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Sathiyarajeswaran P

Siddha Central Research Institute,
Central Council for Research in Siddha,
Ministry of AYUSH, Chennai, Tamil
Nadu-600 106, India

Shree Devi MS

Siddha Central Research Institute,
Central Council for Research in Siddha,
Ministry of AYUSH, Chennai, Tamil
Nadu-600 106, India

Sunil Kumar Koppala Narayana

Siddha Central Research Institute,
Central Council for Research in Siddha,
Ministry of AYUSH, Chennai, Tamil
Nadu-600 106, India

Muthu Tamizh Manoharn

Siddha Central Research Institute,
Central Council for Research in Siddha,
Ministry of AYUSH, Chennai, Tamil
Nadu-600 106, India

Satheesh Durairaj

Siddha Central Research Institute,
Central Council for Research in Siddha,
Ministry of AYUSH, Chennai, Tamil
Nadu-600 106, India

Brindha Sundaramoorthy

Siddha Central Research Institute,
Central Council for Research in Siddha,
Ministry of AYUSH, Chennai, Tamil
Nadu-600 106, India

Dhanaraj K

Siddha Central Research Institute,
Central Council for Research in Siddha,
Ministry of AYUSH, Chennai, Tamil
Nadu-600 106, India

Patturayan R

Former HOD, Dept. of Kuzhanthai
Maruthuvam, National Institute of
Siddha, Chennai, Tamil Nadu-600 047,
India

Correspondence:

Sathiyarajeswaran P

Siddha Central Research Institute,
Central Council for Research in Siddha,
Ministry of AYUSH, Chennai, Tamil
Nadu-600 106, India

Email: siddha2k6[at]gmail.com

Quality Standards for *Urai Mathirai* - A Siddha Immunomodulator Formulation for Children

Sathiyarajeswaran P*, Shree Devi MS, Sunil Kumar Koppala Narayana, Muthu Tamizh Manoharn, Satheesh Durairaj, Brindha Sundaramoorthy, Dhanaraj K, Patturayan R

ABSTRACT

Standardization of Siddha formulations is a major step for establishment of biological activity, consistent chemical profile, or quality control for production and manufacturing of herbal drugs. *Urai Mathirai* (UM) is a Siddha polyherbal preparation comprising of Chukku (*Zingiber officinale* Roscoe), Adimathuram (*Glycyrrhiza glabra* L.), Akkirakaram (*Anacyclus pyrethrum* (L.) Lag.), Vashambu (*Acorus calamus* L.), Catikkai (*Myristica fragrans* Houtt.), Katukkai (*Terminalia chebula* Retz.), Masikkai (*Quercus infectoria* G.Olivier), Acanam (*Allium sativum* L.), Tippili (*Piper longum* L.) and Perunkayam (*Ferula aasa-foetida* L.). UM reduces accumulated *Aiyam* with ingredients having hot potency which increases *Azhal*. As UM is an important medicine in Siddha pediatrics the current study is aimed to standardize UM employing standard testing protocol for AYUSH drugs. Macroscopic authentication, powder microscopy and physico-chemical studies like loss on drying, water soluble ash, acid insoluble ash, ethanol soluble extractive, water soluble extractive, pH and HPTLC were performed as per standard methodology. The current study derived quality indicating botanical and chemical fingerprints routine quality check of UM.

Keywords: Immunomodulator, Monograph, Quality control, Siddha Pediatrics, *Urai Mathirai*.

1. INTRODUCTION

Reproductive and child health is a flagship program of Govt. of India. Immunological diseases and respiratory disease of children are taken in high priority. Increasing antibiotic resistance and awareness on AYUSH drugs have focused on usage of herbal and other AYUSH drugs. World Health Organization (WHO) encourages, recommends and promotes herbal remedies in natural health care [1]. The specific guidelines put forth by WHO serves as a prerequisite for global harmonization by assessing the safety, efficacy and quality of herbal medicines. The quality assessment of herbal formulations is of paramount importance in order to justify their acceptability in modern system of medicine [2]. WHO has also evolved guidelines for the validation of plant based drugs for developing countries like India [3].

