KSCSTE SCHEME

Investigations on antiinflammatory properties of selected underexploited medicinal plants

(002/SRSAGR/2006/CSTE)

FRC No: AMP-06-00-02-2008/ODL(10)/KSCSTE

Project Completion Report

(01-04-2007 to 30-9-2010)

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Thiruvanthapuram

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CONTENTS

No	Topic	Page No
1	Title, Details of participating centers, investigators	1
2	Abstract of the project proposal	2
3	Summary of the work done	4
4	Financial Details	7
5	Introduction	9
6	Experimental set up	13
	Phytochemical studies	13
	In vitro antioxidant assay using DPPH (2, 2 diphenyl 1-picryl hydrazyl radical)	13
	Acute inflammation models	14
	Chronic inflammation models	15
	Lipid peroxidation assay	16
	In vitro human recombinant Cyclooxygenase 2 inhibition assay	17
	In vitro assay 15-lipoxygenase inhibition (kinetic mode)	19
7	Results	22
	Morphological characterization of experimental plants	22
	Harvesting, processing and solvent extraction	26
	Preliminary phytochemical screening	28
	Pectin content in *Artanema sesamoides*	29
	In vitro antioxidant activity assay of ethanol extracts by DPPH method	29
	Protocol for mouse ear erythema model *in vivo* anti inflammatory studies	30
	Screening for anti-inflammatory activity in acute inflammation models	30
	Acute toxicity of *Artanema sesamoides* ethanol extractives	32
	Purification of active compounds from *Artanema sesamoides* root extractives	34
	Purification of compounds/ peaks from butanol fraction by preparative RP HPLC	38
8	Summary	44
9	Contributions made towards increasing the state of knowledge in the subject	45
10	Scope of future work	46

No	List of Tables	Page No
1	Observations on yield parameters	27
2	Percentage recovery of different solvent extractives	27
3	Preliminary phytochemical screening of crude powders	28
4	Phytochemical screening of different solvent extractives	28
5	Quantitative analysis of major phyto- constituents of crude root powders	29
6	Antioxidant activity of crude powders of experimental plants by DPPH method	29
7	Anti-inflammatory activity of different extractives in mouse ear erythema model	31
8	Effect of ethanol extractives of roots on rat paw inflammation	31
9	Effect of ethanol extractives of roots on rat paw inflammation on 7th day of consecutive drug administration	32
10	Lipid peroxidation in kidney and blood of rats under cotton pellet granuloma model	33
11	Percentage recovery of extractives from root and leaf decoctions and infusions of *Artanema sesamoides*	33
12	Effect of water infusion and decoction of *Artanema sesamoides* roots on rat paw inflammation in acute model ie carrageenan model at 3 hrs	34
13	Antiinflammatory activity of root and leaf decoctions and infusions of *Artanema sesamoides* in chronic inflammation model (Adjuvant Arthritis model)	34
14	Effect of different solvent fractions of root ethanol extractives on inflammation in rat paw oedema model	35
15	Antioxidant activity of ethanol fraction and its butanol subfraction of *Artanema sesamoides* by DPPH method	36
16	Antiinflammatory activity of hydrolyzed fraction	37
17	Human recombinant Cox 2 inhibition by *A. sesamoides* root butanol fraction	37
18	Lipoxygenase inhibition by *A. sesamoides* root butanol fraction	37
19	Absorption maxima of peaks in active fraction in Solvent system	40
20	Antioxidant and anti-inflammatory activity of purified peaks by prep HPLC from *A. sesamoides* root butanol fraction	40
21	Base peak and fragmentation pattern of compounds in active root fraction of *A. sesamoides* as per LCMS mass spectrum	41

No	List of figures	Page No
1	Effect of different solvent fraction of root ethanol extractives on rat paw inflammation in rat paw oedema model	35
2	Normal phase TLC of Prep HPLC loaded fraction (EA: Ethanol: water (8:1:1)	38
3	Analytical RP HPLC profile of *Artanema sesamoides* root active fraction ie butanol sub fraction	39

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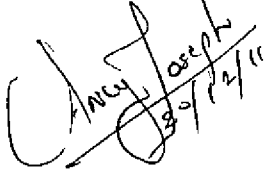

Date: 20/12/11

Ancy Joseph

PROJECT COMPLETION REPORT

1.	**Title of the project**	:	**Investigations on anti-inflammatory properties of selected underexploited medicinal plants** (002/SRSAGR/2006/CSTE)
	FRC No Allotted		**AMP-06-00-02-2008/ODL(10)/KSCSTE**
2.	**Name of Principal Investigator**	:	Dr. Ancy Joseph Associate Professor (Horticulture) Aromatic and Medicinal Plants Research Station, Odakkali, Ernakulam District (Kerala Agricultural University)
	Co-investigators		Dr. Chandrasekharan Nair, A.M. Professor and Head, Dept of Pharmacology & Toxicology, COVAS, Mannuthy (Co PI) Dr, Samuel Mathew, Professor, Soil Sci & Agrl. Chemistry, AMPRS, Odakkali Dr. Baby P. Skaria Professor & Head Aromatic and Medicinal Plants Research Station, Odakkali, Ernakulam District (Kerala Agricultural University)
3.	**Implementing Institution**	:	Kerala Agricultural University
4.	**Date of commencement**	:	0. 04. 2007
5.	**Planned date of completion**	:	30. 09. 2010
6.	**Actual date of completion**	:	30. 09. 2010

7. Objectives as stated in the project proposal:

- Evaluation of anti-inflammatory property of different solvent fractions from different parts of the selected plants *Argyreia speciosa, Ipomoea mauritiana* and *Artanema sesamoides* through *in vivo* studies in albino rats
- Isolation and purification of anti-inflammatory compound/ compounds from these plants through bioassay guided fractionation using acute inflammation models
- Evaluation of anti-inflammatory activity of purified compounds in chronic inflammation models
- Chemical characterization of anti-inflammatory compounds by elemental analysis, FTIR, NMR, and MS analysis

8. Deviation made from original objectives if any: Nil

9. Abstract of the project proposal

The project proposal envisaged the following activities

1. Preliminary screening of selected medicinal plants for anti- inflammatory activity

Under- exploited medicinal plants included in the study were *Argyreia speciosa* Lour. (Samudrapacha), *Ipomoea mauritiana* Jacq. (Palmuthukku), *Artanema sesamoides* Benth. (Neermulli). Authenticated plants from Aromatic and Medicinal Plants Research Station, Odakkali, were used for the studies. Activities such as documentation, conservation and multiplication of each species, extraction of phyto-constituents from different plant parts used in traditional medicine system through sequential soxhlet extraction using a series of solvents of increasing polarity starting from petroleum ether, chloroform, ethanol and water and evaluation of the solvent fractions for anti-inflammatory activity by mouse ear erythema / oedema method etc were envisagedunder the first phase of study.

II. Isolation and purification of anti- inflammatory principles through bioassay guided fractionation.

Qualitative analysis of the solvent fractions with significant anti-inflmmatory activity identified in Phase I for the presence of steroids, terpenoids, phenolics etc by TLC profiling. Isolation of compounds from the effective solvent fractions using column chromatography, prep TLC and prep HPLC. Testing of these compounds *in vivo*

for identification of compounds with anti- inflammatory property using carrageenan induced rat paw oedema model.

III. **Testing of compounds/ fractions in chronic inflammation models.**

Testing of active fractions/ compounds purified through bioassay guided fractionation using acute inflammation models in chronic inflammation models namely adjuvant arthritis model/ cotton pellet granuloma model.

IV. **Characterization of the anti- inflammatory compounds:**

Chemical characterization by elemental analysis, FTIR, NMR and MS analysis.

10. Key words: *Argyreia speciosa, Ipomoea mauritiana, Artanema sesamoides,* antioxidant activity, anti-inflammatory activity

11. Achievements:

i. List of research publications

- Joseph, A., Skaria, B.P., Mathew, S., Joy P.P. and Mathew, G. 2008. Giant Potato- An under-exploited medicinal plant. Indian Journal of Arecanut, Spices and Medicinal plants 10 (1): 8-12

- Ancy Joseph, Chandrasekharan Nair, A.M, Samuel Mathew, Baby P. Skaria, Sheeja E. C. and Savita V. Rajan 2009. 'Preliminary Screening of *Ipomoea mauritiana* tuber extracts for antiinflammatory activity. National seminar '"Molecule to Drugs- Frontiers of Genetic Diversity on Herbs for Better Health Care" held from 21st to -22nd January, 2009 at Mar Athanasius College, Kothamangalam

- Ancy Joseph, Chandrasekharan Nair, A.M. Samuel Mathew, Baby P. Skaria, Sheeja E.C.2010. Antioxidant and antiinflammatory activities of *Argyreia speciosa* Sweet (Hawaiian Baby Woodrose). Proceedings of State level Seminar on Modern Methods in Herbal Drug Development, July 28, & 29, 2010 held at Bharath Matha College, Thrikkakkara, Kochi- 21, Kerala. pp 54-60

- Ancy Joseph, Chandrasekharan Nair, A.M. Samuel Mathew, Baby P. Skaria, Sheeja E.C and Sibil Jose. 2010. Evaluation of antioxidant and anti-inflammatory activity of *Artanema sesamoides*. South Indian Hort. 58: 177- 181.

ii. **Manpower trained on the project:** Two project fellows

iii. **Innovations / Technology developed**

Procedure for extraction of anti-inflammatory fraction from *Argyreia speciosa, Ipomoea mauritiana* and *Artanema sesamoides* were developed. Procedure for purification of anti-inflammatory compounds from leaves and roots of *Artanema sesamoides* standardized.

iv. **Patents taken, if any:** Nil

v. **Application potential**

The study has generated information on antioxidant and anti-inflammatory potential of *Argyreia speciosa, Ipomoea mauritiana* and *Artanema sesamoides*. The data generated is helpful in the development of herbal drug formulation from *Artanema sesamoides* and *Argyreia speciosa* for inflammatory and rheumatic conditions.

12. Summary of the work done.

The KSCSTE funded research project entitled 'Investigations on anti-inflammatory properties of selected underexploited medicinal plants' was carried out at Aromatic and Medicinal Plants Research Station, Odakkali of Kerala Agricultural University during 1-4-2007 to 30-9-2010. Pharmacological studies were carried out at Dept of Pharmacology & Toxicology, College of Veterinary and Animal Sciences, Mannuthy.

The three medicinal plants selected for the study were *Argyreia speciosa* (samudrapachha), *Ipomoea mauritiana* (palmuthukku) and *Artanema sesamoides* (vathomvaretti). The study was intended to evaluate anti-inflammatory activity of these plants and to isolate active fractions/compounds from the potential plants and to characterize them.

Phytochemical studies

Preliminary phytochemical screening and quantitative analysis of major phyto-constituents of crude raw drugs and solvent fractions of *Argyreia speciosa, Ipomoea mauritiana* and *Artanema sesamoides* were done by conventional protocols.

Evaluation of *in vitro* antioxidant activity

Antioxidant activity of crude powders of experimental plants by DPPH method was evaluated and among the three species *Artanema sesamoides* was found to have very high antioxidant activity. *Artanema sesamoides* was also found to have very high phenol content which may contribute to its high antioxidant activity.

Evaluation of antiinflammatory property in different acute inflammation models

Evaluation of antiinflammatory property of different solvent fractions (hexane, chloroform, ethanol and water) from root of *Argyreia speciosa, Ipomoea mauritiana* and *Artanema sesamoides* in acute inflammation model was done by mouse ear erythema method and ethanol extractives of all the three species showed significant anti-inflammatory activity.

Effect of most active solvent fraction obtained by preliminary screening ie; ethanol extractives of roots was studied in another acute inflammation model by rat paw oedema method in different dosages. *Artanema sesamoides* was found to be most effective in reducing acute inflammation among the three species when efficacy of ethanol extractives were studied in rat paw oedema model. Single dose oral administration of root ethanol extract of *A. sesamoides* at 200 mg/kg and 400 mg/kg inhibited inflammation induced by carrageenan injection, by 30.1% and 47.98% respectively compared to negative control (untreated) whereas inhibition was 57.8% in positive control (treated with standard drug).

Effect of ethanol extractives of roots of all the three species on rat paw inflammation on 7^{th} day of consecutive drug administration was evaluated and in this study also *Artanema sesamoides* showed highest activity at a lower dose of 100 mg/ kg of animal.

Evaluation of antiinflammatory property of root ethanol extract of *Artanema sesamoides* in chronic model (cotton pellet induced granuloma model)

In chronic model (cotton pellet induced granuloma), oral administration of ethanol extract of *Artanema sesamoides* showed decreased formation of granuloma tissue by 11.66%, 24.00% and 29.46% ($p < 0.05$) respectively on drug administration for seven consecutive days at doses of 100 and 200 mg/kg of rat and standard drug, diclofenac sodium 10 mg/kg. The root ethanol extract showed high antioxidant activity (EC_{50} = 62.3 ppm) when analysed by DPPH method.

