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# Comparative Standardisation Study of a Siddha Source Herb *Acalypha indica Linn.* Aerial Parts and Marketed Raw Material - An Insight to Develop Monograph

**Research Article**

**Teshini S[1], Swetha T[1], Mangathayaru K[1], Husna Kanswal Qureshi[1], Vinodhini C[1*]**

1. Department of Pharmaceutical Chemistry, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Chennai-600116, India.

## Abstract

Background: *Acalypha indica Linn.*(AI) is a herbaceous annual catkin-like inflorescences belonging to the *Euphorbiaceae* family. AI has a wide range of therapeutic effects including laxative properties, anthelmintic, wound healing, antiseptic etc. Objectives: This study was to carry out the comparative standardisation study for a siddha source herb *Acalypha indica*(Linn) aerial parts and marketed raw material by collection and authentication of aerial parts of AI and to evaluate the physicochemical constants such as LOD, ash values, extractive values as per the standard methods. Materials and Methods: The preparation of hydro alcoholic and ethyl acetate extract was carried out by cold maceration extraction procedure for both fresh leaves and raw material of AI. Results: The preliminary physicochemical screening tests, quantitative determination of total flavonoid and phenolic contents, hydrogel preparation of AI was carried out. The HPTLC finger printing for ethyl acetate extract of fresh leaf was developed in comparison with three standards Quercetin, Gallic acid and Beta sitosterol and were scanned under UV light at 397nm, 280nm, 580nm respectively. The number of peaks obtained in AI extract were observed to be 3, 8 and 28 respectively. Pesticide analysis regulations often require chemical testing using confirmatory techniques such as GC/MS and about 26 compounds were identified under suitable gas chromatographic conditions. Conclusion: The presence of phytoconstituents were analyzed and the studies were performed to aid further investigations for the identification of therapeutic value.

**Keywords:** *Acalypha indica L., Comparative standardization, HPTLC finger printing, UV Assay method, GC/MS analysis*.

## Introduction

A Monograph is a study document which projects a complete information about the specified topic of interest mentioned as a single species/ group/ categories. **World Health Organization** promotes usage of Monograph to create a harmonization in herbal drug consumption with regards to Efficacy, Quality and Safety(1).

Herbal Medicine system have been followed through generations and is still practiced now in various parts of the world (around 80% - according to WHO), yet it fails when compared to the Allopathic system of medicine in terms of its acceptance ratio. Though the herbal drugs can be measured as comparatively safe to allopathic drugs, the possible fear of toxicity cases on usage of herbal drugs is predominant due to the following reasons (2, 3)**.**

1. Method of handling the herbs during manufacturing.
2. Loss of the active constituent.
3. Presence of substandard species.
4. Presence of pesticide content.
5. Adulteration.
6. Mixture of some other species of herbs.

Hence the need for an established quality control profile for the herbal drugs is deemed to be essential (4).

*Acalypha indica L.* is an annual herb present in the tropical regions. Medicinally it is used in siddha polyherbal formulations.

**Plant Uses**

1. Leaves are converted into a decoction and used as a laxative -alternative for senega.
2. When mixed with garlic, it's treated as an anthelmintic.
3. Given internally and topically for scabies treatment.
4. Decoction is instilled into ear for treatment of ear pains, anti-arthritic.
5. It's also used to treat respiratory disease in the form of expressed juice.
6. Dry leaves are powdered and applied for bed sores and methonolic extract as anti-ulcer.
7. For treating congestive headache, a cotton plug is saturated with the expressed juice and inserted into each nostril. Pain was said to be removed by causing hemorrhage from the nose.

*** Corresponding Author:**

**Vinodhini C**

Associate Professor, Department of Pharmaceutical Chemistry, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Chennai-600116, India.

Email Id: vinpharma79@gmail.com

8. According to **PATHARTHA GUNA CHINTHAMANI** it has the ability to cure teeth & gum problem, burns, toxins, wheezing, sinusitis and neutralizes '*Kapha'* (**7**).
9. According to **SIDDHA MATERIA MEDICA,** leaf powder in dose 950 – 1300mg can cure respiratory diseases.
10. It is mainly used to treat burns as a topical formulation[10]and extract acts as anti-oxidant (5-6).

This paper involves the preliminary studies on a traditional siddha source herb *Acalypha indica* in lieu of development of monograph which is unavailable in the herbal pharmacopoeia.

## Materials and Methods

The colour and solubility of *Acalypha india L.* Fresh Leaf (AIFL) and *Acalypha indica L.* Raw Material (AIRM) were observed in different polarity of solvents such as water, ethanol, ethyl acetate and hexane in the unit concentration of 1mg/ml. IR spectrum was recorded for samples by KBr pellet method. Powder microscopy was performed as mentioned in the standard procedure. Physicochemical parameters such as total ash, water soluble ash, acid insoluble acid, moisture content by LOD and Halogen moisture analysis and extractive values such as water soluble and ethanol soluble were determined according to the procedures mentioned in WHO quality control methods for herbal materials (8-10).