Urai mathirai (UM) is a drug used for prevention of recurrent respiratory infections. UM is made from 10 ingredients out of which majority of ingredients possess hot-potency and pungent taste. These drugs in post digestive transformation get converted into fire moiety which in turn increases *Azhal* of the body. *Azhal* is responsible for immunity as per the Siddha concept "*Vathamai Padaithu, Pitha Vanniyai Kathu, Sethuma Seethamai Thudaitu*" meaning immunity is taken care with increase of *Azhal*. UM is a drug used for the past 3 decades in the form of long finger sized bullets which are rubbed and administered with breast milk to children for improving immunity and to get freedom from health hazards such as frequent respiratory infections /gastrointestinal infections and anorexia. The ingredients of Siddha polyherbal formulation UM are Chukku (*Zingiber officinale* Roscoe), Adimathuram (*Glycyrrhiza glabra* L.), Akkirakaram (*Anacyclus pyrethrum* (L.) Lag.), Vashambu (*Acorus calamus* L.), Catikkai (*Myristica fragrans* Houtt.), Katukkai (*Terminalia chebula* Retz.), Masikkai (*Quercus infectoria* G.Olivier), Acanam (*Allium sativum* L.), Tippili (*Piper longum* L.) and Perunkayam (*Ferula aasa-foetida* L.) [4]. This paper deals about the pharmacognostic and phyto chemical standardization of UM to ensure quality of authentic preparation.

2. MATERIALS AND METHODS

2.1 Raw Drugs

The ingredients of UM (Figure 1, Table 1) were collected from the local market of Chennai, Tamil Nadu

India. The collected drugs were identified and authenticated at Department of Pharmacognosy, Siddha Central Research Institute (SCRI), Chennai, Tamil Nadu, India.

2.2 Preparation of UM

The identified raw drugs as per formula composition mentioned in Table 1 are ground together with water and made into tablets.

Table 1: Ingredients of *Urai mathirai*

S. No	Ingredients	Botanical Name	Proportion
1.	Chukku	<i>Zingiber officinale</i> Roscoe	1 part
2.	Adimathuram	<i>Glycyrrhiza glabra</i> L.	1 part
3.	Akkirakaram	<i>Anacyclus pyrethrum</i> (L.) Lag.	1 part
4.	Vashambu	<i>Acorus calamus</i> L.	1 part
5.	Catikkai	<i>Myristica fragrans</i> Houtt.	1 part
6.	Katukkai	<i>Terminalia chebula</i> Retz.	1 part
7.	Masikkai	<i>Quercus infectoria</i> G.Olivier	1 part
8.	Acanam	<i>Allium sativum</i> L.	1 part
9.	Tippili	<i>Piper longum</i> L.	1 part
10.	Perunkayam	<i>Ferula assa-foetida</i> L.	1 part

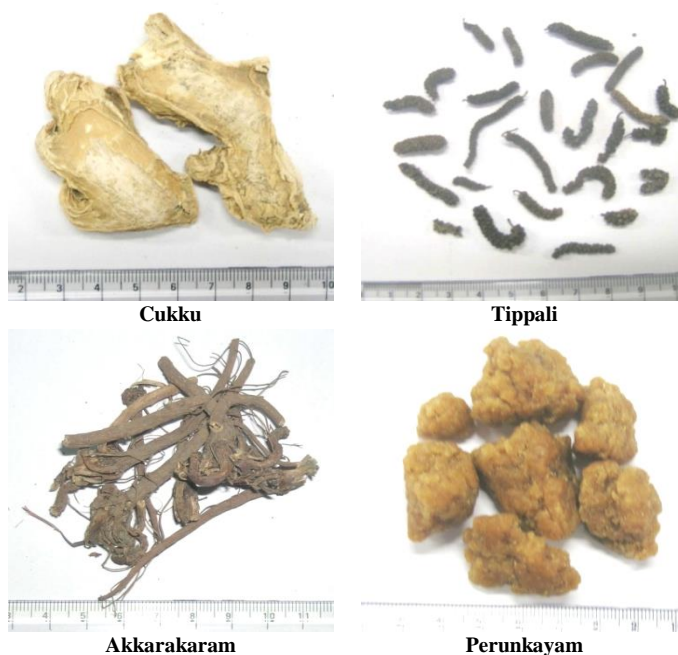


Figure 1: Ingredients of *Urai mathirai*



2.3 Botanical Characterization

2.3.1 Macroscopic authentication

The ingredients were authenticated based on macroscopy and organoleptic features.