Evaluation of antiinflammatory property of decoction and infusion of roots and leaves of *Artanema sesamoides*

Water infusion and water decoction of both root and leaves of *Artanema sesamoides* was evaluated for anti-inflammatory property in rats using carrageenan induced rat paw oedema model and root decoction was found to be most active in curing inflammation but inferior to successive ethanol extract of root. Traditionally root decoction is used in inflammatory condition.

Evaluation of antiinflammatory property of root decoction of *Artanema sesamoides* in chronic model (Adjuvant Arthritis model)

Root water decoction was evaluated for anti-inflammatory property in chronic inflammation model ie adjuvant arthritis model and was found to inhibit inflammation by 9.78 % at a dose of 200 mg/kg and 14.67 % at a dose of 400 mg/kg.

Acute toxicity studies using *Artanema sesamoides* extracts

Root ethanol extract showed no acute toxicity such as death or fatal symptoms up to a dosage of 15 g/kg on oral administration of drug. Acute toxicity of root decoction was studied and no death or fatal symptoms had been noticed up to a dosage of 5000 mg/ kg of animal.

Fractionation, purification and characterization of active compounds

On further activity guided fractionation of ethanol extract, antiinflammatory activity was found to concentrate in butanol fraction (50% inhibition compared to negative control) in rat paw oedema model.

Butanol fraction possessed 5.7 fold higher antioxidant activity compared to ethanol extract. LC- MS analysis of active butanol fraction was done at Sophisticated Analytical Instrument Facility (SAIF), IIT, Mumbai and SAIF, CDRI, Lucknow. Compounds from the active fraction were further purified by preparative HPLC and most active compound was subjected to FTIR, CHNSO analysis, H NMR and C13 NMR analysis at SAIF, IIT, Mumbai.

Anti-inflammatory activity of all the three plant species included in the study is confirmed by in vivo studies. Use of *Argyreia speciosa, Ipomoea mauritiana* and *Artanema sesamoides* for cure of inflammatory conditions in traditional medicine system is scientifically validated. Ethanol extractives of *Artanema sesamoides* and *Argyreia speciosa*

roots were much superior in anti-inflammatory activity. *Artanema sesamoides* root extractives showed highest antioxidant activity and very high total phenolic content compared to other two root extractives. It also showed highest anti-inflammatory activity at a lower dosage at 3 hr observation and also at 7th day of consecutive drug administration. Among infusion and decoction of roots and leaves of *Artanema sesamoides*, root decoction showed highest activity validating use of root decoction in traditional system. Active compounds were chemically characterized from the most potential species ie *Artanema sesamoides* and were found to be iridoid, flavonoids and phenyl ethanoid glycosides. Its root decoction and ethanol extract did not show acute toxicity on oral administration. The root extract possessed high antioxidant activity and was found to act through inhibition of 15- Lipoxygenase and Cyclooxygenase 2 enzymes in inflammatory pathways. The active fraction inhibited lipid peroxidation process in rat blood and kidney tissue confirming its *in vivo* antioxidative power.

13. Financial Details

No.	**Sanctioned Head of Expenditure**	**Amount Allocated* (Rs.)**	**Total expenditure (Rs)**	**Utilization (%)**
1	Manpower	391500	389000	99.3
2	Equipments	150000	56250	37.5
3	Consumables	500000	500000	100.0
4	Travel	30000	25591	85.3
5	Contingencies	30000	29520	98.0
6	Inst. overhead	99800	84782	84.9
	Grand Total	**1201300**	**1085143**	**84.17**

14 a. Procurement / usage of equipment

No.	Name of equipment	Make / Model	Cost (Rs.)	Date of installation	Utilization (%)	Remarks
1	Vertical Deep freezer 170 litre	Rotek-RDFV-175	56250	07/02/2008	100%	In working condition

14 b. Plans for utilizing the equipment facilities in future

The equipment will be utilized for continuing studies on bioactivities of medicinal plants

Name and signature with date

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INTRODUCTION

Inflammation is a non-specific defensive response of the tissues to local aggression. In acute inflammation, blood vessel dilatation, liquid and plasmatic protein exudation and leukocytarian emigration are prevalent. Chronic inflammation results when the acute response is insufficient to eliminate proinflammatory agent. It is characterized by the presence of lymphocytes and macrophages, as well as the proliferation of blood vessels and conjunctive tissue. In both cases, oedema, blush, increase in local temperature and pain are the typical symptoms. Herbal medicines derived from the plant extracts are being utilized to treat a variety of acute and chronic inflammatory conditions though relatively little knowledge is available about their mode of action. There is a growing interest in the pharmacological evaluation of various plants used in traditional system of medicine for inflammatory condition. The project envisaged evaluation of the anti-inflammatory potential of three medicinal plant species in experimental animal models. These plants have good reputation as potent medicaments in the treatment of inflammatory ailments. However, very little scientific backing was available with respect to their pharmacological properties. The project included preliminary screening of these plants for anti-inflammatory properties through different animal models to identify the plant part that possess the highest anti- inflammatory activity and bioassay guided fractionation of the anti-inflammatory principles to homogeneity through various chromatographic procedures. Though these plants are being utilized in ayurveda, unani and folklore medicine system, their anti- inflammatory efficacy was not yet studied by scientific techniques. Conclusive finding from the present study would be of significant impact in the treatment of inflammatory conditions including chronic diseases such as arthritis. Availability only as raw drug and lack of scientific validation is limiting the potential of these natural resources and keeps away a majority from these traditional medicines.

Many of the plants used in traditional system of medicine are under exploited and information is lacking about the active chemical constituents of the economic parts. Scientific validation of the medicinal property of these plants is possible through pharmacological evaluation in animal systems. Plants included in this study namely *Argyreia speciosa, Ipomoea mauritiana* and *Artanema sesamoides* are under exploited and studies on their anti- inflammatory properties are scarce. Purification and identification of functional compound is possible through bioassay guided fractionation. The plants included in the study were the following.

1. *Argyreia speciosa* Lour. (Elephant creeper/ Woolly morning creeper).

Family: Convolvulaceae

Vernacular names: Samudrapacha (Malayalam), Samandar ka pat (Hindi), Samudrapalaka (Sanskrit).

A very large climber with stout stems and large ovate leaves. It is propagated through cuttings and seeds. The root is bitter, aphrodisiac, diuretic, used in gonorrhoea and chronic ulcers in unani system of medicine. It is used in the indigenous systems of medicine for the treatment of inflammatory conditions. In ayurveda, the root is regarded as alterative tonic and useful in rheumatic and nervous diseases. The tuber in the form of a paste is applied externally in abscess of the stomach. The leaves are used as emollient poultices for wounds and externally in skin diseases. In synovitis, the powdered root is given with milk. Mixed with vinegar, the sap is rubbed over the body to reduce obesity.

2. *Ipomoea mauritiana* Jacq. Syn: ***I. digitata* Linn.** (Giant potato).

Family: convolvulaceae

Vernacular names: Palmuthukku (Malayalam), Bidarikand (Hindi), Payasvini (Sanskrit)

It is a perennial with large ovoid or elongated tuberous roots and long thick twining stems. It is distributed throughout tropical India. It is propagated through seeds and tubers. The large tuberous roots are very much used in native medicine, being regarded as a tonic, alterative, aphrodisiac, demulcent and galactogogue, chologogue, diuretic, stimulant, demulcent, useful in leprosy and burning sensation. The root is anthelminthic, stomachic, appetiser, useful in syphilis, gonorrhoea, and inflammations (Unani). The powdered root-stock is given with the milk for the purpose of increasing secretion of milk. It cures rheumatism, diabetis and fatigue, improves bodyweight and regulates mensus. It is an ingredient in Vidaryadi ghrtam, ashwagandhadi ghrtam, Vidari churnam, Dasamoolarishtam, Dhannvatharam thailam etc. Taxaxerol acetate is identified as active principle. Tubers contain resin, carbohydrate, protein and sugars.

3. *Artanema sesamoides* Benth. Syn: ***A. longifolium* (L.) Vatke.**

Family: Scrophulariaceae

Vernacular names: Neermulli (Malayalam), Kokilasha (Sanskrit)

It is an erect herb, 60- 90 cm high, sparingly branched, stem hollow and quadrangular, often tinged with purple. Leaves opposite, simple subsessile, lanceolate.

1. ***Argyreia speciosa*** **Lour. (Samudrapacha)**

Rooted cutting

Field planted

2. ***Ipomoea mauritiana*** **Jacq. (Palmuthukku)**

Raised seedlings

Field planted

3. ***Artanema sesamoides*** **Benth. (Neermulli)**

Rooted cuttings

Field planted

Flowers large, bisexual, zygomorphic, in erect terminal racemes up to 40 cm long; calyx five lobed, 3-10 mm long, persistent in fruit; corolla violet; stamens four, didynamous; ovary superior, 2 celled; style filiform; stigma 2 lobed, capsules globose, up to 1 cm in diameter, many seeded. Seeds 0.6 mm long, ellipsoid globose, yellowish brown. It is distributed in western Peninsula, Ceylon, Malay Peninsula, Sumatra, Java, Philippines, Borneo and Tropical Africa. It occurs in forest, in humid and swampy localities and is common along rice fields and watercourses up to 400 m altitude. In Nigeria and Tanzania, its leaves are collected from wild and used as vegetable. Its roots are diuretic and anti-inflammatory. A decoction of the root is used in rheumatism, syphilis, ophthalmia and diarrhoea, stone and nausea. The seeds cure biliousness, improve vitality and favour conception.

Anti-inflammatory activity is reported in *Argyreia speciosa* but the functional compounds are not yet identified and characterized. Though a lot of information is available on many *Ipomoea* species, work done on anti- inflammatory activity of *Ipomoea mauritiana* are scanty. Though *Artanema sesamoides* is used in traditional medicine system for inflammatory conditions, research reports on pharmacological properties of this crop is meager.

Practically no attempt has been done to study in depth about these under exploited medicinal plants with anti- inflammatory properties and commercial cultivation is not practiced for them. Information available on phyto- chemical composition and *in vivo* studies to confirm their efficacy in curing various inflammatory conditions are scanty. Hence the study was intended to generate information on these aspects. Biochemical profiling and isolation and chemical characterization of anti-inflammatory compounds would enable us to get information on chemical constituents that contribute to the medicinal property of these plants. Conventional anti-inflammatory drugs such as steroidal drugs and non-steroidal drugs like indomethacin and phenylbutazone rely on diverse action mechanisms, yet share severe gastrointestinal side effects. Some act by stimulating the immunological system and some others by inhibiting lipid peroxidation, a phenomenon associated with antioxidant as well as anti-inflammatory activities. Their antioxidant action protects cells from degenerative processes. Some inhibit enzymes which participate in the synthesis of inflammatory substances, prostaglandins and prostacyclines in the body derived from arachidonic acid. Confirmation of anti-inflammatory effect of these medicinal plants and generation of information on their mode of action through pharmacological evaluation could promote their use in traditional medicine system and also could help to

find alternative medicinal uses. The findings would be of potential benefit to pharmaceutical firms in formulating anti-inflammatory drugs. So the studies were taken up with the following objectives

I. Preliminary screening of the selected medicinal plants for anti- inflammatory activity

Sequential extraction with solvents of increasing polarity separately from powdered dry samples of different plant parts used in traditional medicine system and evaluation of each solvent fraction for anti- inflammatory activity by mouse ear erythema method to identify and most suitable solvent for extracting anti-inflammatory prienciples.

II. Isolation and purification of anti- inflammatory principles through bioassay guided fractionation.

Solvent fractions with significant anti- inflammatory property obtained from Phase I experiments would be fractionated through column chromatography, prep TLC and prep HPLC and each fraction/ compound would be tested by mouse ear erythema method for identification of the active compound. Anti- inflammatory activity of these compounds would be further ascertained by rat paw oedema model and would be purified further through HPLC.

III. Testing purified compounds in chronic inflammation models

Testing of the compounds purified through bioassay guided fractionation using acute inflammation models from Phase II experiments in chronic inflammation models namely adjuvant arthritis model.

IV. Chemical characterization of the purified anti- inflammatory compounds

Chemical characterization of purified anti-inflammatory compounds by elemental analysis, FTIR , NMR and MS.

EXPERIMENTAL SET UP

Animals: Sprague Dawley strain of albino rats (150-180 g) and Balb C albino mice (25-30 g) of either sex were procured from the Small Animal Breeding Station of Kerala Agricultural University. They were housed in polypropylene cages and kept under standard environmental conditions. The rats were given uniform laboratory diet and water *ad libitum*. All experimental protocols were approved by the institutional animal ethics committee.