**Preparation of Ethyl Acetate extract and Hydroalcoholic extracts**

About 25g of AIFL, and AIRM powders were weighed and macerated with ethyl acetate and ethanol: water (1:1) solvent mixture (hydroalcoholic) for 48 hours. It was shaken every 6 hours during maceration. It was then filtered, and 25ml of the filtrate was taken in a previously tared china dish and the solvent was evaporated on a hot plate. The ethyl acetate fraction was collected and weighed. The extract thus obtained was designated as Ethyl Acetate extract of Fresh Leaf (EAFL), Ethyl Acetate extract of Raw Material (EARM) and Hydroalcoholic extract of Fresh Leaf (HAFL), Hydroalcolohic extract of Raw Material (HARM) respectively (11).

**Phytochemical Screening**

The tests for terpenoids, flavonoids, steroids, anthraquinones, glycosides, carbohydrates, alkaloids, quinone, phenols, tannins, saponins, proteins and amino acids were performed for all the extracts (12-14).

**UV - Visible Spectrophotometric Analysis**

Standards selected for the analysis include, Gallic acid, Elagic acid, Quercetin and the solvent used was ethanol: water (1:1). The below working standard, mixed standard and sample solutions were scanned in the UV- Visible spectrophotometer in the range 200-800nm and the spectrum was recorded for its absorbance at characteristic wavelength.

**•Preparation of standard stock solution**

About 10mg of the standards were weighed separately and transferred into a 10ml standard flask, dissolved in diluent and the volume was made upto 10ml with diluent (1000µg/ml).

**•Preparation of mixed standards**

About 0.1ml of each standard solutions were pipetted to 10ml standard flask and volume was made up with diluent (10 µg/ml).

**•Preparation of working standard solutions**

Pipette out 0.1 and 0.5ml of the standard stock solution into 10ml standard flask and volume was made up with diluent (10 & 50 µg/ml).

**•Preparation of sample Stock solution**

About 10mg of AIFL, AIRM powder, EAFL, EARM, HAFL, HARM, extracts were weighed separately and transferred into a 10ml standard flask, dissolved with diluent and made up the volume with diluent (1000 µg/ml).

**•Preparation of sample solutions**

Pipette out 0.1 and 0.5ml of the sample stock solution of AIFL, AIRM, EAFL, EARM, HAFL, HARM into 10ml standard flask and volume was made up with diluent (10 & 50 µg/ml) (15-16).

***Quantitative Estimation of Phenolic and Flavonoid Content:***

The estimation of total phenol and flavonoid content for the extracts were performed as per the standard procedure (17-18, 24-25).

**Preparation of Gel formulation**

Carbopol was weighed in a beaker and 50ml distilled water was added. It was allowed to soak for 8 hours allowing the Carbopol to swell. To 5ml of distilled water methyl paraben and propyl paraben was added and dissolved by heating it and cooled. To this propylene glycol and the extracts were added. This solution was poured into Carbopol with continuous stirring and then triethanolamine was added dropwise until a consistent gel was formed. The gel was stored in a stored in a tightly packed container for further evaluation. The composition is given in table 1.

**Table 1: Composition of AI Hydrogel**

| S.No | Ingredient Name | Weight to be taken | Use |
|---|---|---|---|
| 1 | Extracts EAFL, EARM, HAFL, HARM | 0.05g | Traditional medicine. |
| 2 | Carbopol 940 | 1g | Gelling agent. |
| 3 | Methyl paraben | 0.2g | Preservative. |
| 4 | Propyl paraben | 0.1g | Preservative. |
| 5 | Triethanolamine | 1.2ml | pH neutralizer. |
| 6 | Propylene glycol | 5ml | Solubiliser. |

**Evaluation of Gel**

The pH, extrudability, irritancy test was carried out as per the standard procedure. (26-29)

**Drug content by UV Spectroscopy**

A quantity of gel equivalent to 50mg of extracts of HAFL, HARM, EAFL, EARM, were weighed and transferred to a 100ml volumetric flask. 20ml of methanol was added and stirred for 30 minutes. It was made up to 100ml and filtered. 1ml was pipetted and made upto 10ml using alcohol. The above solutions were scanned at 200-400 nm and the absorbance was measured at the wavelength 303nm (22,23).

**HPTLC Analysis**

**Preparation of Standard Marker Solutions**

**CAMAG® TLC Scanner 3** was used for the densitometric evaluation of TLC/HPTLC plates, spectral range of 190 to 900 nm, plate sizes up to 20 x 20 cm, absorbance and fluorescence mode, CAMAG *vision CATS* controlled.

The standard markers quercetin, gallic acid, beta-sitosterol were selected for HPTLC finger printing given in Table 2 and the chromatograms are illustrated in figure 4,5,6. (21)

**Table 2: Requirements for HPTLC finger printing**

| Standards | Concentration | Wave length | Mobile Phase |
|---|---|---|---|
| **Gallic Acid** | 1mg/ml in methanol concentration (100 µg,200 µg,300 µg,400 µg,500 µg) CONTROL: Methanol EXTRACT:10mg/ml methanolic extract | 280 | Toluene: Ethyl acetate: Formic acid: Methanol 3: 3: 0.4: 0.1 |
| **Beta Sitosterol** | 1mg/ml in chloroform concentration (100 µg,200 µg,300 µg,400 µg,500 µg) CONTROL: methanol EXTRACT: 10mg /1ml methanolic extract | 580 | Toluene: Ethyl acetate 8: 2 |
| **Quercetin** | 1mg/1ml in ethanol Concentration (10 µg,50 µg, 100 µg, 150 µg, 200 µg) CONTROL: Methanol+ ethanol EXTRACT: 5 mg/1 ml methanolic exctract | 397 | Hexane: Toluene: Ethyl acetate: Methanol 5: 3: 1.5: 0.5 |