2.3.2 Powder microscopy

Powder microscopic features of the UM were documented as per standard procedures [5]. A pinch of the powdered sample was mounted on a microscopic slide with a drop of glycerin-water. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Nikon COOLPIX 5400 digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented. Characters of all the nine organized drug ingredients have been observed and recorded.

2.4 Chemical characterization

2.4.1 Physico-chemical studies

The procedures recommended by WHO were adopted for the determination of loss on drying at 105°C, total ash, water soluble ash, acid insoluble ash, water soluble extractive, ethanol soluble extractive and pH [6].

2.4.2 Preliminary phyto-chemical studies

The *Urai mathirai* formulation were subjected to preliminary phytochemical screening for identification of phytochemical constituents [7-9].

2.4.3 High Performance Thin Layer Chromatography

HPTLC experiments were performed for hydro-ethanolic extract of powdered plant materials or formulation on aluminum packed silica gel 60F₂₅₄ HPTLC plates (Merck). The mobile phase was toluene: ethyl acetate: formic acid (5 : 2.5 : 0.5) and it was poured into the Camag twin trough glass chamber and allowed to equilibrate for 30 min. The Samples (5 -15 uL) were applied to the plates as sharp bands by using of CAMAG Automatic TLC Sampler 4 (ATS4) applicator. After drying the spots in a current of air the plats were placed in one trough of CAMAG twin trough glass chamber and then developed until the solvent front had travelled a distance of 8 cm above the position of sample application. The developed plate was air dried, visualized under UV 254, 366 nm for documenting the TLC chromatograms; Then scanned in both wavelengths for generating the finger print profiles. The photo documentation and finger printing was also done at 575 nm after dipping the plate in vanillin-sulphuric acid reagent, followed by heating in an oven till the appearance of colour of the spots [10].

3. RESULTS

Standardization tests were performed for UM as per standard testing protocol. Macro-microscopic features of the drug and powder respectively confirmed the authenticity of the raw drugs in UM (Figure 1 and 2). UM is found to be dark green in color with characteristic odor and bitter taste. The physico chemical characteristics are presented in Table 2.

Table 2: Physico-chemical characteristics of *Urai mathirai*

Parameter	Result
Loss on Drying (%)	3.84
Total Ash (%)	4.33
Water soluble Ash (%)	3.17
Acid insoluble Ash (%)	1.05
Ethanol soluble Extractive (%)	9.5
Water soluble Extractive (%)	12.25
pH	5.03

