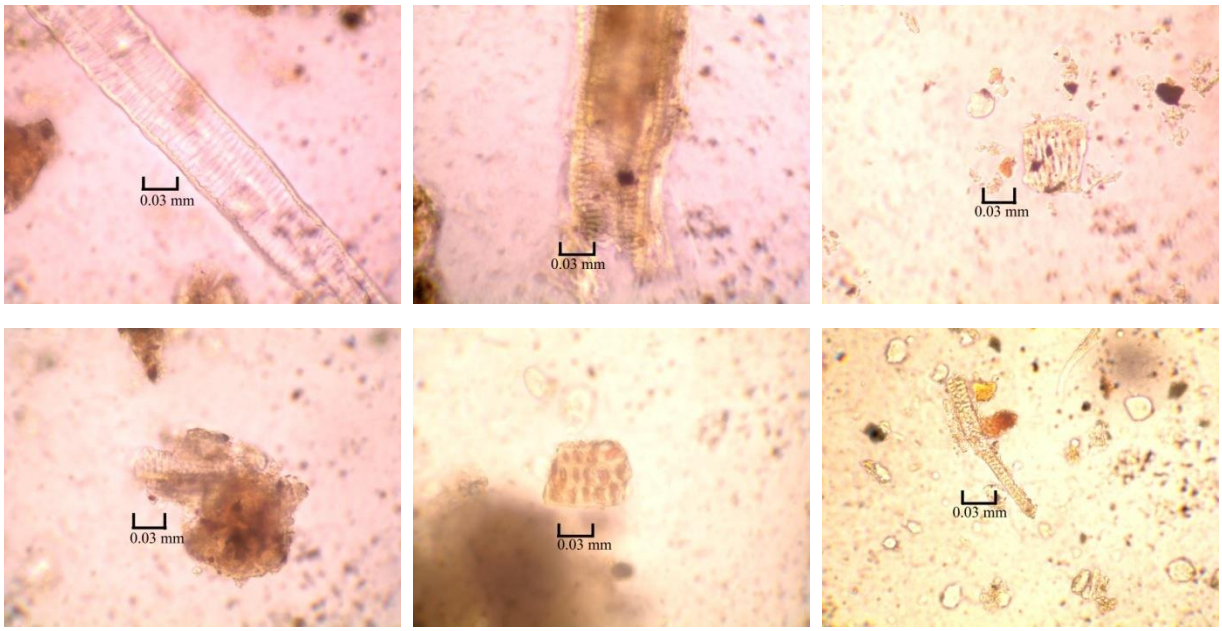


Fig. No 28

PHYSIOCHEMICAL ANALYSIS OF INDIVIDUAL HERBAL DRUGS AND ITS FORMULATION

The results of analytical parameters such as ash values, extractive values, pH and loss on drying content were presented in Table. No.4. The extractive values were carried out by successive extraction with solvents viz. Ethanol and water. Water soluble extractive values were found to be comparatively higher than alcohol soluble extractives in all the individual herbal drugs and its formulation.

Table . No 10

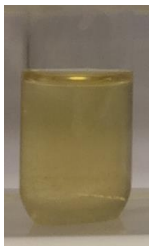
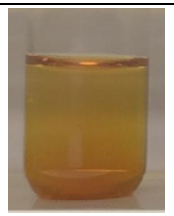
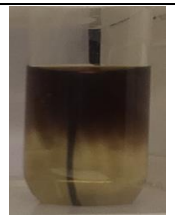
S. No.	Name of the Experiment	Results										
		UM-01	UM-02	UM-03	UM-04	UM-05	UM-06	UM-07	UM-08	UM-09	UM-10	UM-F
1.	Loss on Drying (%)	3.69	9.54	14.85	4.78	6.23	3.78	8.62	4.12	8.39	7.22	3.84
2.	Total Ash (%)	6.33	6.33	4.79	15.25	2.26	1.50	2.91	1.12	4.34	2.35	4.33
3.	Water soluble Ash (%)	2.57	1.62	2.06	9.83	0.61	1.04	1.86	0.15	2.37	1.37	3.17
4.	Acid insoluble Ash (%)	0.17	1.13	1.36	1.04	0.19	0.07	0.20	0.26	0.32	0.10	1.05
5.	Alcohol soluble Extractive (%)	8.42	22.17	10.44	6.73	13.06	74.43	63.20	3.10	9.68	9.46	9.5
6.	Water soluble Extractive (%)	17.71	31.09	27.66	17.26	8.63	65.11	51.76	16.56	12.28	79.30	12.25
7.	pH	6.10	5.69	5.06	7.51	5.71	3.82	3.57	5.60	4.52	3.25	5.03

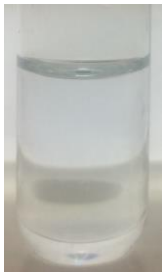
UM -01- *Zingiber officinale,* UM -02- *Glycyrrhiza glabra,* UM -03- *Anacyclus pyrethrum,* UM -04- *Acorus calamus,* UM -05 *Myristica fragrans,* UM -06-*Quercus infectoria,* UM -07-*Terminalia chebula,* UM-08- *Ferulla asafetida,* UM-09-*Allium sativum,* UM-10- *Piper logum,* UM-F- Urai Mathirai Formulation.

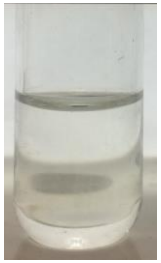
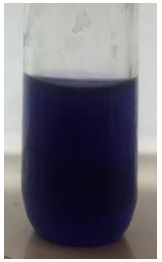
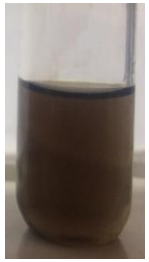
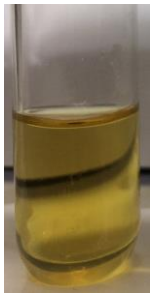
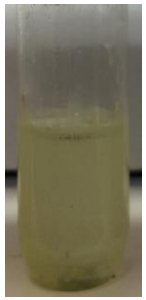
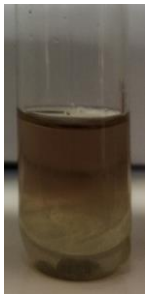
PHYTOCHEMICAL TEST

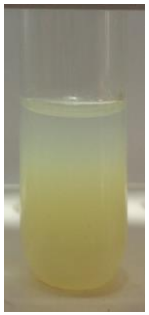
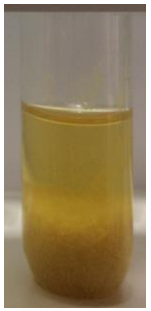
The qualitative phytochemical screening was carried to reveal the presence of bioactive compounds. The table no. 6 showed that the presence of alkaloids, glycosides, phenolic compounds (Flavonoid, Tannin) terpenoids, protein in Urai mathirai formulation.

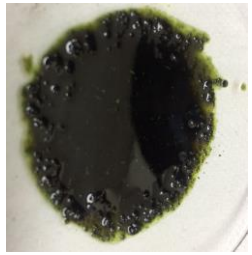
Fig. No 29

S. No	Name of Test	Pictorial observation of results				Color if positive	Observation	Result
1.	**Tests for Alkaloids:**	Test Solution	Blank + Reagent	Standard Solution + Reagent	Test Solution + Reagent			
	1. *Mayer's test*					Cream coloured precipitate Reddish brown precipitate	Less intense cream colour Reddish brown colour	+ +
	2.Dragendorff's test					Yellow precipitate	Less intense yellow colour	
	3.Hager's Test					Cream coloured precipitate Reddish brown precipitate	Less intense cream colour Reddish brown colour	
	4. Wagner's Test					Yellow precipitate	Less intense yellow colour	

2.	**<u>Tests for Sugars and Carbohydrates</u>** 1. ***Molish's test***	Test Solution	Blank + Reagent	Standard Solution + Reagent	Test Solution + Reagent	Yellow precipitate	Less intense yellow colour	
								
	2. **Fehling's test**					Red precipitate Violet ring	Reddish brown precipitate Violet ring	**++**
	3. **Benedict's test**					Brick red precipitate	Brick red precipitate	
3.	**<u>Test for Proteins</u>** 1. ***Biuret Test***					Purple or violet colour Yellow colour	Purple colour Yellow colour	**+**

	2.Xanthoprotei c test	Test Solution	Blank + Reagent	Standard Solution + Reagent	Test Solution + Reagent			
								+
4.	**<u>Test for Amino Acids</u>** **1. *Ninhydrin Test***							+
5.	**<u>Test for Flavonoids</u>** 1. ***Shinoda test***							++
6.	**<u>Test for Terpenoids</u>** 1. ***Noller's Test (or) Salkowski Test***							+

7.	**<u>Tests for Phenolic Compounds</u>** 1. ***Ferric Chloride Solution Test***	Test Solution	Blank + Reagent	Standard Solution + Reagent	Test Solution + Reagent	Blue to blue black white precipitate	Black Off white precipitate	++
								
	2. ***Lead Acetate Solution Test***					Blue to blue black	Black	
8.	**<u>Tests for Tannins</u>** 1. ***Ferric chloride***					greenish blue precipitate white colour precipitate	Black colour precipitate white colour precipitate	++

	2. ***Lead acetate***					greenish blue precipitate	Black colour precipitate	
9.	**<u>Tests for Steroids</u>** 1. ***Liebermann's Burchard test***					Bluish green Red colour	Reddish green Red colour	+
	2. ***Salkowski Test***							
10.	**<u>Tests for Glycosides</u>** 1. ***Anthrone test***	Test Sample	Anthrone	Blank + Reagent	Test Sample + Reagent	Dark green colour	Dark green colour	++
								

<table>
<tr><td rowspan="2">11.</td><td rowspan="2"><u>Detection of Fixed Oils and Fats</u>

1. Spot test</td><td>Test Sample</td><td>Filter Paper Before pressing</td><td>Filter Paper Before pressing</td><td>Test Sample</td><td rowspan="2">Oily stain</td><td rowspan="2"></td><td rowspan="2">+</td></tr>
<tr><td>  </td><td>  </td><td>  </td><td>  </td></tr>
<tr><td rowspan="2">12.</td><td rowspan="2"><u>Test for Saponin</u>

1. Foam test</td><td colspan="2">Extract</td><td colspan="2">Extract after Foam test</td><td rowspan="2">Stable froth</td><td rowspan="2">Stable froth</td><td rowspan="2">++</td></tr>
<tr><td colspan="2">  </td><td colspan="2">  </td></tr>
</table>

HPTLC

The results of HPTLC of Urai mathirai formulation and its individual herbal ingredients were expressed in chromatogram, finger print (TLC Pattern).

HEAVY METAL ANALYSIS OF URAI MATHIRAI FORMULATION

The therapeutic power of elements was recognized in traditional system of Indian and Chinese medicine. In recent years, health care scientist and nutritionist have realized significant benefits of elements in human health. So, in the present study the heavy metals content was analyzed by Atomic Absorption Spectroscopy and the results are tabulated in Table.No.11.

Table No. 11. Heavy metal Analysis of Urai Mathirai formulation

S.NO	HEAVY METALS	PERMISSIBLE LIMIT	Urai Mathirai formulation
			Concentration /100 gm Sample
1.	Lead	10.0ppm	BDL
2.	Cadmium	0.30ppm	BDL
3.	Mercury	1.00ppm	0.3ppm
4.	Arsenic	10.0ppm	Less than 5ppm

*BDL- Below detectable level

MICROBIAL ANALYSIS OF URAI MATHIRAI FORMULATION

In the presented study, Urai Mathirai formulation were tested for microbial limit test, all showed the total aerobic viable count, with in the limits prescribed by WHO for them. Total yeast and mould showed absent and the results are tabulated in Table.No.12.

Table No. 12. Microbial analysis of Urai Mathirai formulation

S.NO	MICROBIAL LOAD	PERMISSIBLE LIMIT	UM-F
1.	Total aerobic viable count	10^5/gm	110cfu/gm
2.	Total yeast and mould	10^3/gm	Absent
3.	*E.coli*	Absent	Absent
4.	*Salmonella spp*	Absent	Absent
5.	*S.aureus*	Absent	Absent
6.	*Pseudomonas aeruginosa*	Absent	Absent
7.	*Coliforms*	Absent	Absent

*UM-F: Urai Mathirai formulation

ANTIMICROBIAL ACTIVITY

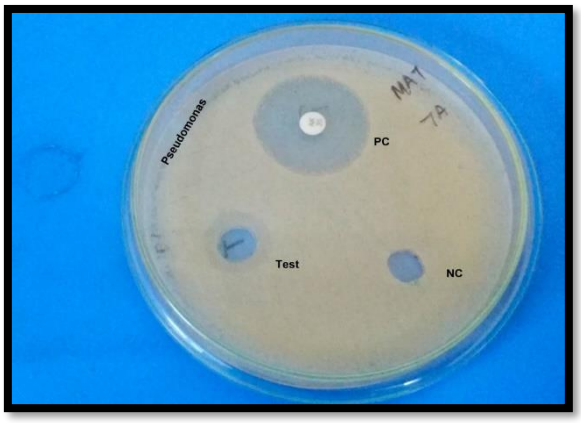
Results obtained in the present study relieved that the tested water Urai Mathirai extracts possess potential antibacterial activity against Pseudomonas, Staphylococcus aureus and E.coli. By well diffusion method, the extracts showed significant activity against all the tested microorganisms (Table 13). The highest antimicrobial activity recorded in Pseudomonas with 2.3 mm, E. coli 2.0mm followed by Staphylococcus aureus with 1.6mm. The extract showed negative results for Bacillus and Klebsiella

Table 13: Antimicrobial activity of pathogenic target strains

Target Strains	Zone of Inhibition
MA7-7A *Pseudomonas*	**2.3**
7A-MA3 *Staphylococcus*	**1.6**
8P 7A *E.Coli*	**2.0**
2A MA6 *Bacillus*	**Negative**
6P 1A *Klebsiella*	**Negative**

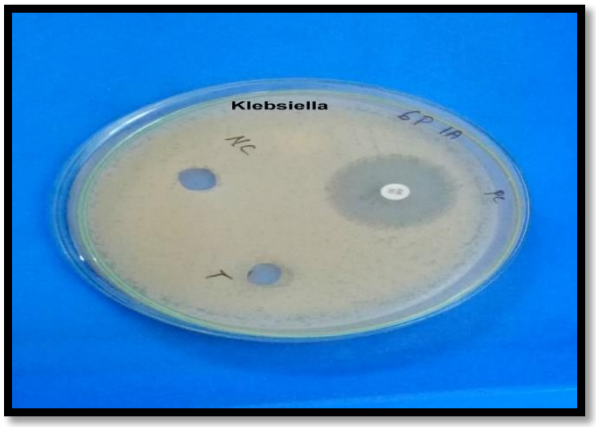
Figure 41: Antimicrobial activity against various pathogen

Pseudomonas aeruginosa **with Zone of inhibition** 2.3mm

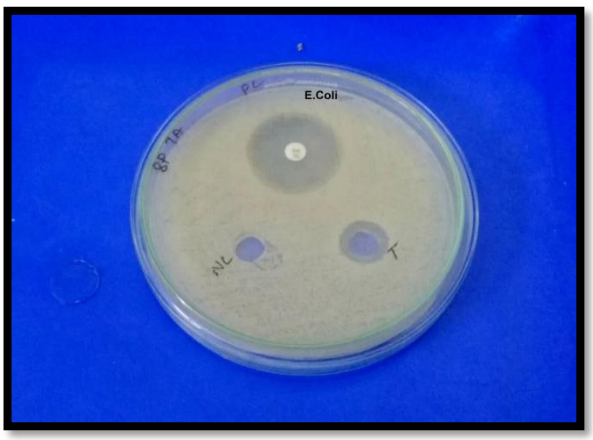


Staphylococcus aureus **with Zone of inhibition 2.3mm**

Klebsiella – Negative



E.coli with Zone of inhibition 2.3mm



PESTICIDE RESIDUE AND AFLATOXIN ANALYSIS

In the present study Urai Mathirai formulation were tested for pesticidal residue and Aflatoxins and the results are tabulated in Table.No.14 & 15.

Table No. 14. Pesticide residue analysis of Urai Mathirai formulation

S.NO	REFERENCE SAMPLE	Chooranam
1.	Chlorpyriphos	ND
2.	DDT	ND
3.	Endosulfan	ND
4.	Malathion	ND
5.	Parathion	ND

*ND – No spots were detected

Table No. 15. Aflatoxin analysis of Urai Mathirai formulation

S.NO	REFERNCE SAMPLE	Chooranam
1.	B1	Traces
2.	B2	Absent
3.	G1	Absent
4.	G2	Absent

PRE-FORMULATION STUDIES

PHYSICAL CHARACTERIZATION

Organoleptic characters

The organoleptic characters of Urai Mathirai were studied. The study showed that the drug was adeep green colour powder and sour taste with herby odour. The results were shown in the table 16.

Table 16: Organoleptic charactersof Urai

S.NO	PROPERTY	RESULTS
1.	Organoleptic character	Color: A deep green colour powder. Odour: Herby Taste: Sour taste Melting point: 50.0ºC-54.0ºC Lod: 6.044%s

BULK CHARACTERIZATION

The bulk density of lubricated blend (F3) was found to be 0.5383 gm/cc and the tapped density was found to be 0.5897 gm/cc. Both the values were similar to the specific standard. The angle of repose was done as per the procedure and the value was 28° 2'.The value indicated that the powder had Good flow property. The results were shown in the table 17.

Table 17: Bulk characterization of lubricated blends of optimized formulation (F3)

Parameters	Results	Reference value	Flow property
Bulk density (gm/cc)	0.5383	-	-
Tapped density (gm/cc)	0.5897	-	-
Compressibility index (%)	9.548	≤10	Excellent
Hausner's ratio	1.095	1.00–1.11	Excellent
Angle of Repose (θ)	28° 2'	20-30	Good

Drug - Excipients compatibility study

The FT-IR spectra of the Urai Mathirai drug and physical mixture of drug– excipients were recorded using FTIR spectrophotometer in order to check interaction between drug and excipients. The characteristic peaksUraiMathirai had appeared in the spectra without any markable change in the position in the spectra of drug-excipients. The results indicated that there was no chemical interaction between Urai Mathirai and excipients and suitability of the excipients in the formulation. The results were shown in the (Table 18).

Table 18: The Drug and Excipients compatibility studies

EXCIPIENTS	COMPATIBLE	INCOMPATIBILE
Urai+ MCC	√	-
Urai+ Polyvinyl pyrollidone	√	-
Urai+Talc	√	--
Urai+ MCC + Polyvinyl pyrollidone + Talc	√	-

