



## Research article

## Formulation and standardisation of a polyherbal cough syrup

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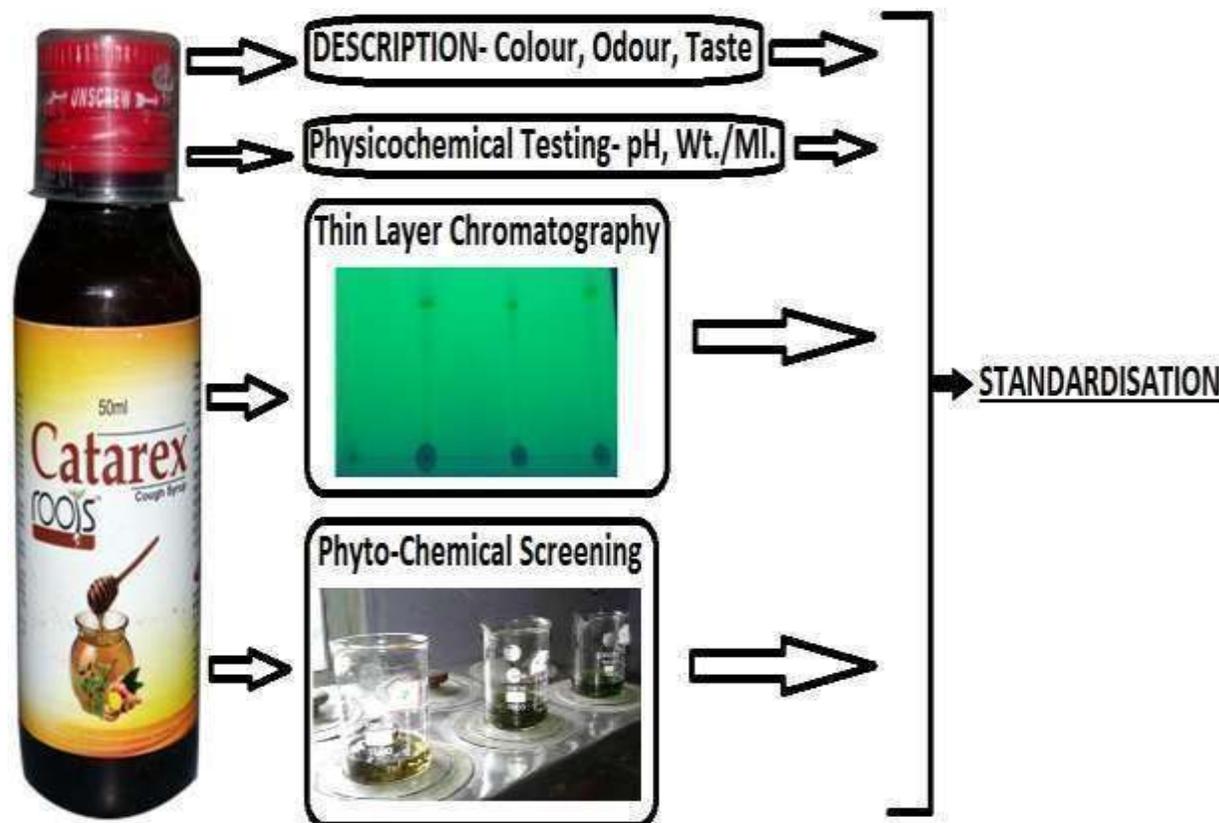
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### ABSTRACT

Catarex Roots syrup is a balanced composition of Ayurvedic natural ingredients for the relief from dry & wet cough due to smoke or season change, throat irritation & cold. The herbal ingredients of cough syrup are rich in alkaloids, tannins, saponins, terpenoids and trace elements. Catarex Roots is formulated with herbal drugs such as Tulsi (*Oscimum sanctum*), Mulethi (*Glycyrriza glabra*), Talish Patra (*Abies webbiana*), Haridra (*Curcuma longa*), Saunf (*Foeniculum vulgare*), Pippali (*Piper longum*), Kalimorich (*Piper nigrum*), Sonth (*Zingiber officinale*), Kalanamak (Black Salt) & Madhu (Honey) that are found widely in the medicinal herbs and are utilised as anti-cough, digestive, rejuvenative, anti-tussive and other biological activities. Standardisation of Catarex Roots syrup using TLC & Phytochemical Screening has been developed.



**Keywords:** Ayurvedic natural ingredients, Wet cough, Anti-cough, Rejuvenative, Anti-tussive.

## INTRODUCTION

A poly-herbal formulation is a medicinal preparation that combines two or more different ingredients from plant species, animal origin & mineral origin to achieve a desired therapeutic effect. This approach, rooted in Ayurveda, aims to leverage the synergistic properties of multiple herbs to potentially enhance efficacy, reduce toxicity, and minimize side effects. An Ayurvedic cough syrup

formulation typically involves a combination of medicinal herbs and a base, often with a sweetening agent like Honey, to address cough and related symptoms. These formulations are designed to be palatable and easily administered, while leveraging the therapeutic properties of the chosen herbs.

## MATERIAL AND METHOD

**Table 1:** Components of a Nanoemulgel with their specific functions

Plant Name	Parts Used	Strength	Qt.
Tulsi ( <i>Ocimum sanctum</i> )	Leave Extract	5:1	35 mg
Mulethi ( <i>Glycyrriza glabra</i> )	Root Extract	5:1	35 mg
Talish Patra ( <i>Abies webbiana</i> )	Leaves Extract	8:1	30 mg
Haridra ( <i>Curcuma longa</i> )	Rhizome Extract	7:1	20 mg
Saunf ( <i>Foeniculum vulgare</i> )	Fruit Extract	7:1	20 mg
Pippali ( <i>Piper longum</i> )	Fruit Extract	4:1	20 mg
Kalimorich ( <i>Piper nigrum</i> )	Fruit Extract	6:1	20 mg
Sonth ( <i>Zingiber officinale</i> )	Root Extract	6:1	20 mg
Kalanamak (Black Salt)	Mineral		40 mg
Madhu (Honey)	Liquid		0.6 gm
Preservative			Q.S.
Flavoured Syrupy Base			Q.S.

### Storage condition

The samples of 3 lab batches were kept into Accelerated (ACC) stability chamber (Condition- Temperature- 40±2° C, Humidity- 75±5 %) [11] & was subjected to analysis on 0 Day, 3 Month, 6 Month, 9 Month, 12 Month.

### Analytical parameters

#### Description

Check the sample's physical appearance. (Colour, Odour, Taste)

#### Determination of weight per millilitre value [12]

The weight per millilitre of a liquid is the weight in g of 1 ml of a liquid when weighed in air at 25°C.

#### Method

Select a thoroughly clean and dry pycnometer. Calibrate the pycnometer by filling it with recently boiled and cooled water at 25 °C and weighing the contents. Assuming that the weight of 1 ml of water at 25°C when weighed in air of density 0.0012 g per ml, is 0.99602 g. Calculate the capacity of the pycnometer. (Ordinary deviations in the density of air from the value given do not affect the result of a determination significantly. Adjust the temperature of the substance to be examined to about 200 and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 25°C, remove any excess of the substance and weigh. Subtract the tare weight of the pycnometer from the filled weight of the pycnometer. Determine the weight per millilitre by dividing the weight in air, expressed in g, of the quantity of liquid which fills the pycnometer at the specified temperature, by the capacity expressed in ml, of the pycnometer at the same temperature.