**GC-MS ANALYSIS OF ETHYL ACETATE EXTRACT OF *Acalypha indica L.***

GC-MS analysis was carried out in a 890B GC 5977B gas chromatograph system (Agilent Technologies) and mass spectrophotometer. Helium gas was used as a carrier gas and was adjusted to column velocity flow of 1.0mL/min. Other GC-MS conditions include injector and transfer line temperature at 280°C; pressure 8.2317 psi with a spectrum purge flow at 3 ml/ min. the oven temperature was fixed at 60°C and reached maximum of 325°C.

**Sample Preparation Procedure**

50µL or 50 mg of liquid or powder sample dissolved in 1mL of GC grade ethyl acetate and vortexed. Filter the sample through 0.45µ filter cartridge & inject in to GC-MS. Volume of 3.0 µL was injected. Pre injection solvent washes were carried out 3 Times and the Post injection solvent washes were carried out 4 Times.

**Table 3: Conditions for Pesticide analysis**

| | Rate °C/ min | Value °C | Hold Time min | Run Time min |
|---|---|---|---|---|
| **Initial** | | 60 | 1 | 1 |
| **Ramp 1** | 40 | 120 | 0 | 2.5 |
| **Ramp 2** | 5 | 190 | 3 | 19.5 |
| **Ramp 3** | 2 | 210 | 0 | 29.5 |
| **Ramp 4** | 10 | 310 | 2 | 41.5 |

## Results and Discussion

The colour and solubility characters of AIFL and AIRM was found to be similar. The IR spectrum of *Acalypha indica L.* FL and RM powder were scanned in the range of 4000 – 400cm$^{-1}$ using FT-IR JASCO, 4100, Model Spectrophotometer and was depicted in Fig 1. The overlay spectra's reveal that the procured raw material of AIRM has the characteristics peaks which coincides with that of the fresh leaf powder of AIFL.

The powder characteristics of *Acalypha indica L.* FL and RM powder has shown the presence of Large, lignified fibers with moderately thickened walls, bordered and spiral shaped xylem vessels, large number of trichomes, multiseriate medullary rays. The results were shown in Fig (2 – 2.7). The ash content was found to be less in AIFL compared to AIRM but the extractive values are on the higher side which may be due to the enriched fraction of FL constituents compared to that of procured raw material which might have lost its constituents during manufacturing process. The moisture content performed by two different techniques revealed relatively low percentage of moisture in AIFL compared to that of AIRM. The extraction of *Acalypha indica L.* using ethyl acetate and hydroalcohol (ethanol:water – 1:1) were performed. The percentage yield of extracts was found to be high in fresh leaf of both the extracts (EAFL and HAFL) compared to raw material of both the extracts (EARM and HARM). On the other way, the percentage yield was found to be comparatively more in hydroalcoholic (ethanol:water - 1:1) extract than ethyl acetate extracts.

The UV- Visible spectrum was recorded for AI samples such as FL, RM, EAFL, EARM, HAFL, HARM and were scanned in the range of 200 - 800nm and their absorption at different wavelengths were tabulated in Table 3.

In comparison to the standards used, the sample powder and extracts of AI has shown characteristics absorption at different wavelengths. In particular AIFL, EAFL, HAFL has characteristic strong absorption at 414nm (Abs- 0.295, 0.144, 0.178 respectively) but wherein this characteristic absorption was missing in case of HARM extracts, still intensely seen in EARM.

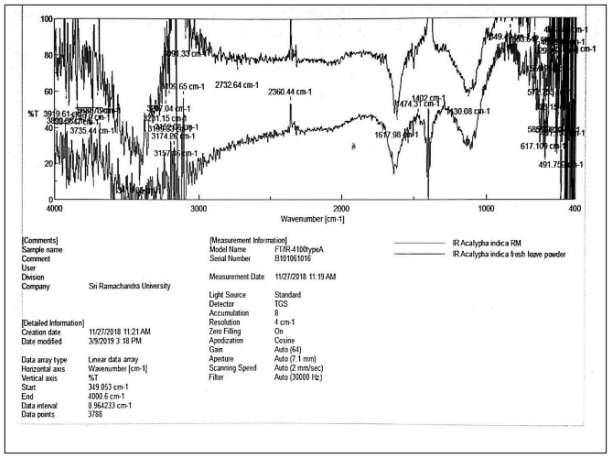
Also the absorption at 303nm is found to be present in all the samples of AI. This could be considered as a vital absorption characteristic in the analytical perspectives. Overall compared to RM, FL has more absorption peaks but intensity at particular wavelengths varied tremendously without supporting to result conclusion.

