

Original Research Article

Volume 9, Issue 4 -2023

DOI: <http://dx.doi.org/10.22192/ijcrms.2023.09.04.005>

Preliminary phytochemical physicochemical and qualitative biochemical evaluation of Thazhuthalai ilai Chooranam (*Clerodendrum phlomidis*)

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Abstract

Background: The Standardization is the essential and initial step to new drug development. It improves the safety and efficacy of herbal medicine to provide best herbal medicines to society. Thazhuthalai ilai chooranam (*Clerodendrum phlomidis*) is a classical Siddha drug that indicated for Kumbavadham (Periarthritis). Periarthritis is a type of arthritis that cause pain and stiffness in single joint. **Aim:** The aim of the study is determined the physicochemical parameters like total Ash and Preliminary phytochemical analysis and Qualitative biochemical analysis, High performance thin layered chromatography of the drug thazhuthalai ilai chooranam. **Methods:** Thazhuthalai leaves were collected and dried then were ground into micronized powder. Physicochemical analysis, preliminary phytochemical analysis and biochemical analysis were carried out by standard procedures. **Results:** Preliminary phytochemical screening found the presence of phytochemicals such as Alkaloids, Saponins, Tannins, Terpenoids, Steroids, and Glycosides. Physicochemical analysis found the Total ash value 11.24, Loss on dry 9.33, swelling index 4.0 etc. Preliminary Biochemical analysis found the presence of calcium, sulphate, tannic acid, ferrous iron from this study. High Performance Thin layered chromatography fingerprinting explore the presence of many phytochemicals with different Rf values & densitometric scan of plates showed numerous bands and peaks. **Conclusion:** The study concluded that the presence of various active components of phytochemicals, and physicochemical properties, biochemical components and HPTLC of Thazhuthalai ilai chooranam.

Keywords: Thazhuthalai, Phytochemicals, kumbavadham, physicochemical analysis, Standardization, Biochemical analysis, HPTLC

Introduction

The Siddha system of medicine deals with physical, psychological social, spiritual well-being of individuals. Medicinal plants are richest bio resources of drugs in traditional system of medicine. Medicinal plants produce the secondary metabolites thus are phytochemicals. Phytochemicals provide health benefits for humans and also protect from diseases, damage, harmful agents such as insects and microbes¹. They contribute to the plant's color, aroma, and flavor. They have biological properties such as Anti-inflammatory activity, Anti-oxidant, modulation of detoxification enzymes, stimulation of immune system. Now a days the need for herbal medicines is increasing daily. It is essential to standardize the herbal medicines for assess the safety and quality of the drug. Thazhuthalai ilai chooranam is a herbal drug mentioned in Siddha materia medica. *Clerodendrum phlomidis* is one of the important are widely used medicinal plant in Siddha system. Thazhuthalai grows as large shrub or small tree belongs to verbenaceae family². Thazhuthalai ilai chooranam is siddha drug that indicated for Kumbavadham (Periarthritis)³. Kumbavadham is one of the vadha diseases. Siddhars classified vadha diseases into 80 types. Periarthritis shoulder is one of the most common musculoskeletal disorders in Indian population. This condition most common in peak age between 40 to 70 years. This plant possess Anti-inflammatory, Anti-arthritic, Analgesic, Anti-microbial, Anti-diabetic activity, Immunomodulatory activity, Anti-cancer activity, Anti-malarial activity^{4,5}. This study will used for further future research studies.

Materials and Methods

Thazhuthalai leaves were collected from in and around the area of Palayamkottai. The raw drug was identified and authenticated by Medicinal Botanist of Govt. Siddha Medical College Palayamkottai. Then, the leaves were dried and ground into micronized powder and filtered to get chooranam. The drug was labelled as Thazhuthalai Ilai chooranam(TIC) which was used for experimental purposes.

Preliminary Phytochemical analysis methods:

TIC Alcohol extracted solvents in order of increasing polarity by using soxhlet method,. The solvents (80%) are removed by distillation over water bath. This carried out to find out the presence of various phytoconstituents.

Physicochemical Analysis :

Physicochemical analysis such as determination of loss on drying, total ash value, acid insoluble ash, water soluble ash, sulphated ash, pH value, volatile oil, alcohol soluble extractives, water soluble extractives were carried out by standard procedures.