#### Calculation

Weight of Pycnometer with Purified water = \_\_\_\_\_ g

Weight of empty Pycnometer = \_\_\_\_\_ g

Capacity of Pycnometer [weight of Purified water] = X g

Weight of Pycnometer with liquid to be tested = \_\_\_\_\_ g

Weight of empty Pycnometer = \_\_\_\_\_ g

Weight of liquid to be tested = Y g

Weight/ml =  $\frac{Y \times 0.99602}{X}$  = \_\_\_\_\_ g

X

#### Determination of pH value [13]

The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in g per litre. For pharmacopoeia pH is defined as the value given by a suitable, properly standardised, pH meter capable of reproducing pH values to 0.05 pH unit using an indicator electrode sensitive to hydrogen ion activity, the glass electrode and a suitable reference electrode. The instrument should be capable of sensing the potential across the electrode pair and for pH standardization purposes, applying an adjustable potential to the circuit by manipulation of “standardization,” “zero,” “asymmetry,” or “calibration” control, and should be able to control the change in millivolts per unit change in pH reading through a “temperature” and/or “slope” control. Measurements are made at 25 ± 20, unless otherwise specified. To standardise the pH meter, select two Buffer Solutions whose difference in pH does not exceed 4 units, such that the expected pH of the material under test falls between them. Commercially available buffer solutions for pH meter standardisation, having traceability to the National Standards, can be used. Fill the cell with one of the Buffer Solutions for Standardisation at the temperature at which the test material is to be measured. Set the “temperature” control at the temperature of the solution, and adjust the calibration control to make the observed pH value identical with that of the declared pH. Rinse the electrodes and cell with several portions of the second Buffer Solution for Standardization, then fill the cell with it, at the same temperature as the material to be measured. The pH of the second buffer solution is within

$\pm 0.07$  pH units of the declared value. If a larger deviation is noted, examine the electrodes and if they are faulty, replace them. Repeat the standardisation until both Buffer Solutions for Standardisation give observed pH values within 0.05 pH units of the declared value without further adjustment of the controls. When the system is functioning satisfactorily, rinse the electrodes and cell several times with a few portions of the test material, fill the cell with the test material, and read the pH value. Use carbon dioxide-free water for solution or dilution of test material in pH determinations. In all pH measurements, allow a sufficient time for stabilisation. Unless otherwise specified in the monograph, prepare 5 per cent w/v of the sample. Filter if it is not soluble completely, and use the filtrate to measure the pH.

Transfer 50 ml of the solution to a 100 ml cleaned beaker. Immerse the electrode of the pH meter in the liquid that has been previously calibrated. Record the pH value.

### Thin Layer Chromatography (TLC) <sup>[14]</sup>

#### Method

Pre-coated TLC plates are used for the whole procedure.

Unless unsaturated conditions are prescribed, prepare the tank by lining the walls with sheets of filter paper; pour into the tank, saturating the filter paper in the process, sufficient of the mobile phase to form a layer of solvent 5 to 10 mm deep, close the tank and allow it to stand for 1 hour at room temperature. Remove a narrow strip of the coating substance, about 5 mm wide, from the vertical sides of the plate. Apply the solutions being examined in the form of circular spots about 2 to 6 mm in diameter, or in the form of bands (10 to 20 mm x 2 to 6 mm unless otherwise specified) on a line parallel with, and 20 mm from, one end of the plate, and not nearer than 20 mm to the sides; the spots should be 15 mm apart. If necessary, the solutions may be applied in portions, drying between applications. Mark the sides of the plate 15 cm, or the distance specified in the monograph, from the starting line. Allow the solvent to evaporate and place the plate in the tank, ensuring that it is as nearly vertical as possible and that the spots or bands are above the level of the mobile phase. Close the tank and allow it to stand at room temperature until the mobile phase has ascended to the marked line. Remove the plate and dry and visualise as directed in the monograph; where a spraying technique is prescribed, the reagent must be evenly applied as a fine spray.

When the method prescribed in the monograph specifies 'protected from light' or 'in subdued light', it is intended that the entire procedure is carried out under these conditions.

#### Visualisation

The phrases *ultra-violet light (254 nm)* and *ultra-violet light (365 nm)* indicate that the plate should be examined under an ultra-violet light having a maximum output at about 254 or at about 365 nm, as the case may be.

The term *secondary spot* means any spot other than the principal spot. Similarly, a *secondary band* is any band other than the principal band.

#### Rf value

Measure and record the distance of each spot from the point of its application and calculate the *Rf* value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

#### Identification of Tulsi

##### Control preparation of Tulsi extract

35 mg of Tulsi extract dissolved in 25 ml of alcohol for 24 hours by the maceration technique with occasional shaking. Filter the extract & make up to 25 ml in a volumetric flask.

##### Sample preparation

5 ml of the sample was dissolved in 25 ml of alcohol for 24 hours by the maceration technique with occasional shaking. Filter the extract & make up to volume to 25 ml.

**Mobile phase:** Toluene: Ethyl Acetate (9:1).

**Volume of solution applied on plate-** 3  $\mu$ l.

#### Identification of Pippali & Kalimorich

##### Standard preparation of piperine for Pippali & Kalimorich

10 mg Piperine dissolved in 25 ml alcohol.

##### Control preparation of Pippali extract

Reflux 20 mg of coarsely powdered Pippali with 25 ml of methanol for 15 minutes, cool & filter. Reflux the residue further for 2 times with 25 ml of methanol, cool & filter. Combine all the filtrates & concentrate under vacuum to 3 ml.

##### Control preparation of Kalimorich extract

Reflux 20 mg of coarsely powdered Kalimorich with 25 ml of methanol for 15 minutes, cool & filter. Reflux the residue further for 2 times with 25 ml of methanol, cool & filter. Combine all the filtrates & concentrate under vacuum to 3 ml.

##### Sample preparation

5 ml of the sample was dissolved in 25 ml of alcohol for 24 hours by the maceration technique with occasional shaking. Filter the extract & make up to volume to 25 ml.

**Mobile phase-** Benzene: Ethyl Acetate: Diethyl ether (6:3:1)

**Volume of solution applied on plate-** 3  $\mu$ l.

#### Identification of Mulethi-

##### Control preparation of Mulethi extract

35 mg of Mulethi extract dissolved in 25 ml of alcohol for 24 hours by the maceration technique with occasional shaking. Filter the extract & make up to 25 ml in a volumetric flask.

##### Sample preparation

5 ml of the sample was dissolved in 25 ml of alcohol for 24 hours by the maceration technique with occasional shaking. Filter the extract & make up to volume to 25 ml.

**Mobile Phase-** Toluene: Ethyl Acetate: Formic Acid (5:4:1).

**Volume of solution applied on plate-** 6  $\mu$ l.

**Identification of Sunthi-****Control preparation of Sunthi extract**

20 mg of Sunthi extract dissolved in 25 ml of alcohol for 24 hours by the maceration technique with occasional shaking. Filter the extract & make up to 25 ml in a volumetric flask.

**Sample preparation**

5 ml of the sample was dissolved in 25 ml of alcohol for 24 hours by the maceration technique with occasional shaking. Filter the extract & make up to volume to 25 ml.

**Mobile phase-** n-Hexane: Diethyl Ether (4:6).

**Volume of solution applied on plate-** 3 µl.

**Identification of Saunf-****Control preparation of Saunf extract**

20 mg of Saunf extract dissolved in 25 ml of alcohol for 24 hours by the maceration technique with occasional shaking. Filter the extract & make up to 25 ml in a volumetric flask.