Chemicals: DPPH (1, 1-diphenyl-2-picryl-hydrazyl) was obtained from Sigma Chemicals, USA and carrageenan from Himedia, Mumbai. All chemicals and organic solvents used were of analytical grade.

Extraction: The roots were sliced, oven dried at 55°C, powdered and extracted successively with hexane, chloroform, ethanol and water.

Phytochemical studies: Freshly prepared extracts were subjected to preliminary phytochemical screening tests for the detection of various constituents using conventional protocol. In the preliminary phytochemical tests, test for phenols (folin ciocalteau), test for flavonoids (flavonoid test, shinoda test, ferric chloride test and lead acetate test), test for saponins (foam test), test for glycosides (Killar Killiani test), test for alkaloids (Mayer's test, Wagner's test and Dragendorff's test) and test for tannins (ferric chloride test) were carried out. Major categories of phytoconstituents were estimated from the dry powder following standard procedures (Peach and Tracey, 1959, Raman, 2006, Harborne, 1998).

Pectin estimation: Pectin content of roots and leaves of *Artanema sesamoides* was estimated as per Harborne, 1998.

***In vitro* antioxidant assay using DPPH (2, 2 diphenyl 1-picryl hydrazyl radical)**

DPPH radical scavenging activity of extractives was determined using the method described by Huang *et al* (2005). The extract was diluted with methanol to prepare a series of five concentrations ranging from 0 to 250µg/ml. To each of 2.9 ml of the dilution taken in a spectrophotometer cuvette, 0.1 ml of 0.1 mM methanolic DPPH solution was added. The contents were mixed, incubated in dark for 20 min and absorbance measured at 517 nm.

1. ***Argyreia speciosa*** **Lour. (Samudrapacha)**

Harvested roots

Root Bark

Drying in wooden panels

Root powder

2. ***Ipomoca mauritiana*** **Jacq. (Palmuthukku)**

Harvested root tubers

Sliced tubers

Artanema sesamoides **Benth. (Neermulli)**

Harvested roots

Root powder

Successive solvent extraction of root powders

Ethanolic extraction of *Ipomoea mauritiana* (palmuthukku) root powder

Chloroform extraction of *Artanema sesamoides* (neermulli) root powder

A graph was drawn with concentration of the extractives in the reaction medium on the x-axis and percentage DPPH quenched on the y-axis. From the relationship, the concentration of extractives resulting in 50% quenching of DPPH was worked out and expressed as EC50.

Antiinflammatory studies in animal models

Acute inflammation models

i). *Mouse ear erythema model*

Preliminary evaluation of the solvent fractions for antiinflammatory activity was carried out by mouse ear erythema method using Balb C mice of either sex. Inflammation was induced on the inner side of both the external ears of the mice by topical application of croton oil (0.5 mg/ear diluted with liquid paraffin in 1:8 ratio). Mice were divided into six groups consisting of eight animals each, one group served as negative control (treated with vehicle only) and second group served as positive control. Positive control received hydrocortisone acetate 0.5mg/ear and third, fourth, fifth and sixth group received 10 mg/ear of hexane, chloroform, ethanol and water extractives, respectively on inner side of the left ear 30 minutes prior to application of croton oil and then at 30 minutes intervals, five times. Degree of cure in left ear of each mouse was scored in comparison to positive and negative control in 0-10 scale at 24 hours and 48 hours.

ii). *Rat paw oedema model*

Confirmation of activity of ethanol extract was done by rat paw oedema model using Sprague Dawley strain of albino rats. Oedema was induced by injection of 0.1 ml carrageenan 1% w/v in 0.9% saline into sub plantar region of left hind paw. The rats were divided into 4 groups (n = 8). Each rat in negative control group received 0.25 ml of 3% Tween 80, positive control group received diclofenac sodium 4mg/kg and third and fourth group received 200 mg/kg and 400 mg/kg of ethanol extractives, respectively one hour prior to injection of carrageenan. Paw volume was measured using plethysmometer at 0 hr and 3 hr of carrageenan injection (Winter *et al.*, 1962).

The antiinflammatory effect was calculated by the following equation

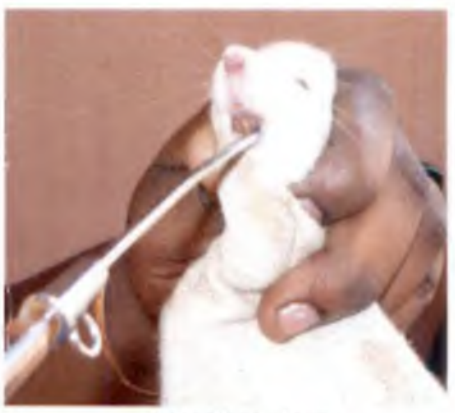
Antiinflammatory activity (%) = (1-D/C) x 100,

where D represents the average increase in paw volume 3 h after carrageenan injection in treatment group and C represents average increase in paw volume in negative control group (Suleyman *et al.*, 1999).

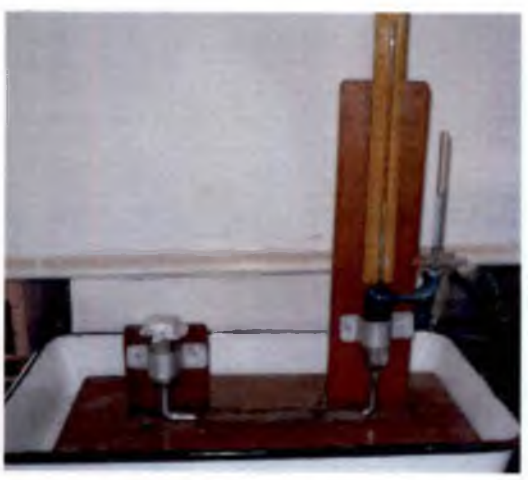
Wistar rat

Paw Odema

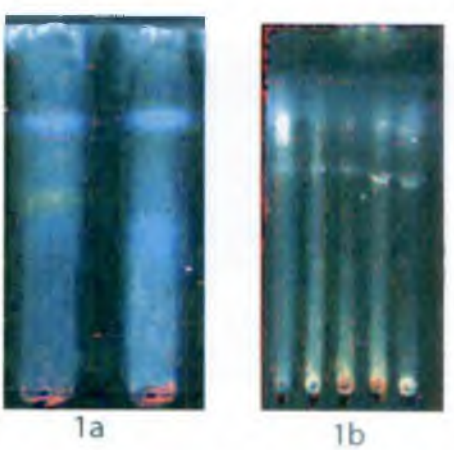
Carrageenan injection



Feeding drug



Plethysmometer



1a 1b

Active butannol fraction :TLC profile
1a. In chloroform methanol 85: 15
1b, In Chloroform methanol 20: 80

Chronic inflammation models

i). *Cotton pellets-induced granuloma model*

The rats were divided into four groups (n = 8). After shaving the fur, the rats were anaesthetized and 20 mg of sterile cotton pellets were inserted, one in each axilla. The ethanol extract (100 and 200 mg/kg, p.o) and diclofenac sodium (10 mg/kg, p.o.) and control vehicle were administered orally for 7 consecutive days from the day of cotton pellet implantation. The animals were anaesthetized on the eighth day and cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 h and dried at 60°C to constant weight. Increase in the dry weight of the pellets was taken as measure of granuloma formation (Winter *et al.*, 1962).

ii). *Complete Freunds Adjuvant Arthritis model*

In this experiment, effect of the test drugs on established arthritis (curative protocol), was studied. Adjuvant arthritis was induced as described by Pearson (1956) and as modified by Woode *et al.* (2008). Sprague Dawley strain of albino rats were obtained from Small Animal Breeding station, Mannuthy, housed in rat cages on a 12 hour light/dark cycle and given free access to standard lab diet. All the rats were injected intraplantar with 0.1 ml Complete Freund's Adjuvant (Sigma Aldrich) suspended in sterile 0.9% PBS into the right hind paw. CFA contained heat-killed *Mycobacterium tuberculosis* strains in paraffin oil as a 3 mg ml^{-1} suspension. Rats were randomly assigned to groups of eight numbers as follows.

1. Arthritic control ie negative control: (intraplantar injection of 0.1 ml CFA only and left untreated)
2. Positive control (intraplantar injection of 0.1 ml of CFA) and administered standard drug diclofenac sodium 10 mg kg^{-1} i.p.., 9 days after intraplantar injection of CFA
3. Test drug group 1(intraplantar injection of 0.1 ml of CFA) administered plant extract 100 mg kg^{-1} p.o., 9 days after intraplantar injection of CFA
4. Test drug group 2(intraplantar injection of 0.1 ml of CFA) administered plant extract 200 mg kg^{-1} p.o., 9 days after intraplantar injection of CFA

Arthritic control group (negative control) received only intraplantar injection of Complete Freund's Adjuvant in sterile paraffin oil (CFA) on day 1, while positive arthritic control group received intraplantar injection of 0.1 ml CFA on day 1 and standard drug Diclofenac sodium, 10mg/ kg, on day 9 with the onset of arthritis. *Artanema sesamoides* extract (100 and 200 mg kg^{-1}) were administered to rats in two treatment groups. The

extract was suspended in 2% gum acacia, while the reference drug (diclofenac sodium) was dissolved in normal saline. Standard drugs and test drugs were administered on day 9 with the onset of arthritis. Test drugs were administered in volumes not exceeding 10 ml kg^{-1}. All drugs were freshly prepared. Inflammation induced untreated (negative control) animals were given 2% gum acacia (10 ml kg^{-1} p o).

In all experiments, foot volume was measured by mercury displacement plethysmography for both the injected paw and the non-injected paw before intraplantar injection of CFA on day 0 and every consecutive days for 14 days. The oedema component of inflammation was quantified by measuring the difference in foot volume between day 0 and the various time points. The extract was administered every day while standard drug was administered every other day from 9^{th} day onwards after the onset of arthritis.

Lipid peroxidation assay:

Lipid peroxidation assay was done in case of rats subjected to the chronic inflammation model ie; cotton pellet induced granuloma model studies. At the end of experiment, three rats from each treatment were sacrificed by euthanasia and the blood was collected in heparin-coated tubes, plasma was separated from it and frozen. The kidney was harvested and washed with cold normal saline and frozen. Thiobarbituric acid reactive substance (TBARS), were measured in the plasma and kidney tissues.

Tissue preparation: As haemoglobin interferes with the assay, blood was removed from tissue sample by perfusion with PBS containing heparin. Tissue was then re-suspended at 100 mg/ml in PBS. The tissue sample was then homogenized on ice, spun at 10,000 g for 5 minutes and the supernatant collected and assayed directly for its TBARS level and results were normalized based on its protein concentration.

Plasma: The plasma sample was prepared as soon as blood sample was drawn to minimize the hemoglobin interference and then assayed directly.

TBARS Assay enables the direct quantification of the end products of lipid peroxidation, specifically malondialdehyde (MDA) in plasma and tissue homogenates and it provides measurement of anti-oxidant characteristics of test drugs. Thiobarbituric acid reacts with malondialdehyde to yield 1:2 adduct which was measured colorimetrically. The unknown

MDA containing samples or MDA standards were first reacted with TBA at 95°C. After a brief incubation, the samples and standards were read spectrophotometrically. The MDA content in unknown samples was determined by comparison with the predetermined MDA standard curve.

Reagents used:

- TBA Reagent: Prepared just before use *as is stable for 24 hours only*. 200 mg of thiobacbituric acid in 30 ml distilled water and 30 ml of acetic acid.
- 20% trichloroacetic acid (TCA)
- Malondialdehyde standard

Plasma 0.1 ml was treated with 2 ml of TCA–TBA–HCL (TBA 0.37%, 0.25 N HCl and 15% TCA) reagent (1: 1: 1) and incubated in boiling water bath for 10 min. After that, the mixture was cooled, mixed with 2 ml of freshly prepared 1 M NaOH. The absorbance was measured at 535 nm. It is expressed as millimoles per deciliter for plasma and as millimoles per gram wet kidney tissue.

In vitro human recombinant Cyclooxygenase 2 inhibition assay

The inhibitory effect of active fraction was tested and expressed as IC50 and percentage of enzyme activity inhibition.

1. Tris-HCl buffer 100 mM (pH 8) - Tris HCl 1.576 g dissolved in 100ml and pH adjusted with 0.1 M NaOH
2. Cox 2 human recombinant enzyme (Sigma), received as aqueous solution. EC 1.14.99.1 Prostaglandin H synthase 2. Product No C 0858.

   COX-2 was received as a solution in 80 mM Tris-HCl, pH 8, with 0.1% Tween 20 and 300 mM diethyldithio-carbamate (DDC) as a preservative. Specific Activity: minimum 8,000 units per mg protein. After initial defrost, the product was aliquoted in 5 microlitre batch and re-frozen at -70 °C to avoid repeated freeze-thaw cycles and never stored in a frost-free freezer. Diluted 1 microlitre supplied solution to 50 microlitre/ or upto 250 microlitre, kept diluted with buffer in ice / chilling water during experiment.