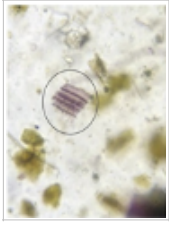
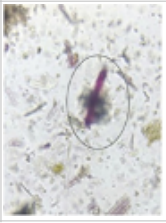
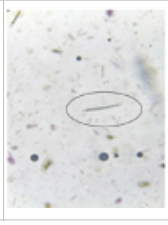
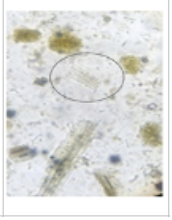
The phenolic content measured at 765 nm revealed that the FL powder and ethyl acetate extract of FL are moderately high compared to that of RM, where in hydroalcoholic extract (ethanol:water) the procured RM has shown high phenolic content as that of FL powder. But overall, the sum of percentage content was found to be 3.6% w/w for FL sample which is moderately high compared to that of 3.15% w/w in procured RM sample as shown in Fig 5.

The flavonoid content measured at 415 nm revealed that the RM powder and ethyl acetate extract of RM are moderately high compared to that of FL, where in hydroalcoholic extract (ethanol: water – 1:1) FL has shown high flavonoid content of that of RM powder. But overall, the sum of percentage content was found to be 0.73% w/w for procured RM sample which is higher compared to that of 0.6% w/w in FL sample as shown in Fig 6.

The colour of AI EAFL and AI EARM gels were observed as dark green and pale green respectively while the AI HAFL and AI HARM gels were pale brown in colour. The pH of the gels AI EARM and AI HARM were slightly higher than that of the AI EAFL and AI HAFL gels but within the range of skin pH (3 - 9). In overall, there were no signs of redness and oedema was observed in various extracts of AI gels. Results for the above tests are given in Table 4.

**Fig 1:IR overlay spectrum of AIFL and AIRM using KBr pellet method**




| Fig 2: Lignified Fibres (AIFL) | Fig 2.1: Xylem Vessels (AIFL) | Fig 2.2: Trichomes (AIFL) | Fig 2.3: Medullary Rays (AIFL) |
|---|---|---|---|
|  |  |  |  |

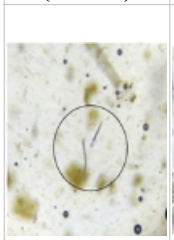
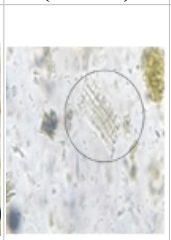
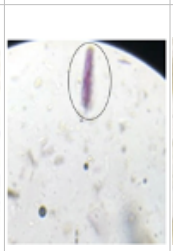
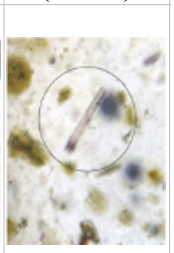
| Fig 2: Lignified Fibres (AIRM) | Fig 2.1: Xylem Vessels (AIRM) | Fig 2.2: Trichomes (AIRM) | Fig 2.3: Medullary Rays (AIRM) |
|---|---|---|---|
|  |  |  |  |