**Sample preparation**

5 ml of the sample was dissolved in 25 ml of alcohol for 24 hours by the maceration technique with occasional shaking. Filter the extract & make up to volume to 25 ml.

**Mobile phase-** Toluene: Ethyl Acetate (9:1).

**Volume of solution applied on plate-** 3 µl.

**Phytochemical screening** <sup>[15,16]</sup>

Phytochemical screening was performed to identify phytochemicals in the water extracts of the plant extracts used in the work. The phytochemicals were detected by colour tests.

**Control solution preparation**

All the ingredients mentioned in the label claim were weighed properly & dissolve in 25 ml of water for 24 hours by the maceration technique with occasional shaking. Filter the extract & make up to volume to 25 ml.

**Sample solution preparation**

10 ml of the sample was dissolved in 25 ml of water for 24 hours by the maceration technique with occasional shaking. Filter the extract & make up to volume to 25 ml.

**Test for alkaloids****Mayer's test**

Treat the test solution with Mayer's reagent, a cream colour appears, which shows the presence of an alkaloid.

**Wagner's test**

Treat the test solution with some acidic solution & Wagner's reagent, brown precipitate will show the presence of Alkaloid.

**Dragendorff's test**

Of each extract, 2 ml was acidified with a few drops of dilute hydrochloric acid. Then 1 ml of Dragendorff's reagent was added. The appearance of orange to red precipitate indicates the presence of alkaloids.

**Test for tannins****Ferric chloride test**

The test solution was a ferric chloride solution, and a dark colour appeared, which shows the presence of Tannin.

**Gelatin test**

The test solution was treated with 1% Gelatin solution containing 10% NaCl, and a white ppt formed, which shows the presence of Tannin.

To 2 ml of each extract, a few drops of 10 % lead acetate were added. The appearance of white precipitate indicates the presence of tannins.

**Test for saponins**

To 1 ml of extract taken in a measuring jar, 9 ml of distilled water was added and shaken vigorously for 15 seconds, and the extract was allowed to stand for 10 min. Formation of stable foam (1 cm) indicates the presence of saponins.

**Test for steroids**

Chloroform (10 ml) was added to 2 ml of all three plant extracts. To these extracts 1ml of acetic anhydride was added, and then 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Colour formation at the junction is noted. The appearance of blue-green colour indicates the presence of steroids.

**Test for triterpenoids**

The test for Triterpenoids is the same as that for steroids. The appearance of red, pink colour or violet colour at the junction indicates the presence of Triterpenoids.

**Test for glycosides**

To 1 ml of each extract, a few drops of glacial acetic acid, ferric chloride and 3-4 drops of concentrated sulphuric acid were added. The appearance of blue-green colour indicates the presence of glycosides.

**Test for flavonoid**

0.5 g of various extracts was shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in 20 ml of 80 % ethanol and filtered. The filtrate was used for the following tests:

3 ml of the filtrate was mixed with 4 ml of 1 % aluminium chloride in methanol in a test tube, and the colour was observed. Formation of yellow colour indicated the presence of flavonols, flavones and chalcones.

3 ml of the filtrate was mixed with 4 ml of 1 % potassium hydroxide in a test tube, and the colour was observed. A dark yellow colour indicated the presence of Flavonoids.

5 ml of the dilute ammonia solution was added to the portion of the aqueous filtrate of each plant extract, followed by the addition of concentrated H<sub>2</sub>SO<sub>4</sub>. The appearance of the yellow colouration indicated the presence of flavonoids.

**Test for phenol**

To 1 ml of various solvent extracts of the sample, 2 ml of distilled water, followed by a few drops of 10 % aqueous ferric chloride solution, were added. Formation of blue or green colour indicated the presence of phenols.

**Test for carbohydrate****Iodine test**

2 ml of iodine solution mixed with crude plant extract.

Purple or dark blue Colours prove the presence of the carbohydrate.

The TLC denotes the presence of phytochemicals in the solution & phytochemical screening confirms the presence of those molecules in the samples.

**RESULT****Inter Referencing****Table 1:** (Batch No.- CTRT/LB/01)

Test Parameters	0 Day	Acc 3 Month	Acc 6 Month	Acc 9 Month	Acc 12 Month
Description ( <b>Specification-</b> Dark Brown Color, Sweet Taste & Characteristic odor)	Dark Brown Color, Sweet Taste, Characteristic odor	Dark Brown Color, Sweet Taste, Characteristic odor	Dark Brown Color, Sweet Taste, Characteristic odor	Dark Brown Colour, Sweet Taste, Characteristic odor	Dark Brown Colour, Sweet Taste, Characteristic odor
pH ( <b>Specification-</b> 3-5)	4.38	4.22	4.18	3.93	3.88
Weight per ml (g/ml) ( <b>Specification-</b> 1.10-1.20 g/ml)	1.17	1.17	1.17	1.17	1.17
Thin Layer Chromatography ( <b>Specification-</b> Complies for Active ingredients)	Complies Figure- 1, 6, 11, 16, 21	Complies Figure- 2, 7, 12, 17, 22	Complies Figure- 3, 8, 13, 18, 23	Complies Figure- 4, 9, 14, 19, 24	Complies Figure- 5, 10, 15, 20, 25
Phytochemical Screening ( <b>Specification-</b> Present/Absent)	Table-2				

**Table 2:** ('+' for Positive & '-' for Negative)

Time duration	Alkaloid	Tannin	Saponin	Steroid	Terpenoid	Phenol
0 Day	+	+	+	+	+	-
3 Month ACC	+	+	+	+	+	-
6 Month ACC	+	+	+	+	+	-
9 Month ACC	+	+	+	+	+	-
12 Month ACC	+	+	+	+	+	-

**Table 3:** (Batch No.- CTRT/LB/02)

Test Parameters	0 Day	Acc 3 Month	Acc 6 Month	Acc 9 Month	Acc 12 Month
Description ( <b>Specification-</b> Dark Brown Colour, Sweet Taste & Characteristic odour)	Dark Brown Colour, Sweet Taste, Characteristic odour				
pH ( <b>Specification-</b> 3.5)	4.58	4.31	4.26	3.81	3.73
Weight per ml (g/ml) ( <b>Specification-</b> 1.10-1.20 g/ml)	1.19	1.19	1.19	1.19	1.19
Thin Layer Chromatography ( <b>Specification-</b> Complies for Active ingredients)	Complies Figure- 1, 6, 11, 16, 21	Complies Figure- 2, 7, 12, 17, 22	Complies Figure- 3, 8, 13, 18, 23	Complies Figure- 4, 9, 14, 19, 24	Complies Figure- 5, 10, 15, 20, 25
Phytochemical Screening ( <b>Specification-</b> Present/Absent)	Table-4				

**Table 4:** ('+' for Positive & '-' for Negative)

Time duration	Alkaloid	Tannin	Saponin	Steroid	Terpenoid	Phenol
0 Day	+	+	+	+	+	-
3 Month ACC	+	+	+	+	+	-
6 Month ACC	+	+	+	+	+	-
9 Month ACC	+	+	+	+	+	-
12 Month ACC	+	+	+	+	+	-