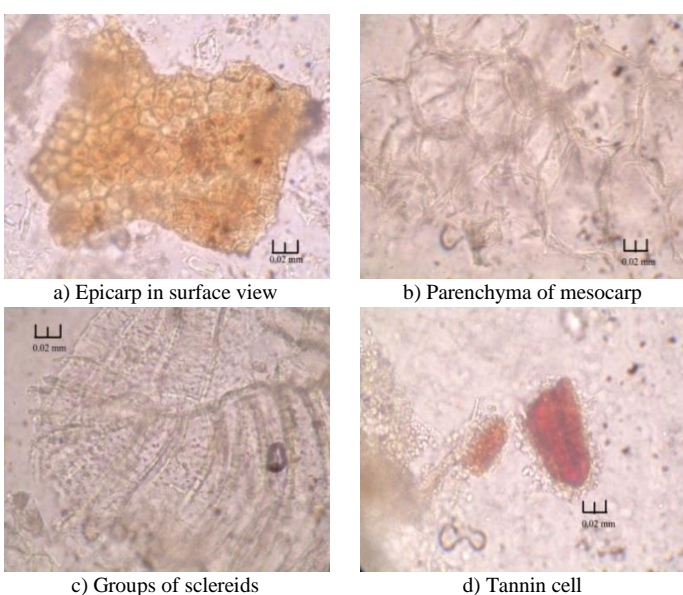


Figure 2.1: Characters from Masikkai - *Quercus infectoria* - gall

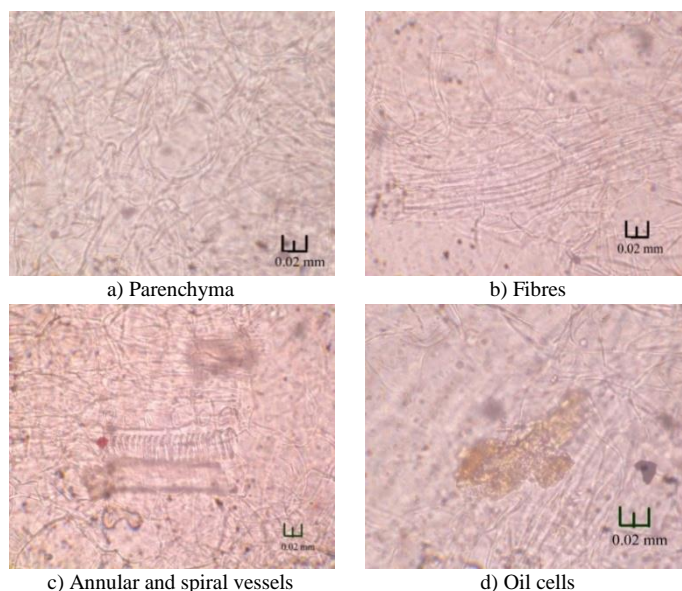


Figure 2.2: Characters from Poondu - *Allium sativum* - bulb

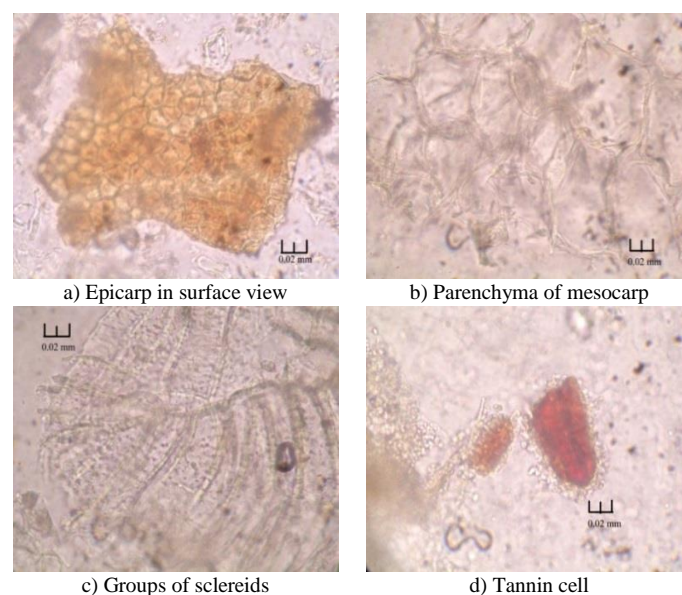


Figure 2.3: Characters from Tanrikkai - *Terminalia chebula* - Dried fruit

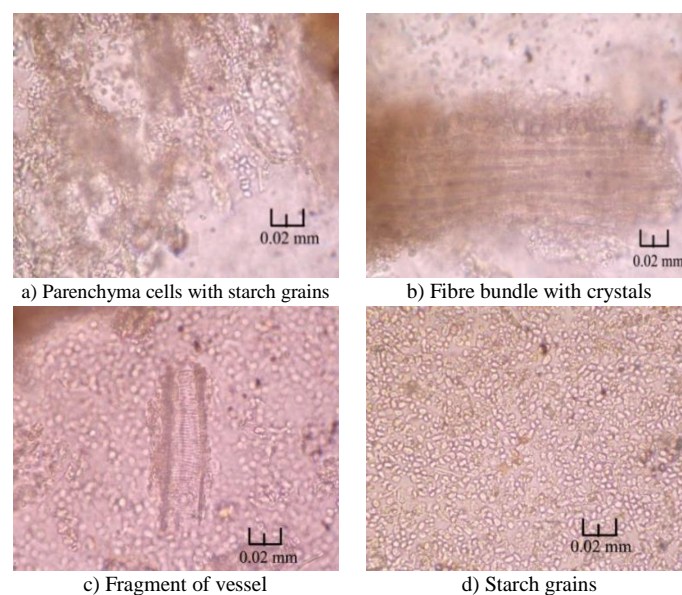