Qualitative Biochemical analysis methods:

5gms of the drug taken and placed in 250 ml clean beaker then 50ml of distilled water is added and dissolved well. Then boiled well for 10minutes. It is cooled and filtered in 100ml flask & then it is made to 100ml with distilled water. This fluid is taken for analysis.

High Performance Thin Layer Chromatography (HPTLC):

HPTLC is one of the sophisticated instrumental techniques for Quantitative and qualitative analysis of herbs and herbal drugs. It can serve as a tool for Identification, Authentication and quality control of herbal medicines. Used with standardized procedures, it guarantees reproducible results, a vital elements in routine identification of complex fingerprints of plant extracts and pharmaceutical products.

Developing solvent system

A number of solvent systems were tried and a system which gave the maximum resolution was selected as the solvent system for the extract. The optimum separations of constituents were achieved using the solvent system: Toluene: Ethyl acetate: (5:2)

Sample application

The extracts were applied as different tracks of different concentrations of width 8 mm each on silica gel 60 F₂₅₄ pre-coated aluminium sheets through CAMAG micro litre syringe using Automatic TLC Sampler 4 (ATS4).

Development of chromatogram

After sample application the plate was introduced vertically in a CAMAG developing chamber (10 cm × 10 cm) pre-saturated with the mobile phase selected.

Documentation

The developed chromatogram was air dried to evaporate solvents from the plate and the plate was kept in CAMAG Vizualizer and the images

were captured under UV light at 254 nm and 366 nm.

Densitometry

The plate was scanned at 254 nm and 366 nm using TLC Scanner 4 and the finger print profiles were documented. The R_f values and finger print data were recorded with win CATS software associated with the scanner.

Post chromatographic derivatisation

The plate was derivatised using vanillin-sulphuric acid reagent, heated at 105⁰ C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and the chromatograms were documented. The plate was scanned at 575 nm and the R_f values and finger print data were documented.

Results and Discussion

Table:1 Preliminary phytochemical analysis:

Phytochemicals	Test	Results
Saponins	Froth test	Present
Tannins	Ferric chloride test	Present
Phenols	Alcoholic Ferric chloride test	Absent
Terpenoids	Liebermann test	Present
Alkaloids	Dragendorff's test	Present
Flavonoids	Alkaline reagent test	Absent
Steroids	Liebermann Burchard test	Present
Glycosides	Anthrone test	Present
Carbohydrates	Molisch's test	Absent
Quinones	Sodium Hydronide test	Absent
Proteins	Biuret test	Absent

Interpretation:

Phytochemicals:

Saponins:

They form a stable foam in aqueous solutions such as soap, hence the name "saponins". They

structurally diverse compounds have been observed to kill protozoa's to be anti-oxidants to impair the digestion of proteins and uptake of vitamins and minerals in gut to cause hypoglycemia and act as anti-fungal, anti-viral. Saponins possess Anti-inflammatory activity that mediated through the inhibition of release and synthesis of agents that produce inflammations⁶.

Tannins :

Tannins are a heterogeneous group of high molecular weight polyphenol compounds with capacity to form reversible and irreversible complexes with proteins, alkaloids, nucleic acid, minerals etc. Tannin containing plant extracts used as Anti-inflammatory, Anti-oxidant, Anti-septic and astringent against diarrhea. Recently some studies found that plant tannins have anti-inflammatory effects by inhibiting NO and prostaglandins E2 (PGE2)⁷.

Alkaloids:

Alkaloids are natural products that contain heterocyclic nitrogen atoms and are basic in character. Alkaloids used as local anesthetics almost all the alkaloids have a bitter taste. Alkaloids have diverse physiological effects. Antibacterial, anti-inflammatory, analgesic, anti-tumor activity. Alkaloids mainly possess anti-inflammatory activity demonstrating inhibition of expression of several pro-inflammatory factors such as cytokines, lipid mediators, histamines involved in inflammatory response⁸.

Glycosides:

Glycoside is a molecule in which a sugar is bound to another functional group via a glycoside bond.

Recent studies, some glycosides were found to induce the most of analgesic effect through cyclooxygenase and lipoxygenase pathways⁹.

Terpenoids:

Terpenoids are a class of natural products which have been derived from five carbon isoprene units. Most of the monoterpenes could inhibit the production of inflammatory factors nitric oxide, IL-6, tumor necrosis factor alpha (TNF) induced by lipopolysaccharides¹⁰.