3. 5 mM glutathione reduced, freshly prepared. MW 307.33, soluble in water/ buffer. For 5mM solution 0.0015g/ ml was used. 50 microlitre/ sample was added.

4. 5 mM haemoglobin, freshly prepared. MW 64500. Soluble in water/ buffer. 0.3225 g/ 1ml was dissolved. 50 microlitre/ sample was added.

5. 200 uM arachidonic acid $C_{20}H_{32}O_2$ MW 304.47 soluble in 50% aqueous ethanol/ aq methanol, DMSO, or dimethyl formamide, is stable up to 6 months when stored at -20 °C when purged and stored with an inert gas such as argon or nitrogen. Dissolved in ethanol only and kept in different aliquot, diluted with equal volume buffer, freshly prepared every day. Aqueous solutions of arachidonic used within 12 hours.

6. 10% trichloroacetic acid in 1N HCl. 200 microlitre/lot, 0.1ml /ml 1N HCl.

7. 1% thiobarbiturate freshly prepared 200 microlitre 0.05 g/ 5 ml buffer/ DW

8. Plant extract 100 microgram in min ethanol (10 microlitre) and 90 microlitre buffer.

In 100 % activity group, the reaction was initiated by the addition of 200 uM arachidonic acid 50 micro litre and terminated after 20 minutes incubation at 37 °C by addition of 10% trichloroacetic acid in 1N hydrochloric acid 200 microlitre . No plant extract was added. Following centrifugal separation and addition of 1% thiobarbiturate, 200 microlitre, COX activity was determined by reading absorbance at 530 nm.

In blank, no cox 2 enzyme and plant extracts were added, but substrate arachidonic acid and plant extract were added and after 20 min incubation at 37°C, reaction was terminated by addition of 10% trichloroacetic acid in 1N hydrochloric acid. Following centrifugal separation and addition of 1% thiobarbiturate, COX activity was determined by reading absorbance at 530 nm.

In inhibitor assay group, add plant extract active fraction, 200 micro gram dissolved in minimum ethanol and diluted in buffer in 100 microlitre buffer, incubated for 1 min. Then reaction was initiated by the addition of 200 uM

arachidonic acid and terminated after 20 minutes incubation at 37°C by addition of 10% trichloroacetic acid in 1N hydrochloric acid. Following centrifugal separation and addition of 1% thiobarbiturate, COX activity was determined by reading absorbance at 530 nm.

Sample preparation for photometric analysis

		Blank Control group	100 % activity group	Inhibitor dose 1	Inhibitor dose 2	Inhibitor dose 3
1	Cox 2 1 microlitre to 50 microlitre	Nil	50µl	50 µl	50 µl	50 µl
2	Tris HCl buffer, pH 8	2.55 ml	2.5 ml	2.45	2.40	2.30
3	Glutathione reduced	50 µl	50 µl	50 µl	50 µl	50 µl
4	Hemoglobin	50 µl	50 µl	50 µl	50 µl	50 µl
	Mix well					
5	Plant extract	nil	nil	5 µl (50 µg)	10 µl(100 µg)	20 µl(200 µg) Leaf AF
	Vortex , incubate for 1 min					
6	Arachidonic acid	50 µl	50 µl	50 µl	50 µl	50 µl
	Incubate at 37 degree centigrade for 20 min					
7	TCA	200 µl	200 µl	200 µl	200 µl	200 µl
	Centrifuge, at 3000 rpm take supernatant					
8	TBA (Light sensitive, fresh)	200 µl	200 µl	200 µl	200 µl	200 µl

In vitro assay 15-lipoxygenase inhibition (kinetic mode)

The enzyme used was lipoxygenase from soybeans («Lipoxidase»; Sigma No. L-7395, ≥50,000 units/mg solid,108 kDa) which has sufficient correlation for mammalian enzyme inhibition. S lox (soyabean lox) is considered quintessential to 15 lox. Linoleic acid/ sodium lineate was used as substrate (L8134 Linoleic acid MW 280.45, Sodium linoleate MW 303.45). One unit will cause an increase in A_{234} of 0.001 per min at pH 9.0 at 25 °C when linoleic acid is the substrate in 3.0 ml volume (1 cm light path). One A_{234} unit is equivalent to the oxidation of 0.12 µmole of linoleic acid. Linoleic acid was dissolved in tween 20/ethanol. Sodium lineolate was dissolved in 2ml borate buffer.

Reagents

A: Borate buffer, 0.2 M, pH 9.00: Prepared 100 ml from boric acid and pH adjusted with 1M sodium hydroxide.

B: Substrate solution 250 uM: Freshly prepared every day. The final concentration was 125 uM.

C: Enzyme solution: sLox (Lipoxidase», Sigma) supplied in buffer was stored at -20°C. Enzyme supplied in buffer was diluted 50 times only. The enzyme was kept on ice throughout the experimental period and used after dilution to 400 U/ml.

D: Inhibitors: The plant extract to be tested was dissolved in dimethyl sulfoxide (DMSO) starting with a fairly strong solution (e.g. 10 mg/ml) and then making a dilution series. Used a final inhibitor concentration of 125 uM (same as substrate).

Measurement of enzyme activity

Quartz cuvette was used for blanks and samples.

Blank: Pipetted 20 microlitre DMSO and 200 µl linoleic acid and 2.55 ul borate buffer and placed in the blank sample compartment of the spectrophotometer throughout the experimental period.

Sample without inhibitor: Pipetted 20 microlitre DMSO and 50 ul enzyme solution into cuvettes. Added rapidly 200 ul substrate (250 uM sodium lineoate in borate buffer to cuvettes.. Absorbance was read at 234 nm for 5 minutes every 30 seconds.

Sample with inhibitor: Pipetted out varying quantity of plant extract (inhibitor) in DMSO, and 50 ul enzyme solution and 2.55 ul borate buffer into the 5 remaining cuvettes. Incubated for 5 minutes. Added rapidly 200 ul substrate in (250 uM sodium lineolate in borate buffer) to each of the 5 cuvettes.. Absorbance was read at 234 nm for 5 minutes every 30 seconds. Inhibitor ie plant extract was prepared @ 10 mg/ml in DMSO ie 10µg/µl from which varying quantities are pipetted out.

Sample preparation of for kinetic mode

No	Reagents	Blank	No Inhibitor	Inhibitor dose 1	Inhibitor dose 2	Inhibitor dose 3
1	s lipoxygenase in 0.2 M borate buffer (2 µl ino 200 µl buffer	Nil	50µl	50 µl	50 µl	50 µl
2	0.2 M borate buffer pH 9.	2.55ml	2.5 ml	2.30	2.30	2.30
	Mix well & keep on ice					
5	Plant extract in DMSO 10mg/ml	nil	Nil	5 µl (50 µg)	10µl (100 µg)	20 µl (200 µg)
	DMSO			15 µl	10 µl	0µl
	Vortexed, incubated for 5- 15 min, observed till absorbance at 234 is constant					
6	Sodium lineolate 250 µM in borate buffer	200 µl	200 µl	200 µl	200 µl	200 µl
	Absorbance was read at 234 nm for 5 minutes every 30 seconds in kinetic mode					

15 - lox assay in photometric mode

No	Reagents	Blank	No Inhibitor	Inhibitor dose 1	Inhibitor dose 2	Inhibitor dose 3
1	15 lipoxygenase in 0.2 M borate buffer. 2 µl enzyme to 200 µl buffer	Nil	50 µl	50 µl	50 µl	50 µl
2	0.2M borate buffer pH 9.	2.55ml	2.5 ml	2.30	2.30	2.30
	Mix well & keep on ice					
3	Plant extract in DMSO 10mg/ml	nil	Nil	5 µl (50 µg)	10µl (100µg)	20 µl (200 µg)
4	DMSO			15 µl	10 µl	0 µl
	Vortexed, incubated for 5-15 min on ice till absorbance at 234 is constant					
5	Sodium lineolate 250 µM in borate buffer	200 µl	200 µl	200 µl	200 µl	200 µl
	Incubation 20 min at room temp 25 degree & then stop reaction					
6.	Ice cold Acetonitrile + Glacial acetic acid 2.5% v/v	400 µl	400 µl	400 µl	400 µl	400 µl
	Vortex, Incubate on ice 10 min, centrifuge at 10000 rpm for 4 min, read ab at 237 nm in constant mode					

Acute toxicity studies: For toxicity studies, groups of 8 Sprague Dawley strain of albino rats were administered orally with ethanol extract in the range of doses 2000-15000 mg/kg and the mortality rates was observed upto 72 hours.

Statistical analysis: The experimental results were expressed as the mean ± S.E.M. Data were assessed by the method of analysis of ANOVA followed by student's *t*-test. P value of < 0.05 was considered as statistically significant.

RESULTS

Results of the study on "Investigations on anti-inflammatory properties of selected under exploited medicinal plants" are presented in this chapter.

Experimental plants were raised in the field to produce genuine raw drugs for extraction: All the three experimental plants were collected from authentic sources, multiplied by vegetative propagation and planted in the field and maintained by providing aftercare. Morphological characterization of all the three experimental plants was done. Morphological traits were documented in detail to serve as a plant descriptor for correct identification of the plant species.

1. Morphological characterization of experimental plants

1. *Argyreia speciosa*

No	Morphological trait description	Biometric observation
1	**Growth habit**: Twining woody climber	Plant height: More than 20 m
2	**Stem**: Stout and woody at the base and ash coloured, young shoots are densely covered with white pubescence, shows close coiling, profuse branching	Diameter : 1.5 cm-7 cm Internodal : 1.3 cm- 38 cm length
3	**Canopy** : Spreading	Diameter: 3.5-4.0cm at one year
4	**Leaf:** simple, large ,ovate, or broadly ovate-cordate, alternate, exstipulate, acute, or shortly acuminate or apiculate, entire, long petioled, margin smooth, the petioles generally shorter than the blade. Blade, fairly large, upper surface dark green, fairly smooth or glabrous, white tomentose beneath and with two large flat scabrous dark coloured glands one on either side at the base, strongly nerved with several large distinct nearly opposite lateral nerves that run parallel	length : 7.5-30 cm width : 6.3-26 cm petiole length : 7-25 cm No of veins on each side of midrib : 14-16 pair :
5	**Inflorescence:** Axillary, sub capitate to umbelliferous, bracteate cymes borne on stout stiff erect whitish tomentose peduncles, bracts large, whitish, foliaceous, deciduous; the outer larger, up to 5 cm long, oblong to ovate-lanceolate, acute or more often with a long acumin, somewhat fleshy, veined, softly wooly	Length :10-15 cm No of flowers/Inf: 6-14 Peduncle length : 7-9 cm

pubescent outside and glabrous inside.

6	**Flower:** large, showy, silky, pubescent, regular, bisexual, hypogynous, funnel shaped, tinted purple or pale to deep rose, sub capitate or nearly sessile or with very short pedicels	Diameter : 6-8 cm Pedicel length : 0.4-0.6 cm
7	**Calyx:** 5 sepals, thick coriaceous (leathery) or slightly fleshy broadly ovate or oblong-elliptic, imbricate, very obtuse persistent sepals, densely white tomentose or wooly outside, reddish within	sepal's length : 1- 1.7 cm width : 1-1.5 cm
8	**Corolla:** . Tubular, infundibuliform, gamopetallous, large and showy, deep purple along inside the corolla tube and deep rose in lobes. Pinkish white along outer side of tube, the tube, somewhat inflate, wooly or pubescent and rosy outside and glabrous within, limb, very shortly lobed plaited or plicate with 5 longitudinal bands or parts exposed in bud, more or less silky pubescent or hirsute outside.	Lobe length : 2-2.5 cm Lobe width : 2.4-2.6 cm Tube length : 3-4 cm Tube width : 2.6-3 cm Total length : 5-6.5 cm Total width : 5-7 cm
9	**Stamens**: 5 stamens, three short and 2 long, epipetalous, filaments free, often enlarged and villous at base and bearing straight oblong anthers, anthers basifixed and 2 celled. A prominent annular disc surrounding the ovary is present. Filaments & anthers white	Filament length Long stamen : 2-2.5cm Short stamen : 1.5-2 cm Anther length: 0.5 cm
10	**Ovary**: Superior, bicarpillary, syncarpous, globose, glabrous, completely four locular and 4 ovuled, one ovule in each cell. Style filiform, Stigma bifid	Style length : 2.2-2.5 cm
11	**Period of flowering**: Throughout the year	Aug- Sept, May- June
12	**Fruit**: dry, globose, small, apiculate, pale or yellowish brown, smooth and shining, indehiscent, irregularly crumbling berry	Diameter : 1.2 - 2 cm No of seeds/fruit : 4
13	**Seeds**: embedded in a mealy pulp, with erect curved embryo with corrugated cotyledons.	
14	**Roots**: long, spreading cylindrical roots, many narrow rootlets at the distal region, brown, smooth, milky latex exudes when wounded. The woody regions are light yellow in colour, thicker roots are with rough surface	Length : 1- 5 m or more Thickness : 1- 1.5 cm for 1 yr old plant