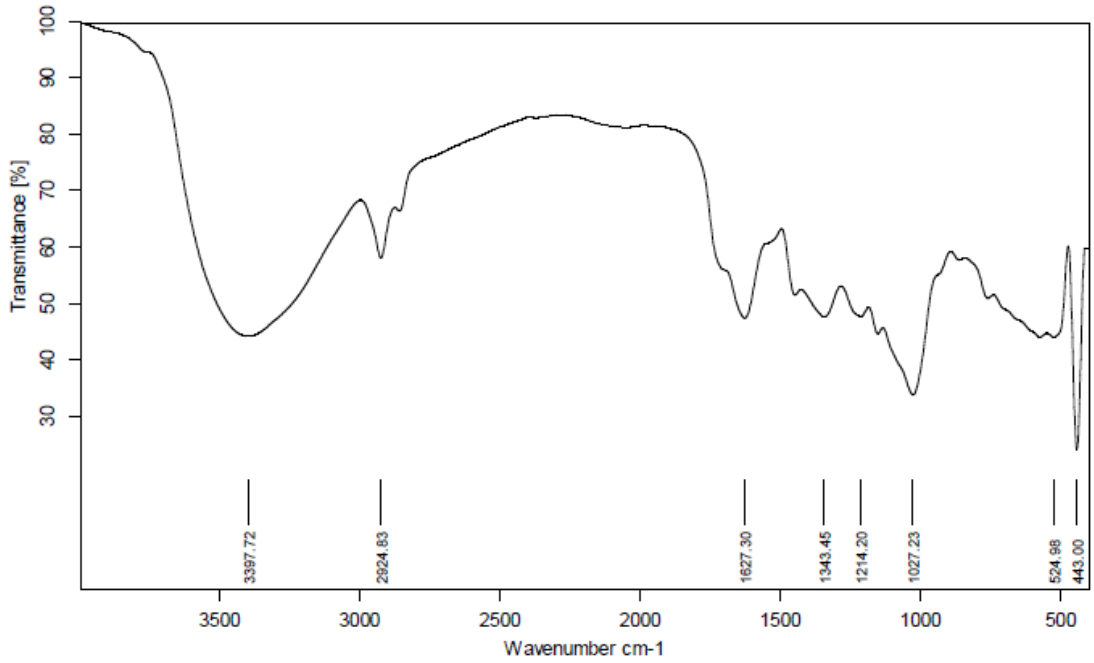


Fig 42: FTIR spectrum of Urai Mathirai

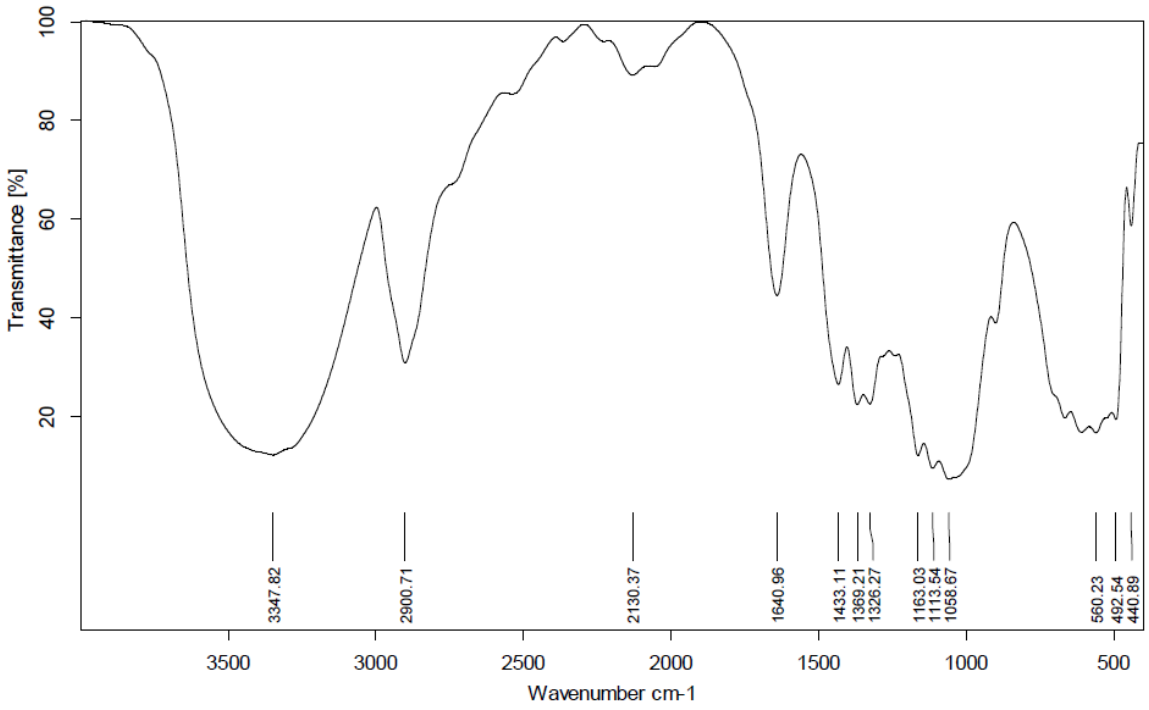


Fig 43: FTIR spectrum of MCC

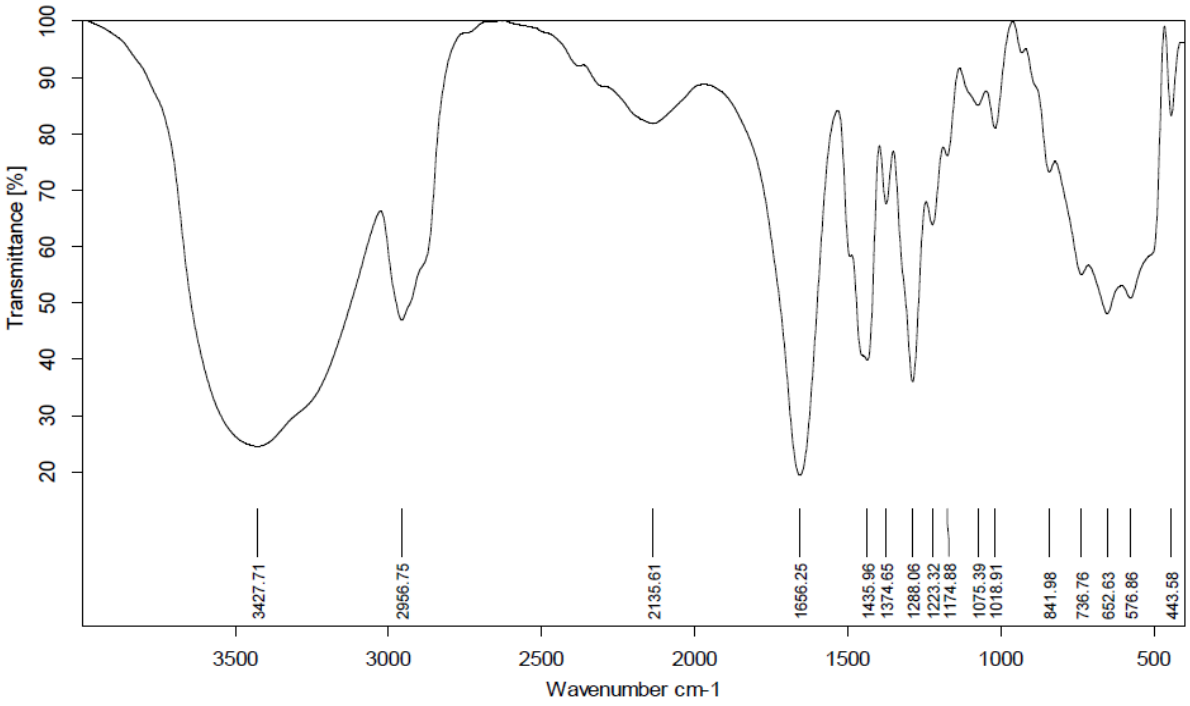


Fig 44: FTIR spectrum of Polyvinyl pyrollidone

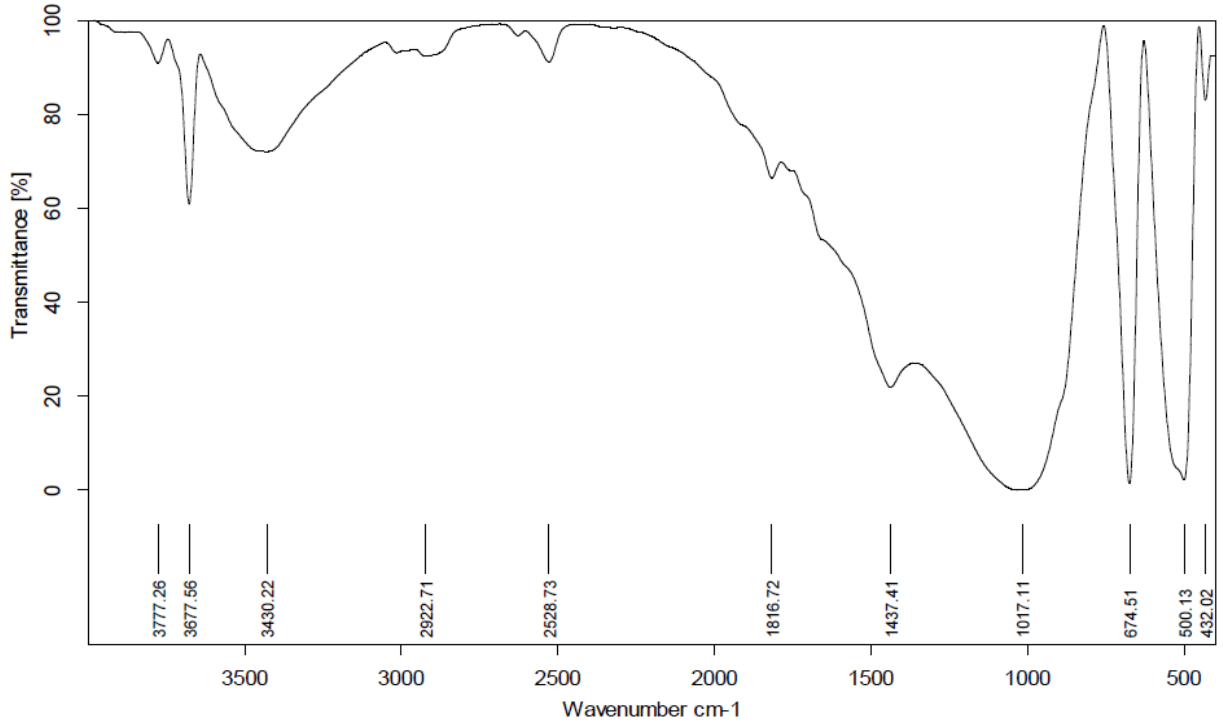


Fig 45: FTIR spectrum of Talc

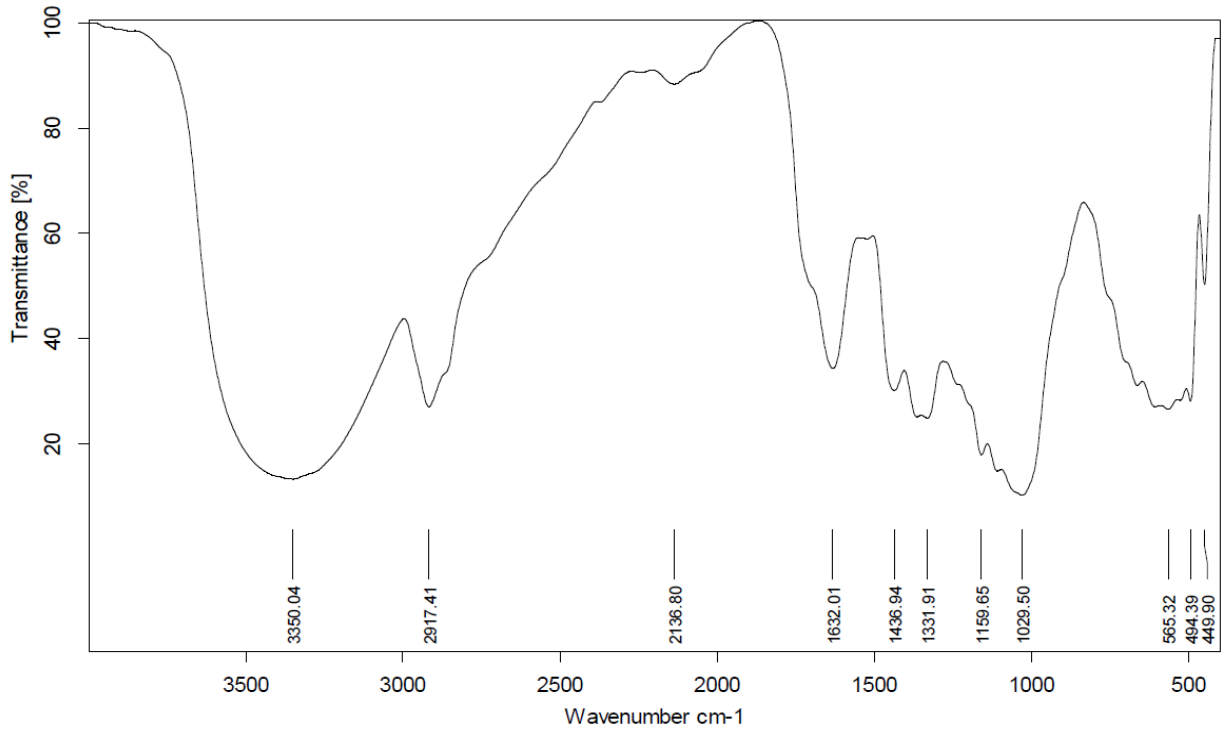


Fig 46: FTIR spectrum of Urai Mathirai + MCC

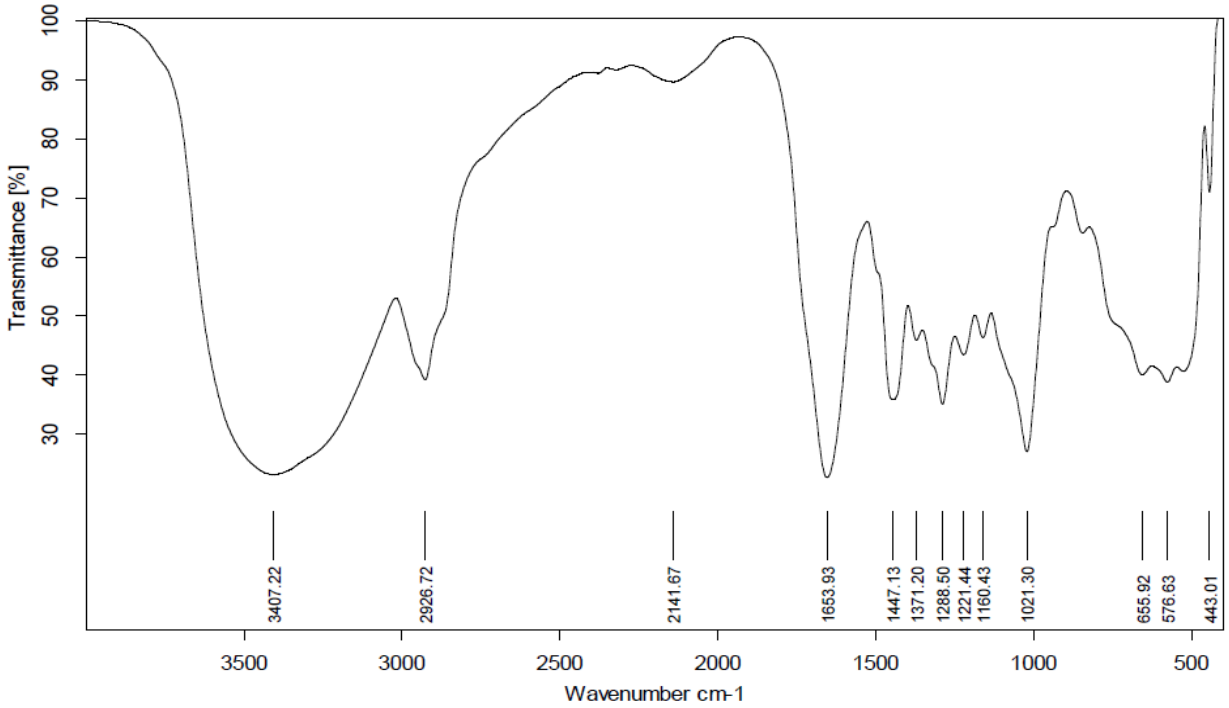


Fig 47: FTIR spectrum of Urai Mathirai+ polyvinyl pyrollidone

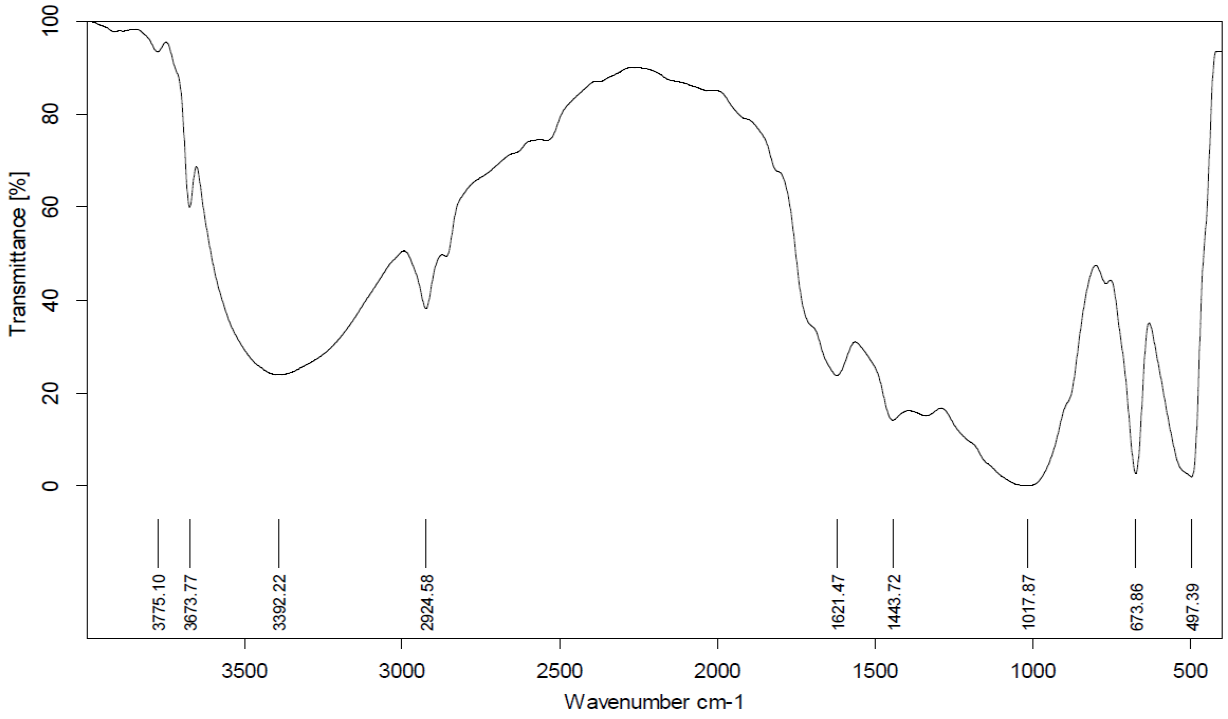


Fig 48: FTIR spectrum of Urai Mathirai + Talc

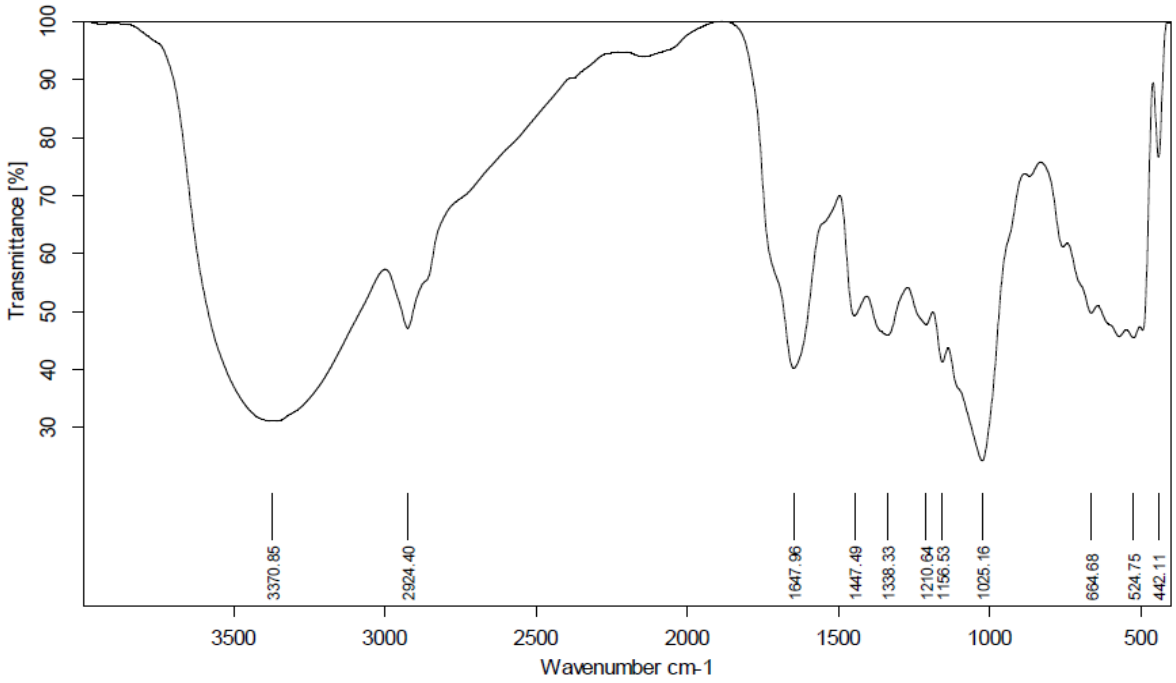


Fig 49: FTIR spectrum of Final lubricated blend (F3)

Post compression parameters of optimized formulations of Urai Mathirai (F3)

The optimized formulations of Urai Mathirai (F3) was evaluated for post compression parameters like the weight variation, thickness, disintegration time, hardness and friability and the values were shown in (Fig.50 and Table19).

Table 19: Post compression parameters of optimized formulation (F3)

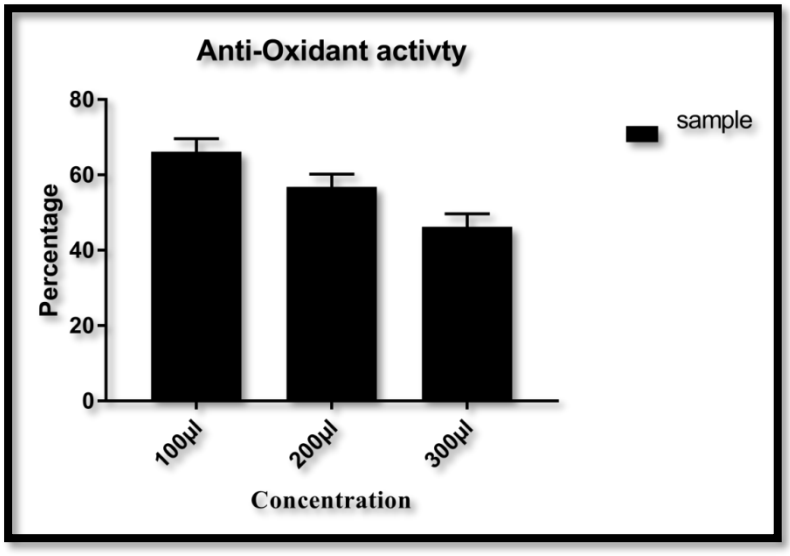
Weight variation	63 ± 0.71 mg
Hardness	3.5 kg/cm^2
Thickness	1±0 .08 mm
Disintegration time	13 mins ± 0.82

Fig 50: Formulation (F3)

ANTI-OXIDANT ACTIVITY:

The DPPH assay constitutes a screening method currently used to provide basic information on the antiradical activity of the extracts. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by loss of colour and reduction in the absorbance. In the present study, the water extract of Urai Mathirai exerted a strong antiradical activity with least IC50 value (100µg; 66.19%) followed by the 200 µg – 56.83% and 300µg – 46.24%) (Table 3). The antiradical activity of the extract was gradually decreased when the concentration of sample is increased. The IC50 value for the positive control ascorbic acid was 5.23 µg. In this study extracts showed adequate IC50 value than the positive control; however, the extracts were able to scavenge the DPPH free radicals suggesting that it may have a role in preventing free radical mediated chain reactions.

Figure 51: Anti-oxidant activity for Urai Mathirai



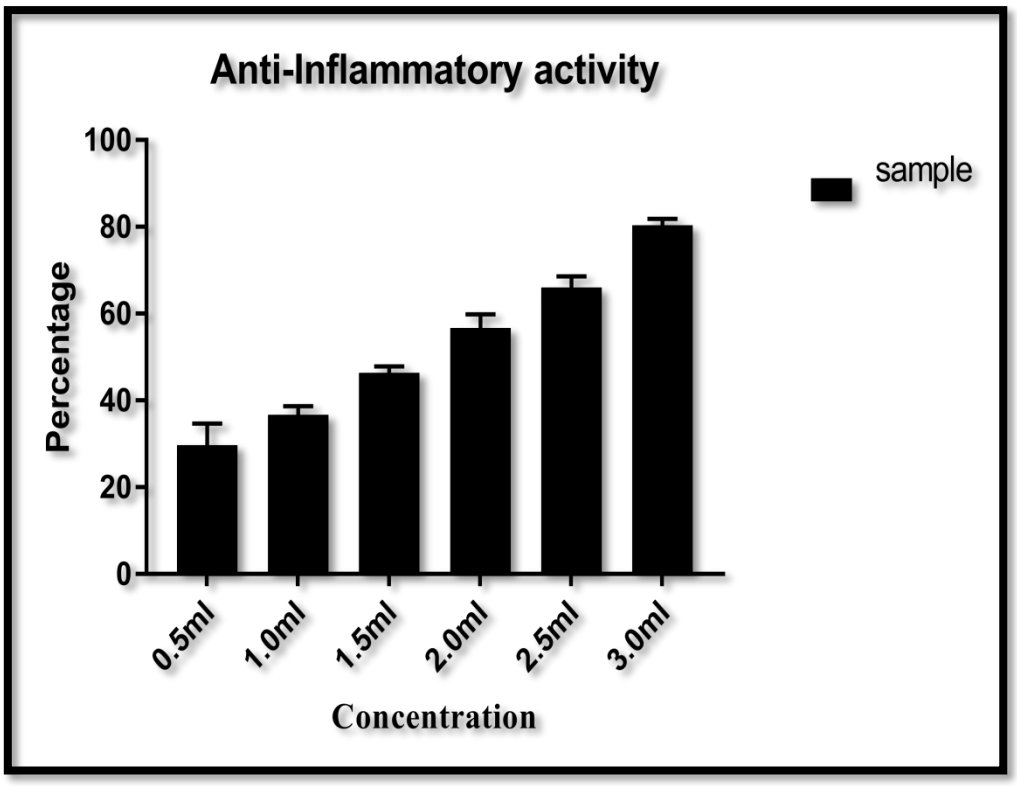
ANTI-INFLAMMATORY ACTIVITY

Denaturation of proteins is the main cause of inflammation. As part of the investigation on the mechanism of the anti-inflammatory activity, ability of the extract to inhibit protein denaturation was studied. Selected extracts were effective in inhibiting heat induced albumin denaturation. Water extract of Urai Mathirai were gradually increased its effective activity when there is increase in the concentration of test sample. The maximum activity were observed as 80% with 3ml of extract respectively. Aspirin was used as a standard anti-inflammation drug. Results are presented.

Table 20: Tabular column of anti-inflammatory activity

Sample (ml)	Test 1	Test 2	Test 3
0.5ml	**25**	**29**	**35**
1.0ml	36	35	39
1.5ml	**46**	**48**	**45**
2.0ml	58	59	53
2.5ml	**63**	**67**	**68**
3.0ml	79	80	82

Figure 52: Anti-inflammatory activity of Urai Mathirai by BSA method.



SAFETY PROFILE STUDY

Acute Toxicity Study

The toxic effect of the formulation was evaluated as per the OECD guidelines 423 respectively by acute method. All the results pooled from these studies were summarized below, showing for each test group the number of animals used, the number of animals displaying signs of toxicity. No mortality in the acute oral toxicity test has been observed. No other toxic symptoms were observed in any of the test dose treated animals. For the usage of all the animals used in the present investigations were approved by the Ethical Committee and the number is 162/Pharma/SCRI/2017.

Body weight

All the survived animals showed again in body weight on day 7 and 14 in comparison to their day 0 body weight (Female & male)) except few animals.

Clinical signs:

All the animals in treatment groups are prepared normal at 30mi, 1,2,3,4,8 and 24 hours observations, following Urai Mathirai administration. Only two animals have shown irritability at hour Post administration and one animal show lacrimation at 24 h of Urai Mathirai administration.

Mortality:

There was no treatment related mortality in the treatment groups

Gross pathology:

No gross lesions were recorded in all the experimental animals of Urai Mathirai.

Macroscopic Findings:

No gross lesions were recorded in all the experimental animals of Urai Mathirai during necropsy.

Conclusion:

Based on the above observations, “Urai Mathirai” was found to be safe at all the tested dose levels.

SUB ACUTE ORAL TOXICITY

Objective

The objective of this study was to evaluate the toxicological profile, the target organs of toxicity, reversibility of toxicological findings and No Observed Effect Level (NOEL) or No Observed Adverse Effect Level (NOAEL) in experimental rats after oral (gavage) administration of ***Urai Mathirai*** **for 28 consecutive** days.

Animal welfare

This study was performed as per the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for Laboratory Animal Facility after approval of Institutional Animal Ethics Committee.

Archiving

The following study material was retained in the Archived in Department of Pharmacology, SCRI, Chennai. This includes: Raw data, Wet tissue samples, (Blood smear, Bone Marrow and Histopathology report), Final report.

Materials and methods

Instruments: Auto analyser, Haematology analyser (BC-2800 Vet)

Test item

Identity	: Urai Mathirai
Physical Appearance	: Greenish powder
Manufactured by	: SCRI- Chennai

Test System

A total 48 no of animals (male and female) of 6 to 7 weeks age, were received from King Institute of preventive Medicine, Guindy, Chennai-600 032. The body weight variation of the animals selected for the study on the day of randomization were not exceeding ± 20 % of the mean body weight of each sex. Only nulliparous and non-pregnant females were used in the experiment.

Acclimatization

Male and female animals were acclimatized for 7 days, respectively before initiation of treatment. Veterinary examination and detailed clinical signs of all the animals were performed on the day of receipt followed by cage side clinical signs observations once daily during the acclimatization period. Body weight of all the animals were recorded on the day of receipt and randomization.

Animal Identification

Each animal was identified uniquely by marking using picric acid within the cage during acclimatization period. Post randomization, animals were identified uniquely using marking throughout the study. Each cage was identified by cage cards properly during the acclimatization period and throughout the study.

Grouping and randomisation of animals

A total 48 animals (24 Male + 24 Female) were randomly distributed to 4 groups (Group I, II, III and IV). Each group consisted of 12 animals each.

Experimental procedure

Study Design

An outline of the study design is presented in the following table.