Steroids:

Steroids have the fundamental structure of four carbon rings called steroid nucleus. Medicinal steroids possess many interesting medicinal activities like anti-tumor, anti-bacterial, anti-inflammatory, cardio tonic activity, hepatoprotective activity. Plant steroids possess ideal structural chemistry for anti-inflammatory activity¹¹.

The above phytochemicals have a vital role to healing the Kumbavadhham (Periarthritis) because the most of alkaloids are good anti-oxidant, anti-inflammatory & analgesic activity.

Table: 2 Physicochemical analysis results:

Parameters	Observed values
Loss on Drying at 105°C	9.33%
Total Ash	11.24%
Acid insoluble Ash	0.99%
Water soluble Ash	4.32%
Sulphated Ash	19.48%
Water Soluble Extract	5.42%
Alcohol Soluble Extract	8.47%
pH(4% Aq.soln.)	5.56
Volatile oil	Nil
Foaming Index	<100
Swelling Index	4.0

Physicochemical analysis interpretation:

pH :

The pH of TIC is 5.56(weak acidic). Acidic Drug is essential for its bioavailability & Effectiveness. Acidic drugs are better absorbed in stomach.

Total ash:

Total Ash value of plant material indicated the amount of minerals & earthy materials present in drug. The total ash value of TIC is 11.4% which determine the absence of inorganic content

Acid soluble ash:

The quality of Drug is better if Acid insoluble Ash value is low. The value of TIC is 0.99%

Water soluble ash:

Decreased Water soluble Ash value indicates easy facillation of diffusion & osmosis mechanism. The value of TIC is 4.32%

Water soluble Extract value:

Water soluble extract value plays an important role in evaluation of crude drugs. The value of TIC is 5.42%

Alcohol soluble extract value:

Alcohol soluble extract value signifies the presence of amount of fats, lipids some steroids in drug. The value of TIC is 8.47%

Swelling index:

Swelling index determine the purity of drug material. Swelling index of TIC is 4.0

Table: 3 Qualitative biochemical analysis results:

Experiment	Observation
Test for Calcium	Present
Test for Sulphate	Present
Test for Chloride	Present
Test for Carbonate	Absent
Test for Starch	Present
Test for Ferric iron	Absent
Test for Ferrous iron	Present
Test for Phosphate	Absent
Test for Albumin	Absent
Test for Tannic acid	Present
Test for Unsaturation	Present
Test for Reducing sugars	Absent
Test for Amino acid	Present
Test for Zinc	Absent

Qualitative biochemical analysis interpretation:

Qualitative analysis of TIC revealed the presence of Calcium, Sulphate, Chloride, Starch, Ferrous iron, Tannic acid, Aminoacid.

Calcium:

Calcium reduces the number of circulating monocytes and also blocked the action of macrophages in order to suppress inflammation. And also, can suppress the platelet aggregating factor production in endothelial cells. The above mechanisms may play a role in the Anti-inflammatory activity of calcium carbonate and calcium gluconate also.

Calcium being an essential element for vascular smooth muscle contract can prevent effusion and

thereby exert its Anti-inflammatory effect. Calcium containing drug prevent osteoporosis. And gives bone strength

Tannic acid:

Tannic acid has Anti-inflammatory, Anti-oxidant activities which reduce inflammation. Tannic acid has also Anti-viral and anti-fungal activities. In recent studies, the Anti-inflammatory effects of tannin rich plant extracts, including tannic acid have been investigated in paw edema model. This study shows tannic acid may contribute to the treatment of inflammation by decreasing MPO activity.

Sulphate:

Sulphate Reduce the increased serum enzymes in the liver and it has liver protection property. Chondroitin sulphate may reduce inflammatory processes by acting on the nuclear translocation of NF- Kb which is closely associated with the blood biomarkers of inflammation, primarily IL-1 , IL-6 and C-reactive protein.

Chloride:

Chloride regulates the amount of fluids in the body that reduce the inflammation in body tissues.

Ferrous iron:

Ferrous iron is essential for oxygen transport, blood production also needed for energy

Recent studies, shows ferrous iron leads to the potentiation of antigen binding and Anti-inflammatory activities of IgG..

Amino acids:

Amino acid essential for grow and repair body tissue. It maintains the healthy skin, hair and nails. Essential amino acid associate reduce inflammation.