2. *Artanema sesamoides*

No	Morphological trait description	Biometric observation
1	**Growth habit**: Herbaceous annual	Plant height: 100-150 cm
2	**Stem**: erect, sparingly branched, sharply quadrangular to almost 4-winged, scabrid on the angles, fleshy, hollow stem, often tinged with purple colour at nodes and in tender shoots	Diameter : 0.5 -1 cm Primary branches : 1 Secondary branches: 3-8 Internodal length: 5-18 cm
3	**Canopy**: Bushy, spreading	Diameter: 80-105 cm
4	**Leaf**: opposite, simple, sessile or short petioled, subsessile, glabrous but rough above because of small bristles, smooth & glabrous on under surface, ovate lanceolate, rarely oblong,, acute or acuminate, slightly serrate, more or less scabrid, slightly thick, purple tinge along the midrib at the basal portion especially in young leaves.	Length : 3.5-25 cm Width : 1-6 cm No/pt : 40-60
5	**Inflorescence**: terminal, erect, many flowered, green coloured, peduncled, erect terminal raceme.	Length : 10-60 cm Diameter : 0.3-0.5 cm Inflorescence No/pl : 3-5 No. of whorls/inf. Stalk: 6-24 No of flowers / inf : 12-48
6	**Flower**: bisexual, zygomorphic, fairly large, short-stalked, bracts beneath, blue or purple, solitary in the upper leaf axils or in terminal bracteate racemes, the bracts especially the lower being foliaceous. Bracteoles absent. The pedicels are shorter than or nearly equal to the calyx.	Diameter : 2-2.5 cm Pedicel length: 0.3-1.6 cm
7	**Calyx**: 5- partite almost to the base, Sepals green, ovate, oblong, broader, acute, nearly glabrous, much imbricate, somewhat leafy, overlapping at anthesis, persistent in fruit.	No of lobes : 5 Sepal length : 0.7-1.0 cm Sepal width : 0.5-0.8 cm
8	**Corolla**: Blue or violet- purple, glandular, hairy, gamopetalous, blue or lilac, sub-companulate, reticulately veined; tube long, broad and 2 lipped, the upper lip which is outer in bud is erect, broad, rounded, and notched or emarginate, lower lip patent, scarcely longer, spreading, rounded, 3 fid.	lobe length : 0.6-0.9 cm lobe width : 1-1.3 cm Tube length : 1.5-2.7 cm Tube width : 0.5-0.7 cm
9	**Stamens**: didynamous, all perfect, epipetalous, the lower posterior pair with short filament	No of stamens: Four Filament length:

	included in the tube of the corolla with a small scale at the base of each filament. Filaments of the upper or the anterior pair are long, arching (connivent at the top under the upper lip), dilated and broadly appendiculate or furnished with a short obtuse discoid appendage at the base. Anther 2 celled, four lobed.	Upper stamens: 1.2-1.5 cm Lower stamens:0.3-0.5 cm
10	**Ovary**: superior, spherical, two celled with many ovules in each loculus on thick axile placenta style simple, slender, ending in a bilamellate stigma.	Style length : 1-1.2 cm
11	**Period of flowering**: Throughout the year if growth conditions are favorable	August- September April- May-June
12	**Days for >90% flowering**:	60 days
13	**Fruit**: septicidally dehiscent, subglobose capsule, many seeded, small, truncate, papillose, glabrous, with the valves when ripe separating from the broadly winged placentiferous axis	Diameter : 0.6- 0.8 cm
14	**Seeds**: ellipsoid-globose, rounded or truncate at the ends, yellowish brown, covered with white tubercles, many, small, papillose or rugose. Seedling with epigeal germination, epicotyl very short. Cotyledons rhomboid, leafy.	Seeds : 0.6 mm long Hypocotyls : 3-7 mm Cotyledons: 4-5 mm long

3. *Ipomoea mauritiana*

No	Morphological trait description	Biometric observation
1	**Growth habit**: Perennial evergreen climber	Plant height: ≥3 m at1 yr
2	**Stem**: glabrous, long, thick, round, light reddish, or light purplish green, smooth twining stems.	Diameter : 0.2-2.5 cm Internodal length : 8-16 cm
3	**Canopy**: Spreading	Diameter : 1.5-2.8 m
4	**Leaf**: simple, alternate, exstipulate, long-petioled, fairly large, often broader than length, broadly ovate in out line, deeply palmately divided, lobes ovate- lanceolate, occasionally spathulate, entire, pale, glabrous, acute or acuminate, nerves prominent beneath glossy green above, and slightly lighter coloured below, smooth margin.	No of lobes : 5-7 lobed Length : 5-10 cm Width : 5.6-18 cm petiole length : 3.5-8 cm

5	**Inflorescence**: axillary, long pedunculate corymbiform cymose panicles.	Inflorescence length : 8 -9 cm No. of flowers/ inflorescence: 8-10 Peduncle length: 15-25 cm
6	**Flower**: Many, bell shaped, large, showy pink or purplish-red in colour, bracteate, bisexual, zygomorphic, lobes of the limb emarginate	Diameter 6-6.5 cm Flower stalk1-2 cm
7	**Calyx**: polygamous, sepals five, free, nearly equal, ovate, or elliptic rotund to orbicular, concave, obtuse or shortly acute, whitish green and slightly enlarging in fruit.	Length : 0.75-1cm Width : 0.6cm
8	**Corolla**: gamopetalus, showy, pink to purple, Deep purple inside the corolla tube, light pink in lobes, light purple outside, widely campanulate, narrowed at base, glabrous	lobes length : 2.5-4.5 cm lobes width : 2.5-3 cm tube length : 1.4-2.3 cm tube width : 2.2-3.8 cm
9	**Stamens**: five, epipetalous three small and two large; filaments free; anthers straight, never twisted	Number : 5 Anther length : 0.5 cm Large filament : 1.5-2 cm Small filament : 0.8-1.2 cm
10	**Ovary**: almost completely four celled except at the top with one ovule in each locule, bicarpellary, syncarpous, superior. Style simple with a capitate or two lobed (bifid) stigma.	Style Length : 2.5cm
11	**Period of flowering**: The plant is in flower usually during the wet season	August –September April- May – June
12	**Fruit**: Ovoid, small, brown, completely four celled to the apex and 4-valved capsules about 8mm in length and enclosing four seeds surrounded by enlarged fleshy sepals	Length : 1.3- 1.5 cm Breadth :1.0-1.2 cm
13	**Seeds**: Small, brown seeds, covered all over with many long yellowish-brown cottony hairs	Seed length : 6 mm

Harvesting, processing and solvent extraction

Roots of the experimental plants were harvested at maturity, pre-processed, dehydrated and ground into a fine powder for solvent extraction and storage. Samudrapacha (*Argyreia speciosa*) roots were processed by peeling the root skin and discarding the fibrous core, chopping the root peel into thin slices, macerating into paste using wet grinder, drying the wet mass by spreading in wooden panels at 45°C and then grinding it in to fine texture in a powder mill. In case of *Artanema sesamoides* (vathomvaretti) and *Ipomoea mauritiana*

(palmuthukku), entire roots were chopped into pieces, dehydrated and powdered. Successive solvent extraction of root powder of three plant species namely *Argyrea speciosa*, *Artanema sesamoides*, and *Ipomoea mauritiana* with hexane, chloroform, ethanol and water were done repeatedly to produce enough quantity of each solvent fractions for *in vivo* studies.

Table 1. Observations on yield parameters

Plant species	Root tuber length	Root tuber diameter	Root fresh weight/ plant	Drying % of root	Total Fresh weight/plant	Harvest index
Ipomoea mauritiana (at 1 year)	15-42 cm	5.5-7.0 cm	630-1180	13.81-16.86	1.85-2.33 kg	0.27-0.34
Argyreia speciosa (at 1 year)	2.5-3.3 m	1.3-2.9 cm	1.15- 1.76 kg	14.79-27.84	8.26-10.37 kg	0.14-0.17
Artanema sesamoides	45-63 cm	0.5- 0.9 cm	122.5-160.5 g	27.6-30.5	675-783 g	0.18-0.21

Table 2. Percentage recovery of different solvent extractives

A. ***Ipomoea mauritiana*** **roots**

Solvent	Weight of sample (g)	Weight of extractives (g) *	% recovery of extractives *
Hexane	250	6.08	2.43
Chloroform	250	2.20	0.73
Ethanol	250	34.96	11.66
Water	250	6.78	2.08

B. ***Argyreia speciosa*** **Root**

Hexane	250	16.34	6.54
Chloroform	250	2.40	0.96
Ethanol	250	12.54	5.01
Water	250	3.18	1.27

C. ***Argyreia speciosa*** **leaves**

Hexane	100	3.78	3.78
Chloroform	100	1.15	1.15
Ethanol	100	3.53	3.53
Water	100	1.30	1.30

D. ***Artanema sesamoides*** **Root**

Hexane	250	5.42	2.17
Chloroform	250	4.78	1.91
Ethanol	250	32.01	12.80
Water	250	4.29	1.72

* Values are means of six replications

Preliminary phytochemical screening

Preliminary phytochemical screening of crude raw drugs of the selected plants ie *Argyreia speciosa, Ipomoea mauritiana* and *Artanema sesamoides* and solvent fractions was done and results are shown below.

Table 3. Preliminary phytochemical screening of crude powders

No	Phytochemical test	Ipomoea		Artanema		Argyriea	
		Root	Leaf	Root	leaf	Root	leaf
a	Alkaloids	++	++	+++	++	++++	+++
b	Carbohydrates	+++	+ +	+	+	+	+
c	Proteins	-	-	-	-	-	-
d	Saponins	++	+	+++	+++++++	+	+
e	Phenolic compounds	+	+	+	+++	+	++
f	Tannins	++	++	++	+++	++	++
g	Flavonoids	+	+	+	+	+	+
h	Phytosterols	+	++	+	+	+	++
i	Gum & mucilage	+	+	++++	+	+	+

Table 4. Phytochemical screening of different solvent extractives

Artanema sesamoides

No.	Phytochemical test	Hexane		Chloroform		Ethanol		water	
		Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
a	Carbohydrates	+	+	+	+	+++	+	+++	+
b	Alkaloid	+++	+++	+++	+++	++++	++++	++	++
c	Phenolics	-	-	-	-	+	++	+	+
d.	Tannins	-	-	-	-	+	++	+	+
e.	Flavonoids	-	-	-	-	+	+	+	+
f	Phytosterols	+	+	+	+	-	-	-	-

Ipomoea mauritiana

a.	Carbohydrate	+	+	+	+	+++	+	+++	+
b.	Alkaloids	+++	+++	+++	+++	++++	++++	++	++
c.	Phenolics	-	-	-	-	+	++	+	+
d.	Tannins	-	-	-	-	+	++	+	++
e.	Flavonoids	-	-	-	-	+	++	+	+
f.	Phytosterols	+	+	+	+	-	-	-	-

Argyreia speciosa

a.	Carbohydrates	+	+	+	+	+++	+++	+++	+++
b.	Alkaloids test	+++	+++	+++	+++	++++	++++	++	++
c.	Phenolic compounds	-	-	-	-	++	+++	++	++
d	Tannins	-	-	-	-	++	+++	++	++
e	Flavonoids	-	-	-	-	++	++	++	++
f	Phytosterols	+	+	+	+	-	-	-	-

Quantitative analysis of major phyto- constituents of crude root powders

Results of quantitative analysis of major phyto- constituents of crude root powders of *Argyreia speciosa, Ipomoea mauritiana* and *Artanema sesamoides* are given below.

Table 5. Quantitative analysis of major phyto- constituents of crude root powders

No	Phytochemical test	Ipomoea		Artanema		Argyriea	
		Root	leaf	Root	leaf	Root	leaf
a	Total Carbohydrates (%)	67.81	18.73	28.21	17.65	35.85	16.85
b	Reducing sugar (%)	4.50	5.34	4.69	12.59	8.11	5.47
c	Non reducing sugar (%)	5.98	8.63	5.77	4.31	13.47	6.77
d	Starch (%)	51.59	4.28	16.20	0.67	12.85	4.15
e	Ash (%)	4.27	8.13	8.86	4.86	5.34	9.90
f	Fibre (%)	10.42	12.58	26.34	9.035	29.24	26.38
g	Total phenolic content (g/kg)	8.21	21.12	16.78	34.17	6.69	21.63

Pectin content in *Artanema sesamoides*

Pectin which is generally reported to contribute to anti inflammatory activity was detected to a level of 3.19% in roots and 5.69 in leaves of *Artanema sesamoides*.The pectin present in leaves were found to be different from that of roots. The leaves contained low methylated pectin. The pectin is reported to have antiinflmmatory effect which might have contributed to its high anti-inflammatory effect.