Species	Animals No.		Age (weeks)	Weight (g)	Duration of experiment Dosing
	Male	Female			
Rat Wistar	24 6x4	24 6x4	6-7	90-120	28 days

The dose levels and groups were as follows:

Group	Treatment	Dose	Animals/ sex (M/F)
I	Vehicle control (Warm		6 male + 6 female
II	water)	10mg/kg	6 male + 6 female
III	*Urai Mathirai* T.D	50mg/kg	6 male + 6 female
IV	*Urai Mathirai* -T.D x 5	100mg/kg	6 male + 6 female
	Urai Mathirai T.D x 10		
Total			= 48 rats

Route, Frequency and Method of Administration

The test item Urai Mathirai and vehicle (Warm water) were administered to the animals once daily up to 28 days by oral (gavage) route using appropriate graduated disposable syringe. The animals from Group II, III and IV received test item whereas the animals from Group I received vehicle.

Justification for Selection of Route

Oral route has been selected, as oral route is the recommended route of administration in human being.

Observations

The following observations were made during the course of study.

Clinical Signs and Mortality

Male and female animals were examined once daily for clinical signs within 1 to 2 hrs post dosing. Mortality and morbidity were recorded twice daily. All the animals were examined for cage side clinical signs once daily except on days of detailed clinical signs observations viz. salivation, fur, lacrimation etc. All the animals were examined for

detailed clinical signs on treatment Day 1 and once weekly thereafter. Mortality and morbidity were recorded twice daily.

Body Weight

Body weight of the animals was recorded once weekly throughout the study.

Feed Consumption

Feed consumption was recorded once weekly throughout the study for study animals. The feed was withdrawn overnight before scheduled blood collection (clinical pathology investigation) and terminal sacrifice respectively.

Clinical laboratory investigations

General

The blood samples were analyzed for clinical chemistry and hematology on Day 15, 29, 43. Blood was withdrawn from retro-orbital plexus (left eye) with light ether anesthesia from overnight fasted animals. Approximately 0.5 ml of blood was collected in each tube containing EDTA-K2 for analysis of hematological parameters. Approximately 1.0 ml of blood was collected in tubes containing heparin [10 µl of heparin/ml of blood (Heparin: 5000 IU/ml)] for analysis of clinical chemistry parameters.

The plasma was separated by centrifugation approximately at 3000 rpm for 15 minutes for clinical chemistry analysis.

Clinical Chemistry

The clinical chemistry parameters were estimated by Automatic Clinical Chemistry Analyzer.

Pathology

Necropsy

All surviving animals were sacrificed on Day 91 by carbon dioxide euthanasia. Complete necropsies were carried out on all animals. All the tissues listed below were collected from each animal. Testes, eyes, optic nerve was preserved in modified Davidson

fixative and all other tissues were preserved in 10% neutral buffered formalin. Viz. adrenals, brain (cerebrum, cerebellum & medulla oblongata), pancreas, epididymis, heart, kidneys, spleen, stomach, lungs, liver, testes / ovaries with oviducts, sciatic nerve, esophagus, duodenum, ileum, cecum, colon, rectum.

Organ Weights

Organs indicated below were taken from all surviving animals at the scheduled necropsies, weighed, recorded and organ/body and organ/brain weight ratios were calculated. Prior to weighing, all organs were carefully dissected and properly trimmed to remove fat and other contiguous tissues in a uniform manner. Organs were weighed as soon as possible after dissection to avoid drying. Viz. adrenals, liver, testes, brain, ovaries with oviducts, thymus, heart, uterus (with cervix), kidneys, spleen, epididymis.

Histopathology

The organs collected from the vehicle control (Group I), Therapeutic dose (Group II), 5 x TD (Group III), 10 x TD (Group IV) of the study animals were submitted to Department of pathology, Veterinary Medical college, Vepery, Chennai.

Statistical analysis

The data were expressed as mean ± SD. Results were analysed statistically by One-Way ANOVA followed by Tukey's multiple comparison using Graph Pad Prism version 6.01. The difference was considered significant if $p<0.05$.

Results

Mortality, body weight and feed consumption

Mortality was observed on 8th day, 17th day and 21st day of the study including the one male (Group-IV) and Two Female animals (Group-III & IV) of all four groups. There are no significance changes were observed in the body weights of animals entire study period in treatment group of animals compared with normal control (Table).

Biochemical parameters for liver and kidney function

Results of the biochemical parameters were given in Table 1 to 25. No significant difference was observed in biochemical (liver and kidney function tests) parameters of Urai Mathirai treated animals on 15, 29 and 43rd days, when compared with control animals. Determination of kidney function tests showed that the Urai Mathirai did not produce any renal dysfunction, as increase in creatinine reveals the impaired renal function or acute renal failure. AST, ALT, ALP and total bilirubin are good indices of liver function; indicate that there were no significant changes in the enzyme levels, in both liver and kidney tissues, when compared with the control animals. Hence the Urai Mathirai did not induce any toxicity to the liver and kidney.

Biochemical parameters observed on 15th day and 29th day, there is no significance changes were observed in the entire study period in treatment group of animals compared with normal control.

Hormonal assays

Thyroid function was analysed using the hormonal assays of T3 and TSH which does not produce any significant changes in the normal control animal with the Urai Mathirai treated groups. It indicates the Urai Mathirai does not have any effect on thyroid gland.

Absolute and relative organ weight

Results of the absolute and relative organ weight parameters were given in Table

Urai Mathirai treated animals did not produce any significant change in organ weight (absolute and relative to body weight and brain weight) of adrenals, brain, heart, spleen, liver, epididymis, testes, thymus and kidneys, when compared with control animals.

Table 53: Effect of *Urai mathirai* on fasting blood glucose levels (mg/dl) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	28.33 ± 9.57	42.00 ± 5.96	33.80 ± 6.34	52.00 ± 8.56	35.33 ± 5.08	43.67 ± 5.37	43.67 ± 3.84	53.33 ± 9.33	48.50 ± 5.00
II	36.00 ± 1.88	50.40 ± 6.88	42.55 ± 3.84	64.50 ± 11.25	53.83 ± 7.67	59.17 ± 6.69	61.00 ± 10.02	45.33 ± 2.67	53.17 ± 5.81
III	39.83 ± 2.23	49.60 ± 7.78	44.27 ± 3.84	66.17 ± 3.28	39.40 ± 9.56	54.00 ± 6.11	71.33 ± 6.17	39.00 ± 7.00	58.40 ± 8.89
IV	36.50 ± 5.70	44.00 ± 5.93	39.91 ± 4.08	95.60 ± 10.80	42.00 ± 9.11	71.78 ± 1.61	56.50 ± 0.50	42.50 ± 2.50	49.00 ± 7.01

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 54: Effect of *Urai mathirai* on Total Cholesterol levels (mg/dl) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	73.60 ± 6.65	87.75 ± 7.59	79.89 ± 5.30	71.50 ± 3.30	91.67 ± 4.14	81.58 ± 3.95	71.67 ± 0.88	83.00 ± 4.04	77.33 ± 3.14
II	70.17 ± 2.59	213.00±107.73	135.09±51.16	77.20 ± 2.27	83.67 ± 3.85	80.73 ± 2.45	65.00 ± 0.58	84.33 ±14.88	74.67 ± 7.94
III	62.33 ± 3.56	67.50 ± 0.65	64.40 ± 2.23	72.60 ± 1.94	67.00 ± 4.97	70.11 ± 2.47	65.00 ± 6.00	82.50 ± 1.50	73.75 ± 5.65
IV	75.40 ± 2.98	72.75 ± 7.87	74.22 ± 3.60	71.20 ± 3.35	85.25 ± 1.89	77.44 ± 3.13	62.50 ± 2.50	79.00 ± 6.00	72.50 ± 6.81

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 55: Effect of *Urai mathirai* on Triglyceride levels (mg/dl) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	86.80 ± 19.18	104.75 ±21.75	94.78 ± 13.82	74.83 ± 12.07	98.67 ±10.55	86.75 ± 8.44	125.33 ± 3.18	139.00 ± 9.71	132.17 ± 5.50
II	92.83 ± 5.64	67.00 ± 4.70	81.09 ± 5.41	27.20 ± 1.59	86.17 ±10.57	81.18 ± 6.67	99.33 ± 11.02	92.00 ± 7.94	95.67 ± 6.29
III	104.17± 10.63	70.25 ± 6.69	90.60 ± 8.62	89.80 ± 12.52	75.00 ± 6.28	83.22 ± 7.54	132.50 ± 16.50	122.50±16.50	127.50±9.95
IV	77.60 ±21.41	70.00 ± 5.61	74.22 ±11.59	89.60 ± 8.42	110.00±30.29	97.25 ±11.73	132.00 ± 17.00	134.00±28.00	103.25±18.02

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 56: Effect of *Urai mathirai* on HDL levels (mg/dl) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	32.84 ± 2.01	32.00 ± 1.35	32.47 ± 1.20	27.83 ± 0.87	32.00 ± 3.91	29.92 ± 2.01	25.33 ± 0.33	28.67 ± 2.91	27.00 ± 1.51
II	30.00 ± 1.51	29.80 ± 1.74	29.91± 1.08	27.20 ± 1.59	29.17 ± 0.75	28.27 ± 0.84	22.67 ± 0.88	27.33 ± 1.33	25.00 ± 1.26
III	27.83 ± 1.11	25.25 ± 1.25	26.80 ± 0.89	26.80 ± 1.16	21.50 ± 2.02	24.44 ± 1.39	30.50 ± 1.50	27..50± 0.50	29.00 ± 1.08
IV	28.40 ± 1.21	28.13 ± 2.31	28.28 ± 1.14	31.40 ± 1.75	28.67 ± 0.33	30.38 ± 1.16	29.50 ± 1.50	31.00 ± 0.00	30.00 ± 0.71

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 57: Effect of *Urai mathirai* on LDL levels (mg/dl) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	23.20 ± 3.46	34.25 ± 9.55	28.11 ± 4.72	28.67 ± 1.43	43.50 ± 5.33	36.08 ± 3.45	21.33 ± 0.333	27.00 ± 4.00	24.17 ± 2.20
II	24.17 ± 2.15	187.20±107.34	98.27 ±52.49	35.00 ± 1.00	37.50 ± 3.48	36.36 ± 1.91	20.50 ± 2.50	38.33±11.89	31.20 ± 7.88
III	13.50 ± 3.12	28.00 ± 3.03	19.30 ± 3.17	26.60 ± 2.99	34.25 ± 9.55	28.44 ± 2.37	8.00 ± 1.00	30.00 ± 2.00	19.00 ± 6.42
IV	31.40 ± 5.02	30.50 ± 5.44	31.00 ± 3.46	22.00 ± 1.58	29.33 ± 1.86	24.75 ± 1.75	6.50 ± 4.50	20.50 ± 0.50	22.00 ± 9.64

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 58: Effect of *Urai mathirai* on SGOT levels (U/L) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	313.65±22.55	284.00 ±26.57	301.79±16.94	301.00 ± 7.31	310.67±18.61	305.83 ±9.64	331.33 ±39.61	266.67±17.52	299.00±24.17
II	300.67 ± 9.79	319.83 ±34.30	310.25±17.25	332.00±25.70	298.83±15.92	315.42±15.25	290.00 ±35.12	321.67 ± 6.44	305.83±17.47
III	227.17±11.53	304.80 ±23.70	262.45±16.97	273.00±16.90	417.00±30.56	338.45±27.60	331.67 ±10.40	317.50±14.50	326.00 ± 8.09
IV	278.50±14.75	265.75 ±12.99	273.40 ± 9.97	247.80±10.08	333.75±21.80	286.00±18.32	285.50 ±19.50	299.00±61.00	299.00±17.01

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 59: Effect of *Urai mathirai* on SGPT levels (U/L) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	66.35 ± 2.10	65.75 ± 6.45	66.11 ± 2.65	61.00 ± 2.22	65.67 ± 4.89	63.33 ± 2.66	58.33 ± 12.44	57.33 ± 8.65	57.83 ± 6.78
II	65.17 ± 3.46	76.83 ±16.21	71.00 ± 8.10	67.50 ± 1.48	58.67 ± 2.73	63.08 ± 1.99	58.33 ± 9.40	55.33 ± 3.84	56.83 ± 4.59
III	62.83 ± 3.13	73.80 ± 2.87	67.82 ± 2.68	69.33 ± 1.93	71.60 ± 2.36	70.36 ± 1.47	77.33 ± 7.26	57.00 ± 6.00	69.20 ± 6.65
IV	56.20 ± 4.58	66.75 ± 7.98	60.89 ± 4.46	64.20 ± 5.21	65.50 ± 2.99	64.78 ± 3.01	65.50 ± 3.50	70.00 ± 9.00	61.75 ± 3.86

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 60: Effect of *Urai mathirai* on ALP levels (U/L) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	326.00 ± 21.67	25.00 ± 21.15	295.60 ± 19.24	240.33 ± 23.78	174.83 ± 7.87	207.58 ± 15.49	217.67 ± 12.67	174.00 ± 27.50	195.83 ± 16.69
II	306.67 ± 10.48	250.17 ± 29.91	278.42 ± 17.34	224.33 ± 18.00	204.50 ± 31.15	214.42 ± 17.41	185.00 ± 7.94	157.33 ± 28.83	171.17 ± 14.74
III	298.00 ± 21.70	422.00 ± 36.28	354.36 ± 27.37	255.83 ± 10.71	316.60 ± 57.81	283.45 ± 27.03	260.50 ± 41.50	205.50 ± 30.50	233.00 ± 26.35
IV	290.20 ± 21.12	264.75 ± 30.97	278.89 ± 17.43	312.00 ± 8.57	207.50 ± 19.96	265.56 ± 20.59	315.00 ± 10.00	276.00 ± 36.00	248.25 ± 39.74

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 61: Effect of *Urai mathirai* on GGT levels (U/L) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	3.17 ± 0.40	3.25 ± 0.75	3.20 ± 0.36	3.33 ± 0.33	4.50 ± 0.43	3.92 ± 0.31	2.67 ± 0.88	3.67 ± 0.88	3.17 ± 0.60
II	4.17 ± 0.54	3.40 ± 0.81	3.82 ± 0.46	2.80 ± 0.92	2.92 ± 0.52	2.86 ± 0.48	3.00 ± 0.58	4.33 ± 0.67	3.67 ± 0.49
III	2.50 ± 0.43	24.75 ±20.09	11.40 ± 8.19	4.40 ± 0.68	5.00 ± 0.91	4.67 ± 0.53	3.50 ± 0.50	4.50 ± 0.50	4.00 ± 0.41
IV	2.80 ± 0.20	4.75 ± 0.48	3.67 ± 0.41	4.40 ± 0.24	4.75 ± 0.48	4.56 ± 0.24	2.50 ± 1.50	3.50 ± 1.50	3.75 ± 1.03

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 62: Effect of *Urai mathirai* on Total Protein levels (g/dl) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	7.22 ± 0.12	7.18 ± 0.19	7.20 ± 0.10	6.87 ± 0.10	3.85 ± 0.14	7.08 ± 0.09	7.27 ± 0.29	7.40 ± 0.12	7.33 ± 0.14
II	6.82 ± 0.23	7.03 ± 0.25	6.93 ± 0.16	6.92 ± 0.13	7.20 ± 0.19	7.06 ± 0.12	6.77 ± 0.26	7.17 ± 0.52	6.97 ± 0.28
III	6.52 ± 0.09	6.82 ± 0.23	6.65 ± 0.12	16.68 ± 9.98	7.02 ± 0.12	11.85 ± 4.97	7.20 ± 0.20	7.20 ± 0.20	7.20 ± 0.12
IV	7.14 ± 0.18	7.13 ± 0.26	7.13 ± 0.14	7.04 ± 0.27	7.55 ± 0.22	7.27 ± 0.19	6.85 ± 0.05	7.20 ± 0.40	7.18 ± 0.19

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 63: Effect of *Urai mathirai* on Albumin levels (g/dl) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	3.55 ± 0.15	3.88 ± 0.03	3.68 ± 0.10	3.47 ± .11	3.85 ± 0.14	3.66 ± 0.10	3.57 ± 0.20	3.97 ± 0.26	3.77 ± 0.17
II	3.32 ± 0.07	3.60 ± 0.04	3.46 ± 0.06	3.43 ± 0.07	3.73 ± 0.08	3.58 ± 0.07	3.27 ± 0.07	3.67 ± 0.18	3.47 ± 0.12
III	3.43 ± 0.06	3.12 ± 0.07	3.29 ±0.07	3.36 ± 0.14	3.08 ± 0.28	3.22 ± 0.15	3.40 ± 0.00	3.55 ± 0.35	3.48 ± 0.15
IV	3.62 ± 0.08	3.53 ± 0.15	3.58 ± 0.08	3.54 ± 0.08	3.85 ± 0.10	3.68 ± .08	3.45 ± 0.15	3.50 ± 0.20	3.48 ± 0.10

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 64: Effect of *Urai mathirai* on LDH levels (U/L) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day		
	MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**
I	384.00 ± 162.34	585.00 ± 158.43	464.40 ± 114.93	505.33 ± 79.88	372.67 ± 56.48	439.00 ± 50.75
II	524.67 ± 103.54	562.60 ± 114.58	541.91 ± 73.12	647.00 ± 156.16	709.50 ± 189.55	681.09 ± 119.70
III	1464.50 ± 138.09	1076.75 ± 339.65	1309.40 ± 160.46	1361.20 ± 136.50	221.75 ± 90.00	854.78 ± 215.87
IV	805.40 ± 208.70	1244.50 ± 157.47	1000.56 ± 148.94	1717.60 ± 77.34	695.75 ± 88.70	1263.44 ± 187.62

Table 65: Effect of *Urai mathirai* on CRP levels (mg/L) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	0.43 ± 0.13	0.43 ± 0.13	0.43 ± 0.09	0.60 ± 0.16	0.32 ± 0.10	0.46 ± 0.10	0.90 ± 0.12	0.63 ± 0.15	0.77 ± 0.10
II	0.45 ± 0.10	0.62 ± 0.15	0.53 ± 0.09	0.55 ± 0.23	0.87 ± 0.10	0.71 ± 0.13	1.10 ± 0.06	0.90 ± 0.12	1.00 ± 0.07
III	0.28 ± 0.07	0.78 ± 0.24	0.48 ± 0.12	0.44 ± 0.14	1.08 ± 0.19	0.72 ± 0.16	0.60 ± 0.50	1.15 ± 0.05	0.88 ± 0.26
IV	0.28 ± 0.05	0.78 ± 0.25	0.50 ± 0.14	0.88 ± 0.20	0.58 ± 0.11	0.74 ± 0.13	0.75 ± 0.55	1.60 ± 0.00	0.98 ± 0.31

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 66: Effect of *Urai mathirai* on Creatinine Kinase levels (U/L) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	1210.50 ± 127.57	1248.50 ± 225.68	1225.70 ± 110.70	1365.00 ± 63.18	1565.00 ± 84.05	1465.00 ± 58.50	1490.00 ± 99.00	1194.33 ± 106.33	1312.60 ± 98.07
II	1243.83 ± 61.10	1357.17 ± 306.50	1300.50 ± 149.97	1325.00 ± 108.08	1429.83 ± 191.50	1382.18 ± 111.35	1550.33 ± 542.70	1539.33 ± 58.33	1544.83 ± 244.11
III	741.83 ± 40.94	545.50 ± 95.05	663.30 ± 52.83	1213.40 ± 106.82	1906.50 ± 270.50	1411.43 ± 158.93	516.00 ± 120.00	550.00 ± 71.00	533.00 ± 57.76
IV	882.80 ± 62.72	729.25 ± 83.30	814.56 ± 54.56	1260.20 ± 221.28	1507.25 ± 155.03	1370.00 ± 139.61	1135.00 ± 184.00	1437.00 ± 0.00	1230.75 ± 103.60

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 67: Effect of *Urai mathirai* on Creatinine Kinase – MB levels (U/L) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	493.67 ± 46.43	449.25 ± 35.12	475.90 ± 30.59	567.00 ± 25.62	625.83 ± 27.04	596.42 ± 19.85	693.00 ± 99.04	479.00 ± 71.67	586.00 ± 72.66
II	486.33 ± 13.66	434.60 ± 29.66	462.82 ± 16.65	553.00 ± 51.07	523.33 ± 51.52	536.82 ± 34.93	515.67 ± 85.84	594.67 ± 40.93	555.17 ± 46.05
III	303.83 ± 10.22	276.75 ± 21.65	293.00 ± 10.81	401.00 ± 15.25	691.25 ± 71.85	530.00 ± 59.37	516.00 ± 120.00	550.00 ± 71.00	533.00 ± 57.76
IV	375.20 ± 29.92	256.25 ± 3.64	322.33 ± 26.22	362.80 ± 15.30	505.75 ± 13.53	426.33 ± 26.95	484.00 ± 70.00	613.00 ± 0.00	500.00 ± 49.70

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 68: Effect of *Urai mathirai* on Urea levels (mg/dl) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	0.53 ± 0.02	0.53 ± 0.03	0.53 ± 0.02	0.37 ± 0.05	0.40 ± 0.04	0.38 ± 0.03	0.60 ± 0.10	0.43 ± 0.03	0.52 ± 0.06
II	0.45 ± 0.04	0.48 ± 0.05	0.47 ± 0.03	0.37 ± 0.03	0.38 ± 0.03	0.38 ± 0.02	0.40 ± 0.10	0.37 ± 0.07	0.38 ± 0.05
III	0.42 ± 0.02	0.44 ± 0.02	0.43 ± 0.01	0.45 ± 0.02	0.46 ± 0.05	0.45 ± 0.02	0.47 ± 0.03	0.35 ± 0.05	0.42 ± 0.04
IV	0.53 ± 0.06	0.40 ± 0.03	0.47 ± 0.04	0.48 ± 0.08	0.43 ± 0.02	0.46 ± 0.04	0.30 ± 0.00	0.40 ± 0.00	0.38 ± 0.05

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 69: Effect of *Urai mathirai* on serum creatinine levels (mg/dl) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	32.60 ± 0.93	34.00 ± 2.65	33.22 ± 1.21	28.00 ± 1.93	38.80 ± 4.59	32.91 ± 2.78	34.00 ± 3.21	32.00 ± 1.00	33.00 ± 1.57
II	35.17 ± 2.30	36.17 ± 2.96	35.67 ± 1.79	29.83 ± 1.56	29.50 ± 1.34	29.67 ± 0.98	33.67 ± 0.88	35.00 ± 2.31	34.33 ± 1.15
III	30.50 ± 1.06	41.00 ± 4.00	35.27 ± 2.44	29.17 ± 1.76	40.00 ± 3.30	34.09 ± 2.39	28.00 ± 2.65	37.50 ± 4.50	31.80 ± 3.09
IV	32.00 ± 1.61	30.80 ± 1.98	31.45 ± 1.21	30.80 ± 1.59	37.25 ± 3.52	33.67 ± 2.01	28.50 ± 0.50	35.00 ± 0.00	31.75 ± 1.89

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 70: Effect of *Urai mathirai* on Total Bilirubin levels (mg/dl) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM								
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	0.20 ± 0.03	0.21 ± 0.03	0.20 ± 0.02	0.15 ± 0.02	0.20 ± 0.03	0.18 ± 0.02	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.02
II	0.18 ± 0.01	0.23 ± 0.02	0.20 ± 0.01	0.18 ± 0.02	0.17 ± 0.02	0.18 ± 0.01	0.13 ± 0.03	0.10 ± 0.00	0.12 ± 0.02
III	0.15 ± 0.01	0.16 ± 0.03	0.15 ± 0.01	0.13 ± 0.02	0.22 ± 0.04	0.17 ± 0.02	0.10 ± 0.00	0.15 ± 0.05	0.12 ± 0.02
IV	0.18 ± 0.02	0.19 ± 0.02	0.19 ± 0.02	0.12 ± .02	0.20 ± 0.00	0.16 ± 0.02	0.10 ± 0.00	0.20 ± 0.00	0.15 ± 0.03

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 71: Effect of *Urai mathirai* on Uric acid levels (mg/dl) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	1.22 ± 0.19	1.28 ± 0.33	1.24 ± 0.16	0.82 ± 0.14	1.65 ± 0.19	1.23 ± 0.17	12.07 ± 11.37	1.00 ± 0.26	6.53 ± 5.66
II	0.92 ± 0.11	1.42 ± 0.21	1.17 ± 0.13	0.93 ± 0.12	1.33 ± 0.16	1.13 ± 0.11	1.20 ± 0.21	1.40 ± 0.12	1.30 ± 0.12
III	0.77 ± 0.03	1.18 ± 0.23	0.93 ± 0.11	0.62 ± 0.14	1.94 ± 0.07	1.28 ± 0.23	1.05 ± 0.05	0.95 ± 0.05	1.00 ± 0.04
IV	1.14 ± 0.11	1.48 ± 0.25	1.29 ± 0.13	1.30 ± 0.39	1.43 ± 0.15	1.36 ± 0.22	0.75 ± 0.05	1.40 ± 0.00	1.43 ± 0.48