**Table 5:** (Batch No.- CTRT/LB/03)

Test Parameters	0 Day	Acc 3 Month	Acc 6 Month	Acc 9 Month	Acc 12 Month
Description ( <b>Specification-</b> dark brown colour, sweet taste & characteristic odour)	Dark brown colour, sweet taste, characteristic odour				
pH ( <b>Specification-</b> 3-5)	4.66	4.42	3.86	3.81	3.72
Weight per ml (g/ml) ( <b>Specification-</b> 1.10-1.20 g/ml)	1.16	1.16	1.16	1.16	1.16
Thin Layer Chromatography ( <b>Specification-</b> Complies with Active ingredients)	Complies Figure- 1, 6, 11, 16, 21	Complies Figure- 2, 7, 12, 17, 22	Complies Figure- 3, 8, 13, 18, 23	Complies Figure- 4, 9, 14, 19, 24	Complies Figure- 5, 10, 15, 20, 25
Phytochemical Screening ( <b>Specification-</b> Present/Absent)	Table-6				

**Table 6:** ('+' for Positive & '-' for Negative)

Time duration	Alkaloid	Tannin	Saponin	Steroid	Terpenoid	Phenol
0 Day	+	+	+	+	+	-
3 Month ACC	+	+	+	+	+	-
6 Month ACC	+	+	+	+	+	-
9 Month ACC	+	+	+	+	+	-
12 Month ACC	+	+	+	+	+	-

Figure 1: Mulethi 0 Day (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

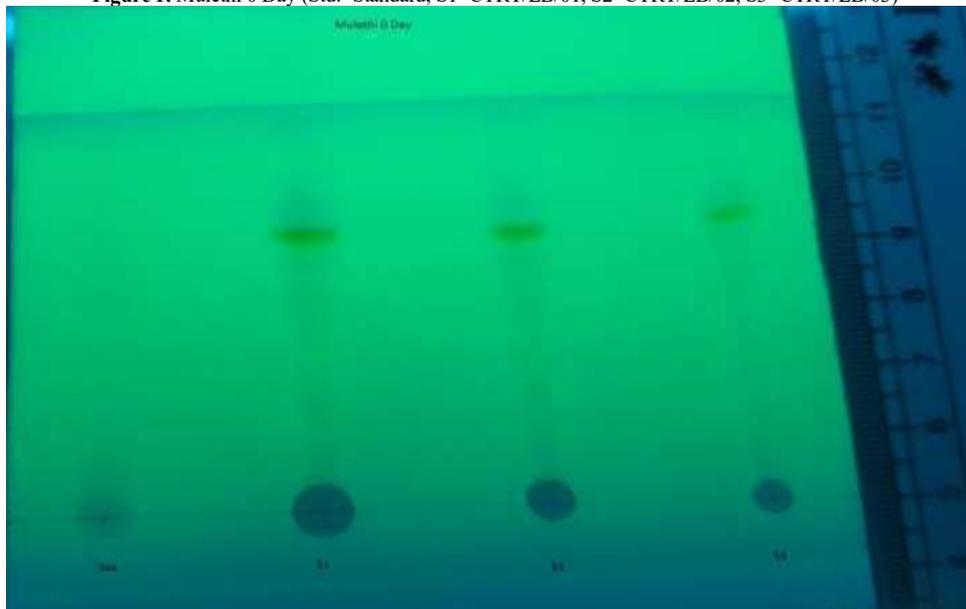


Figure 2: Mulethi ACC 3 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

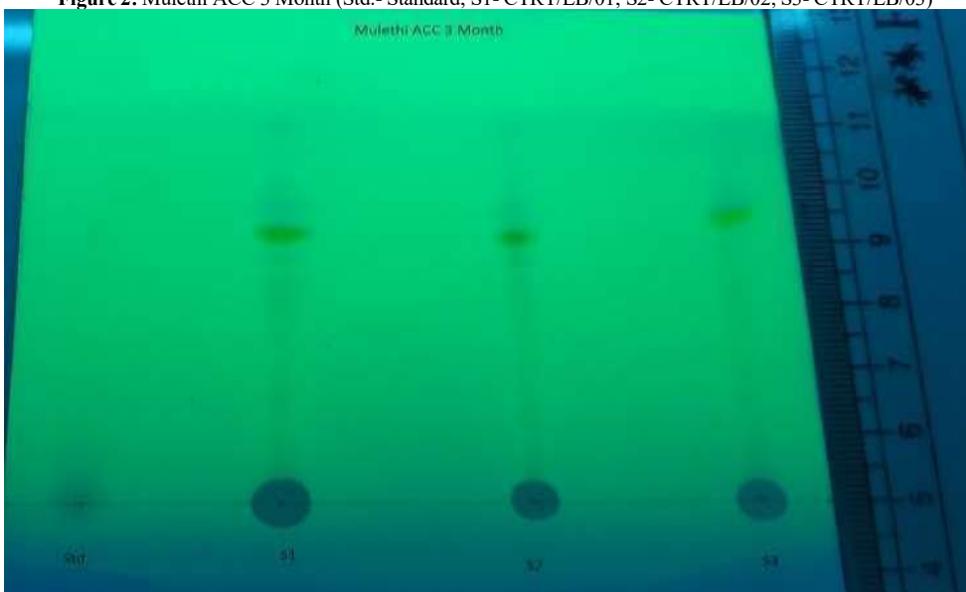
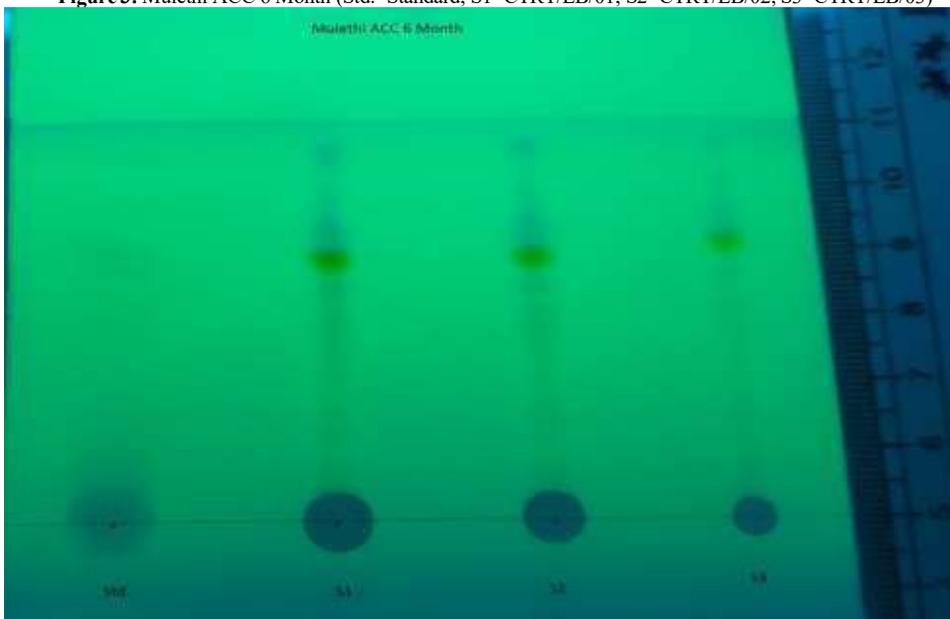
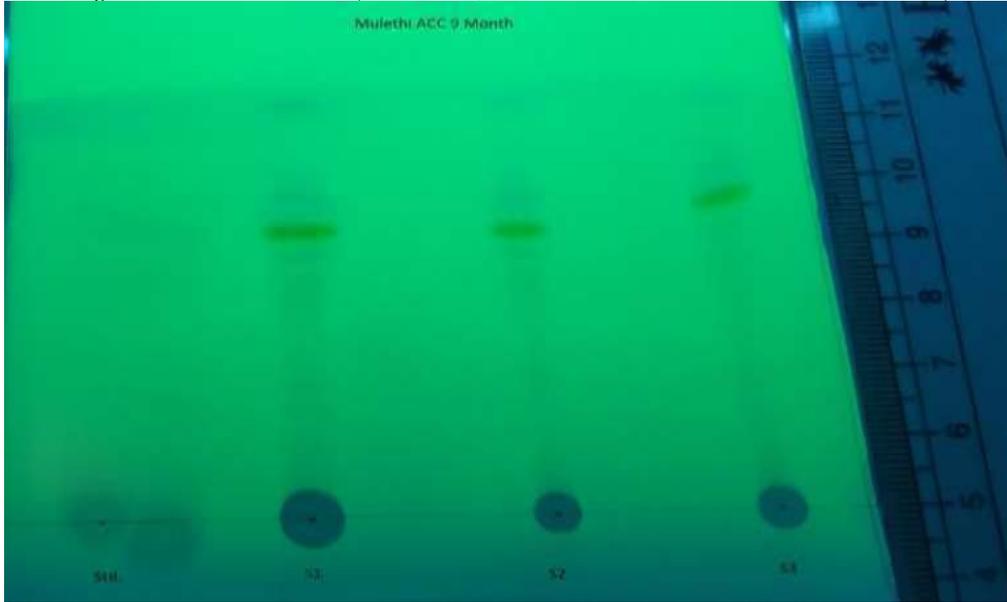