**Figure 3: Test for Flavonoids and Phenols for AIFL, AIRM, EAFL, EARM, HAFL, HARM**

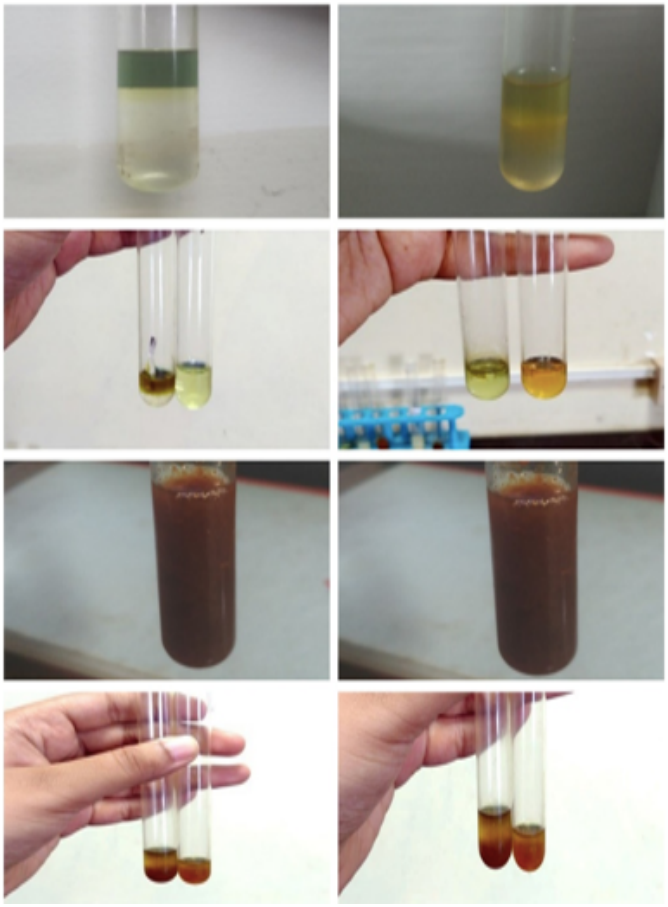


**Figure 4: UV - Visible overlay spectrum of various AI gel (EAFL, EARM, HAFL, HARM)**

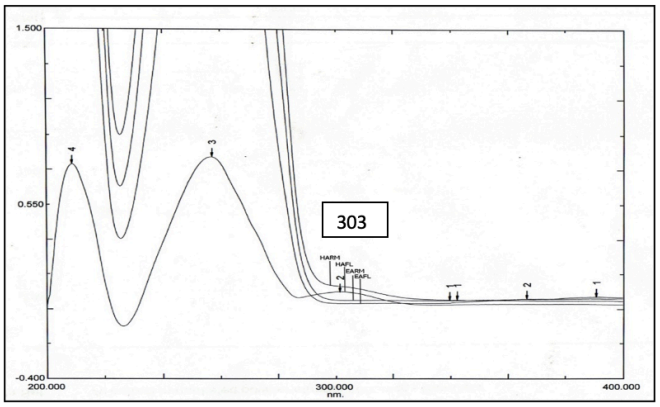


**Table 3: Characteristics Absorption of AI Samples**

| | FL | | RM | | EAFL | | EARM | | HAFL | | HARM | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | λ | Abs | λ | Abs | λ | Abs | λ | Abs | λ | Abs | λ | Abs |
| ***Acalypha indica L.* Samples** | 666 | 0.128 | 666 | 0.040 | 780 | 0.027 | 666 | 0.062 | 666 | 0.069 | **303** | **0.215** |
| | 613 | 0.053 | **414** | **0.153** | 701 | 0.037 | 608 | 0.016 | 608 | 0.037 | 237 | 0.296 |
| | 536 | 0.053 | **303** | **0.840** | 667 | 0.058 | 536 | 0.017 | | | 209 | 0.616 |
| | **414** | **0.295** | 267 | 1.125 | 607 | 0.038 | 503 | 0.020 | **412** | **0.178** | | |
| | **303** | **0.869** | | | 536 | 0.043 | **412** | **0.166** | 320 | 0.201 | | |
| | 269 | 0.952 | | | 475 | 0.083 | 357 | 0.236 | **303** | **0.192** | | |
| | | | | | **414** | **0.144** | **303** | **0.226** | 239 | 0.250 | | |
| | | | | | 326 | 0.159 | | | | | | |
| | | | | | **303** | **0.170** | | | | | | |