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 72: Effect of *Urai mathirai* on Serum Calcium levels (mg/dl) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	11.16 ± 0.13	10.98 ± 0.34	11.08 ± 0.15	9.00 ± 0.24	9.20 ± 0.17	9.10 ± 0.14	9.67 ± 0.62	9.57 ± 0.12	9.62 ± 0.28
II	10.28 ± 0.18	10.67 ± 0.33	10.48 ± 0.19	8.80 ± 0.13	12.50 ± 3.38	10.65 ± 1.71	8.73 ± 0.28	9.47 ± 0.52	9.10 ± 0.31
III	9.98 ± 0.24	10.30 ± 0.21	10.11 ± 0.16	8.92 ± 0.22	8.30 ± 0.24	8.61 ± 0.18	9.55 ± 0.55	9.05 ± 0.05	9.30 ± 0.27
IV	10.54 ± 0.14	10.95 ± 0.46	10.72 ± 0.21	9.88 ± 0.33	9.23 ± 0.12	9.59 ± 0.22	8.65 ± 0.05	9.40 ± 0.00	9.23 ± 0.37

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 73: Effect of *Urai mathirai* on T3 levels (ng/ml) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	0.65 ± 0.05	0.60 ± 0.06	0.62 ± 0.04	1.55 ± 0.06	2.63 ± 0.18	2.09 ± 0.22	2.03 ± 0.79	1.30 ± 0.23	1.67 ± 0.40
II	0.32 ± 0.11	0.42 ± 0.14	0.36 ± 0.08	1.38 ± 0.21	1.53 ± 0.20	1.44 ± 0.14	1.17 ± 0.59	0.90 ± 0.10	1.06 ± 0.33
III	0.27 ± 0.06	0.33 ± 0.05	0.29 ± 0.04	1.04 ± 0.18	1.18 ± 0.14	1.10 ± 0.11	1.60 ± 0.60	INS	1.27 ± 0.48
IV	0.35 ± 0.12	1.93 ± 0.83	1.03 ± 0.45	0.98 ± 0.23	1.60 ± 0.25	1.24 ± 0.20	0.90 ± 0.00	0.90 ± 0.00	1.23 ± 0.33

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 74: Effect of *Urai mathirai* on T4 levels (µg/ml) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	4.10 ± 0.10	3.23 ± 0.03	3.58 ± 0.22	8.43 ± 0.65	14.40 ± 1.74	11.41 ± 1.42	9.83 ± 1.00	9.50 ± 0.21	9.67 ± 0.46
II	3.42 ± 0.14	2.54 ± 0.54	3.02 ± 0.28	8.20 ± 0.58	8.77 ± 0.78	8.44 ± 0.44	8.17 ± 0.55	7.45 ± 0.45	7.88 ± 0.38
III	2.65 ± 0.57	4.53 ± 0.50	3.40 ± 0.49	8.28 ± 0.66	9.88 ± 0.46	8.99 ± 0.49	5.25 ± 0.35	INS	6.37 ± 1.13
IV	3.88 ± 0.48	4.53 ± 2.29	4.16 ± 0.91	7.88 ± 0.70	14.63 ± 1.19	10.77 ± 1.48	8.15 ± 1.55	16.00 ± 0.00	10.38 ± 1.99

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 75: Effect of *Urai mathirai* on TSH levels (mIU/ml) of albino rats – Sub Acute Toxicity Study

Groups	15th day			29th day			43rd day (Post Treatment)		
	MEAN ± SEM			MEAN ± SEM			MEAN ± SEM		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male***	Female***	M + F*
I	0.25 ± 0.15	0.13 ± 0.03	0.18 ± 0.06	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.02	0.10 ± 0.00	0.13 ± 0.03	0.12 ± 0.02
II	0.13 ± 0.02	0.12 ± 0.02	0.13 ± 0.01	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.20 ± 0.00	0.14 ± 0.02
III	0.80 ± 0.37	0.15 ± 0.03	0.54 ± 0.24	0.12 ± 0.02	0.10 ± 0.00	0.11 ± 0.01	0.15 ± 0.05	INS	0.13 ± 0.03
IV	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.02	0.80 ± 0.55	0.30 ± 0.20	0.59 ± 0.32	0.15 ± 0.05	0.10 ± 0.00	0.13 ± 0.03

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 76: Effect of *Urai mathirai* on Feed intake (gm) of albino rats – Sub Acute Toxicity Study

Groups	Week 1 (MEAN ± SEM)			Week 2 (MEAN ± SEM)			Week 3 (MEAN ± SEM)		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male*	Female*	M + F**
I	107.45 ± 2.38	106.9 ± 2.30	214.35 ± 0.08	102.20 ± 5.77	75.75 ± 10.82	177.95 ± 5.05	120.00 ± 0.00	120.00 ± 0.00	240.00 ± 0.00
II	117.00 ± 1.60	82.55 ± 2.65	199.55 ± 1.04	81.00 ± 20.85	61.20 ± 1.18	142.20 ± 19.67	120 .00 ± 0.00	120.00 ± 0.00	240.00 ± 0.00
III	110.10 ± 5.29	79.70 ± 4.97	189.80 ± 0.32	120.00 ± 0.00	70.05 ± 5.21	190.05 ± 5.21	120.00 ± 0.00	104.80 ± 4.22	224.80 ± 4.22
IV	108.75 ± 3.82	82.70 ± 10.53	191.45 ± 6.71	120.00 ± 0.00	74.70 ± 6.90	194.70 ± 6.90	120.00 ± 0.00	92.05 ± 2.22	212.05 ± 2.22
Groups	**Week 4 (MEAN ± SEM)**			**Week 5 (MEAN ± SEM)**			**Week 6 (MEAN ± SEM)**		
	Male*	**Female***	**M + F****	**Male***	**Female***	**M + F****	**Male***	**Female***	**M + F****
I	120.00 ± 0.00	102.60 ± 9.30	222.60 ± 9.30	60.00 ± 0.00	47.75 ± 4.09	107.75 ± 4.09	53.30 ± 3.58	46.58 ± 3.94	99.88 ± 7.52
II	120.00 ± 0.00	111.00 ± 4.81	231.00 ± 4.81	60.00 ± 0.00	36.75 ± 0.24	96.75 ± 0.24	54.50 ± 2.94	34.55 ± 0.29	89.05 ± 2.65
III	120.00 ± 0.00	72.90 ± 11.28	192.90 ± 11.28	60.00 ± 0.00	28.15 ± 0.61	88.15 ± 0.61	60.00 ± 0.00	24.90 ± 2.83	84.90 ± 2.83
IV	117.65 ± 1.26	81.45 ± 9.33	199.10 ± 10.58	34.65 ± 1.15	24.85 ± 2.06	59.50 ± 0.91	36.25 ± 2.00	30.60 ± 3.80	66.85 ± 5.80

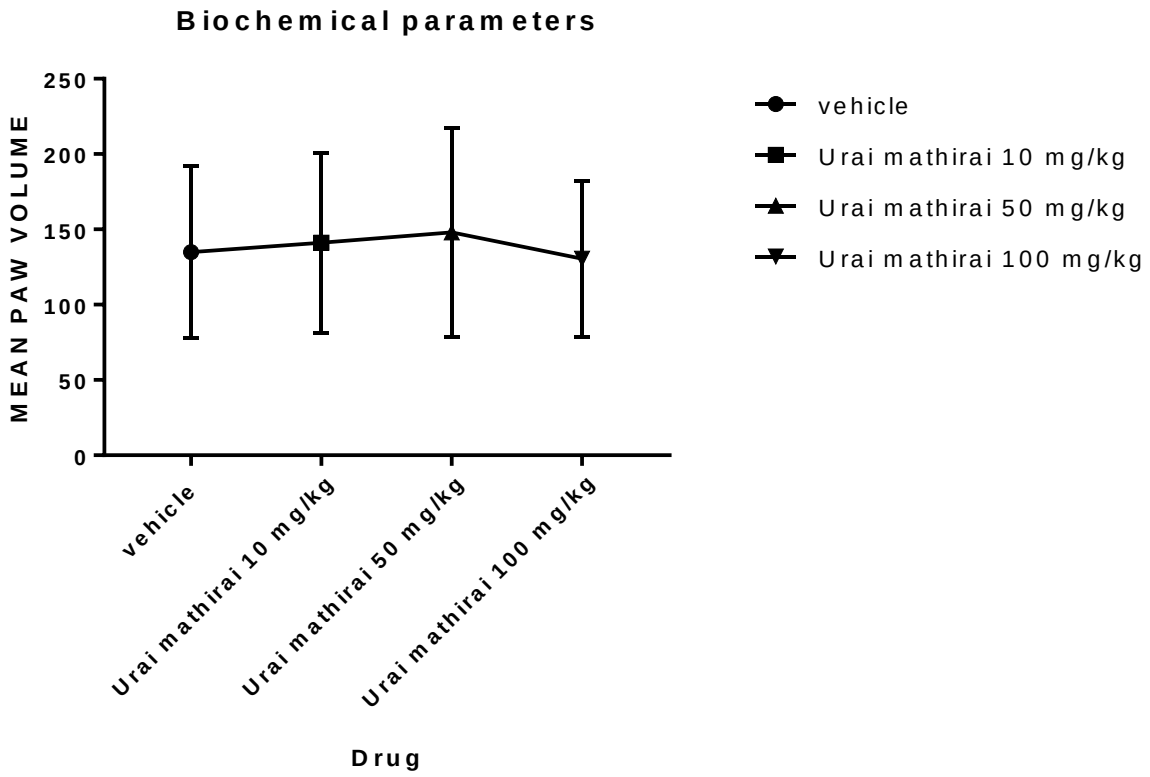
Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Table 77: Effect of *Urai mathirai* on water intake (ml) of albino rats – Sub Acute Toxicity Study

Groups	Week 1 (MEAN ± SEM)			Week 2 (MEAN ± SEM)			Week 3 (MEAN ± SEM)		
	Male*	Female*	M + F**	Male*	Female*	M + F**	Male*	Female*	M + F**
I	50.00 ± 16.04	165.00 ± 4.81	215.00 ± 11.22	154.00 ± 18.17	115.00 ± 11.22	269.00 ± 29.40	176.50 ± 33.94	159.50 ± 43.56	336.00 ± 77.51
II	95.00 ± 20.85	124.00 ± 12.83	219.00 ± 8.02	83.00 ± 1.60	173.00 ± 43.30	256.00 ± 41.69	217.00 ± 1.60	143.00 ± 40.09	360.00 ± 38.49
III	92.00 ± 39.55	129.00 ± 2.67	221.00 ± 36.88	165.00 ± 42.23	82.00 ± 11.76	247.00 ± 53.99	235.00 ± 5.88	167.00 ± 21.38	402.00 ± 27.26
IV	90.00 ± 31.00	186.00 ± 7.48	276.00 ± 38.49	142.00 ± 8.55	78.00 ± 5.35	220.00 ± 13.90	155.00 ± 29.93	106.50 ± 42.49	261.50 ± 72.43
Groups	**Week 4 (MEAN ± SEM)**			**Week 5 (MEAN ± SEM)**			**Week 6 (MEAN ± SEM)**		
	Male*	**Female***	**M + F****	**Male***	**Female***	**M + F****	**Male***	**Female***	**M + F****
I	127.00 ± 9.09	135.00 ± 8.02	262.00 ± 1.07	70.00 ± 7.48	57.00 ± 1.60	127.00 ± 9.09	108.00 ± 22.45	55.00 ± 9.09	163.00 ± 13.36
II	139.00 ± 0.53	126.50 ± 1.34	265.50 ± 1.87	79.00 ± 0.53	52.00 ± 0.00	131.00 ± 0.53	92.00 ± 1.07	69.00 ± 14.43	161.00 ± 15.50
III	174.50 ± 44.10	90.00 ± 10.69	264.50 ± 54.79	86.00 ± 10.69	41.00 ± 1.60	127.00 ± 12.29	70.00 ± 5.35	25.00 ± 1.60	95.00 ± 3.74
IV	140.00 ± 2.14	121.50 ± 12.03	261.50 ± 9.89	40.00 ± 2.14	32.00 ± 7.48	72.00 ± 9.62	40.00 ± 1.07	38.00 ± 6.41	78.00 ± 5.35

Values are expressed as Mean ± SEM *N=6, **=N=12 ***N=3

Fig. No. 53 Biochemical parameters of sub-acute toxicity



PHARMACOLOGICAL STUDIES

Anti-Inflammatory activity

Table 78: Effect of *Urai mathirai* on carrageenan induced paw edema in rats

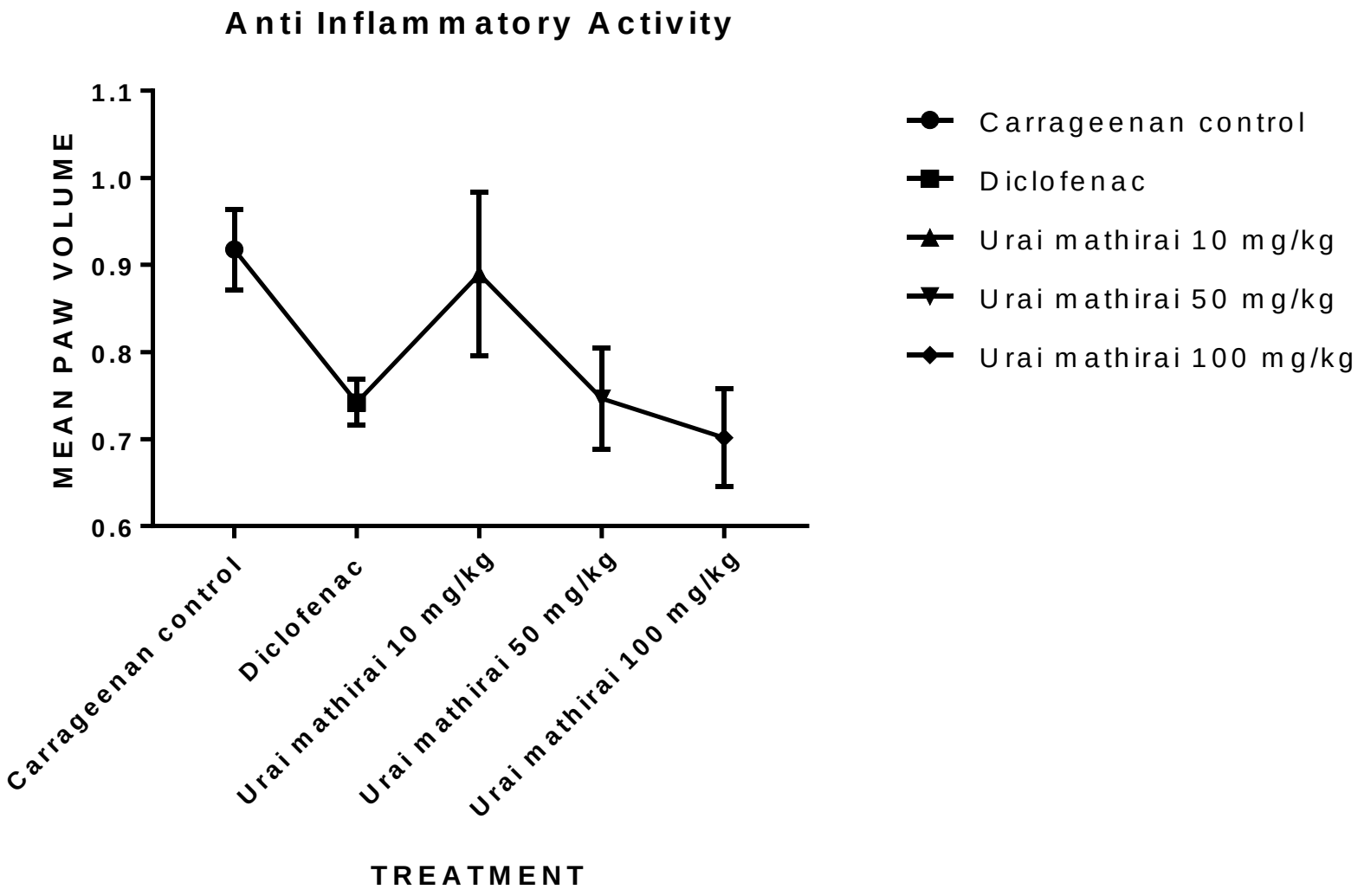
S.No	Groups	Paw Volume in ml Mean + SEM (%Inhibition of Paw edema)			
		1st hour	2nd hour	3rd hour	4th hour
1	Carrageenan control	0.9060 ± 0.06120	0.7990 ± 0.1808	0.9460 ± 0.1847	1.020 ± 0.1469
2	Diclofenac control	0.6760±0.07554(25.39)	0.7520 ± 0.1028 (5.88)	0.8040 ± 0.08358 (15.01)	0.7360 ± 0.09250 (27.84)
3	*Urai mathirai* 10 mg/kg	0.8020 ± 0.1499 (11.48)	0.7760 ± 0.09595 (2.88)	1.170 ± 0.1686 (-23.68)	0.8100 ± 0.1445 (20.59)
4	*Urai mathirai* 50 mg/kg	0.8000 ± 0.05187 (11.70)	0.8360 ± 0.05391 (-4.63)	0.7740 ± 0.1238 (18.18)	0.5760 ± 0.06234 (43.53)
5	*Urai mathirai* 100 mg/kg	0.7520 ± 0.05054 (17.00)	0.5700 ± 0.06964 (28.66)	0.6560 ± 0.05154 (30.66)	0.8280 ± 0.08952 (18.82)

Values are expressed as Mean ± SEM. (N=6)

Statistical significance test is carried out by Two-way ANOVA followed by comparison between multiple groups. The p-values less than 0.05 were considered to be statistically significant.

Data was analyzed by using Graph Pad Prism software version 7.0.3.

Fig. No. 53


Anti Inflammatory Activity
MEAN PAW VOLUME
1.1
1.0
0.9
0.8
0.7
0.6
Carrageenan control
Diclofenac
Urai mathirai 10 mg/kg
Urai mathirai 50 mg/kg
Urai mathirai 100 mg/kg
TREATMENT
Carrageenan control
Diclofenac
Urai mathirai 10 mg/kg
Urai mathirai 50 mg/kg
Urai mathirai 100 mg/kg

Analgesic activity

Table No. 79

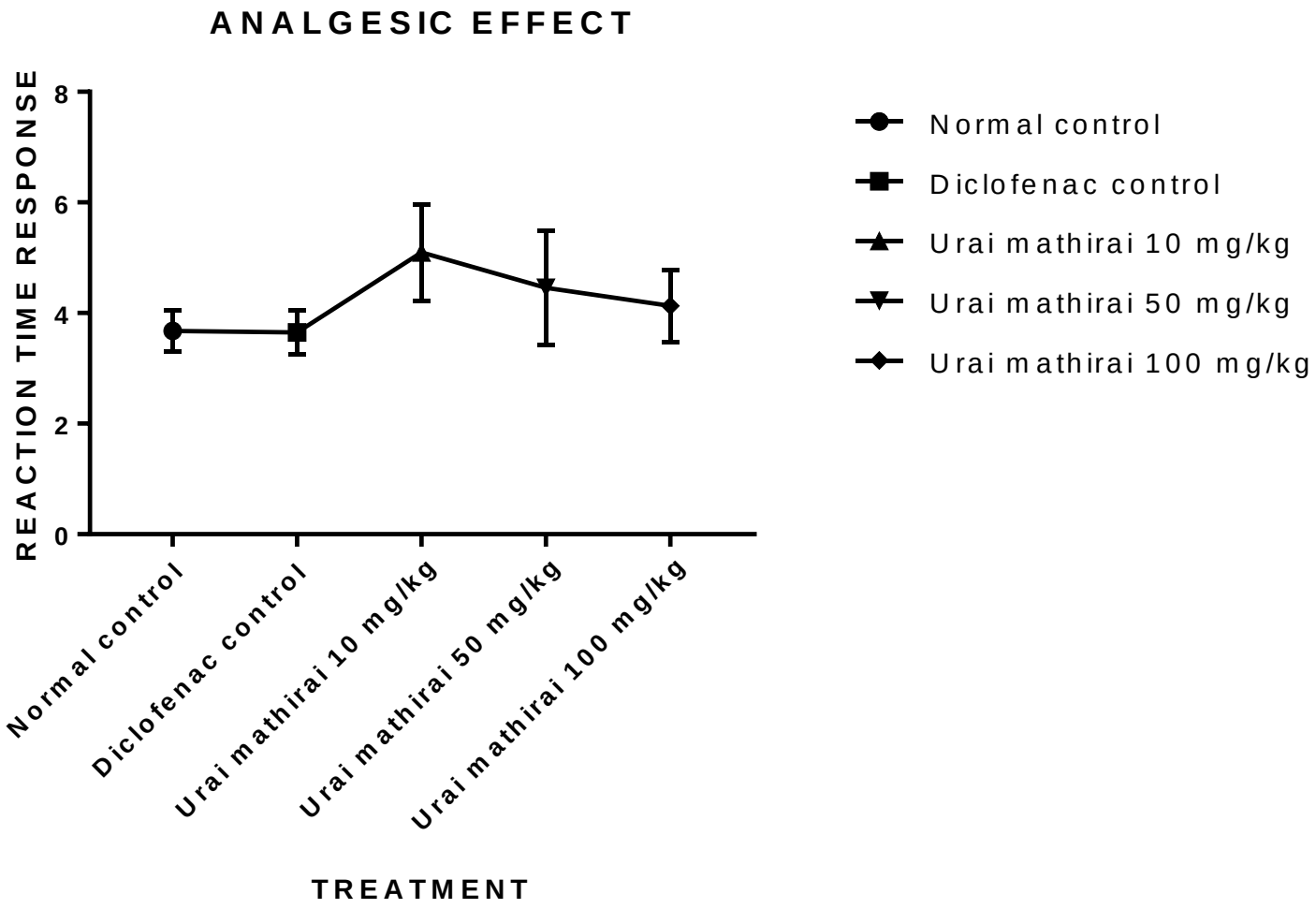
S.No	Groups	Reaction time (Sec)				
		0 hour	**1/2 hour**	**1st hour**	**2nd hour**	**4th hour**
1	Normal control	3.45 ± 0.35	3.65 ± 0.22	5.05 ± 0.54	3.4 ± 0.70	2.85 ± 0.22
2	Diclofenac control	3.25 ± 0.03	3.5 ± 0.32	4.15 ± 1.11	2.5 ± 0.13	4.85 ± 0.35
3	*Urai mathirai* 10 mg/kg	3.25 ± 0.22	5.05 ± 0.16	7.6 ± 1.39	6.45 ± 0.73	3.125 ± 0.43
4	*Urai mathirai* 50 mg/kg	2.95 ± 0.66	3.45 ± 0.03	4.55 ± 0.66	8.45 ± 0.60	2.885 ± 0.12
5	*Urai mathirai* 100 mg/kg	2.2 ± 0.57	4.3 ± 0.70	5.55 ± 0.47	5.45 ± 0.41	3.15 ± 0.47

Values are expressed as Mean ± SEM. (N=6)

Statistical significance test is carried out by Two-way ANOVA followed by comparison between multiple groups. The p-values less than 0.05 were considered to be statistically significant.

Data was analyzed by using Graph Pad Prism software version 7.0.3.