In vitro antioxidant activity assay of ethanol extracts by DPPH method

Antioxidant activity of crude powders of experimental plants was evaluated by DPPH method and *Artanema sesamoides* root ethanol extract was found to have very high antioxidant activity comparable to other two species (Table 8).

Table 6. Antioxidant activity of crude powders of experimental plants by DPPH method

Sample	EC50 value (ppm) *
Ipomoea root	209.45
Ipomoea leaf	209.44
Artanema root	62.3
Artanema leaf	154.8
Argyreia root	151.6
Argyreia leaf	188.24

*Each value is mean of four observations

Artanema sesamoides was also found to have very high phenol content (Table 7) which may contribute to its high antioxidant activity observed compared to other two plant species.

Protocol for mouse ear erythema model *in vivo* anti inflammatory studies

Standardization of protocol for in vivo studies were done to initiate anti-inflammatory screening. Dosage of croton oil, its dilution ratio, dilution method, dosage of standard drug required etc were standardized prior to the start of anti-inflammatory studies in animal.

- Experimental animal used: Balb C mice
- Irritant used: Croton oil (diluted with liquid paraffin in 1:8 ratio)
- Standard drug used: Hydrocortisone acetate
- Method of application: Topical application
- Total number of animals used for standardization: 18

Approximately 0.5mg of irritant was applied on inner side of both external ears of mice. After the application of irritant, the standard drug was applied on the outer side of left ear. The standard drug, Hydrocortisone acetate is applied 6 times at time intervals of 30 min. During the initial 4-5 hrs, there was no difference between two ears. But after 20 hrs observable difference was seen. The untreated right ear showed higher inflammation and reddening than the left ear. After 46 hrs also detectable difference between treatment and control were observed.

Screening for anti-inflammatory activity in acute inflammation models

i). Croton oil induced mouse ear erythema model

Evaluation of anti-inflammatory property of different solvent fractions (hexane, chloroform, ethanol and water) from different parts of *Argyreia speciosa, Ipomoea mauritiana* and *Artanema sesamoides* in acute inflammation model by mouse ear erythema method was done and ethanolic extractives of all three species showed significantly superior antiinflammatory activity compared to other solvent extractives. Comparison of anti-inflammatory activities of three species was done using ethanol extractives alone after this preliminary screening in mice.

Table 7. Anti-inflammatory activity of different extractives in mouse ear erythema model

No	Treatment	Average score of cure of inflammation of 8 animals					
		Argyreia speciosa		*Artanema sesamoides*		*Ipomoea mauritiana*	
		at 24 hrs	at 48 hrs	at 24 hrs	at 48 hrs	at 24 hrs	at 48 hrs
1	Negative control	0	0	0	0	0	0
2	Positive control (Hydrocortisone acetate)	10	10	10	10	10	10
3	Hexane extract	5	5	2	2	6	6
4	Chloroform extract	2	2	1	1	**2**	**3**
5	Ethanol extract	7.5	8	8	8	9	9
6	Water extract	4	4	4	4	3	3

ii). Carrageenan induced rat paw oedema model

Effect of ethanol extractives of roots of all the three species was studied at two different doses in rat paw oedema model for comparison of anti-inflammatory activity. *Artanema sesamoides* was found to be most effective in reducing acute inflammation among the three species studied.

Table 8. Effect of ethanol extractives of roots on rat paw inflammation

Sl. No.	Treatments	Dosage mg/kg	Paw volume	% inhibition
1	Negative control	-	1.186 ± 0.16*	
2	Positive control	5 mg/ kg	0.513 ± 0.07*	56.74
3	Argyreia root EE	200 mg / kg	0.833 ± 0.13*	29.76
4	Artanema root EE	200 mg /kg	0.829 ± 0.06*	30.10
5	Ipomoea root EE	200 mg/ kg	1.257 ±0.08	-5.99
6	Argyreia root EE	400 mg / kg	0.671 ± 0.08*	43.42
7	Artanema root EE	400 mg /kg	0.617 ± 0.05*	47.98
8	Ipomoea root EE	400 mg/ kg	0.683 ± 0.19*	42.41

Values are mean ± SE; n= 8;* ANOVA $p< 0.05$ vs negative control ie vehicle control

Effect of ethanol extractives of roots of all the three species on rat paw inflammation on 7th day of consecutive drug administration was also evaluated to evaluate the preventive action of the herbal drugs.

Table 9. Effect of ethanol extractives of roots on rat paw inflammation on 7th day of consecutive drug administration

Sl. No.	Treatments	Dosage mg/kg	Paw volume	% inhibition
1	Negative control	-	1.175±0.12	
2	Positive control	4 mg/ kg	0.613±0.07*	47.83
3	Argyreia root EE	200 mg / kg	0.783±0.11*	33.36
4	Artanema root EE	200 mg /kg	0.413±0.05*	64.85
5	Ipomoea root EE	200 mg/ kg	0.975±0.10	17.02
6	Argyreia root EE	400 mg / kg	0.363±0.08*	69.10
7	Artanema root EE	400 mg /kg	0.533±0.08*	54.63
8	Ipomoea root EE	400 mg/ kg	0.725±0.14*	38.30

Values are mean ± SE; n= 8; *ANOVA $p < 0.05$ vs negative control ie vehicle control

In this study also *Artanema sesamoides* showed highest activity at a lower dose of 200 mg/ kg of animal.

Acute toxicity of *Artanema sesamoides* ethanol extractives

No adverse effect or mortality was detected in Sprague Dawley strain of albino rats up to the dose of 15 g/kg, *p.o.* of root ethanol extract during the 72 h observation period when tested at doses of 5, 10, 15 g//kg in groups of 8 rats for every dose. Its use as leafy vegetable in tropical Africa is also suggestive of the relative safety of the plant for human consumption (Grubben and Denton, 2004)

Acute toxicity of root decoction

No acute toxicity was found up to a dose of 5000 mg/kg of animal in case of root decoction extractives when tested in Sprague Dawley strain of albino rats.

Evaluation of anti-inflammatory activity in chronic inflammation model, Cotton pellet granuloma model

In order to evaluate the efficacy in healing chronic inflammation, the root extractives of *A. sesamoides* were tested at two different doses in a chronic inflammation model and the results are presented below.

Table 10. Lipid peroxidation in kidney and blood of rats under cotton pellet granuloma model

Sl. No	Treatment	Dosage	Con. Of MDA in mM/	
			Kidney/100g wet tissue	Plasma/ 100ml
1	Artanema sesamoides root ethanol extractives	100 mg	3.0542±0.217*	1.995±0.078*
2	Artanema sesamoides root ethanol extractives	200 mg	2.3776±0.138*	0.379±0.019*
3	Negative control	No drug	5.7044±0.307	2.5805±0.116
4	Positive control (diclofenac)	10 mg	3.72965±0.203*	0.2818±0.031*

Values are mean ± SE; n= 8;* ANOVA $p< 0.05$ vs negative control ie vehicle control

Evaluation of antiinflammatory activity of root and leaf decoctions and infusions of *Artanema sesamoides*

Traditionally root decoction of *A. sesamoides* is used for rheumatic conditions and hence comparative evaluation of both root and leaf decoction and infusions were done. Percentage recovery of extractives in both decoctions and infusions and results of anti-inflammatory activity studies in acute model are given in tables 11-12. Percentage recovery of extractives was more in leaf water decoction but anti-inflammatory activity was more in root decoction validating the use of roots in traditional medicine for oral administration. Root decoction was more effective compared to leaf decoction and both root and leaf infusion. However successive ethanol extract was found to be better than both decoction and infusion

Table 11. Percentage recovery of extractives from root and leaf decoctions and infusions of *Artanema sesamoides*

Sl. No	Parts Used	% recovery of extractives
1	Root water decoction	10.66
2	Root water infusion	10.29
3	Leaf water decoction	20.89
4	Leaf water infusion	14.51

Table 12. Effect of water infusion and decoction of *Artanema sesamoides* roots on rat paw inflammation in acute model ie carrageenan model at 3 hrs

Sl. No.	Treatments	Dosage mg/kg	Paw volume	% inhibition
1	Negative control	-	1.1167	
2	Positive control	4 mg/ kg	0.605	45.823
3	Root infusion	400 mg / kg	0.800	28.360
4	Root decoction	400 mg /kg	0.733	34.330
6	Leaf infusion	400 mg / kg	0.867	22.361
7	Leaf decoction	400 mg /kg	0.867	22.361
8	Root ethanol extractives	400 mg/kg	0.667	40.300

Values are mean ± SE; n= 8; *ANOVA $p< 0.05$ vs negative control ie vehicle control

In chronic model, root extractives were tested in two different doses and it was found to be effective in curing chronic inflammation also as shown in table 13 below..

Table 13. Antiinflammatory activity of root and leaf decoctions and infusions of *Artanema sesamoides* in chronic inflammation model ie, Adjuvant Arthritis model

Sl.No	Treatments	Dosage mg/kg	Paw volume	Percentage of inhibition	
				12th day	13th day
1	Negative control				
2	Root decoction extractives	100mg/kg		2.4	9.78
3	Root decoction extractives	200mg/kg		8.63	14.67*
4	Positive control	10 mg/ kg		21.6*	28.2*

Values are mean ± SE; n= 8; *ANOVA $p< 0.05$ vs negative control ie vehicle control

Purification of active compounds from *Artanema sesamoides* root extractives

Different fractions (ethyl acetate, butanol, methanol and water) partitioned from root ethanol extractives of *Artanema sesamoides* were evaluated for anti- inflammatory activity by carrageenan induced rat paw inflammation model and among these fractions, butanol fraction showed highest activity. The butanol fraction was further concentrated in active compounds by repeated liquid- liquid partitioning using a five solvent mixture hexane: butanol: ethyl acetate: methanol: 0.5 % acetic acid in the ratio 1:0.5:3.5:1:4. The solvent mixture was separated into two layers in separating funnel and lower layer was collected and upper layer discarded. It was then loaded into preparative HPLC after analyzing the antioxidant peaks in preparative HPLC. TLC profile of active butanol fraction in different solvent system revealed the presence of four major compounds by in situ antioxidant

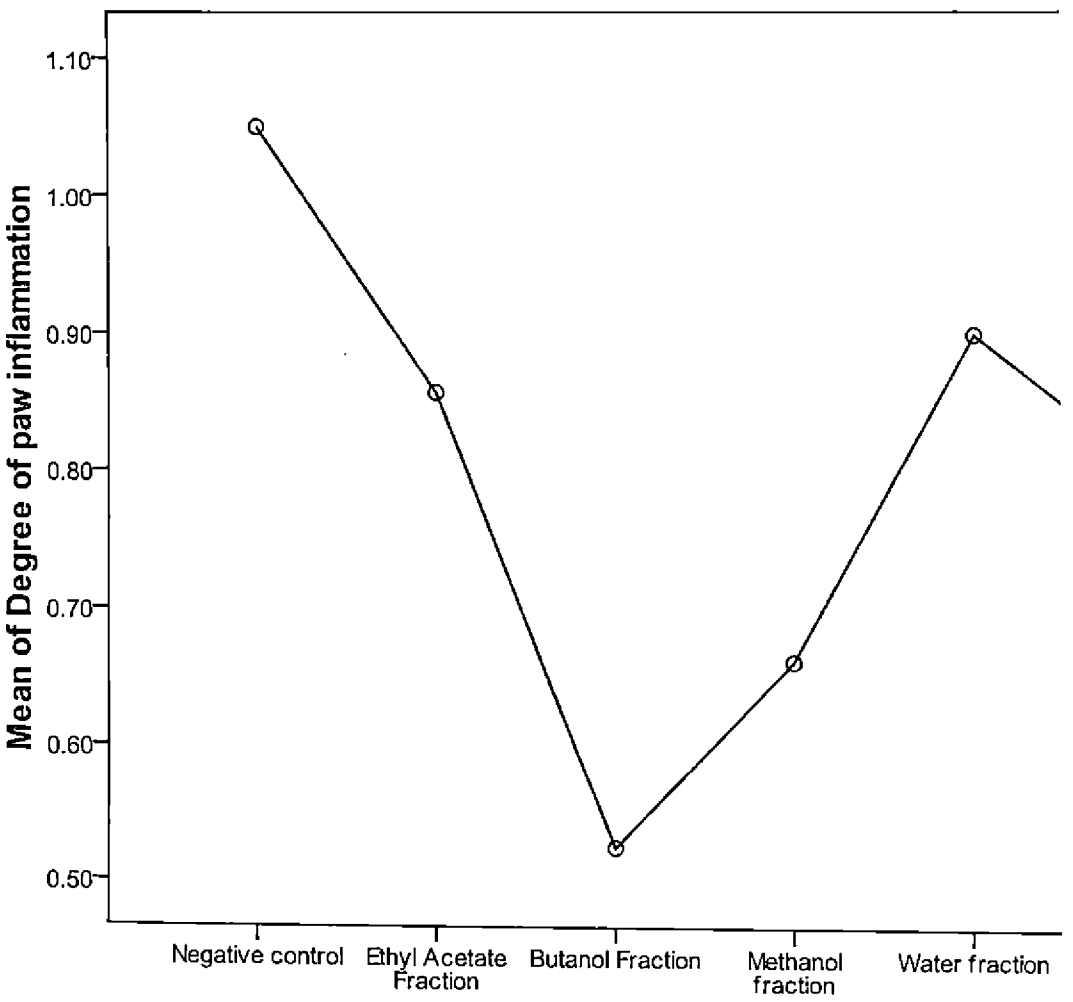
activity testing in developed TLC plates using DPPH solution whereas in analytical HPLC, 8 active peaks were detected by quenching with $FeCl_3$.