HPTLC Results:

HPTLC Plate was developed using Toluene , Ethyl acetate. After development allow the plate to dry in air, and record the fingerprinting and densitometric chromatogram of the two batch samples of single compound scanned at 254 nm , 366nm and 575nm. HPTLC Study on Thazhuthalai ilai chooranam showed a detailed finger printing analysis of drug with the presence of different phytochemical constituents and different components. HPTLC results was interpreted based on area coverage of peak and number of peaks and Rf value of peaks.

The HPTLC fingerprinting patterns of alcoholic extract of Thazhuthalai ilai Chooranam was developed at 254 nm, 366 nm and after derivatisation with vanillin sulphuric acid at 575 nm. The solvent system, Toluene : Ethyl acetate (5:2) efficiently resolved the components.

HPTLC photo documentation profile of the Thazhuthalai ilai Chooranam at 254 nm ,366nm and after derivatisation is given in figure 1,2,3.

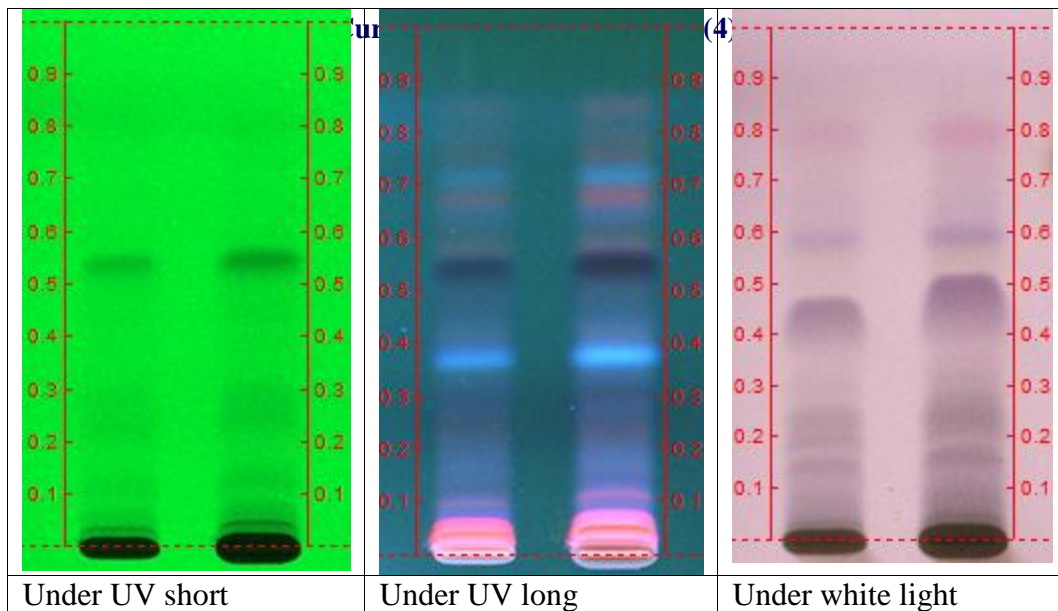
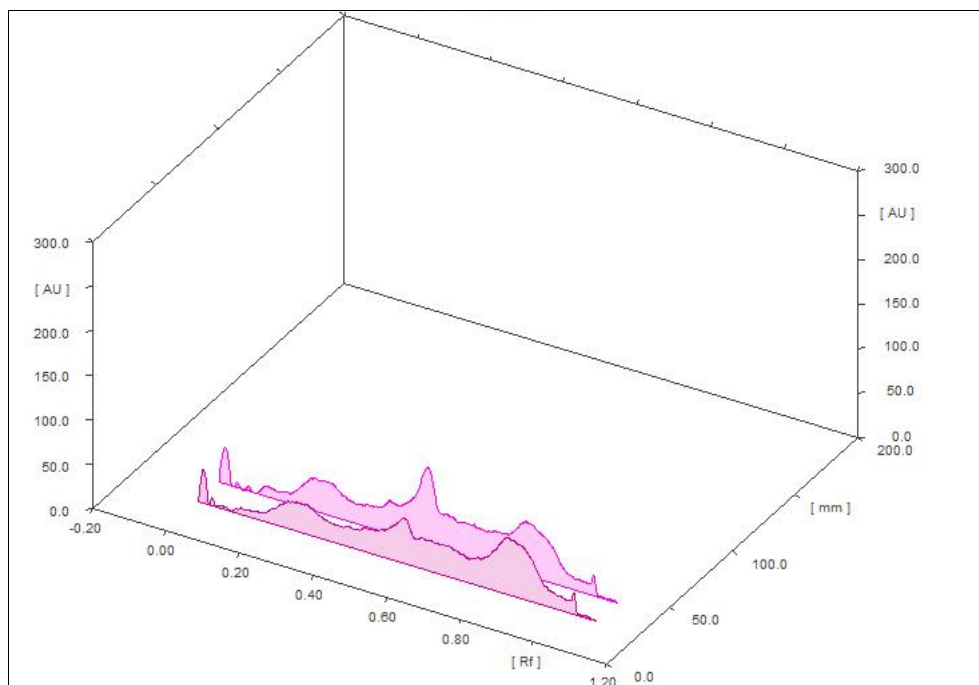