Fig. No. 54


ANALGESIC EFFECT
REACTION TIME RESPONSE
0
2
4
6
8
Normal control
Diclofenac control
Urai mathirai 10 mg/kg
Urai mathirai 50 mg/kg
Urai mathirai 100 mg/kg
TREATMENT
Normal control
Diclofenac control
Urai mathirai 10 mg/kg
Urai mathirai 50 mg/kg
Urai mathirai 100 mg/kg

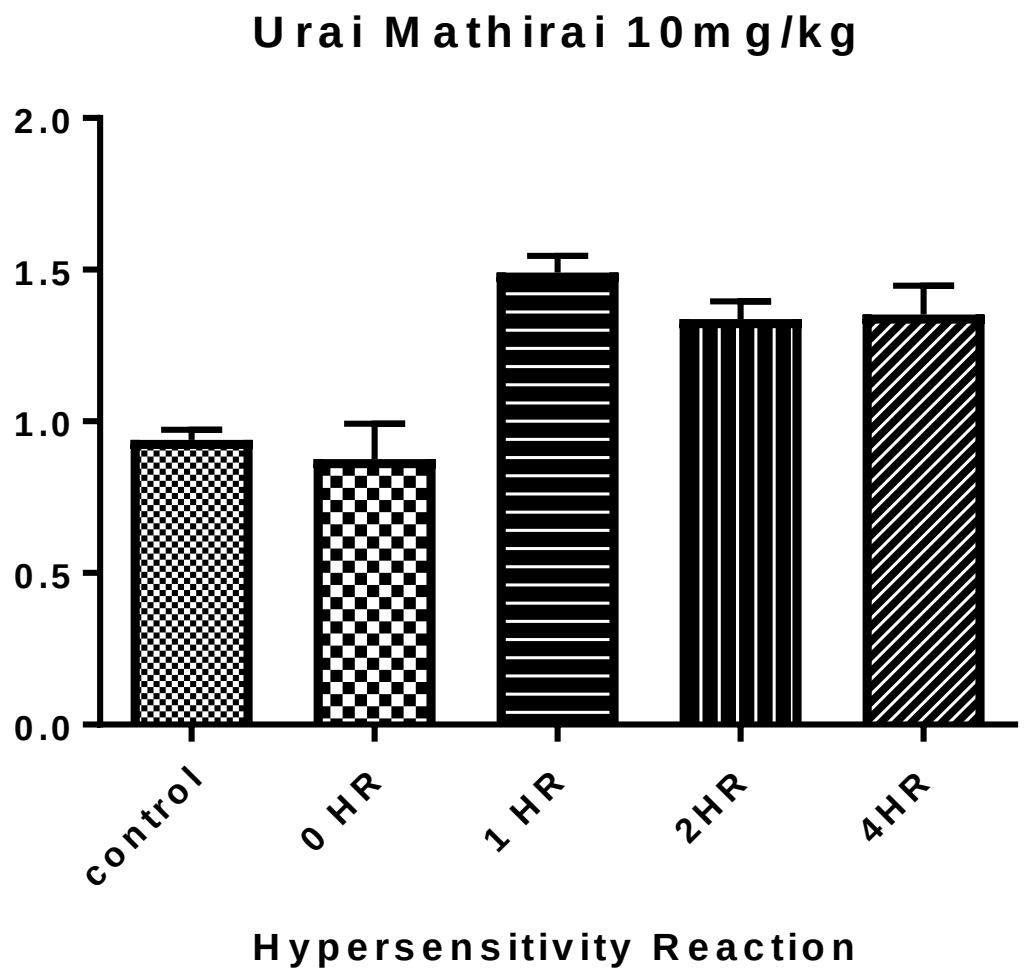
SRBC induced Immunomodulatory activity

Table 80: Effect of *Urai mathirai* on SRBC induced Immunomodulatory in rats

S. No	Groups	Paw Volume in ml Mean + SEM (%Inhibition of Paw edema)				
		Individual control	0th hour	1st hour	2nd hour	4th hour
1	SRBC	0.85 ± 0.07	1.04 ± 0.09	1.66 ± 0.04***	1.93 ± 0.03***	2.13 ± 0.05***
2	*Urai mathirai* 10 mg/kg	0.93 ± 0.03	0.87± 0.11	1.49 ± 0.05***	1.33 ± 0.05**	1.35 ± 0.09**
3	*Urai mathirai* 50 mg/kg	0.94 ± 0.03	1.15± 0.04	1.38± 0.05***	1.25± 0.06**	1.21± 0.06**
4	*Urai mathirai* 100mg/kg	0.84 ± 0.06	1.03 ± 0.08	1.41 ± 0.05***	1.28 ± 0.06**	1.23 ± 0.06**

Values are Mean ± SE, n=6 in each group. *P<0.05, **P<0.01 and ***P<0.001when compared with respective control group.

Fig. No 55


Urai Mathirai 10mg/kg
2.0
1.5
1.0
0.5
0.0
control
0 HR
1 HR
2HR
4HR
Hypersensitivity Reaction

CLINICAL STUDY REPORT

Sex distribution:

The study participants of Urai Mathirai includes 18 male child (60%) and 12 female child (40%) and Thaleesathy chooranam includes 16 male child (55%) and 14 female child (45%).

1. Ratio of Urai Mathirai male child and female child was M:F: 3:2
2. Ratio of Thaleesathy chooranam male child and female child was M:F: 8:7

Age distribution:

Minimum age of patient recruited: 6 months, Maximum age of patient recruited: 5 years.

Gender distribution:

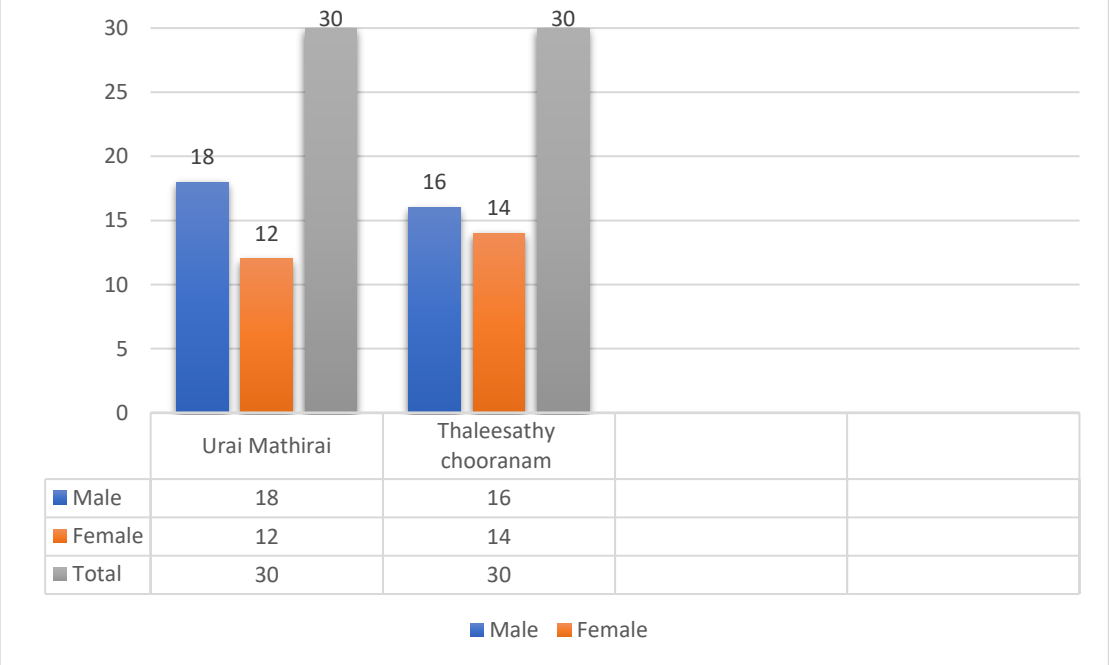
Urai Mathirai

The study participants include 18 male (60%) and 12 female (40%). Ratio of male and female patients was M:F = 3:2.

Thaleesathy chooranam

The study participants include 16 male (55%) and 14 female (45%). Ratio of male and female patients was M:F = 8:7. Show in below diagram.

Fig No.81



Educational status:

Regular school, Pre-School and Non school.

Outcome measures

Primary outcome:

- Reduction in Recurrence of Respiratory Infections
- Clinical Improvement
- No Antibiotic treatment

Secondary outcome:

- Increase in Anti bodies IgA/IgG
- Decrease in Phagocytosis

Clinical features at the time of enrolment:

The clinical features like Rhinitis, Pharyngitis, Cough, Hoarseness, Temperature More than 38.5°C, Prescription of an antibiotic for a URTI, occurring after an asymptomatic period of at least 1 week without antibiotics.

All the patients (100%, n=60) had upper respiratory tract infection at the time of enrolment.

The patients who were to be enrolled were informed about the study, trial drug, possible outcomes and the objectives of the study in the language and terms understandable to them. After ascertaining the patient's willingness, informed consent was obtained in writing in the consent form (Form IV A). All these patients were given unique registration card in which patient's registration number of the study, address, phone number and doctor' s phone number etc., were entered so as to report easily if any complication arises. Filled in screening form (Form 1) was filed separately. Form I A, Form II and Form III was used for recording the patient's history, clinical examination, laboratory investigations respectively.

Details of patients who received treatment:

Urai Mathirai Treatment period:

The date of start of the treatment and the date in which the treatment was completed are tabulated below:

Table 56: Details of Urai Mathirai treatment period each patient in main trial

P. No	Start Date	End Date	Treatment
1.	03.06.2017	29.11.2017	180 days
2.	03.06.2017	29.11.2017	180 days
3.	03.06.2017	29.11.2017	180 days
4.	03.06.2017	29.11.2017	180 days
5.	03.06.2017	29.11.2017	180 days
6.	03.06.2017	29.11.2017	180 days
7.	03.06.2017	29.11.2017	180 days
8.	03.06.2017	29.11.2017	180 days
9.	10.06.2017	06.12.2017	180 days
10.	10.06.2017	06.12.2017	180 days
11.	10.06.2017	06.12.2017	180 days
12.	10.06.2017	06.12.2017	180 days
13.	10.06.2017	06.12.2017	180 days
14.	10.06.2017	06.12.2017	180 days
15.	10.06.2017	06.12.2017	180 days
16.	10.06.2017	06.12.2017	180 days
17.	17.06.2017	13.12.2017	180 days
18.	17.06.2017	13.12.2017	180 days

19.	17.06.2017	13.12.2017	180 days
20.	17.06.2017	13.12.2017	180 days
21.	17.06.2017	13.12.2017	180 days
22.	17.06.2017	13.12.2017	180 days
23.	17.06.2017	13.12.2017	180 days
24.	17.06.2017	13.12.2017	180 days
25.	17.06.2017	13.12.2017	180 days
26.	17.06.2017	13.12.2017	180 days
27.	17.06.2017	13.12.2017	180 days
28.	17.06.2017	13.12.2017	180 days
29.	17.06.2017	13.12.2017	180 days
30.	17.06.2017	13.12.2017	180 days

Thaleesathy chooranam Treatment period:

The date of start of the treatment and the date in which the treatment was completed are tabulated below:

Table 57: Details Thaleesathy chooranam treatment period each patient in main trial

P. No	Start Date	End Date	Treatment
1.	03.06.2017	29.11.2017	180 days
2.	03.06.2017	29.11.2017	180 days
3.	03.06.2017	29.11.2017	180 days
4.	03.06.2017	29.11.2017	180 days
5.	03.06.2017	29.11.2017	180 days
6.	03.06.2017	29.11.2017	180 days

7.	03.06.2017	29.11.2017	180 days
8.	03.06.2017	29.11.2017	180 days
9.	10.06.2017	06.12.2017	180 days
10.	10.06.2017	06.12.2017	180 days
11.	10.06.2017	06.12.2017	180 days
12.	10.06.2017	06.12.2017	180 days
13.	10.06.2017	06.12.2017	180 days
14.	10.06.2017	06.12.2017	180 days
15.	10.06.2017	06.12.2017	180 days
16.	10.06.2017	06.12.2017	180 days
17.	17.06.2017	13.12.2017	180 days
18.	17.06.2017	13.12.2017	180 days
19.	17.06.2017	13.12.2017	180 days
20.	17.06.2017	13.12.2017	180 days
21.	17.06.2017	13.12.2017	180 days
22.	17.06.2017	13.12.2017	180 days
23.	17.06.2017	13.12.2017	180 days
24.	17.06.2017	13.12.2017	180 days
25.	17.06.2017	13.12.2017	180 days
26.	17.06.2017	13.12.2017	180 days
27.	17.06.2017	13.12.2017	180 days
28.	17.06.2017	13.12.2017	180 days
29.	17.06.2017	13.12.2017	180 days
30.	17.06.2017	13.12.2017	180 days

Clinical symptoms score before and after treatment:

The clinical symptoms before and after treatment is given in table.

The table shows the scores and symptoms before and after treatment. A paired sample t test was conducted to compare the scores of the clinical signs/symptoms before and after treatment.

Table No 58: Urai Mathirai

	A		B		C		D		E		F		G		H		I		J		K		L		M		N		O		P		Q	
	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES
S1	3	2	2	0	2	1	3	0	2	1	2	1	2	0	2	1	2	1	3	2	2	1	3	3	3	2	3	2	2	1	2	1	2	1
S2	3	1	3	1	1	0	0	0	1	0	1	0	3	1	1	0	1	0	3	1	1	0	3	1	3	1	3	1	1	0	1	0	1	0
S3	3	2	3	0	2	1	1	1	3	0	2	1	3	0	3	0	2	1	3	2	2	0	3	3	3	2	3	2	2	0	1	0	2	1
S4	3	3	3	1	3	3	3	3	1	0	3	3	3	1	1	0	3	3	3	3	1	0	2	0	3	3	3	3	1	0	1	0	3	3
S5	3	1	3	0	1	0	3	1	0	0	1	0	3	0	1	0	1	0	3	1	2	1	2	1	3	1	3	1	2	0	3	2	1	0
S6	2	0	3	1	2	1	3	3	3	0	2	0	3	1	3	0	2	1	2	0	3	0	3	3	2	0	1	0	3	0	3	0	1	0
S7	3	3	3	0	3	0	2	0	3	0	3	0	3	0	2	0	3	0	3	3	2	0	3	2	3	3	3	3	2	0	2	0	3	0
S8	3	1	3	2	1	0	3	1	2	1	1	0	3	2	2	0	1	0	3	1	1	0	3	1	3	1	3	1	1	0	1	0	1	0
S9	2	1	3	1	1	0	2	1	1	1	1	0	3	1	1	1	1	0	2	1	1	1	3	3	2	1	2	1	1	1	1	1	1	0
S10	3	2	3	2	2	1	1	0	2	1	2	1	3	2	2	0	2	1	3	2	1	0	3	1	3	2	3	2	2	1	3	2	2	1
S11	3	3	3	1	3	1	3	0	1	1	3	2	3	2	1	1	3	3	3	0	1	1	2	0	3	3	3	1	1	1	1	1	2	1
S12	3	1	3	1	1	0	2	1	1	0	1	0	3	1	2	0	1	0	3	1	3	2	1	0	3	1	3	1	2	1	2	0	1	0
S13	3	1	2	0	3	1	1	0	1	1	3	0	2	0	1	1	2	0	3	3	1	1	2	1	3	0	3	3	1	1	1	1	3	0
S14	2	0	3	3	2	0	3	0	1	0	2	1	3	3	2	0	3	2	2	0	2	1	3	0	2	0	2	0	2	1	2	0	1	0
S15	3	1	3	3	1	0	3	0	0	0	1	0	3	3	2	1	1	0	3	1	2	1	2	0	3	1	2	1	2	1	2	1	1	0
S16	3	1	3	2	3	3	1	0	1	1	3	3	3	2	2	1	2	1	3	0	2	1	1	0	3	2	3	1	2	1	3	1	2	1
S17	3	2	3	1	1	0	1	1	3	3	2	1	3	1	2	1	2	1	3	2	2	1	1	1	3	2	3	2	1	1	2	1	2	1
S18	3	1	3	3	1	1	0	0	3	3	1	0	3	3	1	0	1	0	3	1	1	0	0	0	3	1	3	1	1	0	1	0	1	0

S19	3	1	3	1	3	0	1	1	2	0	2	0	3	1	2	1	3	0	3	3	2	1	1	1	3	3	3	3	2	1	2	1	3	0
S20	3	1	2	0	3	3	0	0	3	1	1	0	2	0	3	3	1	0	3	1	3	3	1	0	3	1	3	1	3	3	2	1	1	0
S21	2	0	3	3	2	0	1	1	3	0	2	0	3	1	1	0	2	1	2	0	1	0	2	0	2	0	2	0	1	0	1	0	1	0
S22	3	2	2	1	3	1	1	0	1	0	3	0	3	2	2	1	3	0	3	3	1	0	3	0	3	0	2	1	3	2	2	0	3	0
S23	3	1	3	1	2	0	3	3	1	1	3	0	3	1	3	0	2	0	3	3	3	0	2	0	3	1	2	0	3	0	2	0	3	1
S24	2	0	2	1	3	1	3	1	2	0	3	2	2	1	1	0	1	0	2	0	1	0	2	1	2	0	2	0	1	0	1	0	1	0
S25	3	1	3	2	1	1	3	3	3	1	1	1	3	2	1	0	1	1	3	1	1	0	1	1	3	1	3	1	1	0	1	0	1	1
S26	3	1	2	1	2	0	2	0	3	2	1	0	3	3	2	1	2	0	3	0	2	1	1	0	3	0	2	1	2	1	2	1	1	0
S27	3	1	2	1	3	2	3	1	3	1	1	1	3	1	2	1	1	1	3	1	2	0	1	1	3	1	3	1	1	0	2	0	1	1
S28	1	0	3	1	3	2	3	0	3	3	1	0	3	2	1	0	1	0	2	1	1	0	1	0	2	0	3	2	0	0	1	0	2	0
S29	3	1	2	0	3	1	0	0	2	0	1	1	2	0	2	0	1	1	3	1	2	0	1	1	3	1	3	1	2	0	2	0	1	1
S30	1	0	3	1	2	1	1	1	3	1	2	0	3	1	3	2	1	0	2	0	3	2	2	0	3	0	3	0	1	0	2	0	1	0

A - Poor appetite

B - Not sleeping well

C - Irritable, cranky, fussy

D - Feels unwell

E - Low energy, tired

F - Not playing well

G - Crying more than usual

H - Clinginess

I - Headache

J - Sore throat

K - Muscle aches or pains

L - Fever

M - Cough

N - Nasal congestion, runny nose.

O - Vomiting

P - Not interested in what's going on

Q - Unable to get out of bed

Table No 59 Thaleesathy chooranam

	A		B		C		D		E		F		G		H		I		J		K		L		M		N		O		P		Q	
	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES
S1	3	2	3	2	3	2	2	0	2	1	3	0	2	1	2	1	2	0	2	1	2	1	3	2	2	1	3	3	3	2	3	2	2	1
S2	3	1	3	1	3	1	3	1	1	0	0	0	1	0	1	0	3	1	1	0	1	0	3	1	1	0	3	1	3	1	3	1	1	0
S3	3	2	3	2	3	2	3	2	2	1	1	1	3	0	2	1	3	0	3	0	2	1	3	2	2	0	3	3	3	2	3	2	2	0
S4	3	3	3	2	3	3	3	1	3	3	3	3	1	0	3	3	3	1	1	0	3	3	3	3	1	0	2	0	3	3	3	3	1	0
S5	3	1	3	1	3	1	3	0	1	0	3	1	0	0	1	0	3	0	0	0	1	0	3	1	0	0	2	1	3	1	3	1	0	0
S6	2	0	2	0	2	0	3	1	0	0	3	3	3	2	0	0	3	1	3	0	0	0	2	0	3	0	3	3	2	0	2	0	3	0
S7	3	3	3	3	3	3	3	2	3	2	2	0	3	2	3	2	3	0	2	0	3	0	3	3	2	0	3	2	3	3	3	3	2	0
S8	3	1	3	1	3	1	3	2	1	0	3	1	0	0	1	0	3	2	0	0	1	0	3	1	0	0	3	1	3	1	3	1	0	0
S9	2	1	2	1	2	1	3	1	1	0	2	1	1	1	1	0	3	1	1	1	1	0	2	1	1	1	3	3	2	1	2	1	1	1
S10	3	2	3	2	3	2	3	2	2	1	1	0	0	0	2	1	3	2	0	0	2	1	3	2	0	0	3	1	3	2	3	2	0	0
S11	3	3	3	3	3	3	3	3	3	3	3	0	1	1	3	3	3	3	1	1	3	3	3	3	1	1	2	0	3	3	3	2	1	1
S12	3	1	3	1	3	1	3	1	1	0	1	0	0	0	1	0	3	1	0	0	1	0	3	1	0	0	1	0	3	1	3	1	0	0
S13	3	3	3	3	3	3	3	0	3	0	0	0	1	1	3	0	2	0	1	1	2	0	3	3	1	1	0	0	3	3	3	3	1	1
S14	2	0	2	0	3	2	3	3	0	0	3	0	0	0	0	0	3	3	0	0	0	0	2	0	0	0	3	0	2	0	2	0	0	0
S15	3	1	3	1	3	1	3	3	1	0	3	0	0	0	1	0	3	2	2	1	1	0	3	1	2	1	2	0	3	1	2	1	2	1
S16	3	3	3	3	3	3	3	2	3	3	3	0	1	1	3	3	3	2	2	1	2	1	3	2	2	1	0	0	3	3	3	2	2	1
S17	3	2	3	2	3	2	3	1	0	0	1	1	3	3	2	1	3	1	2	1	2	1	3	2	2	1	1	1	3	2	3	2	1	1
S18	3	1	3	1	3	1	3	3	1	1	3	0	3	3	1	0	3	2	1	0	1	0	3	1	1	0	0	0	3	1	3	1	1	0
S19	3	3	3	3	3	3	3	1	3	3	1	1	2	0	3	2	3	1	2	1	3	0	3	3	2	1	1	1	3	3	3	3	2	1
S20	3	1	3	1	3	1	2	0	3	3	0	0	3	1	1	0	2	0	3	3	1	0	3	1	3	3	0	0	3	1	3	1	3	3
S21	2	0	2	0	2	0	3	3	2	0	1	1	3	2	0	0	3	3	1	0	0	0	2	0	1	0	0	0	2	0	2	0	1	0
S22	3	3	3	3	3	3	3	3	3	1	3	0	0	0	3	0	3	3	0	0	3	0	3	3	0	0	3	0	3	3	3	2	0	0
S23	3	3	3	3	3	3	3	1	3	2	3	2	1	1	3	2	3	1	3	0	2	0	3	2	3	0	2	0	3	3	3	2	3	0

S24	2	0	2	0	3	2	3	1	2	0	3	1	2	0	0	0	2	1	1	0	0	0	2	0	1	0	0	0	2	0	2	0	1	0
S25	3	1	3	1	3	1	3	2	1	1	3	3	3	1	1	1	3	2	1	0	1	1	3	1	1	0	1	1	3	1	3	1	1	0
S26	3	0	3	0	3	0	3	3	2	0	2	0	3	0	0	0	3	2	2	1	0	0	3	0	2	1	0	0	3	0	3	0	2	1
S27	3	1	3	1	3	1	3	1	3	1	3	1	3	2	1	1	3	1	3	3	1	1	3	1	3	3	1	1	3	1	3	1	3	3
S28	3	2	3	0	3	0	3	3	3	0	3	0	3	3	0	0	3	3	1	0	0	0	3	0	1	0	0	0	3	0	3	0	1	0
S29	3	1	3	1	3	1	2	0	3	1	0	0	2	0	1	1	2	0	2	0	1	1	3	1	2	0	1	1	3	1	3	1	2	0
S30	3	0	3	2	3	0	3	1	3	1	1	1	3	2	0	0	3	2	0	0	0	0	2	0	0	0	2	0	3	0	3	0	1	0

A - Poor appetite

B - Not sleeping well

C - Irritable, cranky, fussy

D - Feels unwell

E - Low energy, tired

F - Not playing well

G - Crying more than usual

H - Clinginess

I - Headache

J - Sore throat

K - Muscle aches or pains

L - Fever

M - Cough

N - Nasal congestion, runny nose

O - Vomiting

P - Not interested in what's going on

Q - Unable to get out of bed

Table No 60: Laboratory investigation results of patients before treatment Urai Mathirai

Variable	IgA	IgG	IgE	TC	Neutrophil	Lymphocytes	Eosinophils	ESR	Hb
Sb1	122	772	920	10400	48	38	14	19	13.2
Sb2	204	1826	560	8900	49	41	10	03	12.8
Sb3	116	1205	44	10600	49	33	18	09	11.7
Sb4	33	849	125	9300	63	30	07	13	13.0
Sb5	81	1420	94	9100	57	33	10	09	12.6
Sb6	48	901	237	9800	66	24	10	09	12.9
Sb7	112	1315	1154	7800	41	52	07	11	12.2
Sb8	116	932	137	3200	47	42	11	12	11.2
Sb9	113	761	5	9700	53	37	10	18	10.4
Sb10	76	800	24	9500	65	19	16	0.6	12.6
Sb11	64	978	909	5900	38	51	11	17	10.8
Sb12	141	1546	135	7700	60	32	08	10	12.1
Sb13	169	1048	182	10600	44	45	11	05	13.1
Sb14	130	784	78	6000	22	70	08	31	10.7
Sb15	30	1106	248	5400	75	49	13	03	13.3
Sb16	101	1713	48	5500	54	51	16	09	12.9
Sb17	90	1462	125	7100	61	33	07	13	11.8
Sb18	125	1048	345	10700	59	29	08	09	13.1
Sb19	36	849	171	8400	45	37	11	09	12.6
Sb20	116	761	111	5900	49	43	14	11	12.9
Sb21	130	1826	40	4300	39	59	08	12	12.2
Sb22	33	1205	146	8400	61	49	16	18	11.3
Sb23	76	772	237	2500	53	51	18	0.7	10.5
Sb24	204	932	1154	7500	41	36	13	17	12.7
Sb25	116	784	137	4000	39	31	07	10	10.9
Sb26	122	1713	48	3100	47	43	17	05	12.2
Sb27	30	1021	248	5300	55	59	12	31	13.2
Sb28	90	1003	919	8900	45	57	09	03	10.8
Sb29	85	1462	125	10500	29	49	11	09	13.4
Sb30	112	800	182	5100	33	39	14	13	12.10

Table No 61: Laboratory investigation results of patients after treatment Urai Mathirai