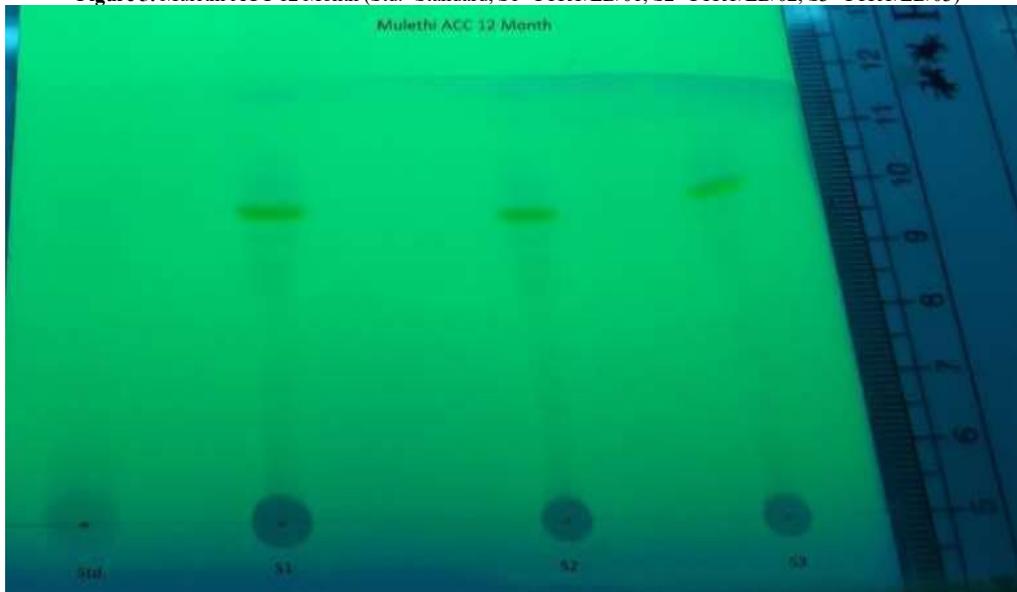
Figure 3: Mulethi ACC 6 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)



**Figure 4:** Mulethi ACC 9 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)



**Figure 5:** Mulethi ACC 12 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)



**Figure 6:** Saunf ACC 0 Day (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

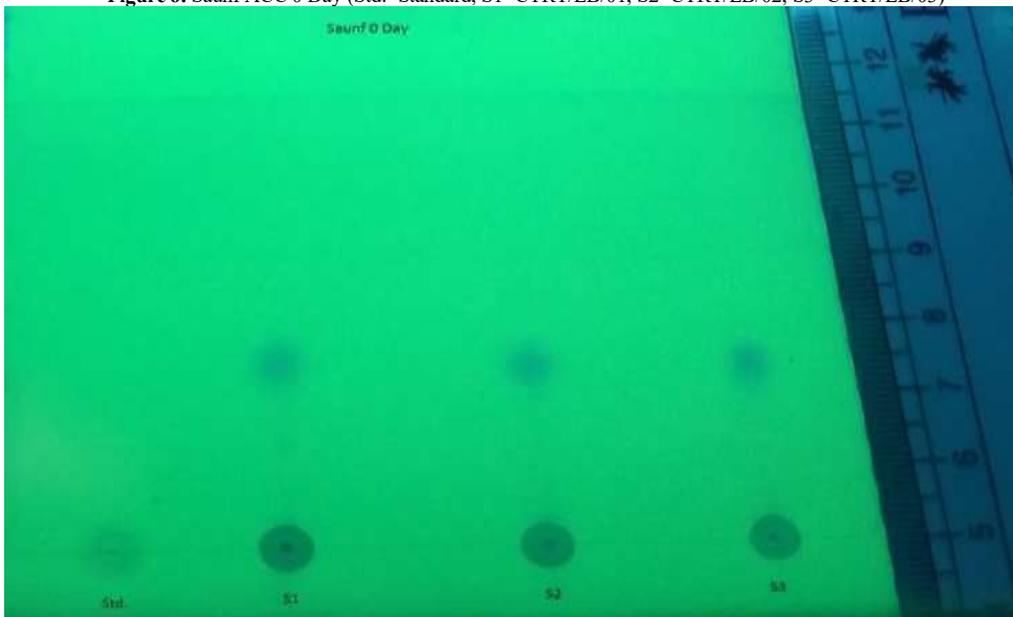


Figure 7: Saunf ACC 3 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)



Figure 8: Saunf ACC 6 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

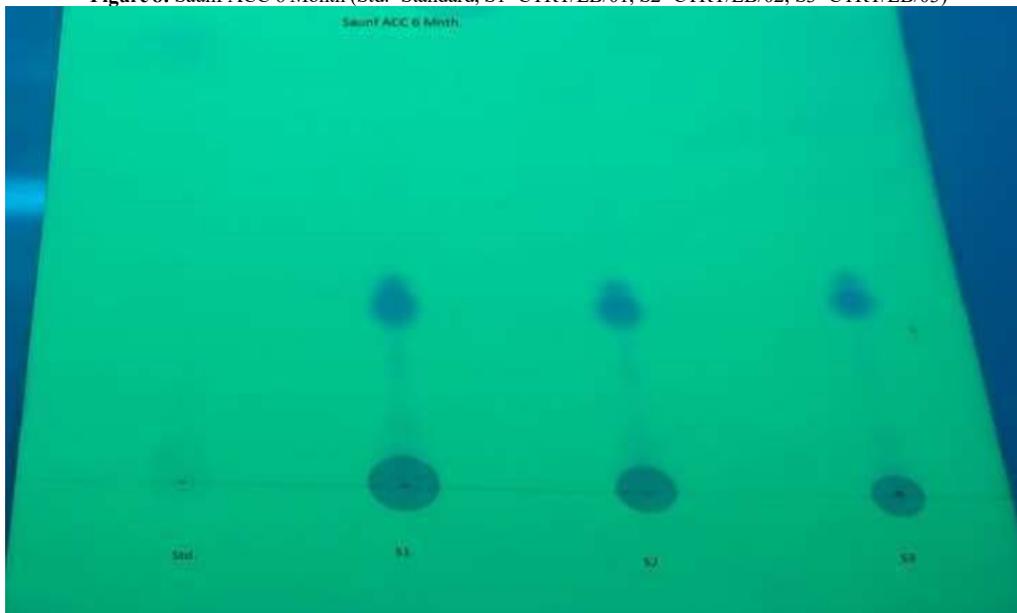


Figure 9: Saunf ACC 9 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)