Figure 2.4: Characters from Vasambu - *Acorus calamus* - rhizome

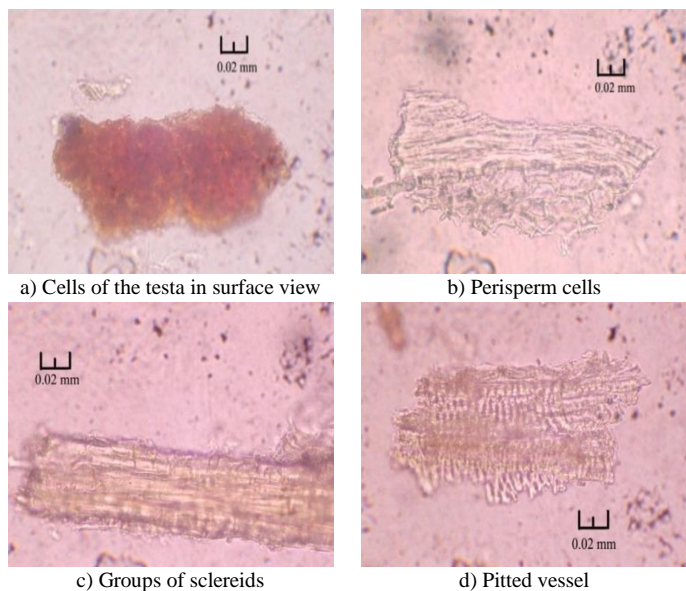


Figure 2.5: Characters from Jatikkai – *Myristica fragrans* – kernel

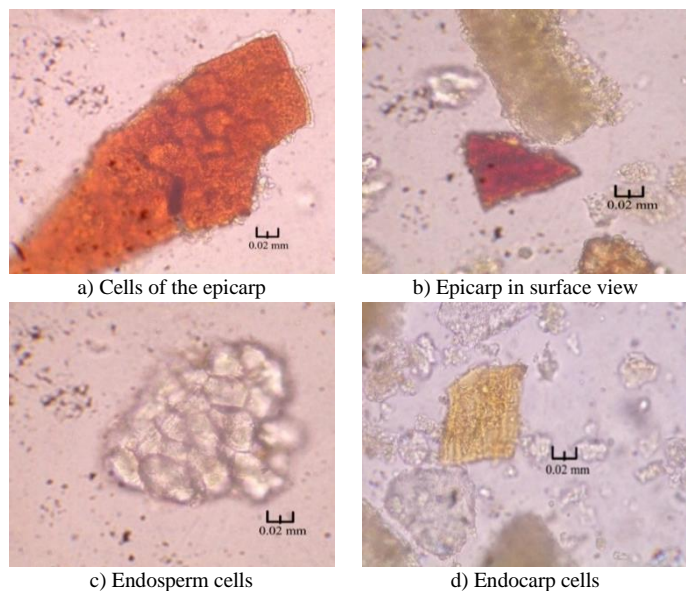


Figure 2.8: Characters from Tippali – *Piper longum* - Fruits

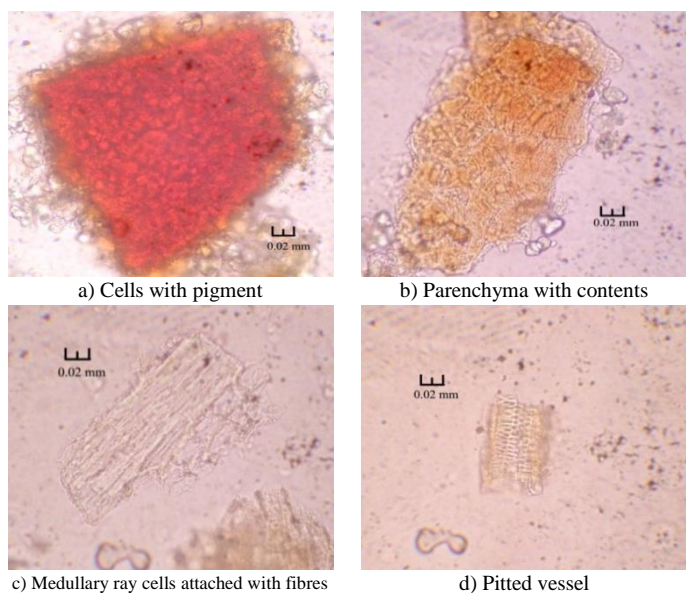


Figure 2.6: Characters from Atimathuram – *Glycyrrhiza glabra* – root and stolon