**Fig 5: Linearity graph of Gallic acid standard**

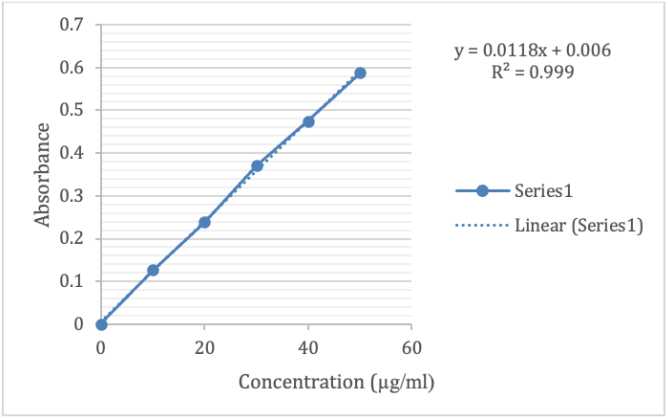



**Fig 6: Linearity graph of Quercetin standard**

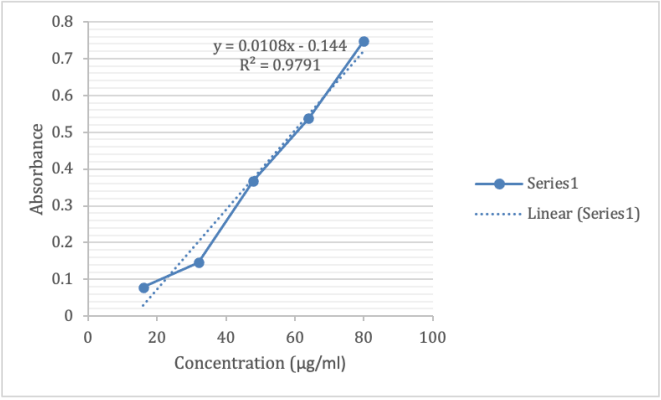



**Table 4: Summary of AI samples and extracts**

| S.No | Parameters | Fresh leaf powder | Raw material powder | EAFL | EARM | HAFL | HARM |
|---|---|---|---|---|---|---|---|
| 1 | **Colour** | Green | Green to pale brown | Dark green | Dark green | Dark brown | Dark brown |
| 2 | **Solubility** | Soluble in Ethanol, ether but insoluble in water and hexane | Soluble in Ethanol, ether but insoluble in water and hexane | Soluble in ethanol | Soluble in ethanol | Soluble in ethanol | Soluble in ethanol |
| 3 | **Infra Red Spectroscopy** | The fresh leaf IR spectrum overlayed with the raw material IR spectrum of *Acalypha indica L.* | | | | | |
| 4 | **Powder Microscopy** | Lignified fibres, xylem vessels, trichomes, medullary rays | Lignified fibres, xylem vessels, trichomes, medullary rays | - | - | - | - |
| 5 | **PHYSICO-CHEMICAL ANALYSIS** | | | | | | |
| | **Total ash** | 9.6%w/w | 13.28%w/w | - | - | - | - |
| | **Water soluble ash** | 8.2%w/w | 10.88%w/w | - | - | - | - |
| | **Acid insoluble ash** | 1.4%w/w | 7.47%w/w | - | - | - | - |
| | **Alcohol extractive value** | 2% | 1.5% | - | - | - | - |
| | **Water extractive value** | 5% | 3% | - | - | - | - |
| | **Loss on drying at 105ºC for 3 hrs.** | 6.48% | 9.12% | - | - | - | - |
| | **Moisture content (Halogen moisture Analyser)** | 4.33% | 7.68% | - | - | - | - |
| 6 | **Extraction by Cold Maceration (% Yield)** | - | - | 0.332% | 0.216% | 0.768% | 0.464% |
| 7 | **PRELIMINARY PHYTOCHEMICAL SCREENING** | | | | | | |
| | Terpenoids | - | - | - | - | - | - |
| | Flavonoids | + | + | + | + | + | + |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Steroids | | - | + | - | + | - | - |
| | Carbohydrates | | + | + | - | - | - | - |
| | Alkaloids | | + | + | - | - | - | - |
| | Phenols | | + | + | + | + | + | + |
| | Tannins | | + | + | + | + | + | + |
| 8 | **UV VISIBLE SPECTROSCOPY** | | | | | | | |
| | **Scannin of standards and mixed standards (200-800 nm)** | | **QUERCETIN** | | **GALLIC ACID** | | **ELLAGIC ACID** | |
| | | | 373, 301, **256**, 203 | | **259,** 214 | | 360, **255** | |
| | | | **MIXED STANDARDS ( Quercetin, Galic acid and Ellagic acid)** | | 369, **256,** 213 | | | |
| | **Scanning of smaples (200-800 nm)** | | **AIFL** | **AIRM** | **EAFL** | **EARM** | **HAFL** | **HARM** |
| | | | 666, 613, 536, 414, **303,** 269 | 666, 414, **303,** 267 | 780,701,667,607,536, 475,414,326, **303** | 666,608, 536, 503, 412, **303** | 666, 608, 412, 372, 320, **303, 239** | **303,** 237, 209 |
| **9** | **Quantitative estimation of total phenols by colorimetry** | **Gallic acid standarad** | **Content (% w/w)** | | | | | |
| | | | 0.79 | 0.58 | **2.02** | 0.51 | 0.79 | **2.06** |
| **10** | **Quantitative estimation of total flavonoid by colorimetry** | **Quercetin standard** | **Content (% w/w)** | | | | | |
| | | | 0.06 | 0.27 | 0.07 | 0.12 | **0.47** | **0.34** |
| **11** | ***Acalypha indica L.* Gel formulation and evaluation** | | | | **EAFL GEL** | **EARM GEL** | **HAFL GEL** | **HARM GEL** |
| | **Colour** | | - | - | Dark green | Pale green | Pale brown | Pale brown |
| | **pH** | | - | - | 6.05 | 6.18 | 6.07 | 6.40 |
| | **Extrudability (%)** | | - | - | 93.64 | 96.03 | 95.68 | 95.80 |
| | **Drug content by UV spectroscopy at 303 nm (%w/w)** | | - | - | 9.73 | 29.16 | 87.05 | 93.95 |

## HPTLC Figerprinting

Natural product substances have historically served as the most significant source of new leads for pharmaceutical development. The TLC chromatogram was run for AI along with standards for various profiles such as Gallic acid, Quercetin, Beta-sitosterol. The results of densitogram of *Acalypha indica L.* aerial parts in fresh leaf extract is given in Table 5

**Table 5: Summary of estimated concentration of standards in AIFL**

| Standard | Rf value | Peak area(AU) | Concentration (µg/ml) |
|---|---|---|---|
| Quercetin | 0.76 | 6544.3 | 157.26 |
| Gallic acid | 0.26 | 1255.3 | 31.64 |
| Beta sitosterol | 0.39 | 2001.7 | 208.65 |

**Fig 4 Densitogram of Quercetin**

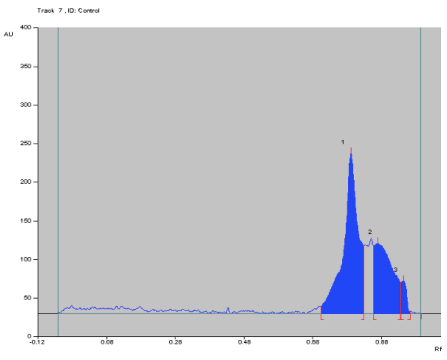


**Fig 5 Densitogram of Gallic acid**

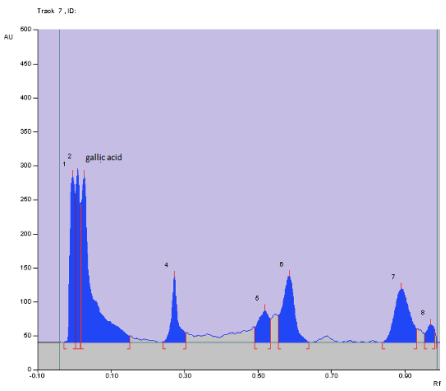
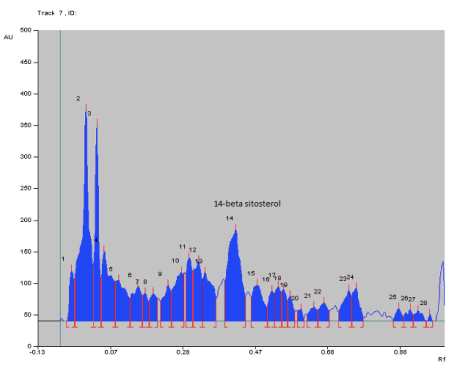
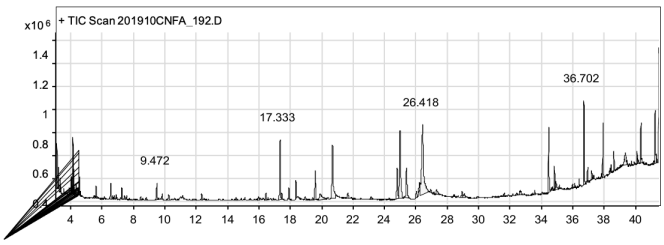