Figure 10: Saunf ACC 12 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)



Figure 11: Pippali & Kalimorich 0 Day (Piperine, Kalimorich Ext., Pippali Ext., S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

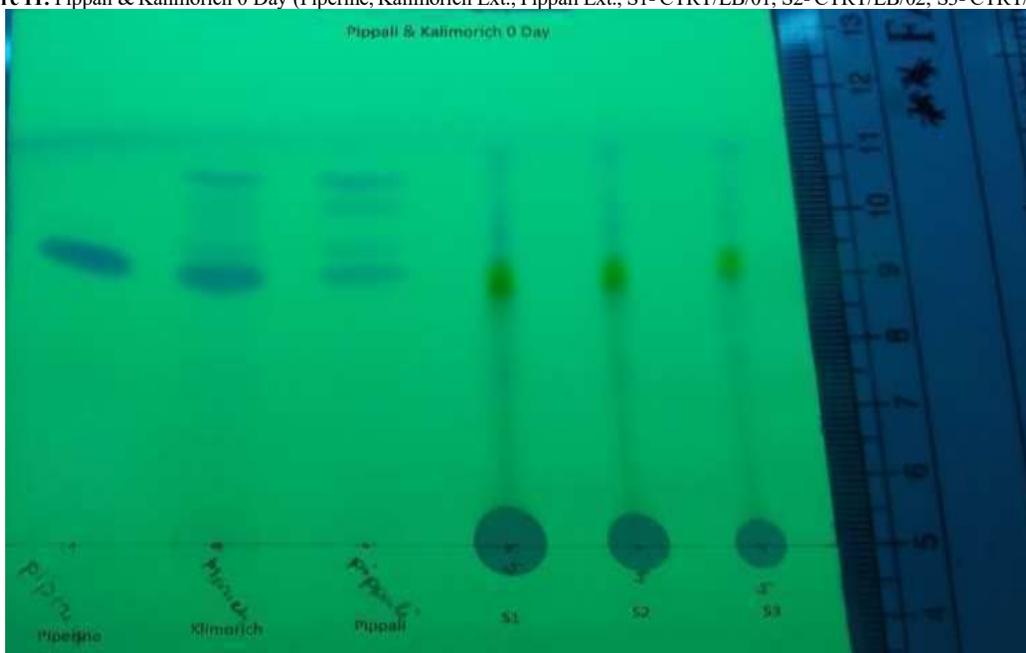


Figure 12: Pippali&Kalimorich ACC 3 Month (Piperine, Kalimorich Ext., Pippali Ext., S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

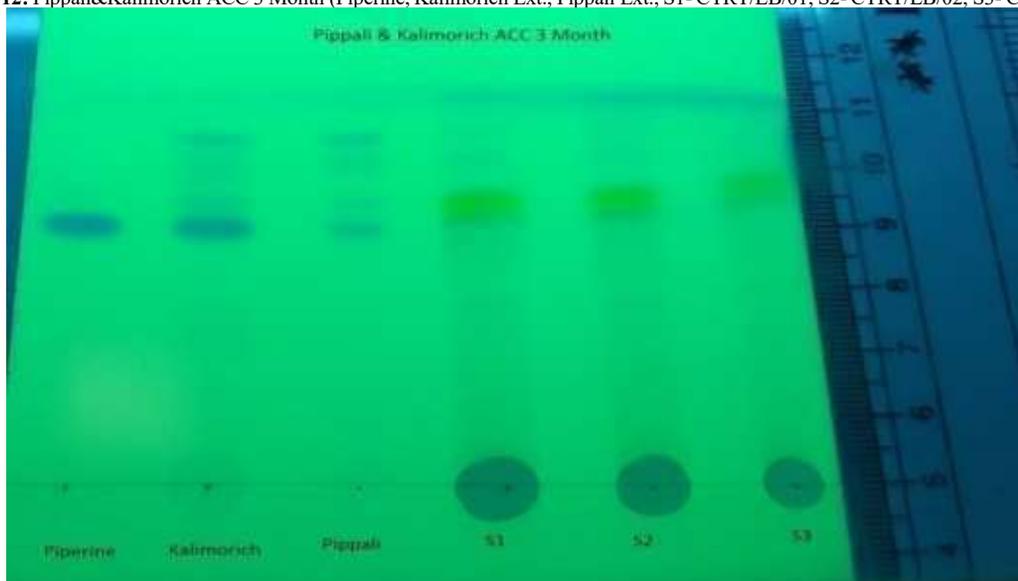


Figure13: Pippali & Kalimorich ACC 6 Month (Piperine, Kalimorich Ext., Pippali Ext., S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

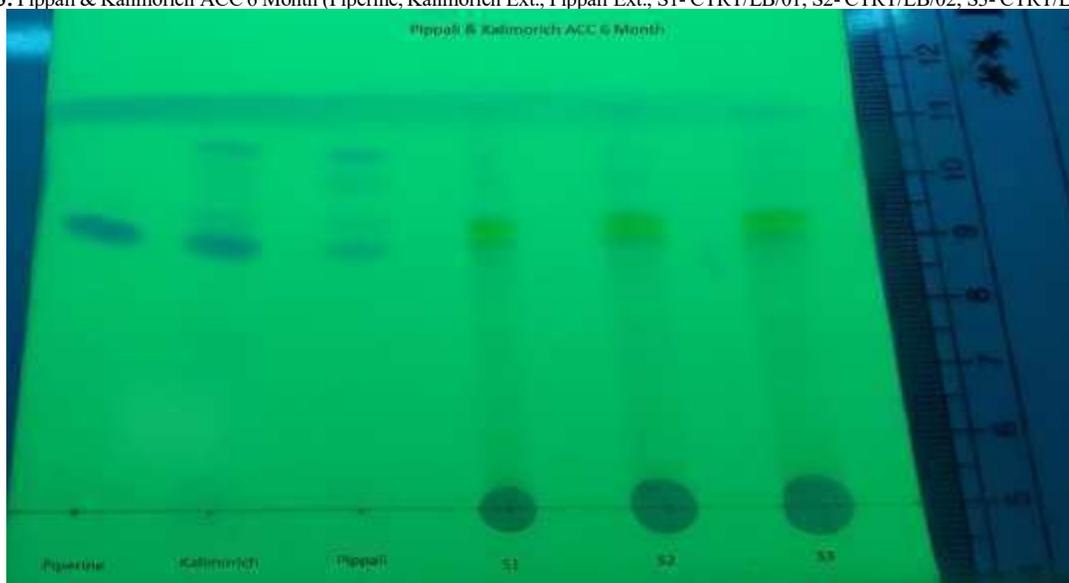


Figure 14: Pippali & Kalimorich ACC 9 Month (Piperine, Kalimorich Ext., Pippali Ext., S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