Variable	IgA	IgG	IgE	TC	Neutrophil	Lymphocytes	Eosinophils	ESR	Hb
Sb1	130	772	124	10000	49	42	9	18	13.9
Sb2	215	1524	119	9000	50	42	8	04	13.2
Sb3	116	1254	48	10400	51	40	9	10	12.5
Sb4	103	867	125	9300	61	34	05	15	13.5
Sb5	141	1405	98	9100	57	33	10	08	12.9
Sb6	91	914	135	9500	66	30	04	10	13.6
Sb7	112	1325	132	7800	43	50	07	14	12.8
Sb8	116	968	137	5100	48	45	07	13	10.5
Sb9	113	803	40	9500	53	41	06	16	10.9
Sb10	80	798	24	9700	65	25	10	09	13.0
Sb11	94	989	132	6000	47	45	08	18	10.8
Sb12	141	1559	135	7900	59	35	06	11	13.5
Sb13	169	1101	132	10400	44	45	11	08	13.9
Sb14	130	868	78	7500	48	45	07	19	14.5
Sb15	121	1119	129	5400	75	21	04	06	15.0
Sb16	101	1489	52	5500	54	36	10	08	14.5
Sb17	90	1478	125	7100	61	30	09	14	16.1
Sb18	125	1124	141	10400	59	35	06	10	14.0
Sb19	129	879	129	8400	54	40	06	10	14.5
Sb20	116	789	111	5900	49	41	10	13	13.5
Sb21	130	1458	133	4300	48	45	07	14	12.8
Sb22	145	1205	137	8400	61	25	14	17	11.9
Sb23	154	789	139	5300	53	40	07	09	11.3
Sb24	204	923	129	7500	53	40	07	15	13.1
Sb25	116	799	137	4800	40	50	10	13	12.1
Sb26	122	1524	128	4700	47	43	10	09	13.6
Sb27	156	1087	129	5300	55	41	04	20	13.5
Sb28	141	1024	139	8900	45	51	04	06	11.8
Sb29	85	1505	125	10200	39	49	12	08	14.6
Sb30	112	816	129	5700	48	39	13	15	13.9

Table No62: Laboratory investigation results of patients before treatment Thaleesathy Chooranam

Variable	IgA	IgG	IgE	TC	Neutrophil	Lymphocytes	Eosinophils	ESR	Hb
Sb1	90	849	146	3200	55	51	16	03	13.3
Sb2	122	1420	237	9700	45	36	11	09	12.9
Sb3	204	901	1154	9500	29	49	08	13	11.8
Sb4	116	1315	137	5900	57	39	11	09	13.1
Sb5	33	932	78	7700	66	37	08	09	12.6
Sb6	85	761	182	10600	41	19	13	11	12.9
Sb7	141	800	125	6000	47	51	16	12	12.2
Sb8	169	978	94	7100	53	32	07	18	11.3
Sb9	130	772	237	10700	65	45	08	0.6	10.5
Sb10	30	1826	1154	8400	38	70	11	17	12.6
Sb11	101	1205	182	5900	60	49	14	10	10.8
Sb12	90	1462	248	4300	44	51	08	05	12.1
Sb13	125	1021	48	8400	22	33	16	0.7	13.1
Sb14	36	1713	125	2500	48	29	18	17	11.2
Sb15	116	1462	345	3100	75	37	13	10	10.4
Sb16	130	772	171	5300	54	43	07	05	12.6
Sb17	81	932	920	8900	61	59	09	31	10.7
Sb18	116	784	560	10500	59	49	08	03	12.7
Sb19	113	1713	44	10400	45	57	13	09	10.9
Sb20	76	1205	18	8900	49	38	18	13	12.2
Sb21	33	761	24	10600	39	41	07	09	13.2
Sb22	76	1048	909	9300	47	33	10	13	10.8
Sb23	204	849	135	9100	53	30	10	09	13.4
Sb24	116	1003	137	9800	41	33	07	09	12.10
Sb25	122	1826	48	7800	39	24	11	11	12.2
Sb26	30	1546	248	5400	63	52	10	18	10.8
Sb27	64	1048	919	5500	49	42	10	12	12.1
Sb28	48	784	125	4500	49	31	11	31	13.1
Sb29	112	1106	111	7500	42	43	17	03	13.7
Sb30	90	955	40	4000	57	59	12	17	12.9

Table No63: Laboratory investigation results of patients after treatment

Thaleesathy chooranam

Variable	IgA	IgG	IgE	TC	Neutrophil	Lymphocytes	Eosinophils	ESR	Hb
Sb1	80	798	24	9700	65	25	10	06	13.9
Sb2	94	989	132	6000	47	45	8	08	11.9
Sb3	141	1559	135	7900	59	35	6	14	11.3
Sb4	169	1101	132	10400	44	45	05	10	13.1
Sb5	130	868	78	7500	48	45	11	10	12.1
Sb6	121	1119	129	5400	75	21	04	13	12.9
Sb7	101	1489	52	5500	54	36	10	16	13.6
Sb8	90	1478	125	7100	61	30	07	09	12.8
Sb9	125	1124	141	10400	59	35	06	18	10.5
Sb10	129	879	129	8400	54	40	06	11	10.9
Sb11	116	789	111	5900	49	41	10	08	13.0
Sb12	130	1458	133	4300	48	45	07	19	10.8
Sb13	145	1405	98	9100	57	33	10	18	13.6
Sb14	103	914	135	9500	66	30	04	04	13.2
Sb15	141	1325	132	7800	43	50	07	10	12.5
Sb16	91	968	137	5100	48	45	07	15	13.5
Sb17	112	803	40	9500	53	41	06	08	13.9
Sb18	116	772	124	10000	49	42	09	10	14.5
Sb19	113	1524	119	9000	50	45	05	14	15.0
Sb20	130	1254	48	10400	51	40	09	08	14.5
Sb21	215	1205	125	9300	61	35	04	15	16.1
Sb22	116	789	137	8400	61	37	02	09	14.0
Sb23	154	923	139	5300	53	43	04	20	14.5
Sb24	204	799	129	7500	53	41	06	06	13.2
Sb25	116	1524	137	4800	40	51	09	13	12.1
Sb26	122	1087	128	4700	47	49	04	14	13.5
Sb27	156	1024	129	5300	55	39	06	17	11.8
Sb28	141	1505	139	8900	45	48	07	09	14.6
Sb29	85	816	125	10200	53	41	06	15	13.9
Sb30	112	867	129	5700	48	49	03	13	13.5

Fig No. 82

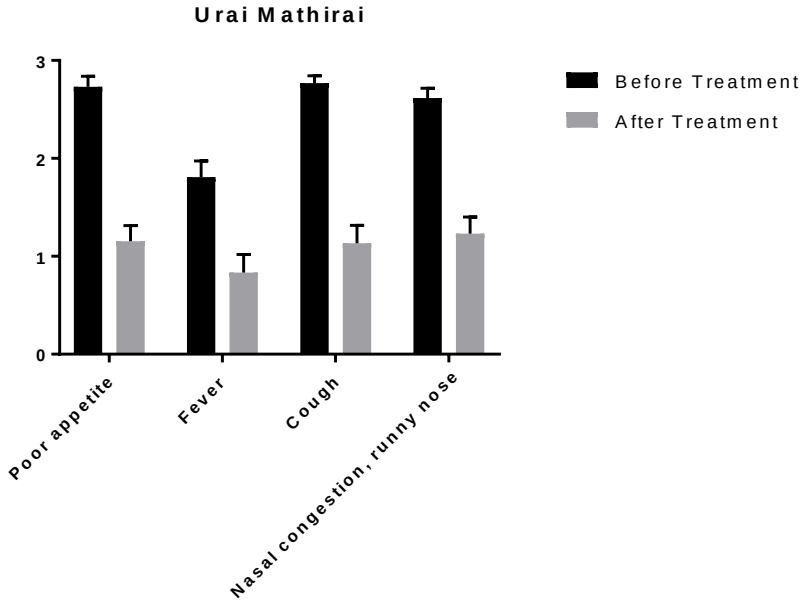
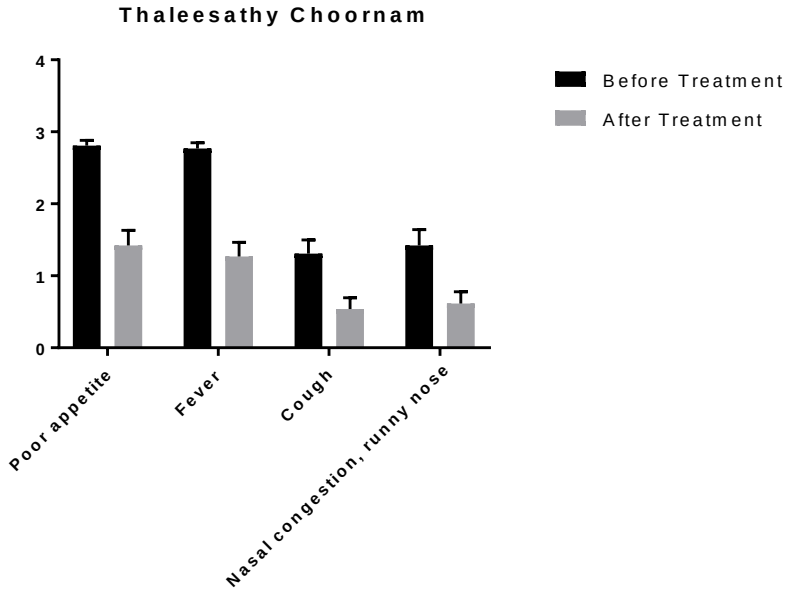


Fig. No 83



General Assessment – Disease Burden

Base line (Depicted in BLUE,) End of the Study (Depicted ORANGE)

Fig. 84

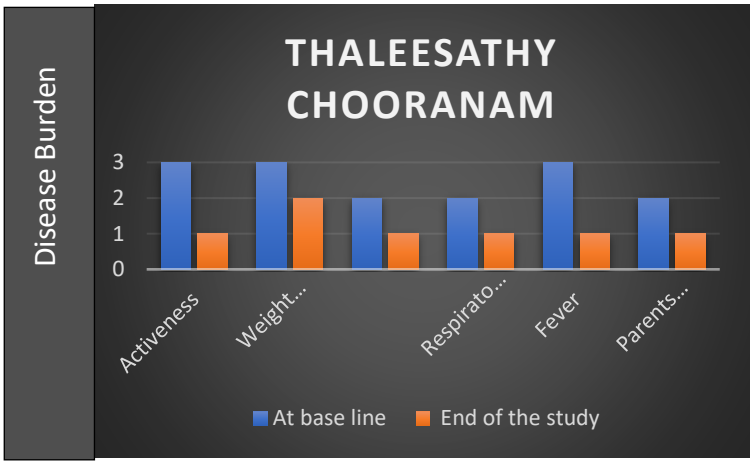
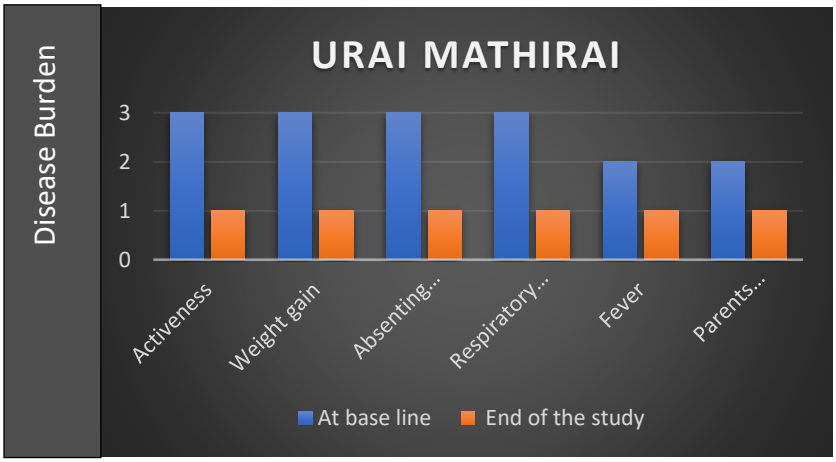


Fig.85



Discussion

Siddha attributes diseases of children to their parents. Immunity is compared to udal vanmai in siddha. Children immunity is directly connected to mother's health. A dietary change in mother, especially consuming non digestive food materials affects the health of kid who consumes her breast milk. This will create Mantham which is turn will pre dispose kanam. Kapham is the main uyir thathu which is increased in Mantham and kanam causing recurrent respiratory infections, gastric upset, loss of appetite, and dullness to children.

Urai Mathirai is a drug finding its place in hospital pharmacopeia of Indian medicine which has 10 major ingredients - majority of them pungent, which help in increase the decreased Pitham. Function of pitham in Siddha is prevention and all immuno globulins are compared to pitham. Phlegm in the throat is one of the deficits equated to Udalkuraivu identified in siddha as explained in T V Sambasivam pillai medical dictionary. Step wise study is discussed in detail.

Identification of raw drugs:

The Raw Drugs were identified by Pharmacognosist of Siddha Central research institute to avoid adulteration. Organoleptic characters and special external characters were compared with that of literary evidences and authenticated.

Organoleptic characters:

The Raw as well as prepared drug was examined for the colour(Green), appearance, odour(Herby), taste(Sour) and texture as organoleptic identification is the first step of Pharmacognostic identification.

Qualitative analysis:

Qualitative Phyto chemical screening revealed the presence of bioactive compound such as alkaloids, glycosides phenolic compounds such as flavonoids, tannins, terpenoids, and proteins in uraimathirai formulation. Flavanoids may be the reason for the drug antibacterial activity. Phenols in the drug reflect antioxidant beneficial increasing tissue damage and reducing inflammation.

Physico-chemical analysis:

The moisture content measurement is absolutely necessary for quality assurance. Loss on Drying is a routine procedure for determining moisture content of a solid or semisolid substance.

3.84 % by loss on drying at 105ºC indicates lesser moisture content of the drug preventing contamination. Acid insoluble ash is 1.05% and indicates lesser silica content.

The extractive value of a drug helps in choosing the better solvent for extraction. The water Soluble extractive (12.25%) is slightly high when compared to alcohol soluble extractive (9.25%).So water could be taken as better solvent of extraction which will contain more active constituents. The pH of UM is 5.5 Slight acidic nature of the drug may be due to high carminative content useful it may improve the appetite and hence resulting in more intake and weight gain.

Preliminary phyto-chemistry:

Flavanoids generally exhibit antibacterial activity. Phenols show antioxidant activity and this may be helpful in Anti-inflammatory activity. HPTLC finger printing revealed different constituents in the formulation. The drug has been subjected for analysis of heavy metals and results revealed that everything is within limits prescribed. The drug is free from Aflatoxins and no microbial load was deducted.

Tablet formulation parameters

Bulk density was tested and free flow property of the powder has been proved. Drug excipient compatibility revealed there were no chemical interaction between the drug and excipients and the excipient is suitable for the formulation. Post compression parameters like weight variation, Thickness, Disintegration time, hardness and friability were with in Normal limits.

Invitro Antioxidant activity

DPPH assay has been carried out to measure the antioxidant property of Uraimathirai and results revealed that Uraimathirai exerted strong antiradical activity of 66.19%, 56.83% and 46.24 % with least IC Values of increasing concentrations from 100 µg, 200 µg, and 300 µg respectively. However the increase in sample and antiradical activity are inversely proportional.

Invitro Anti-inflammatory activity

Protein denaturation is the main cause of inflammation. The ability of the Water extract to inhibit protein denaturation has been studied. On increase in sample of water extract of Urai mathirai. The maximum activity was observed at 80% in 3 ml of extract.

Antimicrobial studies

An antimicrobial study of water extract of Uraimathirai assures antibacterial activity against pseudomonas, Staphylococcus and E.Coli. However the drug could not inhibit Bacillus subtilis and klebsiella.

Toxicity studies:

Urai Mathirai is being used and dispensed in all Siddha hospitals for the past 4 decades and so far no adverse reactions are reported and to have documentary evidence the toxicity study was conducted.

Acute toxicity and 28 Days repeated oral toxicity study:

In acute toxicity a single oral dose (10 times the therapeutic dose) was administered. The observations were made for 14 days. All the survived animals showed again in body weight on day 7 and 14 in comparison to their day 0 body weight. There was no treatment related mortality in the treatment groups. No gross lesions were recorded in all the experimental animals of Urai Mathirai. No gross lesions were recorded in all the experimental animals of Urai Mathirai during necropsy (Tab-3) Urai Mathirai" was found to be safe at all the tested dose levels. Sub-acute toxicity has been carried out as per OECD-407 - *Urai Mathirai* at the dose of 10 mg/kg, 50 mg/kg and 100 mg/kg were considered to be safe as they did not cause any adverse changes in the general behaviour in 28 days repeated dose oral toxicity studies in experimental rats. No alterations were observed in haematological and biochemical parameters of *Urai Mathirai* treated experimental rats. This shows the nontoxic nature of the drug *Urai Mathirai* at the dose level of up to 100 mg/kg and thus the drugs can be used for long-term administration.

Anti-Inflammatory activity.

Carrageenan induced paw edema as an in vivo model of inflammation has been frequently used to assess the anti-edematous effect of the contribution of mediators involved in vascular changes associated with acute inflammation. Oedema formation in the carrageenan-induced paw edema model is a biphasic response. In the early hyperemia, 0-2 hrs after carrageenan injection, there is a release of histamine, serotonin and bradykinin on vascular permeability. The inflammatory edema reached its maximum level at the 1 hr and after that it started declining. The late phase of the inflammatory response has been shown to be due to potentiating effect of bradykinin on mediator release and prostaglandins, producing edema after mobilization of the leukocytes. Nitrous oxide (NO) is a potent vasodilator and is also involved in

carrageenan-induced edema, which may be related to its ability to increase vascular permeability and edema through changes in local blood flow.

When comparing the percent inhibition of paw edema the diclofenac standard control group has decreased in comparison with the standard group. Urai mathirai at higher dose level at 50 and 100 mg/kg has reduced the paw volume at 3rd and 4th hour when compared to the normal control and standard control groups. Urai mathirai at lowest dose have mild effect when compared to the control and standard drug controls.

ANALGESIC ACTIVITY

Analgesic effect of Uraimathirai is tested by Tail flick response method. The reaction time has been increased in diclofenac treated control groups at 4 th hour when compared to the normal control groups shows the analgesic activity of the standard drug. In urai mathirai treated groups at three different doses 0f 10, 50,100 mg/kg there was a increased reaction time at 1st and 2 nd hour shows the effect of analgesia but the reaction time has decreased in the 4 th hour shows the time dependent decrease in analgesic effect of urai mathirai.

Immuno modulatory activity

Uraimathirai had a greater effect on the delayed hypersensitivity reaction in SRBC induced immuno modulation in rats.

Clinical trial:

An open nonrandomized two arm clinical trial was conducted to register documentary evidence on clinical evaluation of Immuno modulatory effect of Uraimathirai Vs Thaleesathy chooranam official formulation. Even though Uraimathirai is widely prescribed and even included in Public health delivery no clinical study documentation exists. The trial has been conducted strictly adhering the AYUSH-GCP Guidelines. Proper permission has been obtained in IHEC and trial is registered in CTRI. ASthe age group is between 6 months to 5 yrs,Assent form is obtained and the same has been informed in Patient information sheet. Total sample size is 60 (n= 30 in every arm . 50 mg of Uraimathirai (once daily in Luke warm water) is compared with standard Siddha formulation Thaleesathy chooranam 500 mg B.D.With honey continuously for a period of Six months.

Age and gender:

The patients between the age group of 6 months to 5 years were included in the trial. Regular school, preschool and Non-school going children of both the sexes are the generalized characteristics of trial population.All the patients in both the arms have complaints of recurrent respiratory infections. Male female ratio in Uraimathirai is 60%:40% while male female ratio in Thaleesathi chooranam is 55%:45%.

Outcome Assessment tool

CARIFS (Canadian acute respiratory illness and Flu Scale) is the assessment tool to measure the respiratory illness and burden created. Along with that General assessments are also taken into consideration. Among the 18 variables in the CARIF scale Nasal congestion, Cough, Fever and loss of appetite are taken into account as they coincide with symptoms of Mantham, Kanam and Pasiyinmai.

Both the drugs were beneficial among children and have reduced clinical symptoms. Based on assessment of above said 4 main variables P Value is derived using T paired test and Significance is calculated. Uraimathirai has a Significant (P – Value 0.0026 / T Value – 9.318) versus Thaleesathy chooranam treatment arm had a (P value significance 0.0099 / T Value- 5.859). Other than this recurrent respiratory infection precipitates lot of burden to parents which are also measured which has reduced both in the arms, however the reduction in Uraimathirai arm is larger. Every time the tablets are counted in Uraimathirai arm for drug compliance checking and Thaleesathy chooranam pockets are verified for proper intake of drug. No adverse events were reported through bout the study. 2 Subjects in Uraimathirai arm (6%) and 3 subjects (10%) in Thaleesathy chooranam reported usage of antibiotics during trial.

It was very clear that lot of hesitation exists among parents to put their children for invasive investigations and X-ray irradiation. In the end of the study the mean of the Reduction in IgE and reduction in eosinophil counts were noticed. IgA, IgG were normal range among both the groups in two arms. The mean values of Hb are slightly increased among the mean of both the arms before and after treatment which shows the effectiveness of the drug in increasing appetite.

Summary and Conclusion

SUMMARY

The question of efficacy of Urai Mathirai as Immunomodulator and its usage to children was tested with a Null hypothesis that Urai Mathirai is not beneficial as an Immunomodulator. To test this *Urai Mathirai* was prepared meticulously as per Hospital pharmacopeia of Indian medicine. However, the Bullet shaped rubbing pills were replaced with 50mg tablets as per specific SOP.

The tablet matched with the standards prescribed by PLIM and also other parameters for tablets. While preparing all the drugs were authenticated. Pharmacognosy, Physico chemical and phytochemical testing was done as per guidelines. The results revealed the purity of the drug as it is free from heavy metals, Aflatoxins, and Microbial load.

In vitro studies for Antioxidant, Anti- Inflammatory and Immunomodulatory effects proved the basic claim of the drug and anti-microbial activity proved the efficacy of drug against certain bacteria. In vivo Safety and Pharmacological studies revealed Nontoxic nature and effectiveness of drug. Anti-inflammatory analgesic and Immunomodulator activity of the drug reassured its traditional claim.

Clinical trial carried out helped in proving the efficacy of Urai Mathirai on par with the official siddha formulation Thaleesathi chooranam in controlling repeated respiratory infections. The outcome measures clearly assured the efficacy of Urai Mathirai and proved that Urai Mathirai decreases the disease burden. Four main variables tested helps in proving the claim of the drug's use in Mantham, Kanam and Loss of appetite.

CONCLUSION

Its concluded that preclinical and clinical studies carried over prove that *Urai Mathirai* possess immuno modulator activity evinced through in vivo Immunomodulatory studies and also by reduction in repeated respiratory infections and disease burden reduction in Clinical trials.

Recommendations

- Chronic toxicity study is the limitation and is to be carried out.
- Large sample randomized and Blinded trials will help to mainstream this drug.
- The efficacy of Urai Mathirai in Bronchial asthma and COPD is to be evaluated.

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Annexures

ANNEXURE

ASSESSMENT FORMS

FORM l	**SCREENING AND SELECTION PROFORMA**
FORM l A	**HISTORY PROFORMA**
FORM ll	**CLINICAL ASSESSMENT DURING AND AFTER TRAIL**
FORM ll A	**GENERAL CLINICAL ASSESSMENTS**
FORM lll	**LABORATORY INVESTIGATIONS**
FORM IV	**INFORMATION SHEET**
FORM IV A	**INFORMED CONSENT FORM**
FORM IV B	**WITHDRAWAL FORM**
FORM IV C	**DRUG COMPLIANCE FORM**
FORM IV D	**ADVERSE REACTION FORM**

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CHENNAI 106

DEPARTMENT OF CLINICAL RESEARCH

EVALUATION OF CLINICAL EFFICACY OF URAI MATHIRAI AS IMMUNO MODULATOR

FORM -I

SCREENING AND SELECTION PROFORMA

(ENTER √ IN THE APPROPRIATE BOX)

1. Code No. (of clinical trial) ☐☐☐☐☐☐☐☐☐☐

2. Centre _____

3. Name of the subject _____

4. Gender Male ☐ Female ☐

5. Date of Birth ☐☐ ☐☐ ☐☐☐☐ Age (In Yrs) ☐☐

6. Address: Permanent postal address with phone number / e-mail if any

CRITERIA FOR INCLUSION

- Children of either gender aged 6 months to 6 Years with a history of recurrent URTIs and presenting with URTI at hospital treatment. **Yes / No**
- The definition used for recurrent URTIs was three or more such episodes during the last 12 months. **Yes / No**
- The current episode required for study eligibility was Defined by the presence of at least two of the following

  Rhinitis **Yes / No**

  Pharyngitis **Yes / No**

  Cough **Yes / No**

  Hoarseness, **Yes / No**

  Temperature More than 38.5°C, **Yes / No**

Prescription of an antibiotic for a URTI, occurring after an asymptomatic Period of at least 1 week without antibiotics **Yes / No**

- Parents Understanding and being willing to sign the informed consent form. Yes / No

CRITERIA FOR EXCLUSION

- Occurrence of otitis media and/or sinusitis **Yes / No**
- Infection of the lower respiratory tract **Yes / No**
- (ie, bronchitis, pneumonia) **Yes / No**
- Proven group A streptococcal angina at the enrollment **Yes / No**
- Allergic asthma **Yes / No**
- Significant systemic disease (eg, hepatic and/orrenal disease, malignancy), **Yes / No**
- Immune system disorders, **Yes / No**
- Major surgical procedure within 3 months of commencement of the study **Yes / No**
- Recent immunosuppressive or immunostimulant therapy, **Yes / No**
- Corticosteroids. **Yes / No**

A subject is eligible for admission if yes is the answer for Inclusion and exclusion criteria.