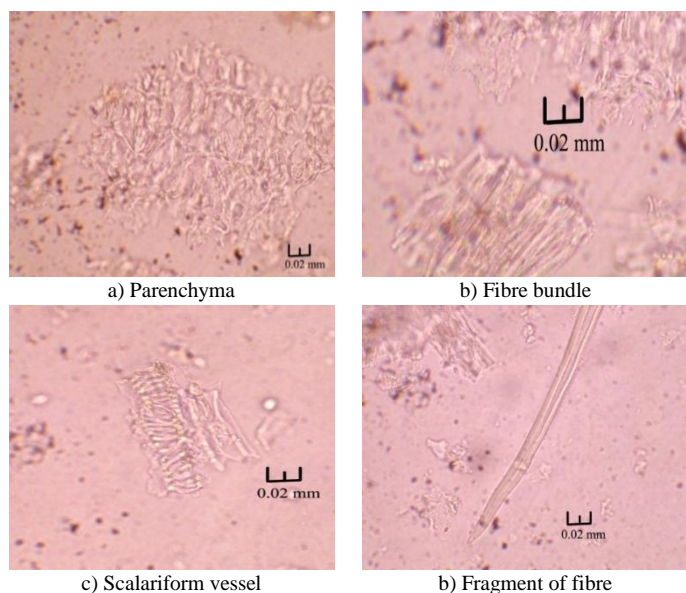


Figure 2.9: Characters from Akkarakaram – *Anacyclus pyrethrum* - root

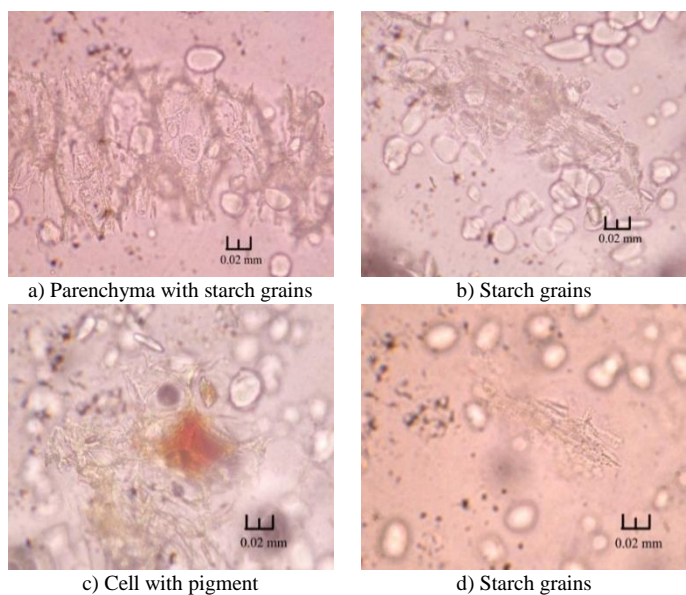


Figure 2.7: Characters from Cukku – *Zingiber officinale* - rhizome

Figure 2: Powder microscopy of *Urai mathirai*

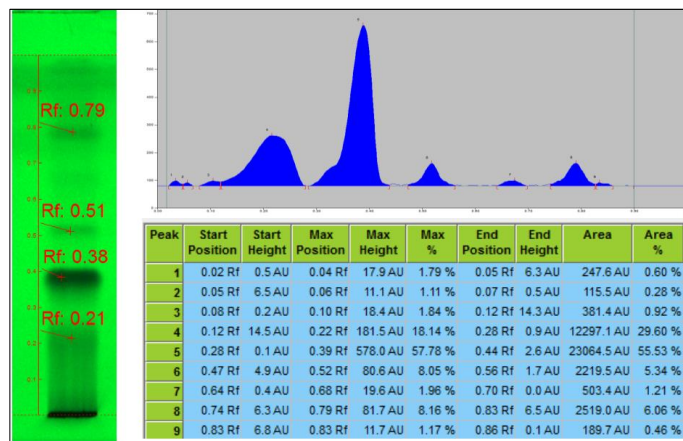


Figure 3: HPTLC fingerprinting of hydro-ethanolic extract of *Urai mathirai* at 254 nm