Table 14. Effect of different solvent fractions of root ethanol extractives on inflammation in rat paw oedema model

No.	Treatments	Dosage mg/kg	Paw volume	% inhibition
1	Negative control	0	1.050±0.035	
2	Ethyl Acetate fraction	100 mg/kg	0.857±0.048	18.38*
3	Butanol fraction	100 mg/kg	0.525±0.045	50.00*
4	Methanol fraction	100 mg/kg	0.663±0.046	36.90*
5	Water fraction	100 mg/kg	0.900±0.045	14.29*

Values are mean ± SE; n= 8; *ANOVA $p< 0.05$ vs negative control (vehicle control)

Figure 1. Effect of different solvent fraction of root ethanol extractives on rat paw inflammation in rat paw oedema model



*Extractive dosage 100 mg/ kg of rat; paw inflammation as increase in paw volume.

Antioxidant activity of ethanol fraction and its butanol subfraction of *A. sesamoides* by DPPH method

Free radical scavenging is considered to be one among the various mechanisms of antioxidation. Positive DPPH test suggests that the extracts are free radical scavengers. The scavenging effects of ethanol extract and its sub fraction butanol on DPPH radical was compared with that of ascorbic acid and BHA (Table 4). The root ethanol extract of *A. sesamoides* was found to have high antioxidant activity (EC_{50} = 62.3 ppm) when analysed by DPPH method. Of the four different sub fractions of ethanol extracts, the butanol fraction in which antiinflammatory activity is concentrated possessed 5.7 fold higher antioxidant activity. The ethanol extract and its butanol sub fraction from the officinal part, root was significantly superior in antioxidant activity compared to corresponding leaf extracts. There are many earlier reports about antioxidant and antiinflammatory effects in case of many herbal drugs rich in free radical scavengers like phenolics (Suleyman *et al.*, 1999, Ravishankara *et al.*, 2002 & Bagul *et al.*, 2003).

Table 15. Antioxidant activity of ethanol fraction and its butanol subfraction of *Artanema sesamoides* by DPPH method

Sl. No	Sample	IC50 value (ppm) *
1	Root ethanol extract	62.30
2	Butanol sub fraction	10.97
3	Leaf ethanol extract	154.80
4	Butanol sub fraction	32.38
5	Ascorbic acid	1.78
6	Butylated hydroxy anisole (BHA)	5.98

*Each value is mean of three observations

Evaluation of anti-inflammatory activity of hydrolyzed butanol fraction

Artanema root ethanol extract was water washed with distilled water. Water soluble fractions separated and water insoluble portions partitioned in to Ethyl acetate, Butanol, methanol fraction. Water soluble fraction was partitioned into butanol recovered fraction from water soluble portion and the left over water soluble fraction. Butanol fraction was found to be most active. This fraction was hydrolyzed anti-inflammatory activity was further tested for hydrolyzed fraction.

Table 16. Antiinflammatory activity of hydrolyzed fraction

Sl. No	Treatments	Average Increase in paw volume (ml)	Percentage inhibition of inflammation
1	Negative control	0.283±0.019	
2	Hydrolysed butanol fraction 20 mg/ kg of animal, recovered by ethyl acetate.	0.207±0.015	26.86*
3	Positive control Diclofenac sodium (4 mg/kg of animal)	0.178±0.008	37.10*

Values are mean ± SE; n= 8; *ANOVA $p< 0.05$ vs negative control (vehicle control)

***In vitro* inflammatory enzyme inhibition by *A. sesamoides* root extractives**

The butanol fraction was found to inhibit both cyclooxygenase 2 and lipoxygenase enzymes in vitro as detailed below. These results indicates that the extract acts through inhibition of these enzymes in the inflammation pathways because its high antioxidant power.

Table 17. Human recombinant Cox 2 inhibition by *A. sesamoides* root butanol fraction

No	Treatments	% inhibition of cox 2 activity (Photometric mode)
1	No inhibitor	-
2	5 µg/ ml	48.035
3	10 µg/ ml	70.940
4	20 µg/ ml	78.625

*Mean of three observations

Table 18. Lipoxygenase inhibition by *A. sesamoides* root butanol fraction

No	Treatments	% inhibition of 15 lox activity
1	No inhibitor	-
2	5 µg/ ml	11.954
3	10 µg/ ml	20.835
4	20 µg/ ml	23.15%

*Mean of three observations

Purification of compounds/ peaks from butanol fraction by preparative RP HPLC

Compounds / peaks were purified from active butanol fraction by preparative HPLC under the following conditions. Active fraction was separated on prep RP HPLC in **C 18** column.

Column: Lichrospher C18, 150x4.6 mm, 5 microns

Solvent system: A = Acetic acid 0.1% v/v, B = Acetic acid 0.1% + Methanol in the ratio 10:90 (v/v)

Solvent System Gradient:

Time (min)	% A	% B	Flow ml/min
0	80	20	1
40	50	50	1
50	40	60	1
60	80	20	1

Temperature = 25 deg C, Detection wavelength = 200 nm, Injection volume = 20 mic lit

Fig.2. Normal phase TLC of Prep HPLC loaded fraction (EA: Ethanol: water (8:1:1)

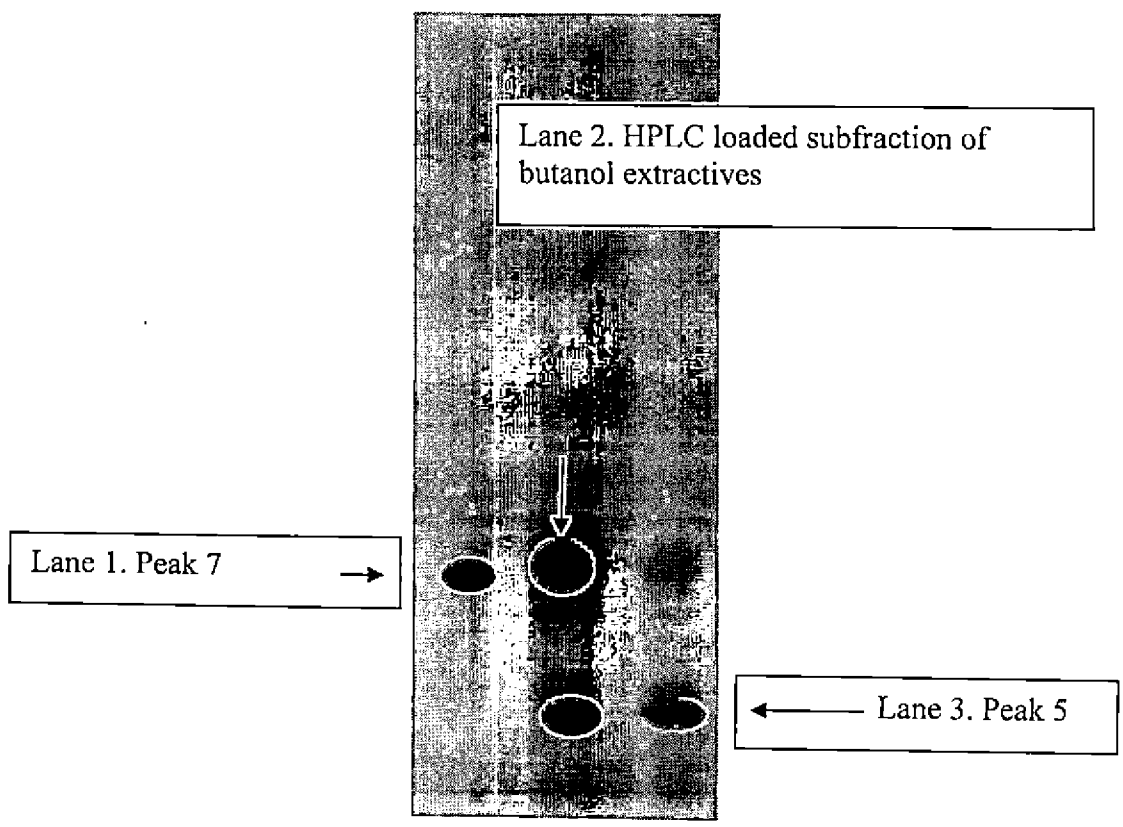
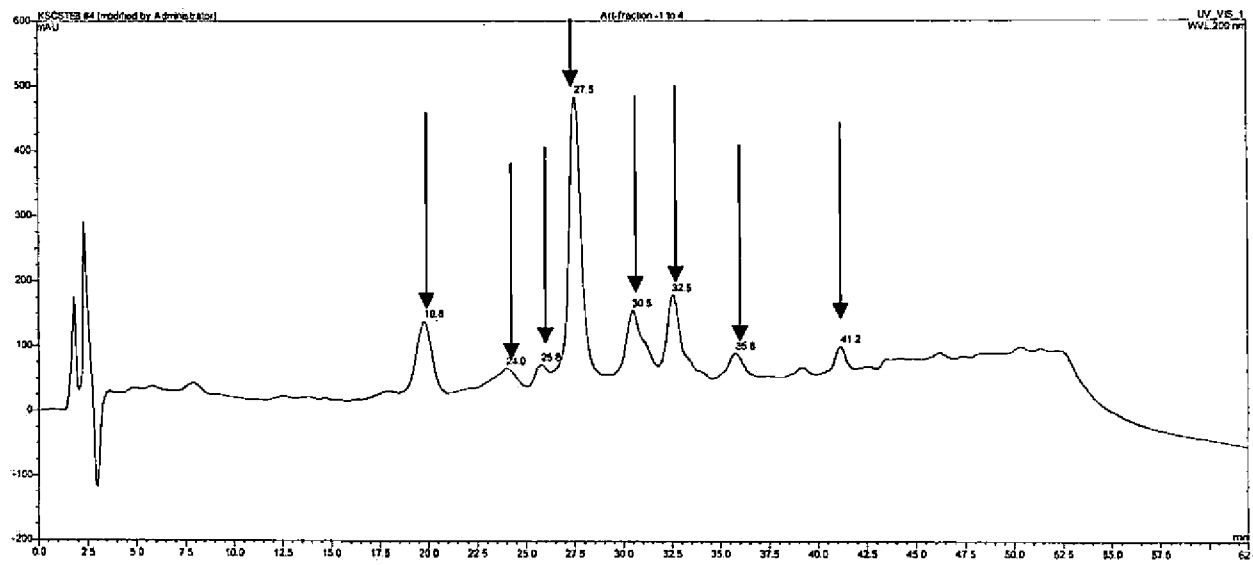


Fig. 3. Analytical RP HPLC profile of *Artanema sesamoides* root active fraction ie butanol sub fraction

Lichrospher C 18 column 150x4.6 mm, 5 microns in solvent system: A = Acetic acid 0.1% v/v, B = Acetic acid 0.1% + Methanol in the ratio 10:90 (v/v).



Active peaks as revealed by analytical RP HPLC

Peak name	Ret.Time	Area	Height	Rel.Area
	min	mAU*min	mAU	%
1	19.8	121.063	114.03	11.87
2	24.0	44.1874	30.893	4.33
3	25.8	12.1593	21.004	1.19
4	27.5	401.1239	448.376	39.33
5	30.5	153.8906	117.671	15.09
6	32.5	149.5075	139.244	14.66
7	35.8	63.5781	46.649	6.23
8	41.2	74.454	51.746	7.3
Total:		1019.964	969.614	100

All the eight peaks marked by arrows were quenched by Ferric chloride illustrating antioxidant activity *in vitro* in RP HPLC system when Ferric chloride is injected together with active fraction in to RP HPLC column under conditions specified above. Of these peaks 4,5 and, 6 were dominant as revealed by percentage relative area of the peaks in table above. The peaks 1-3 eluted merged together in prep RP HPLC and these 1-3 peaks could not be collected separately for further studies.