If Yes Serial Number: _____

Date: Signature of Investigator

Station:

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EVALUATION OF CLINICAL EFFICACY OF URAI MATHIRAI AS IMMUNO MODULATOR

FORM l A

HISTORY PROFORMA

(ENTER √ IN THE APPROPRIATE BOX)

1. Code No. (of clinical trial) ☐
2. Centre _____
3. Name of the subject _____
4. Serial No. of the subject ☐
5. Gender ☐ Male ☐ Female
6. Date of Birth ☐☐ ☐☐ ☐☐☐☐ ☐☐ Age (In Yrs)
7. Address: Permanent postal address with phone number / e mail if any
8. Educational status of parent :(Enter √ IN THE APPROPRIATE BOX)

   Illiterate ☐ Matriculation ☐ Graduate ☐ Post graduate ☐
9. Annual income ☐ 60,000 (enter <, >)
10. Occupation ☐
11. The History of previous illness and

    Treatment

12. History of present illness: Present/absent

- Cough ☐
- Pharyngitis ☐
- Fever ☐
- Hoarseness ☐
- Fever ☐
- Rhinitis ☐

Duration of above registered symptoms ____ days

13. Personal history:

- Mile stone achieved
- Head circumference
- Weight in kgs

Udaliyal ☐

Vali Azhal ☐

Iyam ☐

Thontham ☐

14. Physical examination

1. Built
2. Gait
3. Body Weight kgs
4. Height
5. BMI
6. Temperature

7. Blood Pressure mm/Hg

8. Pulse rate /min

9. Respiratory rate

10. Pallor	Present	☐	☐	Absent
11. Jaundice		☐	☐	
12. Koilonychia		☐	☐	
13. Lymphadenopathy		☐	☐	

Date: Signature of Investigator

Station:

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FORM II CLINICAL ASSESMENT DURINGANDAFTER TRIAL (CARIF Scale)

	No Problem (Score 0)		Minor Problem (Score 1)		Moderate Problem (Score 2)		Major Problem (Score 3)		Don't know or Not Applicable (Score 0)	
	BL	ES	BL	ES	BL	ES	BL	ES	BL	ES
1. Poor appetite										
2. Not sleeping well										
3.Irritable, cranky, fussy										
4. Feels unwell										
5.Lowenergy, tired										
6. Not playingwell										
7. Cryingmorethan usual										
9. Clinginess										
10.Headache										
11.Sorethroat										
12.Muscle aches or pains										
13.Fever										
14.Cough										
15.Nasal congestion, runnynose										
16.Vomiting										
17.Notinterestedinwhat'sgoing										
18.Unable toget outof bed										

Please mark on this line how sick your child is today:

Date:

Station: Signature of Investigator

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EVALUATION OF CLINICAL EFFICACY OF URAI MATHIRAI AS IMMUNO MODULATOR

FORM-II A

GENERAL CLINICAL ASSESSMENTS

S.NO	Assessment end points	At base line	End of the study
1.	Activeness		
2.	Weight gain		
3.	Absenting from School		
4.	Respiratory infection Relapse		
5.	Fever		
6.	Parents Anxiety		

Date: Signature of Investigator

Station:

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DEPARTMENT OF CLINICAL RESEARCH

EVALUATION OF CLINICAL EFFICACY OF URAI MATHIRAI AS IMMUNO MODULATOR

FORM-III

LABORATORY INVESTIGATIONS

(On Day 1 and 180)

1. Centre: …… ☐
2. Code No. (of clinical trial)
3. Name of the subject: …….
4. Date of Birth: ☐☐ ☐☐ ☐☐☐☐ ☐☐ Age (in yrs) :
5. Address …….
6. Date of Assessment : ☐☐ ☐☐ ☐☐ ☐☐
7. Blood Examination
8. TC (Cells/Cmm.): _____
9. DC: P ___ (%) L _____ % E _____(%) M _____ (%) B _____ (%)

10. ESR (At 30 min.) _____mm ESR (At 60 min.) _____mm

11. Hb _____gms%

12. Liver function test

13. Renal function test

14. IgA, IgG, IgM, IgE

15. 14. X-Ray – PA View.

Date: Signature of Investigator

Station:

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EVALUATION OF CLINICAL EFFICACY OF URAI MATHIRAI AS IMMUNO MODULATOR

LABORATORY PARAMETERS-CHART

1. OP/IP NO: _____

2. NAME: _____

3. AGE: _____ years

4. GENDER: Male / Female

BLOOD INVESTIGATION		**NORMAL VALUES**	**Before Treatment (with date)**	**After Treatment (with date)**
HB(gms%)		MC:13-18 FC:11-16		
T.RBC(milli/cu.mm)		MC:4.5-6.5 FC:3.5-5.5		
ESR (mm)	½ hr.	-		
	1 hr.	MC:0-10 FC:0-20		
T.WBC (/cu.mm)		4000- 11000		
Differential Count (%)	Lymphocutes	20-45		
	Neutrophil	40%-75%		
	Esonophils	1-6		

IgA	700-1600mg/dl		
IgG	70-400 mg/dl		
IgE	37-144		

Date: Signature of Investigator

Station:

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EVALUATION OF CLINICAL EFFICACY OF URAI MATHIRAI AS IMMUNO MODULATOR

FORM IV

INFORMATION SHEET

1. Study Information

Protocol Title:

Evaluation of Clinical efficacy of Urai mathirai as Immuno modulator.

Principal Investigator & Contact Details:

Dr. P. Sathiyarajeswaran, M.D(Siddha),M.Phil.(Siddha),

Assistant Director,

Siddha Central Research Institute,

Anna Hospital Campus,

Arumbakkam,Chennai -600106. Mobile:9443579540

2. Purpose of the Research Study

You are invited to participate in a research study. It is important to us that you first take time to read through and understand the information provided in this sheet. Nevertheless, before you take part in this research study, the study will be explained to you and you will be given the chance to ask questions. After you are properly satisfied that you understand this study, and that you wish to take part in the study, you must sign this informed consent form. You will be given a copy of this consent form to take home with you.

You are invited because you have expressed to your treating Physician / Pediatrician that your Child suffers from or often get attack of cold. This study is designed to evaluate the

efficacy of Urai mathirai which is indicated to be efficacious in Repeated respiratory infections and also said to increase the immunity of Children. By participating in this study your Kid will have benefit of His/ Her illness treated or study results of your Kid may be used in future for developing drug for Repeated respitaory infection.

This study will recruit *60* subjects from Siddha central research institute hospital over a period of two months and study period will be six months will be the treatment period.30 subjects will be allotted to Uraimathirai and 30 subjects for Thaleesathy chooranam.

3. What procedures will be followed in this study

If you take part in this study, you will be allocated to either one of the arm by the Physician.

If you take part in this study, you will be asked to Sign parental consent as your children will not be autonomous to take decisions. Initially after signing informed consent your children will be subjected for blood investigations and Clinical examination. After found to be fit to inclusion as per the criteria Urai mathirai tablets (50 mg once a day) / Thaleesathi chooranam(250 mg twice a day with honey) will be dispensed and you will be asked to follow once in every month upto six months. At the end of Six months once again there will be a blood checkup and routine Clinical examination.

If you agree to take part in this study, the following will happen to you:

S.NO	Activities	Important events	Out come
1.	Day 0	Screening / Lab.inv	
2.	Day 3 (Enrollment)	Reports / Inc- Exc Criteria / History – Consent signing.	Exclusion – General OPD
3.	Day 30	Follow up	Drug Compliance form
4.	Day 60	Follow up	Drug Compliance form
5.	Day 90	Follow up	Drug Compliance form
6.	Day 120	Follow up	Drug Compliance form
7.	Day150	Follow up	Drug Compliance form
8.	Day 180	Follow up / Assessment	Study completion.

4. Your Responsibilities in This Study

If you agree to participate in this study, you should follow the advice given to you by the study team. You should be prepared to visit the hospital (six times totally) and undergo all the procedures that are outlined above.

5. What Is Not Standard Care or is Experimental in This Study

The study is being conducted because (Urai Mathirai) is not yet proven to be a standard (Treatment) in subjects with (Repeated respiratory infections). We hope that your participation will help us to determine whether (Uraimathirai) is equal or superior to existing (Thaleesathy Chooranam).

Although (Investigation or Treatment) may be part of standard medical care, in this study this/these procedure(s) are only being performed for the purposes of the research, and are not part of your routine care.

6. Possible Risks and Side Effects

As of now with the available data no side effects are reported.

No Allergic reaction is Reported to the drug as of now.

Allergic reactions can occur with any drug. Common symptoms may include: rash, itching etc. Rarely, a severe and possibly life-threatening allergic reaction can occur. Symptoms of a severe reaction include: swelling of the face, difficulty breathing, or a sudden drop in blood pressure that may cause dizziness. If you have any of these symptoms, call your doctor at once. (Intervention or investigation) is still being tested; therefore, you may experience other side effects that have not yet been reported. However, you will be kept informed of any significant new findings that may relate to your willingness to continue to take part in this study.

If you experience any new symptoms, you should contact your doctor or the Principal Investigator as soon as possible.

Obtaining blood can cause pain, bleeding, bruising, or swelling at the site of the needle stick. Fainting sometimes occurs and infection rarely occurs.

In addition, you can use Antibiotics if you could not get cleared of your illness and usage of Antibiotics will be taken as failure of Intervention and you are requested to report Investgator about this event.

7. Possible Benefits from Participating in the Study

If you participate in this trial you may reasonably expect to benefit from the trial (Uraimathirai) in the following way that your children health condition improve as the immunity gets enhanced and Repeated respiratory infections got reduced.

OR

There is no known benefit from participation in this study. However, your participation in this study may add to the medical knowledge about the use of this (Uraimathirai / Thaleesathy Chooranam)

8. Alternatives to Participation

If you choose not to take part in this study or you are excluded as per the criteria fixed , you will receive standard care for your condition from RCH-OPD of Siddha Central research Institute.

9. Costs & Payments if Participating in the Study

If you take part in this study, Investigations and Treatment will be free of Cost. These costs will be borne by Siddha Central Research Institute.

10. Voluntary Participation

Participation of your Kid in this study is voluntary .Your Kid may stop participating in this study at any time. Your decision not to take part in this study or to stop your participation will not affect your kids medical care or any benefits to which you are entitled. If you decide to stop taking part in this study, you can withdraw at any part of time. However you are requested to

inform the PI the reason for quitting the trial as it may help in the process of recruitment or to correct any flaw in the conduct of the study.

However, the data that have been collected until the time of your withdrawal will be kept confidential and analyzed. The reason is to enable a complete and comprehensive evaluation of the study.

Your doctor, the Investigator and/or the Sponsor of this study may stop your participation in the study at any time if they decide that it is in your best interests. They may also do this if you do not follow instructions required to complete the study adequately by which the subject’s health may be deteriorated. In the event of any new information becoming available

that may be relevant to your willingness to continue in this study, you *(or your legally acceptable representative, if relevant*) will be informed in a timely manner by the Principal Investigator or his/her representative.

11. Confidentiality of Study and Medical Records

Information collected for this study will be kept confidential. Your records, to the extent of the applicable laws and regulations, will not be made publicly available.

"Personal Data" means data about you which makes you identifiable (i) from such data or (ii) from that data and other information which an organisation has or likely to have access. This includes medical conditions, medications, investigations and treatment history.

Research arising in the future, based on this "Personal Data", will be subject to review by the relevant institutional review board.

Data collected and entered into the Case Report Forms are the property of Siddha Central Research Institute. In the event of any publication regarding this study, your identity will remain confidential.

12. Who To Contact if You Have Questions

If you have questions about this research study, you may contact the Principal Investigator,

Dr .P.Sathiyarajeswaran, M.D(Siddha),M.Phil.(Siddha),
Assistant Director,
Siddha Central Research Institute,
Anna Hospital Campus,
Arumbakkam,Chennai -600106.
9443579540

The study has been reviewed and approved by the Institutional ethics Committee of SCRI and has been registered in the CTRI with Number CTRI/2017/06/008723 .

If you have any complaints or feedback about this research study, you may contact the Principal Investigator.

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DEPARTMENT OF CLINICAL RESEARCH

EVALUATION OF CLINICAL EFFICACY OF URAI MATHIRAI AS IMMUNO MODULATOR

FORM IV-A

INFORMED CONSENT FORM

Protocol Title:

Evaluation of Clinical efficacy of Urai mathirai as Immuno modulator.

Principal Investigator & Contact Details:

Dr .P.Sathiyarajeswaran,
M.D(Siddha),M.Phil.(Siddha), Assistant Director,
Siddha Central Research Institute, Anna Hospital
Campus, Arumbakkam,Chennai -600106.
Mobile:9443579540

I voluntarily consent to take part in this research study on behalf of my Child.. I have fully discussed and understood the purpose and procedures of this study. This study has been explained to me in a language that I understand. I have been given enough time to ask any questions that I have about the study, and all my questions have been answered to my satisfaction. I have also been informed and I have understood the alternative treatments or procedures available and their possible benefits and risks. All have been provided in the patient

information
Sheet.

Name of Participant	Signature	Date

(Legal representative)

Impartial Witness Statement

I, the undersigned, certify to the best of my knowledge that the participant signing this informed consent form had the study fully explained in a language understood by him / her and clearly understands the nature, risks and benefits of his / her participation in the study.

_____	_____	_____
Name of Impartial Witness	Signature	Date

Investigator Statement

I, the undersigned, certify that I explained the study to the participant and to the best of my knowledge the participant signing this informed consent form clearly understands the nature, risks and benefits of his / her participation in the study.

_____	_____	_____
Name of Investigator	Signature	Date

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EVALUATION OF CLINICAL EFFICACY OF URAI MATHIRAI AS IMMUNO MODULATOR

FORM IV-B

WITHDRAWAL FORM

NAME: ---------- **OPD/ IPD NUMBER:** ----------

AGE: ---------- **REGISTRATION NO:** ----------

DATE OF TRIAL COMMENCEMENT: ----------

DATE OF WITHDRAWAL FROM TRIAL: ----------

REASONS FOR WITHDRAWAL:

- Long absence at reporting: Yes/ No
- Irregular treatment: Yes/ No
- Shift of locality: Yes/No
- Increase in severity of symptoms: Yes/No
- Development of severe adverse drug reactions: Yes/No

DATE:

SIGNATURE OF THE INVESTIGATOR

SIGNATURE GUIDE

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DEPARTMENT OF CLINICAL RESEARCH

EVALUATION OF CLINICAL EFFICACY OF URAI MATHIRAI AS IMMUNO MODULATOR

FORM IV –C
(DRUG COMPLIANCE FORM)

Name:	**Reg. No.**	**Date:**	**OPD No:**

Name Of The Drug :

Drugs issued: (Grams)

Drugs returned: (Grams)

S.NO	**DATE**	**DRUG TAKEN TIME**	
		MORNING/TIME	**EVENING/TIME**
Day 1			
Day 2			
Day 3			
Day 4			
Day 5 till Day 180			

Date :

Station : Signature of the patient

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EVALUATION OF CLINICAL EFFICACY OF URAI MATHIRAI AS IMMUNO MODULATOR

FORM-IV D

ADVERSE REACTION FORM

1. S. No. 2. OP No. 3. I.D No 4. Name

6. Age 7.Gender 8.Occupation 9. Income

10. Address 11. Contact No.

Sl.no	Particulars	Details
1	Study site	
2	Brief description of the event	
3	Date of onset	
4	Time of onset	
5	Date of administration of 1st dose of study drug	
6	Time of administration of 1st dose of study drug	
7	Date of administration of last dose of study drug	
8	Time of administration of last dose of study drug	
9	Severity of the AE	
10	Did the subject hospitalized	
11	Relationship to the study drug	

To befilled bythe investigator

12	Did the event require to stop the study drug	
13	Outcome of the event	
14	Date and time of report	
15	Signature of the investigator	

The Adverse Effects will be intimated to the Institution Ethical Committee and the Pharmaco vigilance committee of SCRI within 48 hours of time**.**

Date :

Station : Signature of the Patient

சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம்

சென்னை – 600 106

<u>தகவல் படிவம்</u>

படிவம் - IV

ஆய்வு தலைப்பு:

உடல் வன்மையை கூட்டுவதில் உரை மாத்திரையின் பங்கு – குழந்தைகளுக்கான மருந்தாய்வு

தலைமை ஆராய்ச்சியாளர் மற்றும் தகவல் தொடர்புக்கு:

Dr. P.சத்தியராஜேஸ்வரன் M.D(S), M.phil(Siddha),
உதவி இயக்குநர்
சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம்,
அண்ணா மருத்துவமனை வளாகம்,
அரும்பாக்கம் , சென்னை – 600 106.
கைபேசி : 9443579540

1.ஆய்வின் நோக்கம்:

தங்களை ஆய்வில் பங்கேற்க அழைக்கிறோம். தாங்கள் முதலில் முழு தகவலையும் படித்து புரிந்து கொள்வதற்கான நேரம் ஒதுக்குவது எங்களுக்கு மிகவும் அவசியமானது. இருந்தபொழுதும், ஆய்விற்கு உட்படுத்துவதற்கு முன்பாகவே ஆய்வைப்பற்றி விளக்கமாகவும், அதன் தொடர்பான கேள்விகளுக்கும் விளக்கம் அளிக்கப்படும். ஆய்வைப்பற்றிய முறையான திருப்தி ஏற்பட்டப் பிறகே ஆய்விற் பங்கேற்க விருப்பம் தெரிவிக்கப்பட்டவுடன் தகவல் ஒப்புதல் படிவத்தில் ஒரு பதிவை தாங்கள் எடுத்துச் செல்லவும் கொடுக்கப்படும்.

தங்கள் குழந்தைக்கு சளித் தொந்தரவு ஏற்ப்பட்டது அல்லது அடிக்கடி சளித் தொந்தரவு ஏற்பட்டதற்காக குழந்தை மருத்துவரை அணுகியதன் காரணமாகவே தாங்கள் பங்கேற்க அழைக்கப்பட்டீர்கள். இந்த ஆய்வானது அடிக்கடி ஏற்படும் சுவாசத் தொடர்பான தொந்தரவுகள் மற்றும் குழந்தைகளின் குறை நோய் எதிர்ப்பு சக்தி இவற்றில் "உரை மாத்திரை" என்ற மருந்திற்கான திறன் எவ்வாறு உள்ளது என்பதை பற்றி மதிப்பிடுவதற்காக வடிவமைக்கப்பட்டது. தாங்கள் இந்த ஆய்வில் பங்கேற்றால் தங்கள் மகள்/மகன் சிகிச்சை பெற்றுகொள்வதுடன் அவருடைய சிகிச்சைக்கான முடிவைக் கொண்டு மேற்கூறிய மருந்தை எதிர்காலத்தில் சுவாச சம்பந்தமான வியாதிகளுக்கு கையாள்வதற்காக பரிந்துரைக்க ஆலோசிக்கப்படும்.

இந்த ஆய்வில் இரண்டு மாத காலத்திற்குள் நோயாளிகள் (பங்கேற்பாளர்கள்) சித்த மருத்துவ மைய ஆராய்ச்சி நிலைய மருத்துவமனையில் சேர்த்துக் கொள்ளப்படுவர் மற்றும் 6 மாதத்திற்கும் சிகிச்சை செய்யப்படுவர். 30 நோயாளிக்களுக்கு உரைமாத்திரையும் 30 நோயாளிகளுக்கு தாளிசாதி சூரணமும் கொடுக்கப்படும்.

2. ஆய்வில் மேற்கொள்ளப்படும் முறைகள்:

ஆய்வில் பங்கேற்றவுடன் தங்களுக்கான ஒரு மருத்துவர் ஒதுக்கப்படுவார். தங்கள் மகன்/மகள் தன்னிச்சையாக முடிவு எடுக்கப்பட முடியாத காரணத்தால் நீங்கள் ஒப்புதல் படிவத்தில் கையெழுத்து போட கேட்டுக்கொள்ளப்படுவீர்கள். கையெழுத்து போட்ட பிறகு முதலில் தங்கள் மகன்/மகள் / குழந்தைக்கு இரத்தம் மற்றும் பொதுமருத்துவப் பரிசோதனை மேற்கொள்ளப்படும். தங்கள் குழந்தைக்கு ஆய்வில் சேர்த்துக் கொள்வதற்கான நிபந்தனைகள் இருக்கும் பட்சத்தில் தங்கள் குழந்தைக்கு உரைமாத்திரை (50மி.கி 1/நாள்) / தாளிசாதி சூரணம் (250மி.கி 2முறை / 1 நாள் தேனுடன்) ஒரு மாதத்திற்கு 1 முறை வீதம் ஆறு மாதங்களுக்கு என கொடுக்கப்படும். ஆறு மாதத்திற்கு பிறகு மீண்டும் இரத்தம் மற்றும் பொதுமருத்துவ பரிசோதனை மேற்கொள்ளப்படும்.
தாங்கள் ஆய்வில் பங்கேற்றவுடன், கீழ்கண்டவாறு பரிகரிக்கப்படுவீர்கள்

வ.எண்	செயல் பாடு	முக்கிய நிகழ்வுகள்	வெளிப்பாடு
1	நாள் 0	தேர்வுசெய்தல் & இரத்தப் பரிசோதனை	
2	நாள் 3 (சேர்த்துக்கொள்ளல்)	ஆய்வக முடிவு / சேர்த்துக் கொள்வதற்கான நிபந்தனை /	சேர்த்துக்கொள்ளப் படவில்லை – பொது வெளி நோயாளர் பிரிவு
3	நாள் 30	சிகிச்சை தொடர்தல்	மருந்து அட்டவணை
4	நாள் 60	சிகிச்சை தொடர்தல்	மருந்து அட்டவணை
5	நாள் 90	சிகிச்சை தொடர்தல்	மருந்து அட்டவணை
6	நாள் 120	சிகிச்சை தொடர்தல்	மருந்து அட்டவணை
7	நாள் 150	சிகிச்சை தொடர்தல்	மருந்து அட்டவணை
8	நாள் 180	சிகிச்சை தொடர்தல் / மதிப்பீடுதல்	ஆய்வு முடிதல்

3. இந்த ஆய்வில் தங்களுடைய பொறுப்பு:

ஆய்வில் பங்கேற்க ஒப்புதல் அளித்த பின்னர், தாங்கள் ஆய்வுக் குழுவினரால் கொடுக்கப்படும் ஆலோசனைகளை முறையாக கடைபிடிக்க வேண்டும். ஆறு மாதங்களுக்கு தொடர்ந்து மேற்கூறிய சிகிச்சைக்கு தங்களை தயார் செய்து கொள்ள வேண்டும்.

4. இந்த ஆய்வின் பாதுகாப்பு மற்றும் ஆய்வின் நன்மைகள்:

அடிக்கடி ஏற்படும் சுவாசக் கோளாறுகளுக்காக "உரை மாத்திரை" என்ற மருந்து இதுவரை நிரூபிக்கப்படாத காரணத்தால் இந்த ஆய்வு மேற்கொள்ளப்படுகிறது. தங்கள் பங்கேற்பின் பயனாக உரைமாத்திரையானது தாளிசாதி சூரணத்திற்கு நிகராக (அ) அதைவிட மேன்மையாக செயல்படுவதாக நிரூபிக்கப்படும், என்பதை நம்புகிறோம். முறையான மருத்துவ பாதுகாப்புடன் இந்த ஆய்வு மேற்கொள்ளப்பட்டாலும் மேற்கூறிய பரிசோதனைகள் ஆய்விற்கான நோக்கத்திற்காக மட்டுமே ஒழிய உடல் பாதுகாப்பிற்குகாக பரிசோதிக்கப்படுவது இல்லை.

5. பக்க விளைவுகள் மற்றும் ஆபத்திற்கான சாத்தியங்கள்:

இதுவரையில் இந்த மருந்திற்கு எந்த பக்க விளைவுகளோ (அ) ஒவ்வாமையோ ஏற்படும் என்ற தகவல் இல்லை.

எந்த மருந்தினாலும் ஒவ்வாமை ஏற்படலாம். பொதுவாக தடிப்புகள், அரிப்புகள் ஏற்படலாம்.

மிகவும் அரிதாக, அச்சுறுத்தும்படியான ஒவ்வாமை ஏற்படலாம் இதில் முகம் வீங்குதல், சுவாசத்தில் சிக்கல் ,இரத்த அழுத்த குறைவு காரணமாக ஏற்படும் மயக்கம் ஏற்படலாம். இதில் எந்த அறிகுறிகள் ஏற்பட்டாலும் உடனே மருத்துவரை அணுகவும். மருத்துவ ஆய்வு தொடர்ந்து நடைபெற்று கொண்டிருப்பதால் தங்கள் மகன் / மகள் -க்கு புதிய அறிகுறிகள் (அ) விளைவுகள் ஏற்படலாம் எனினும் தங்கள் விருப்பத்தின் பேரில் மட்டுமே ஆய்வில் தொடர அனுமதிக்கப்படுவீர்கள்.