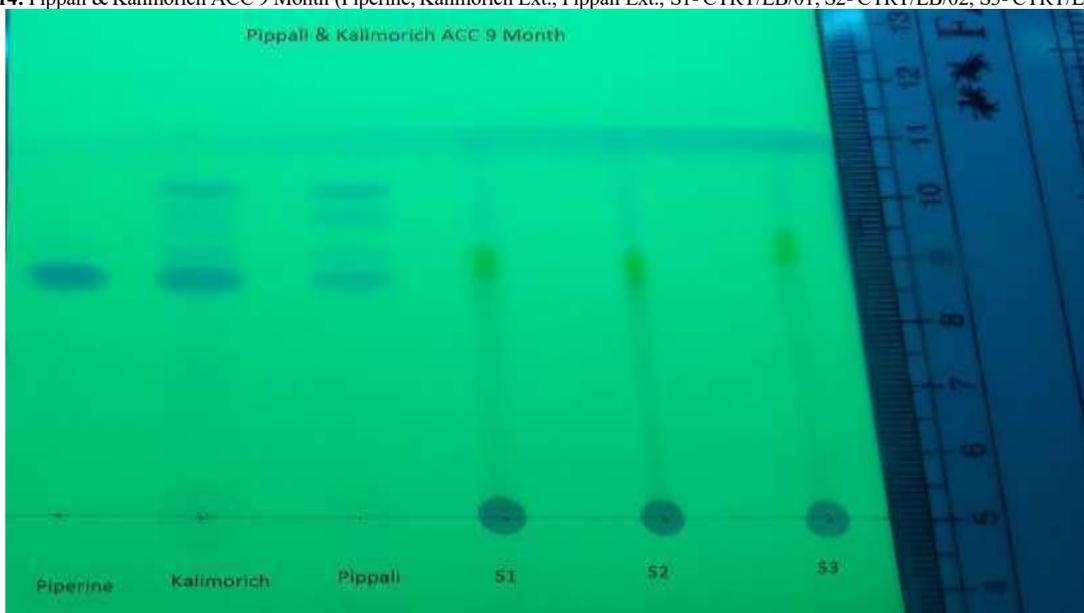


Figure15: Pippali & Kalimorich ACC 12 Month (Piperine, Kalimorich Ext., Pippali Ext., S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