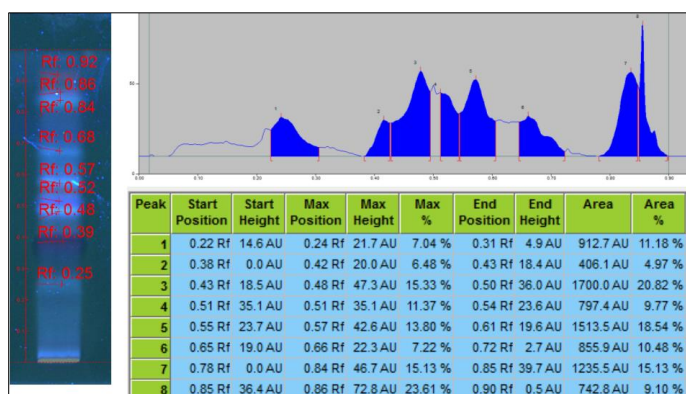


Figure 4: HPTLC fingerprinting of hydro-ethanolic extract of *Urai mathirai* at 366 nm

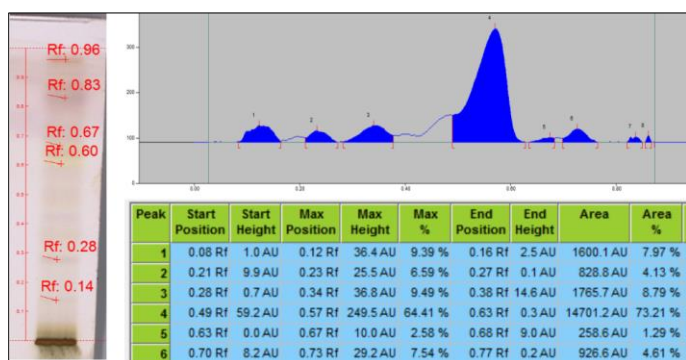


Figure 5: HPTLC fingerprinting of hydro-ethanolic extract of *Urai mathirai* at 520 nm after derivatization with 2% vanillin in ethanolic sulphuric acid

Photo documentation of hydro-ethanolic extract of UM showed 9, 8 and 6 spots under 254, 366 and white light (after derivatisation) respectively (Figure 3). Densitometric scan at 254 nm revealed 9 peaks corresponding to 9 different compounds in the hydro-ethanolic extract, compounds with R_f 0.21 (29.6 %), 0.38 (55.5 %), 0.51 (5.3 %) and 0.79 (6.1 %) were the major peaks (Figure 3). At 366 nm there were 8 peaks, peaks with R_f 0.25 (11.0 %), 0.48 (20.8 %), 0.57 (18.5 %), 0.84 (15.1 %) and 0.86 (9.1 %) being the major peaks detected (Figure 4). After derivatisation and visualization of TLC at 520 nm showed 6 bands. Densitometric scan at 520 nm revealed 6 peaks R_f 0.14 (7.9 %), 0.28 (8.8 %), 0.60 (73.2 %) and 0.83 (4.6 %) were the major peaks (Figure 5).

4. DISCUSSION

Standardisation is a mandate when a medicine is formulated as a proprietary medicine or classical drug [11]. UM showed 3.84% of moisture which is relatively very low. Moisture plays important role in shelf life of a medicine, every drug retains a certain amount of moisture in it based on the hydrophilic nature of the compounds present. The total ash is indicative of inorganic contents including silica; 4.33% is the ash content of UM. The water soluble part of ash was found to be 3.17 while the acid insoluble ash which is mainly silica content is found to be 1.05%. Ethanol and water soluble extractives which are indications of active constituents were found to be 9.5 and 12.254% respectively. pH of UM was found to be 5.03, that is in the weakly acidic range. Most drugs are either weak acids or weak bases. Weak electrolytes, in addition to lipid solubility, depend upon its degree of ionization which is influenced by pH of the area. Weak acids become less ionized (charged) in an acidic medium and

weak bases become less ionized in an alkaline medium. UM showed pH of 5.03 which is weakly acidic. HPTLC is an important tool in standardisation and quality control of polyherbal formulations. Qualitative HPTLC fingerprinting can be used for development of quality standards for polyherbal formulations [12]. These macro-microscopic and physico-chemical constants along with HPTLC can be used as fingerprint to check quality of UM.

CONCLUSION

The purpose of standardization of medicinal plants is to warrant the therapeutic efficacy since the active constituents may differ according to the geographical and sessional source of the drug. Thus, it may not be easy to standardize drug chemically and hence maintaining the quality of these plant products is an essential factor. The constituents of *Urai Mathirai* are endowed with various biological properties and hence the polyherbal preparation UM prepared from these ingredients will have combined goodness of all the individual herbs. The quality indicating tests for UM reported from this study can be used as routine quality check parameter for this polyherbal preparation.

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