Fig 1: HPTLC profile of alcohol extract of Thazhuthalai ilaiChooranam viewed in UV short; UV long; White light after derivatisation using vanillin-sulphuric acid ; Solvent system –Toluene:Ethyl acetate –(5:2); volume applied;Track 1-5 ul: Track 2-10 ul

254 nm



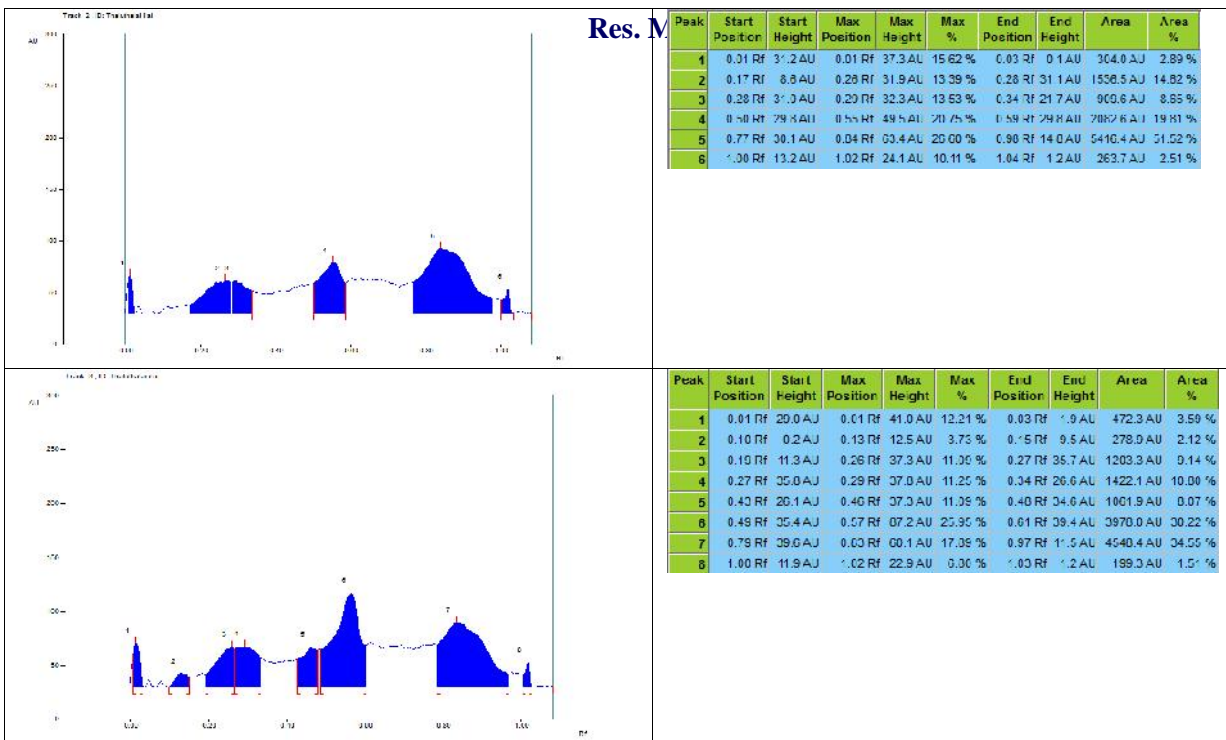
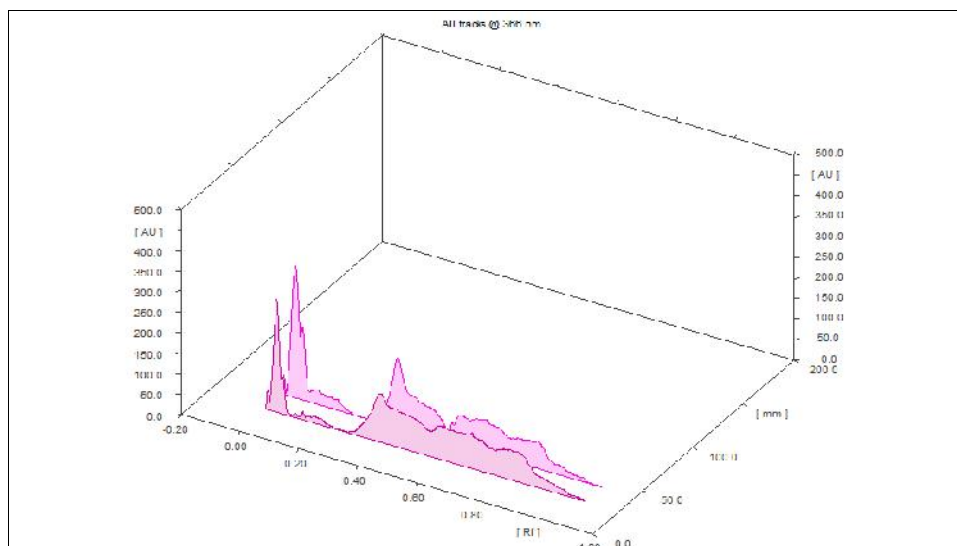