ஏதேனும் புதிய அறிகுறிகள் ஏற்பட்டால் முடிந்தவரை உடனே மருத்துவரையோ (அ) தலைமை ஆய்வாளரையோ தொடர்பு கொள்ள வேண்டும்.

இரத்தம் எடுக்கும் போது அவ்வித்தில் வலி, கசிவு, வீக்கம், சிராய்ப்பு ஏற்படலாம். அரிதாக மயக்கமோ (அ) வேறு தொந்தரவுகளோ ஏற்படலாம்.

உங்களுடைய சிகிச்சைக்காக வேறு ஏதாவது நோய் எதிர்ப்பு மருந்துகள் எடுத்துக்கொண்டால் அது ஆய்வின் நோக்கத்தை பாதிக்கும் எனவே உடனே மருத்துவரிடம் அதை தெரிவித்து விட வேண்டும்.

6. ஆய்வில் பங்கேற்பதால் ஏற்படும் பலன்கள் :

நீங்கள் தங்கள் குழந்தையை ஆய்வில் பங்கேற்க வைப்பதால் உரைமாத்திரையின் பயனாக தங்கள் குழந்தைக்கு சளித்தொந்தரவு ஏற்படாமல் இருக்கலாம் மேலும் நோய் எதிர்ப்பு சக்தி அதிகப்படலாம். வேறு எந்த பலன்களும் பங்கேற்பாளர்க்கு இல்லை எனினும் உரைமாத்திரை / தாளிசாதி சூரணம் பற்றிய மருத்துவ அறிவை பங்கேற்பாளர்கள் பெற்றுக் கொள்ளலாம்.

7. பங்கேற்பாளருக்கு மாற்று முறை:

நீங்கள் ஆய்வில் பங்கேற்கவில்லை என்றாலோ (அ) சேர்க்கப்பட்ட நிபந்தனையிலிருந்து விடுவிக்கப்பட்டாலோ உங்கள் குழந்தையின் சிகிச்சைக்காக நீங்கள் சித்த மருத்துவ ஆராய்ச்சி நிலைய ,RCH – OPD க்கு அனுப்பப்படுவீர்கள்.

8. ஆய்வில் பங்கேற்பவர்களுக்கான ஆய்வு செலவுகள்:

ஆய்வில் பங்கேற்பவர்களுக்கான பரிசோதனைகள் மற்றும் மருத்துவச் செலவுகள் இலவசமாக செய்யப்படும் ஆய்விற்கான செலவுகளை சித்த மருத்துவ ஆராய்ச்சி நிலையம் ஏற்றுக்கொள்ளும்.

9. தன்னார்வ பங்கேற்பாளர்கள் :

தன்னார்வமாக பங்கேற்ற பங்கேற்பாளரின் குழந்தைகள் எப்பொழுது வேண்டுமானாலும் ஆய்விலிருந்து விடுபடலாம். தங்களுடைய முடிவு ஆய்வில் எடுத்துக்கொள்ளப்படாது. ஆனாலும் தங்கள் குழந்தைகளின் மருத்துவப் பாதுகாப்பு மற்ற பலன்கள் பாதிக்கப்படாது. நீங்களாக விடுபட முடிவு எடுத்தாலும் எப்பொழுது வேண்டுமானலும் விடுபடலாம். எனினும் மருத்துவருக்கு எந்த காரணத்தினால் விடுபட விரும்புகிறார்கள் என்பதை தெரிவிக்கப்படுத்த வேண்டப்படுவீர்கள். இது ஆய்வை வழிநடத்த ஏதுவாக இருக்கும். எனினும், தங்கள் தகவல்கள் சேகரிக்கப்படும் நீங்கள் விடுவிக்கப்பட்டாலும், ரகசியமாக வைக்கப்படும். இதன் காரணங்கள் ஆய்வில் முழுமையாகவும் விரிவாகவும் செயல்படுத்தப்படும்.
உங்கள் பங்கேற்பை, மருத்துவர் / ஆய்வாளர் / நிதியாளர் முடிவெடுத்தால் எந்தநேரத்திலும் ஆய்விலிருந்து நிறுத்தப்படலாம். ஆய்வின் விதிமுறைகளை முழுமையாக பின்பற்றாமல் தங்கள் உடல் நிலை மோசமாகும் பட்சத்தில் தங்களின் பங்கேற்றல் நிறுத்தப்படலாம். ஆய்வின் மத்தியில் ஏதேனும் புதிய விபரம் கிடைத்தால் தங்களுக்கு தொடர்புடையதாகயிருக்கும் பட்சத்தில் உரிய நேரத்தில் தெரிவிக்கப்படும்.

10. ஆய்வின் குறிப்புகள் பாதுகாத்தல் :

ஆய்வின் தகவல்கள் ரகசியமாக பாதுகாக்கப்படும். வரையறுக்கப்பட்ட சட்டதிட்டங்களுக்கு உட்பட்டு, பங்கேற்பாளர்களின் குறிப்புகள் வெளியிடப்படாமல் ரகசியமாக பாதுகாக்கப்படும்.

"personal Data" – என்பது உங்களை அடையாளப்படுத்தி அறிவதற்கான தகவல்கள், தங்களுக்கு உள்ள மருத்துவ அறிகுறிகள், மருத்துவ சிகிச்சைகள், பரிசோதனைகள், எடுத்துக் கொண்ட மருத்துவ பரிகார வரலாறு ஆகும்.

இந்த 'personal Data' வை கொண்டே வருங்கால மருத்துவ ஆய்வுகள் மேற்கொள்ளப்படும்.சேகரித்த தகவல்கள் மற்றும் நோயாளர் தகவல் சேகரிப்பு படிவத்தில் உள்ள அனைத்து தரவுகளும் சித்தமருத்துவ மைய ஆராய்ச்சி நிலையத்துக்கு சொந்தமானது. இந்த ஆய்வின் நிகழ்வுகள் குறித்த பிரசுரிக்கப்படும் போது உங்கள் அடையாளம் ரகசியமாக காக்கப்படும்.

11. ஏதாவது கேள்விகள் இருப்பின் யாரை அணுகுவது

இந்த ஆய்வைபற்றி ஏதாவது கேள்விகள் இருப்பின் தலைமை ஆய்வாளர்(PI) அணுகவும்
Dr. P.சத்தியராஜேஸ்வரன் M.D(S), M.phil(Siddha),
உதவி இயக்குநர்
சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம்,
அண்ணா மருத்துவமனை வளாகம்,
அரும்பாக்கம் , சென்னை – 600 106.
கைபேசி : 9443579540

இந்த ஆய்வானது சித்த மருத்துவ மைய ஆராய்ச்சி நிலையத்தில் உள்ள IEC *ஆல் மதிப்பாய்வுக்கு உட்பட்டு பரிந்துரைக்கப்பட்டு* CTRI *யில் பதிவு செய்யப்பட்டுள்ளது.*
இந்த ஆய்வு தொடர்பான ஏதாவது முறையீடுகள் (அ) வமர்சனங்கள் இருப்பின் தெரிவிக்க தலைமை ஆய்வாளரை அணுகவும்.

சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம்

சென்னை – 600 106

ஒப்புதல் படிவம்:

படிவம் - IV

ஆய்வு தலைப்பு:

உடல் வன்மையை கூட்டுவதில் உரை மாத்திரையின் பங்கு – குழந்தைகளுக்கான மருந்தாய்வு

தலைமை ஆராய்ச்சியாளர் மற்றும் தகவல் தொடர்புக்கு:

Dr. P.சத்தியராஜேஸ்வரன் M.D(S), M.phil(Siddha),
உதவி இயக்குநர்
சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம்,
அண்ணா மருத்துவமனை வளாகம்,
அரும்பாக்கம் , சென்னை – 600 106.
கைபேசி : 9443579540

தலைமை ஆய்வாளர் தொடர்புக்கு

நான் என் மகன் / மகள் சார்பாக இந்த ஆய்வில் தன்னார்வத்துடன் பங்குகொள்கிறேன். இந்த ஆய்வைப் பற்றிய நோக்கம் மற்றும் நடைமுறைகளை படித்து, ஆலோசித்து முழுமையாக புரிந்துகொண்டுள்ளேன். இந்த ஆய்வைப்பற்றி எனக்கு தெரிந்த மொழியில் விளக்கப்பட்டுள்ளது. எனக்கு இந்த ஆய்வைப்பற்றி கேள்வி கேட்க போதுமான நேரம் கொடுக்கப்பட்டு அதற்கான திருப்திகரமான பதில் தெரிவிக்கப்பட்டு இருக்கிறது. இந்த ஆய்வில் வரும் பலன்கள் மற்றும் பாதிப்புகளும் விளக்கப்பட்டு விளக்கப்பட்டு அதற்கான மாற்று பரிகாரமுறைகளும் தெரிவிக்கப்பட்டுள்ளது. இதை பற்றிய எல்லா தகவல்களும் தகவல் படிவத்தில் கொடுக்கப்பட்டுள்ளது.

பங்கேற்பாளரின் பெயர்

சட்டப்பிரதிநிதியின் பெயர் *கையொப்பம்* *தேதி*

நடுநிலை சாட்சி அறிக்கை,

நான், கீழே கையொப்பமிட்ட சாட்சிக்காரர் என் சுய அறிவுடன் தெரிவிப்பது என்னவென்றால் இந்த ஆய்வில் பங்கேற்று தகவல் படிவத்தில் கையெழுத்திட்டவர்க்கு அவர் புரிந்து கொள்ளும் மொழியில் ஆய்வைப்பற்றிய பலன்கள் மற்றும் பாதிப்புகள் முழுமையாக விளக்கப்பட்டது.

நடுநிலை சாட்சியாளரின் பெயர் *கையொப்பம்* *தேதி*

ஆய்வாளர் அறிக்கை,

நான், கீழே கையொப்பமிட்ட ஆய்வாளர், என் சுய அறிவுடன் தெரிவிப்பது என்னவென்றால் இந்த ஆய்வில் பங்கேற்று தகவல் படிவத்தில் கையெழுத்திட்டவர்க்கு அவர் புரிந்து கொள்ளும் மொழியில் ஆய்வைப்பற்றிய பலன்கள் மற்றும் பாதிப்புகள் முழுமையாக விளக்கப்பட்டது.

ஆய்வாளரின் பெயர் *கையொப்பம்* *தேதி*

सिद्ध केंद्रीय अनुसन्धान संस्थान

(सी.सी.आर.एस., चेन्नई, आयुष मंत्रालय, भारत सरकार)

अण्णा सरकारी अस्पताल परिसर, अरुम्बाक्कम, चेन्नई - 600106

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Anna Govt. Hospital Campus, Arumbakkam, Chennai - 600106
E-mail: crisiddha@gmail.com Phone: 044-26214925, 26214809

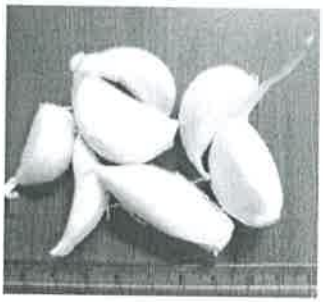
25.03.2017

AUTHENTICATION CERTIFICATE FOR 12.25031701-10

Certified that the drugs submitted by Dr. P. Sathiyarajeswaran, Assistant Director In-charge, Siddha Central Research Institute, Arumbakkam, Chennai-106 are identified as:

SN	Botanical Name	Tamil Name	Part	Code
1.	*Quercus infectoria* G. Olivier	Masikkai	Gall	Q25031701I
2.	*Allium sativum* L.	Poondu	Bulb	A25031702S
3.	*Terminalia chebula* Retz	Katukkai	Fruit	T25031703C
4.	*Acorus calamus* L.	Vasambu	Rhizome	A25031704C
5.	*Myristica fragrans* Houtt.	Catikkai	Kernel	M25031705F
6.	*Glycyrrhiza glabra* L.	Atimathuram	Root & Stolon	G25031706G
7.	*Zingiber officinale* Roscoe	Cukku	Rhizome	Z25031707O
8.	*Piper longum* L.	Tippili	Fruit	P25031708L
9.	*Anacyclus pyrethrum* (L.) Lag.	Akkarakaram	Root	A25031709P
10.	*Ferula foetida* (Bunge) Regel	Perunkayam	Gum resin	F25031710F

Q25031701I



A25031702S

T25031703C

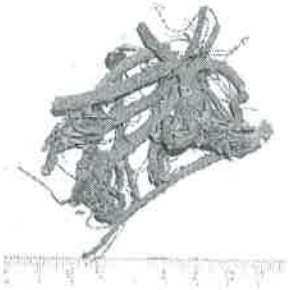
A25031704C

M25031705F

G25031706G

Z25031707O

P25031708L



A25031709P

F25031710F

25/3/17

Dr. K.N. Sunil Kumar
Research Officer and HOD
Department of Pharmacognosy

डॉ. के.एन. सुनील कुमार/Dr. K.N. Sunil Kumar
अनुसंधान अधिकारी (फार्माकोग्नॉसी)/RO(Pharmacognosy)
सिद्ध केंद्रीय अनुसंधान संस्थान,
(सी.सी.आर.एस.,चेन्नई, आयुष मंत्रालय, भारत सरकार)
अण्णा सरकारी अस्पताल परिसर, अरुम्बाक्कम, चेन्नई-600 106
SIDDHA CENTRAL RESEARCH INSTITUTE
(Central Council for Research in Siddha, Ministry of AYUSH, Govt. of India)
Anna Govt. Hospital Campus, Arumbakkam, Chennai 600106

for

Dr. P. Sathiya Rajeswaran
Assistant Director In Charge

சித்தமருத்துவ மைய ஆராய்ச்சி நிலையம்
(மத்திய சித்த மருத்துவ ஆராய்ச்சிக் குழுமம், ஆயுஷ் அமைச்சகம், இந்திய அரசு)
सिद्ध केंद्रीय अनुसन्धान संस्थान
(सी.सी.आर.एस., चेन्नई, आयुष मंत्रालय, भारत सरकार), अण्णा सरकारी अस्पताल परिसर, अरुम्बाक्कम, चेन्नई - 600106
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Anna Govt. Hospital Campus, Arumbakkam, Chennai – 600106, E-mail: crisiddha@gmail.com
Phone: 044-26214925, 26214809, Web: http://crisiddha.tn.nic.in

Certificate

This is to certify that the project titled "Safety evaluation studies and Analgesic, Anti-inflammatory, Immunomodulatory activities of *Urai mathirai*" has been approved by the IAEC.

NO: 162/ Pharma/ SCRI/ 2017.

Dr. P. SATHIYARAJESWARAN — Name of Chairman/ ~~Member Secretary~~ IAEC:

Shri. K.R. NAVANEETHA KRISHNAN — Name of CPCSEA nominee:

Signature with date

3/3/17 — Chairman/ Member Secretary of IAEC:

31/3/2017 — CPCSEA nominee:

சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம், அரும்பாக்கம், சென்னை - 600106
सिद्ध केन्द्रीय अनुसंधान संस्थान, अरुम्बाक्कम, चेन्नै - 600106
Siddha Central Research Institute
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Arumbakkam, Chennai – 600106
[Ph: 044-26214925, 26214809, Fax: 26214809, Email: crisiddha@gmail.com, Web: www.siddhacouncil.com]

Communication of the decision of Institutional Ethics Committee (IEC)

IEC No: CCRS/SCRI-1/2014-15/04

Protocol title: EVALUATION OF CLINICAL EFFICACY OF URAI MATHIRAI AS IMMUNO MODULATOR
Principal Investigator: Dr. P. Sathiyarajeswaran
Name & Address of Institution: Siddha Central Research Institute, (Central Council for Research in Siddha, Ministry of AYUSH, Govt. of India.) Arumbakkam, Chennai - 600106
[ ] New Review [√] Revised review [ ] Expedited review
Date of review (DD/MM/YY): 12-01-2015 Date of previous review, if revised application:
Decision of the IEC: [√] Approved [ ] Approved with recommendations [ ] Revision [ ] Rejected
Suggestions / Reasons/ Remarks: Enclosed as annexure Revised approval for Ph.D. studies
Recommended for a period of: 3 years from date of Completion of preclinical studies

Please note:

- **Inform IEC immediately in case of any Adverse events/ Serious drug reaction**
- **Seek IEC approval in case of any change in the study procedure, site and investigator**
- **This approval is valid only for period mentioned above.**
- **IEC members have the right to review the trial with prior intimation.**

Dr. K. Gopakumar
Member Secretary

Clinical Trial Details (PDF Generation Date :- Fri, 02 Jun 2017 10:14:01 GMT)

CTRI Number	CTRI/2017/06/008723 [Registered on: 01/06/2017] - **Trial Registered Prospectively**
Last Modified On	31/05/2017
Post Graduate Thesis	No
Type of Trial	Interventional
Type of Study	Drug Siddha
Study Design	Non-randomized, Active Controlled Trial
Public Title of Study	Effect of Uraimathirai in Increasing children Immunity
Scientific Title of Study	Evaluation of Clinical efficacy of Uraimathirai as Immunomodulator

Secondary IDs if Any

Secondary ID	Identifier
NIL	NIL

Details of Principal Investigator or overall Trial Coordinator (multi-center study)

Details of Principal Investigator	
Name	DRPSATHIYARAJESWARAN
Designation	ASST DIRECTOR
Affiliation	SIDDHA CENTRAL RESEARCH INSTITUTE
Address	SIDDHA CENTRAL RESEARCH INSTITUTE ANNA HOSPITAL CAMPUS ARUMBAKKAM CHENNAI Thiruvallur TAMIL NADU 600106 India
Phone	04426214809
Fax	04426214809
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Details Contact Person (Scientific Query)

Details Contact Person (Scientific Query)	
Name	DRPSATHIYARAJESWARAN
Designation	ASST DIRECTOR
Affiliation	SIDDHA CENTRAL RESEARCH INSTITUTE
Address	SIDDHA CENTRAL RESEARCH INSTITUTE ANNA HOSPITAL CAMPUS ARUMBAKKAM CHENNAI Thiruvallur TAMIL NADU 600106 India
Phone	04426214809
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Source of Monetary or Material Support

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Primary Sponsor Details	
Name	CENTRAL COUNCIL FOR RESEARCH IN SIDDHA
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Type of Sponsor	Research institution

Details of Secondary Sponsor

Name	Address
NIL	NIL

Countries of Recruitment

List of Countries
India

Sites of Study

Name of Principal Investigator	Name of Site	Site Address	Phone/Fax/Email
DR P SATHIYARAJESWARAN	Siddha central research Institute	Anna Hospital Campus Arumbakkam Chennai Thiruvallur TAMIL NADU	09443579540 siddha2k6@gmail.com

Details of Ethics Committee

Name of Committee	Approval Status	Date of Approval	Is Independent Ethics Committee?
SCRI ETHICS COMMITEE	Approved	12/01/2015	No

Regulatory Clearance Status from DCGI

Status	Date
Not Applicable	No Date Specified

Health Condition / Problems Studied

Health Type	Condition
Patients	Children of either gender aged UPTO 6 Years with a history of recurrent URTIs and presenting with URTI at hospital treatment

Intervention / Comparator Agent

Type	Name	Details
Comparator Agent	THALEESATHI CHOORANAM	1 GRAM TWICE DAILY FOR SIX MONTHS
Intervention	URAI MATHIRAI	50 MG DAILY UPTO SIX MONTHS

Inclusion Criteria

Inclusion Criteria	
Age From	6.00 Month(s)
Age To	6.00 Year(s)
Gender	Both
Details	Children of either gender aged 6 MONTHS to 6 Years with a history of recurrent URTIs and presenting with URTI at hospital treatment. The definition used for recurrent URTIs was three or more such episodes during the last 12 months. The current episode required for study eligibility was Defined by the presence of at least two of the following Rhinitis Pharyngitis

	cough hoarseness, Temperature More than 38.5°C, Prescription of an antibiotic for a URTI, occurring after an asymptomatic Period of at least 1 week without antibiotics

Exclusion Criteria

Exclusion Criteria	
Details	occurrence of otitis media and/or sinusitis and/or infection of the lower respiratory tract (ie, bronchitis, pneumonia)and/or proven group A streptococcal angina at the enrollment Further main exclusion criteria were allergic asthma, significant systemic disease (eg, hepatic and/or renal disease, malignancy), immune system disorders, suspected Malabsorption, major surgical procedure within 3 months of commencement of the study recent immunosuppressive or immunostimulant therapy, or corticosteroids.

Method of Generating Random Sequence	Not Applicable
Method of Concealment	Not Applicable
Blinding/Masking	Not Applicable

Primary Outcome

Outcome	Timepoints
Reduction in Recurrence of Resp Infections Clinical Improvement No Antibiotic treatment Increase in Anti bodies IgA/IgG Decrease in Phagocytosis	6 months

Secondary Outcome

Outcome	Timepoints
Safety of trial drug	6 months

Target Sample Size	**Total Sample Size=**60 **Sample Size from India=**60
Phase of Trial	Phase 2
Date of First Enrollment (India)	01/06/2017
Date of First Enrollment (Global)	No Date Specified
Estimated Duration of Trial	**Years=**0 **Months=**6 **Days=**0
Recruitment Status of Trial (Global)	Not Applicable
Recruitment Status of Trial (India)	Not Yet Recruiting
Publication Details	NIL
Brief Summary	Urai mathirai is a Siddha formulation found in Hospital pharmacopoeia of Govt siddha medical college Palayamkottai and in use for the past 3 decades. It is observed in Pediatric general practice that Children nursed with Uraimathirai are free from repeated respiratory infection and Uraimathirai is claimed to have Immuno modulating properties. However this Claim is left untested and this PhD

study will help to establish the efficacy of the drug comparing with Traditional Siddha formulation Thaleesathy Chooranam 1 gm Bid and Uraimathirai 50 mg daily for continuously for Six months.Before entering into Clinical study Preclinical studies are being carried out to establish the safety and standard of the Drug.

EVALUATION OF CLINICAL EFFICACY OF URAI MATHIRAI AS IMMUNE MODULATOR This is submitted to The Tamil Nadu Dr. M.G.R. Medical University In partial fulfilment for the award of the degree of Doctor of Philosophy – EXII(I)/30184/2011 Research Scholar: Dr. P. Sathiyarajeswaran M.D. (S), M.phil (Siddha)., Research Guide / Supervisor Dr. R. P. Pattarayan M.D. (Siddha) Professor & HOD (Rtd), Dept. of Kuzhanthai Maruthuvam, National Institute of Siddha, Tambaram, Chennai

INTRODUCTION Page 1 1. INTRODUCTION From time immemorial, man depends on plants as medicine. From a historic attitude, it is obtrusive that the fascination for plants is

Publications

WORLD JOURNAL OF PHARMACEUTICAL RESEARCH

Impact Factor 7.523 ICV 90.8

Acceptance Letter

Manuscript No: WJPR/10575/6/2017 **Date: 24/12/2017**

TITLE: DESIGN OF SOLID ORAL DOSAGE FORM AND ITS QUALITY CONTROL ASSESSMENT OF URAI MATHIRAI – A TABLET FROM SIDDHA FORMULATION FOR IMMUNO MODULATION IN PEDIATRIC COMMUNITY

Dear Sathiyarajeswaran P., Dhanaraj K., Shree Devi M.S., Muthu Tamizh M. and Patturayan R.

We are pleased to inform you that out of various research articles submitted in WJPR, Experts/ Referees Panel of WJPR has recommended your manuscript for publication, so World Journal of Pharmaceutical Research has been accepted your manuscript for publication in Current (December) issue of WJPR.

World Journal of Pharmaceutical Research publishes all its article in full open access format which are easily accessible for scientific community.

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WJPR

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Dr. P. Sathiyarajeswaran: Manuscript Acceptance- PHYTO-2017-6641

2 messages

Phytopharmacology Journal <phytopharmajournal@gmail.com> Fri, Dec 29, 2017 at 12:14 PM
To: Sathya Rajeswaran <siddha2k6@gmail.com>
Cc: shreemd@gmail.com, muthutamizh@gmail.com, Sunil Kumar <sunilkumarnarayanan@gmail.com>

Dear **Dr. P. Sathiyarajeswaran,**

Greetings, Your manuscript entitled **"Quality Standards for Urai Mathirai - A Siddha Immunomodulator Formulation for Children"** is very well written. After careful consideration, your manuscript has been accepted for publication in **The Journal of Phytopharmacology**.

Manuscript ID: *PHYTO-2017-6641*

List of Authors: *Sathiyarajeswaran P*, Shree Devi MS, Sunil Kumar Koppala Narayana, Muthu Tamizh Manoharn, Satheesh Durairaj, Brindha Sundaramoorty, Dhanaraj K, Patturayan R*.

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DrSunil Kumar <sunilkumarnarayanan@gmail.com> Sat, Dec 30, 2017 at 3:03 PM
To: Phytopharmacology Journal <phytopharmajournal@gmail.com>
Cc: Sathya Rajeswaran <siddha2k6@gmail.com>, Shree Devi <shreemd@gmail.com>, Muthu Tamizh Manoharan <muthutamizh@gmail.com>

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