**Fig 6 Densitogram of Beta sitosterol standards**



**GC-MS Analysis:**

| *GC-MS Spectrum of AIFL:* | GC-MS spectrum of AIRM: |
|---|---|
| **Fig 7: GC-MS of pesticides in AIFL extract** | **Fig 8 GC-MS of pesticides in AIRM extract** |



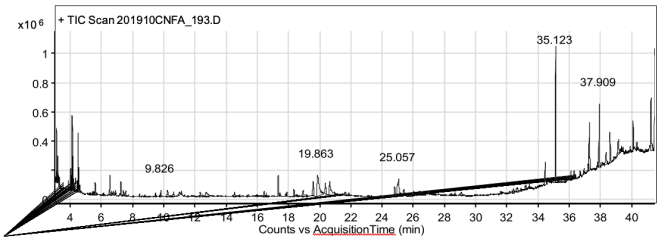




**Table 6: Pesticides commonly observed in both AIFL and AIRM extracts**

| COMPOUND NAME | RT IN AIFL | RT IN AIRM |
|---|---|---|
| p-Xylene | 3.116 | 3.116 |
| Benzene, (azidomethyl)- | 3.223 | 3.223 |
| D-Limonene | 4.023 | 4.023 |
| Dodecane, 1-fluoro- | | |
| Nonane, 4,5-dimethyl- | 4.501 | 4.497 |
| Naphthalene | 5.606 | 5.601 |
| (R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol | 8.796 | 8.796 |
| Quinoline, 1,2-dihydro-2,2,4-trimethyl- | 9.472 | 9.476 |
| 2,5-di-tert-Butyl-1,4-benzoquinone | 9.823 | 9.826 |
| Heptadecane, 2,6,10,15-tetramethyl- | 10.247 | 10.247 |
| 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 17.897 | 17.901 |
| Hexadecanoic acid, methyl ester | 19.575 | 19.575 |
| n-Hexadecanoic acid | 20.672 | 20.634 |
| Phytol | 25.391 | 25.391 |
| Methyl stearate | 26.216 | 26.026 |
| 8-Hexadecenal, 14-methyl-, (Z)- | 28.932 | 21.5 |
| 7-Hexadecenal, (Z)- | 31.62 | 27.378 |
| 11,13-Dimethyl-12-tetradecen-1-ol acetate | 34.047 | 39.146 |
| 9-Octadecenoic acid, (E)- | 34.925 | 35.96 |
| i-Propyl 9-tetradecenoate | 35.985 | 36.702 |
| 1-Monolinoleoylglycerol trimethylsilyl ether | 38.359 | 38.355 |
| Dasycarpidan-1-methanol, acetate (ester) | 38.437 | 37.205 |
| Squalene | 37.91 | 37.909 |
| 1,1,3,6-tetramethyl-2-(3,6,10,13,14-pentamethyl-3-ethyl-pentadecyl)cyclohexane | 38.619 | 38.618 |
| Campesterol | 41.24 | 41.24 |

## Conclusion

Standardization of the selected siddha source herb *Acalypha indica L.* fresh leaf, procured raw material powder and their extracts were carried out as per the standard procedures. The study shows that the investigations that are reported are not specified in the standard literatures such as Ayurvedic Pharmacopoeia. This could help in authentication of *Acalypha indica L.* and also serves as reference monograph in the preparation of Ayurvedic Formulations.

From the Pharmacognostic and Phytochemical investigations it is quite possible to set the standards for this plant as per the Pharmacopoeial guidelines and further additional contributions like HPTLC Finger printing, Isolation of active constituents and Wound healing property are required for the fulfillment of development of herbal Monograph.

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**Conflict of interest:** Declared none

**Abbreviations**

**AI:** *Acalypha indica L.*; **EAFL:** Ethyl Acetate Fresh Leaf; **EARM:** Ethyl Acetate Raw Material; **HAFL:** Hydroalcoholic Fresh Leaf; **HARM:** Hydroalcoholic Raw Material; **LOD:** Loss on Drying; **UV:** Ultra violet; **HPTLC:** High Performance Thin Layer Chromatography; **GC-MS:** Gas Chromatography Mass Spectroscopy

## References

1. Guidelines for the assessment of herbal medicine in Quality assurance of Pharmaceuticals: A compendium of guidelines and related materials, World Health Organization, 1997 Vol. 1.
2. Shailesh.L, Patwekar, Suryawanshi Arvind B Gaikwad Manoj S, Pedewad Snehal R, Potulwar Ashwini P, Standardization of herbal drugs: An overview. The Pharma Innovation Journal. 2015; 4(9); 100-104.
3. The Ayurvedic Pharmacopoeia of India, Part – I, Volume IX, Government of India, Ministry of AYUSH, 2016 Published by Pharmacopoeia Commission for Indian Medicine & Homoeopathy Ghaziabad, First Edition, (Extracts); Appendices, 99-101.
4. Hariharan P, Subburaju T, Medicinal Plants and its Standardization – A Global and Industrial Overview. Global Journal of Medicinal Plant Research. 2012; 1(1);10-13.