Fig 2: HPTLC fingerprint profile of 5ul and 10 ul of alcohol extract of Thazhuthalai ilai Chooranam of 254 nm after derivatisation

HPTLC finger printing analysis of alcoholic extract at 254 nm, the sample explore the presence of 8 prominent peaks corresponds to the presence of 8 versatile phytochemicals present within it. Rf value of the peaks ranges from

0.01Rf – 1.00Rf. Then, the peak 6 and 7 occupies the major percentage of area of 30.22% and 34.55% which denotes the abundant existence of such compound.

366nm



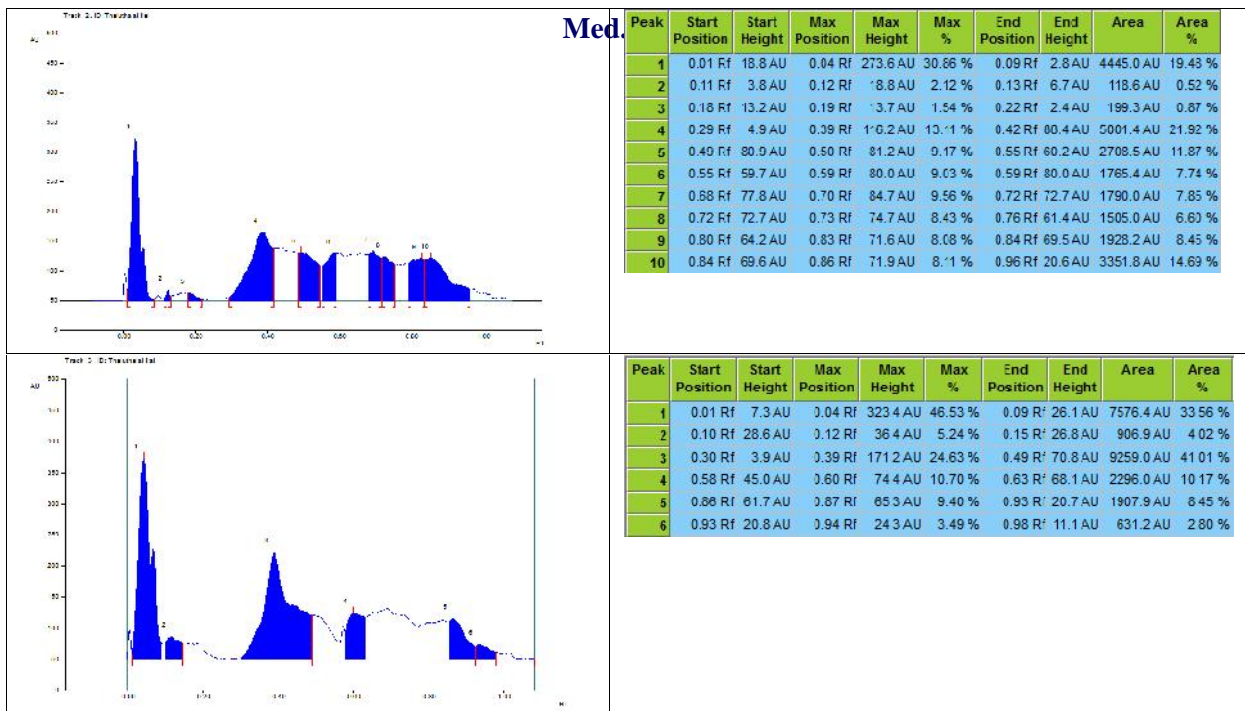