Table 19. Absorption maxima of peaks in active fraction in Solvent system: A = Acetic acid 0.1% v/v, B = Acetic acid 0.1% + Methanol in the ratio 10:90 (v/v).

Peak	Retention Time	Rel.Area	Absorption peaks nm		
	min	%			
1	19.8	11.87	197.1	***218.5***	***330.7***
2	24.0	4.33	207.8	232.1	322.7
3	25.8	1.19	199.3	***219.1***	***331.1***
4	27.5	**39.33**	200.5	***217.8***	***331.0***
5	30.5	15.09	197.7	***218.9***	***330.9***
6	32.5	14.66	196.8	290.9	327.7
7	35.8	6.23	195.8	200.1	328.7
8	41.2	7.3	198.1	***218.3***	***329.9***
Total:		100			

Absorption maxima indicate that peak 1, 3, 4, 5 and 8 may probably be iridoids and peak 2, 6, and 7 flavonoids. Broad band at 3424 cm-1 and between 1025 and 1100 cm-1 in IR spectrum is indicative of glycosidic nature of active compounds.

Evaluation of antioxidant and anti-inflammatory activity of purified peaks collected through prep HPLC

Peaks eluted from prep HPLC is collected and pooled together and subjected to both antioxidant and antiinflmmatory activity studies and the results are shown below (table 19). Only peaks 4 to 8 could be collected from prep HPLC as peaks 1-3 eluted together in prep HPLC though these peaks were well separated in analytical HPLC. Antiinflammatory study could be done only for peaks 4, 5, and 6 since only these peaks were received in sufficient quantity for animal studies. In the case of peaks 7 and 8 turnover was meager as evident from table 19.

Table 20. Antioxidant and anti-inflammatory activity of purified peaks by prep HPLC from *A. sesamoides* root butanol fraction

HPLC Peak	Fraction collected	Antioxidant activity EC 50 value	Anti-inflammatory activity % inhibition @20mg/kg
4	0	22.61	14.35
5	1	6.53	25.05
6	2	45.35	7.93
7	3	17.75	
8	4	32.26	

Chemical characterization

LCMS profiling of Active butanol sub fraction from *Artanema sesamoides* root has been done at SAIF, CDRI, Lucknow and SAIF, IIT, Mumbai and active compounds purified by preparative HPLC were subjected to H NMR and C^{13} NMR, FTIR and CHNSO analysis at SAIF, IIT, Mumbai.

The detailed chemical characterization have shown that the active fraction contains phenyl ethanoid glycosides, flavonoid glycosides and iridoid glycosides of which some are reported from other plant species under the same family Scrophulariaceae. Antiinflammatory activities are reported for these categories of compounds in many studies. The LCMS pattern of most of these base peaks showed presence of one, two and three sugar moiety and presence of flavones such as luteolin, apigenin and hesperitin as aglycones. Glycosides were found to undergo both alkali and acid hydrolysis even under ambient conditions. HPLC analysis along with standards revealed presence of caffeic acid, ferulic acid, hesperitin, syringic acids etc. All these compounds are reported to be bioactive with antioxidant power.

Table 21. Base peak and fragmentation pattern of compounds in active root fraction of *A. sesamoides* as per LCMS mass spectrum

i). LCMS done at CDRI, Lucknow for root active fraction

Peak	M-H m/z	Fragmentation pattern
1	222	205, 187
2	360	343, **325, 289, 259, 331, 198**
3	214	197, 177,181
4	325	231, 214
5	283	241,234, 216,203, 180
6	414	370,270,216,214, 198
7	458	414, 257
8	502	443, 435, 378, 323, 196, 180
9	670	508, 485, 339
10	797	753, 709, 664, 629, 620, 607, 594, 576, 550, 509, 506, 421,399, 325, 302, 214, 198

ii). LCMS done at SAIF, IIT, Mumbai for root active fraction

a. Negative mode

Peak	m/z value (M-H)	Fragmentation pattern
1	189.0	171, 127.2
2	623.2	461.2
3	777.4	623.1, 548.5, 505.2, 480.1, 401.339, 282.6
4	325.1	261.3, 183.1
5	399.3	388.7, 382.9, 379, 371, 352.9, 311.4, 269.1, 242.2
6	339.2	183.1
7	349.0	305.1, 302.9,285.2, 270, 259, 194.9
8	433	414, 372, 270.9, 254.8, 227.2, 171.2

b. Positive mode

Peak	m/z value (M+H)	Fragmentation pattern
1	241.3	225.5, 126.2, 108.2, 98.1
2	263.2	241.3, 164.5, 146.8
3	263.2	242.3, 104.7
4	263.1	196, 101.6
5	263.1	216.6, 104.6
6	263.1	217.1, 199, 189.2, 165.2, 145.3, 137, 131.2, 119.3, 109.1
6	498	451.1, 437.3, 392,382.6, 371.2, 346.2, 329.2, 302.1, 277.1, 224.2, 174.1157.4, 150.7
7	568	550.2, 522.3, 507.2, 494, 479.1, 451.1, 442.2, 424.3, 393.4, 358.3, 331.3, 324, 309.5, 296, 284.1, 277.4, 270..4, 230.1, 207.1, 195.1, 175.9
8	683	597.4, 557.2, 463.1, 419.4, 401.1, 375.4, 332, 287, 257, 243, 231, 213

The preliminary analysis of the mass spectrum of active peaks and absorption maxima and FTIR analysis indicates that the active fraction is rich in iridoid glycosides, flavonoid glycosides and phenyl ethanoid glycosides. The base peak 623.2 in negative ion mode and its fragmentation pattern reveals that one of active the compound in butanol

fraction is acteoside (verbascoside) with molecular formula $C_{29}H_{36}O_{15}$ which is reported in other plant species of the same family.

Iridoid glycosides, flavonoid glycosides and phenyl ethanoid glycosides are commonly reported in scrophulariaceae plants and have been reported to display marked *in vitro* and *in vivo* anti-inflammatory properties. Some of them has been shown to selectively inhibit 5-LO and COX-2activity and ameliorates several inflammatory symptoms. There is evidence of presence of plant-derived flavones chrysin, apigenin, and luteolin which are effective in preventing COX-2 expression in *Artanema* root extract. Active fraction also contains hesperitin.

SUMMARY

Summary of the project on "Investigations on anti-inflammatory properties of selected under exploited medicinal plants" carried out at Aromatic and Medicinal Plants Research Station, Odakkali, Ernakulam District (Kerala Agricultural University) during 2007-10 is presented below.

- Anti-inflammatory activity of all the three plant species included in the study is confirmed by in vivo studies. Ethanol extractives of *Artanema sesamoides* and *Argyreia speciosa* roots were much superior in anti-inflammatory activity compared to *Ipomoea mauritiana.*
- *Artanema sesamoides* root extractives showed highest antioxidant activity and very high total phenolic content compared to other two root extractives.
- High antioxidant activity was observed in fractions with anti inflammatory activity
- Phenolic content was found to be high in fractions showing high anti inflammatory activity
- Pectin which is generally reported to contribute to anti inflammatory activity was detected to a level of 3.19% in roots and 5.69 in leaves of *Artanema sesamoides*
- Different fractions (ethyl acetate, butanol, methanol and water) partitioned from root ethanol extractives of *Artanema sesamoides* were evaluated for anti- inflammatory activity by carrageenan induced rat paw inflammation model and among these fractions, butanol fraction showed highest activity. TLC profile of active butanol fraction in different solvent system revealed the presence of four major compounds.
- Ethanolic extractives of Artanema sesamoides showed anti-inflammatory activity in chronic inflammation model ie in cotton pellet granuloma model.
- Antioxidant activity was found to be the highest in butanol fraction ie an EC 50 value of 62.3 ppm. The butanol fraction after acid hydrolysis for 3 hrs showed antioxidant activity equivalent to 14 ppm (EC 50 value) .
- Water decoction of roots showed higher anti-inflammatory activity compared to water infusion of roots and water decoction and water infusion of leaves.
- Artanema sesamoides root water decoction showed anti-inflammatory activity in chronic inflammation model ie in adjuvant arthritis model. Root water decoction inhibited inflammation by 9.77 % at a dose of 200 mg/kg and 14.66 % at a dose of 400 mg/kg.

- Root and leaf extractives were found to contain pectin which is reported to contribute to anti-inflammatory activity
- No acute toxicity was found up to a dose of 15000 mg / kg of animal in case of ethanolic extractives and up to 5000 mg/kg of animal in case of aqueous methanolic extractives when tested in albino rats.
- Eleven anti oxidant compounds/ peaks were observed in water decoction and aqueous methanolic extractives which may contribute to anti- inflammatory activity. LC–MS profiling of this compounds was done at CDRI, Luknow. and SAIF, Mumbai
- Most active compound which showed highest antioxidant activity and anti-inflammatory activity and yielded in sufficient quantity was subjected to FTIR, and C NMR and H NMR and the major compounds in the active fraction were found to be iridoid glycosides, flavonoid glycosides and phenyl ethanoid glycosides.

CONTRIBUTIONS MADE TOWARDS INCREASING THE STATE OF KNOWLEDGE IN THE SUBJECT

Anti-inflammatory activity was reported in *Argyreia speciosa, Ipomoea mauritiana* and *Artanema sesamoides* used in traditional medicine system but practically no attempt has been done to study in depth about these under exploited medicinal plants. Information available on phyto- chemical composition and *in vivo* studies to confirm their efficacy in curing various inflammatory conditions were scanty. The present study helped to generate information on these aspects. Anti-inflammatory activity of all the three plant species included in the study is confirmed by in vivo studies. Use of *Argyreia speciosa, Ipomoea mauritiana* and *Artanema sesamoides* for cure of inflammatory conditions in traditional medicine system is scientifically validated. Ethanol extractives of *Artanema sesamoides* and *Argyreia speciosa* roots were much superior in anti-inflammatory activity.

Biochemical profiling and isolation and chemical characterization of anti-inflammatory compounds generated information on chemical constituents that contribute to the medicinal property of *Artanema sesamoides*. Mode of action of *Artanema sesamoides* root extract is studied in detail. This traditional anti-inflammatory drug relies on diverse action mechanisms, ie inhibit lipid peroxidation, a phenomenon associated with antioxidant as well as anti-inflammatory activities. Their antioxidant action protects cells from degenerative processes. Root extract also inhibit enzymes 15- lipoxygenase and

Cyclooxygenase 2 which participates in the synthesis of inflammatory substances, prostaglandins and prostacyclines. Confirmation of anti-inflammatory effect of these medicinal plants and generation of information on their mode of action through pharmacological evaluation could promote their use in traditional medicine system and also would help to find alternative medicinal uses. The outcome of the study will be of potential benefit to pharmaceutical firms in formulating anti-inflammatory drugs.

Artanema sesamoides root extractives showed highest antioxidant activity and very high total phenolic content compared to other two root extractives. It also showed highest anti-inflammatory activity at a lower dosage at 3 hr observation and also at 7^{th} day of consecutive drug administration. Antiinflammatory activity of ethanol extract of *A. sesamoides* root is confirmed by *in vivo* studies both in chronic inflammation models also. Antiinflammatory and antioxidant activity was found to concentrate in butanol sub fraction of root ethanol extractives of *A. sesamoides* on further partitioning and LC MS profiling of active compounds from butanol fraction of this most effective plant was done. The peaks which showed maximum activity and yielded in sufficient quantity in preparative HPLC were purified and subjected to FTIR, CHNSO analysis, H NMR and C NMR.

Comparative evaluation of decoction and infusion from leaves and roots of *Artanema sesamoides* validated the better efficacy of root decoction in anti-inflammatory compared to others. Studies also affirmed the safety of this drug as decoction or ethanol extract. Its root decoction and ethanol extract do not possess acute toxicity. Most effective solvent fraction for inflammatory condition from *Argyreia speciosa*, *Ipomoea mauritiana* and *Artanema sesamoides* was proven to be ethanol fraction compared to hexane, chloroform and water fraction. Procedure for purification of anti-inflammatory compounds from leaves and roots of *Artanema sesamoides* by solvent fractionation and preparative HPLC were standardized. Studies revealed that the root ethanol extract is a good source of natural antioxidant and antiinflammatory phytoconstituents.

SCOPE OF FUTURE WORK

Development of ayurvedic drug formulation from *Artanema sesamoides* and *Argyreia speciosa* for inflammatory and rheumatic conditions is to be undertaken. Different kind of

formulations, dosage optimization, ayurvedic drug development etc is to be attempted. Presence of active compounds in leaf fraction and other plant tissues such as stem, floweres etc are also tobe evaluated. Seasonal variation in activity and variation due to wet land and upland cultuivation are also to be evaluated. Other potential uses of these plant also is to be evaluated.