5. http://envis.frlht.org/plantdetails/c638e1e36a66bf46763b77bc49f83773/e8e1ea5edb9ab68fdab058de95594e8b, accessed on 03-04-2020.
6. http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=242300253,accessed on 03-04-2020.
7. Pravin H. Nikam, Joseph Kareparamban, Aruna Jadhav and Vilasrao Kadam, Future trends in standardization of herbal drugs. Journal of Applied Pharmaceutical Science. 2012; 2 (06); 2012; 38-44.
8. Indian Materia Medica by Nadkarni K. M., Vol 1, Bombay Popular Prakashan, reprinted 2000;17-19p.
9. Acalypha indica – Wikipedia https://en.m.wikipedia.org/wiki/Acalypha indica, accessed on 02.02.2019.
10. Patharthagunachinthamani- moolamum, uraiyum, 1932, 179p.
11. Suresh Reddy J, Rajeswara Rao P, Mada S Reddy. Wound healing effects of Heliotropium indicum, Plumbago zeylanicum and Acalypha indica in rats, Journal of Ethnopharmacology. 2002; Volume 79; Issue2; 249-251.
12. https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Spectroscopy/Vibrational_Spectroscopy/Infrared_Spectroscopy, accessed on 23-12-2018.
13. https://www.chromacademy.com/lms/sco533/01-infrared-spectroscopy-introduction.html?fChannel=14&fCourse=77&fSco=533&fPath=sco533/01 infrared-spectroscopy-introduction.html accessed on 23-12-2018.
14. Sharma Y.R., Chand.S publications, Elementary Organic Spectroscopy – Principles and Chemical Applications, Fifth revised edition, 2013; 81-89.
15. Vilash V, Suja SR, Latha PG, Aneesh Kumar AL, Ragesh R Nair and Rajasekharan S, Physicochemical Evaluation and Pharmacognostical Standardisation of Pellionia heyneana Wedd. Leaf, Pharmacognosy Journal.Nov-Dec, 2016; 8(6);51-556.
16. Pulok Mukherjee. K, Quality Control of Herbal drugs - An approach to evaluation of botanicals, Business Horizons Pharmaceutical Publisher, 2002; Edition 1, 186-196p.
17. Vinod Rangari. D, Pharmacognosy and Phytochemistry, Career Publications. 2002; edition 1, 98-99p.
18. Anonymous Indian Pharmacopoeia, 1996. Vol 2, 53-54p.
19. Trease and Evans, Pharmacognosy, 15th edition, Saunders Elsvier, 2005. 336p.
20. Kokate C.K, Practical Pharmacognosy, 4th edition, Vallabha Prakashan, 2002. 123-129p.
21. Kokate C.K, Purohit A.P, Gokhale S.B, Pharmacognosy, 50th edition, NiraliPrakashan, 2015. 7.18-7.19p.
22. Rutuja S Shah, Rutuja R Shah, Rajashri B Pawar, Pranit P Gayakar, UV-Visible Spectroscopy- A review, International Journal of Institutional Pharmacy and Life Sciences. September- October, 2015; 5 (5); 490-506.
23. Tony Owen, Fundamentals of UV – Visible Spectroscopy, Hewlett – Packard Company, 38-67p.
24. Savitree Mongkolsilp, Isara Pongbupakit, NittayaSae-Lee, Woarapan Siithithaworn, Radical Scavenging Activity and Total Phenolic Content of Medicinal Plants Used In Primary Healthcare, J. Pharm Sci.2004;9;32-35.
25. Kalita Pallab, Barman Tapan K, Pal Tapas K, Kalita Ramen, Estimation of Total Flavonoid Content (TFC) and Antioxidant Activities of methanolic whole plant extractions of Biophytum sensitivum Linn, Journal of Drug Delivery and Therapeutics. 2013; 3 (4); 33-37.
26. Seema Yuvraj Mandhekar, Pratik Bharat Thorat, Nikita Nivruti Bodke, Jadhav S.L and Gaikwad D.D, Formulation and Evaluation of Gel containing Neem, Turmeric, Aloe Vera, Green tea, and Lemon extract with activated Charcoal and Honey, European Journal of Pharmaceutical and Medical Research. 2017;4(12): 439-443.
27. Jayapal Karthikeyan, Damineni Saritha, "Formulation development and evaluation of gel containing herbals with Saindhava", International Journal of Pharmacy and Pharmaceutical Analysis. 2014;1(1); 26-43.
28. Ashish Aswal, Mohini Kalra and Abhiram Rout, "Preparation and evaluation of polyherbal cosmetic cream", Der Pharmacia Lettre. 2013; 5(1); 83-88.
29. Niyaz Basha, Kalyani Prakasam, Divakar Goli. Formulation and Evaluation of Gel containing Fluconazole-Antifungal Agent, International Journal of Drug Development and Research. October-December, 2011; 3(4);109-128.

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