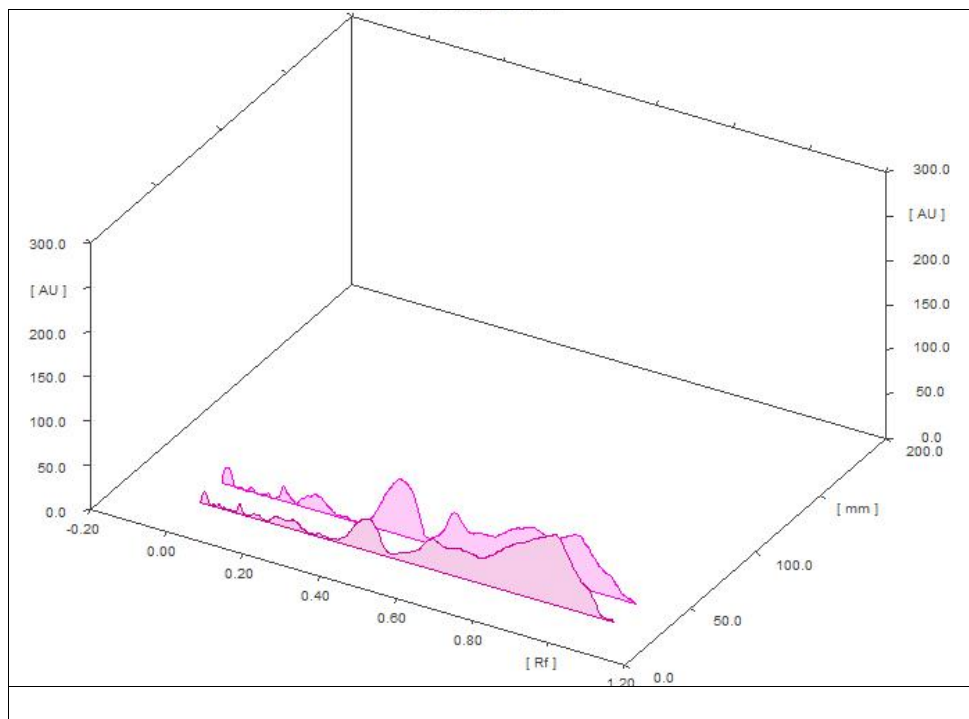


Fig 3: HPTLC fingerprint profile of 5 ul and 10 ul of alcohol extract of Thazhuthalai ilai chooranam at 366 nm after derivatisation

HPTLC finger printing analysis of alcoholic extract at 366 nm, the sample reveals the presence of 6 prominent peaks corresponds to presence of 6 versatile phytochemicals present within it. Rf value of the peak ranges from 0.01Rf –

0.93Rf. Further, the peak 3 and 1 occupies the major percentage of area of 41.01% and 33.56%, which denotes the abundant existence of such compound.

575 nm



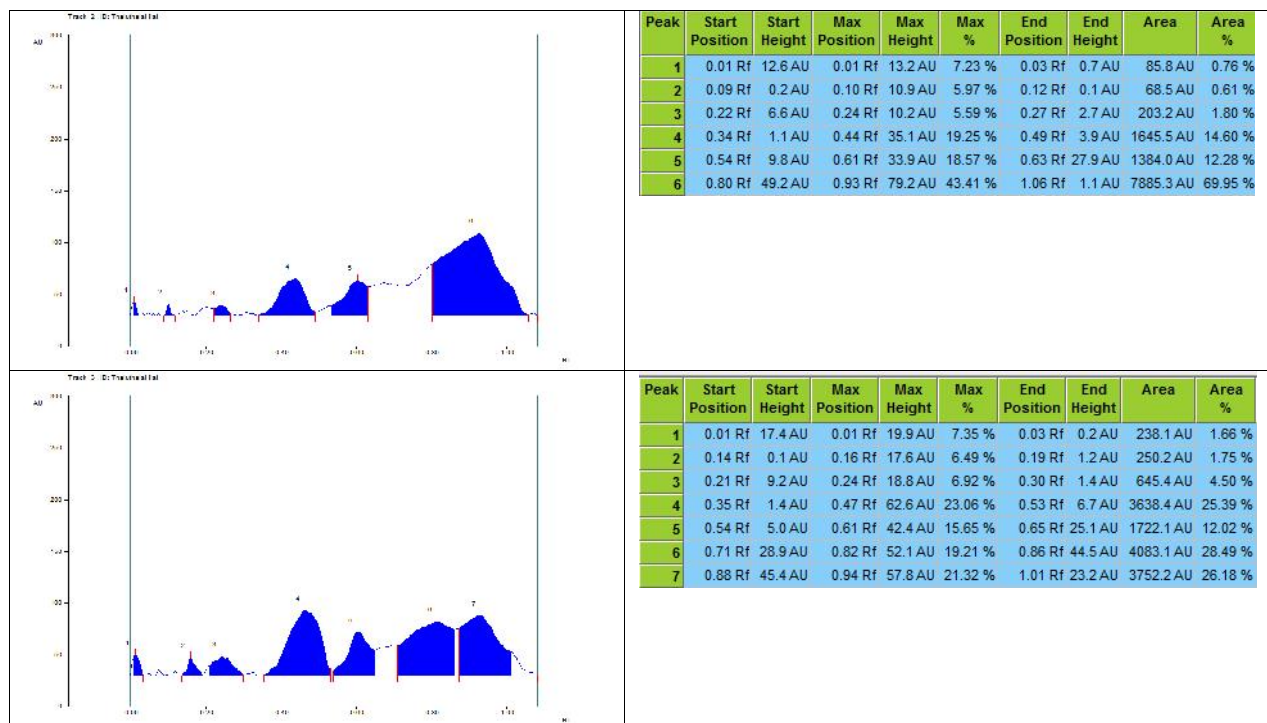


Fig 4:HPTLC fingerprint profile of 5 ul and 10 ul of alcohol extract of Thazhuthalai ilai chooranam at 575 nm after derivatisation

HPTLC finger printing analysis of alcoholic extract at 575 nm, the sample reveals the presence of 7 prominent peaks corresponds to the presence of 7 versatile phytochemicals present within it. Rf value of the peak ranges from 0.01Rf – 0.88 Rf. Further the peak 6 and 7 occupies the major percentage of area of 28.49% and 26.18%, which denotes the abundant existence of such compound.

Conclusion

The Qualitative phytochemical and biochemical analysis from studies showed that Thazhuthalai ilai chooranam is highly responsible and therapeutic value of treating Kumbavadhnam (peri-arthritis). High Performance Thin layered chromatography fingerprinting explore the presence of many phytochemicals with different Rf values & densitometric scan of plates showed numerous bands and peaks. HPTLC results provide important information that can be used as a fingerprint of further study of the drug. From the above analysis come to know that the presence of active ingredients responsible for medicinal efficacy especially treating inflammatory and degenerative diseases.

Acknowledgements

I express my sincere thanks to Dr. A.Manoharan, M.D(s). Ph.D., Professor and HOD, Department of Pothu Maruthuvam, GSMC&H, Palayamkottai for valuable guidance. I thanks to Siddha Regional Research Institute, Poojappura, Trivandrum, Kerala for completing this Research work.

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How to cite this article:

M. Manibharathi, A. Manoharan. (2023). Preliminary phytochemical physicochemical and qualitative biochemical evaluation of Thazhuthalai ilai Chooranam (Clerodendrum phlomidis). Int. J. Curr. Res. Med. Sci. 9(4): 36-46.

DOI: <http://dx.doi.org/10.22192/ijcrms.2023.09.04.005>