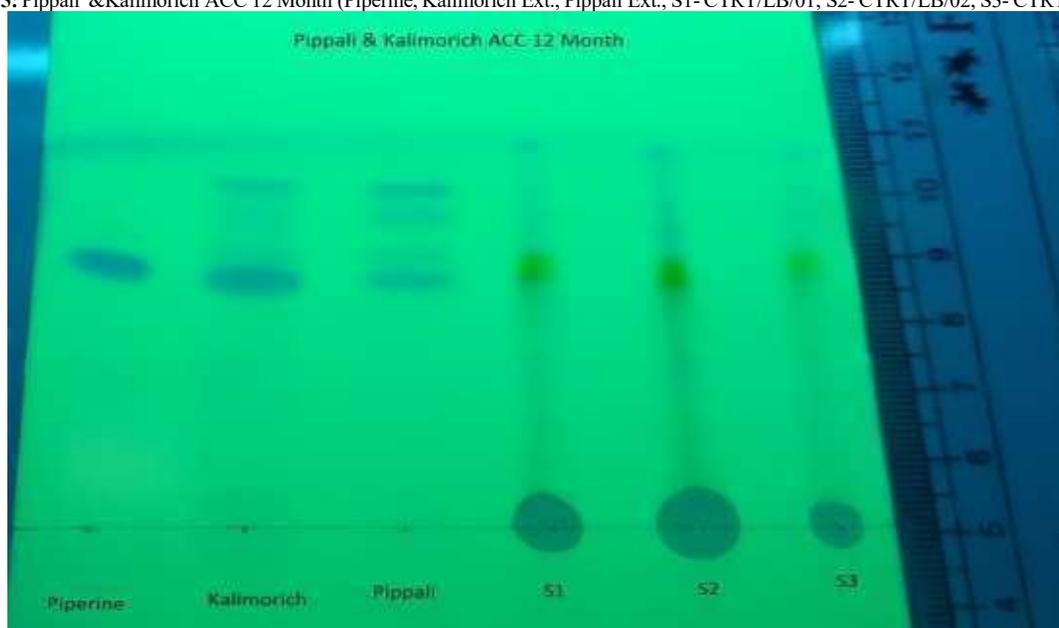


Figure 16: Sunthi 0 Day (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

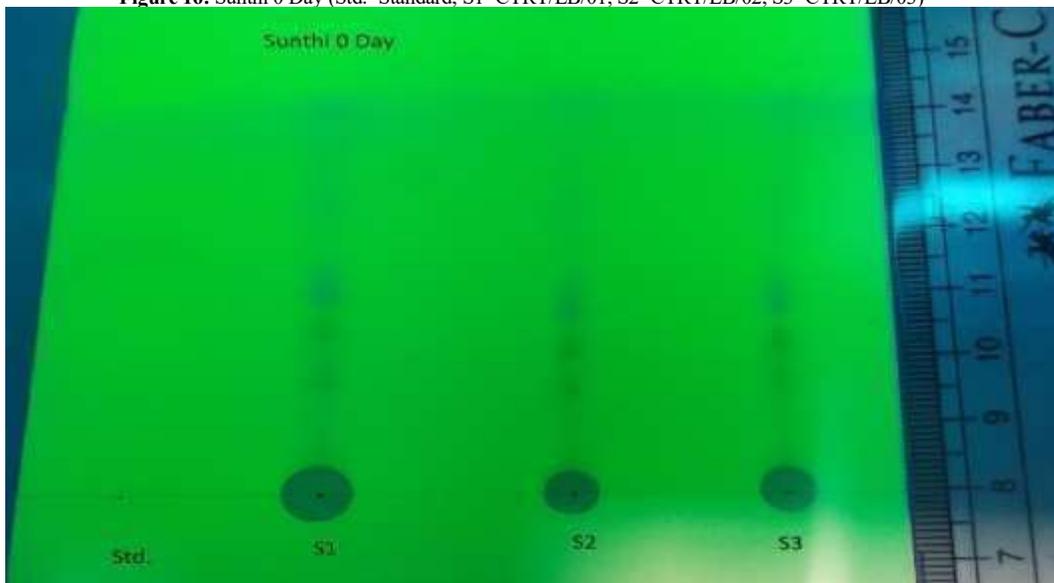


Figure 17: Sunthi ACC 3 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

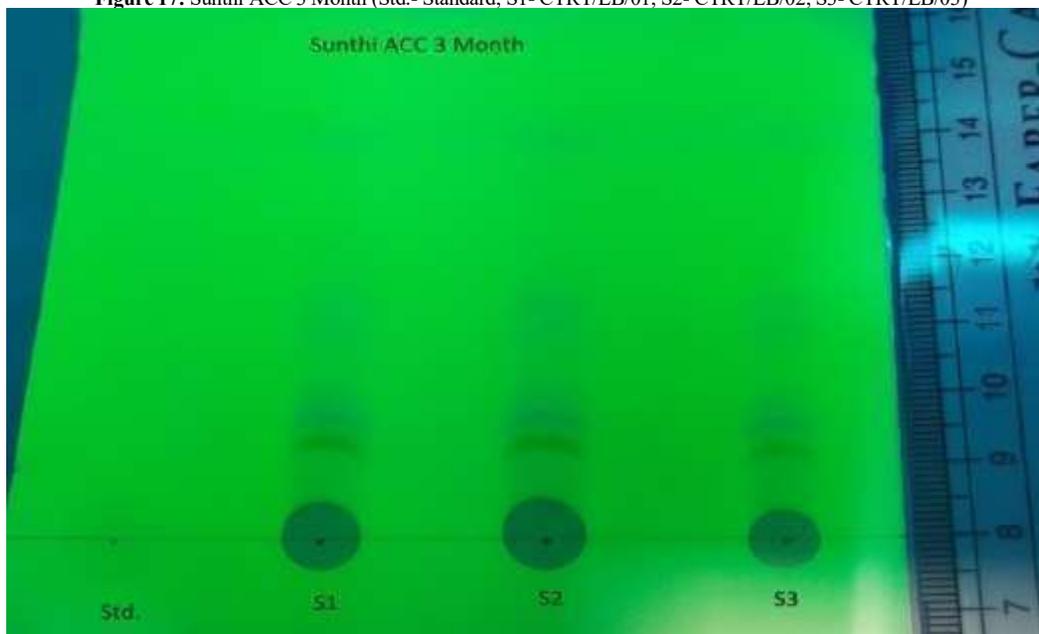


Figure 18: Sunthi ACC 6 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

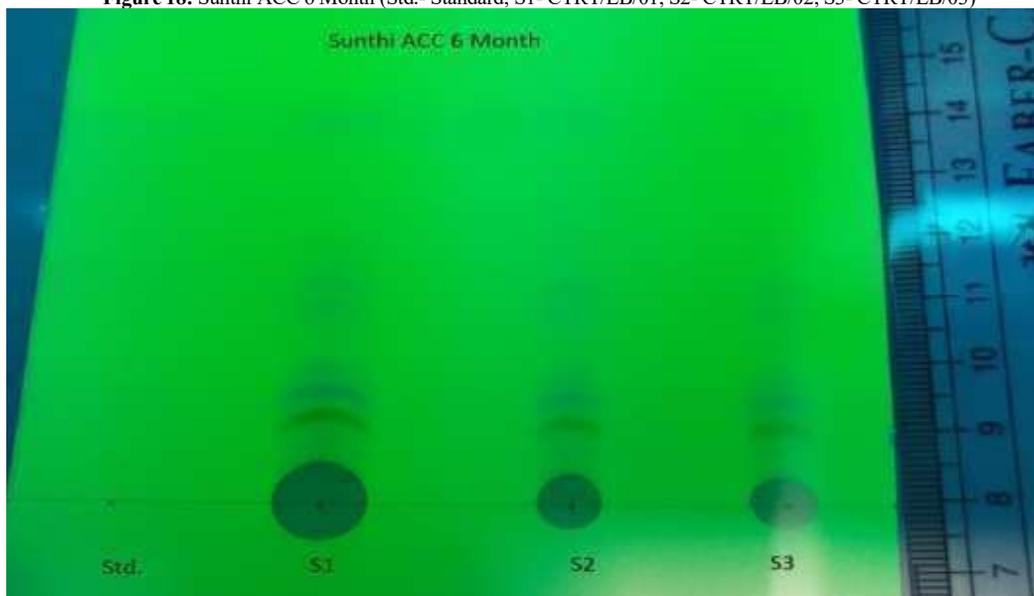


Figure 19: Sunthi ACC 9 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

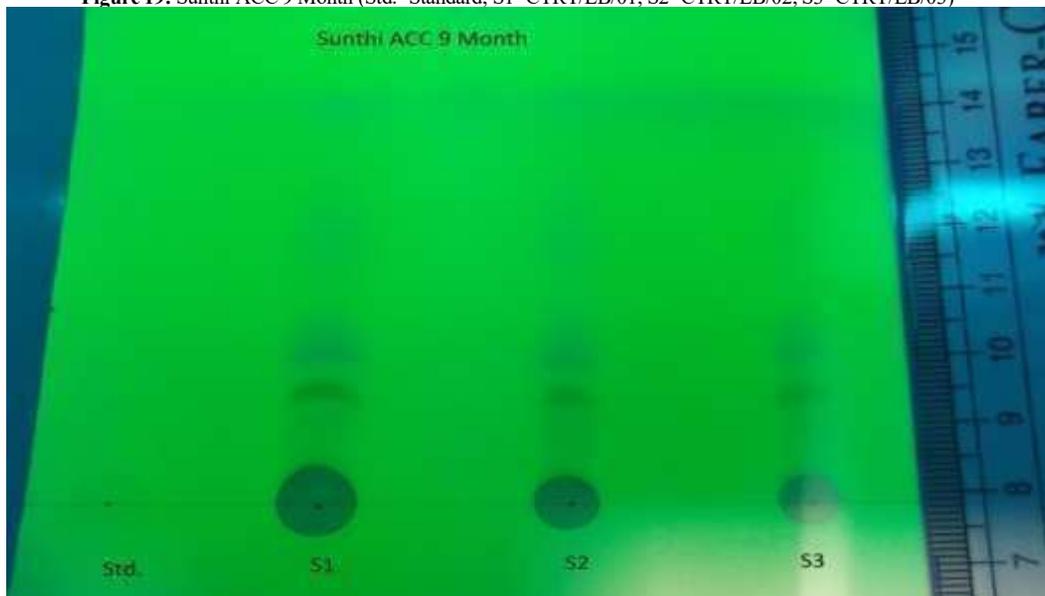


Figure 20: Sunthi ACC 12 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

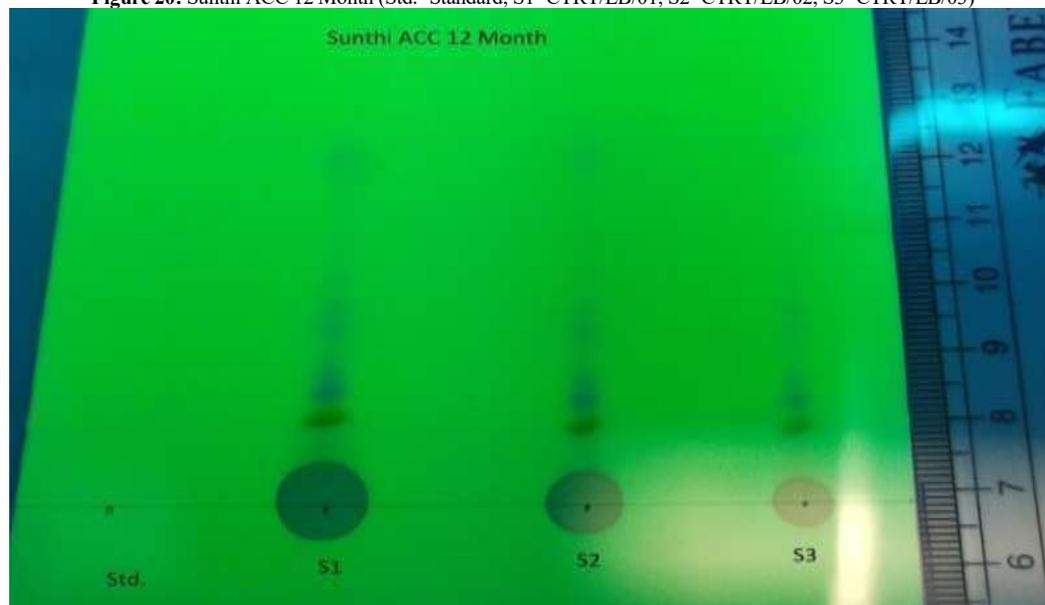


Figure 21: Tulsi 0 Day (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

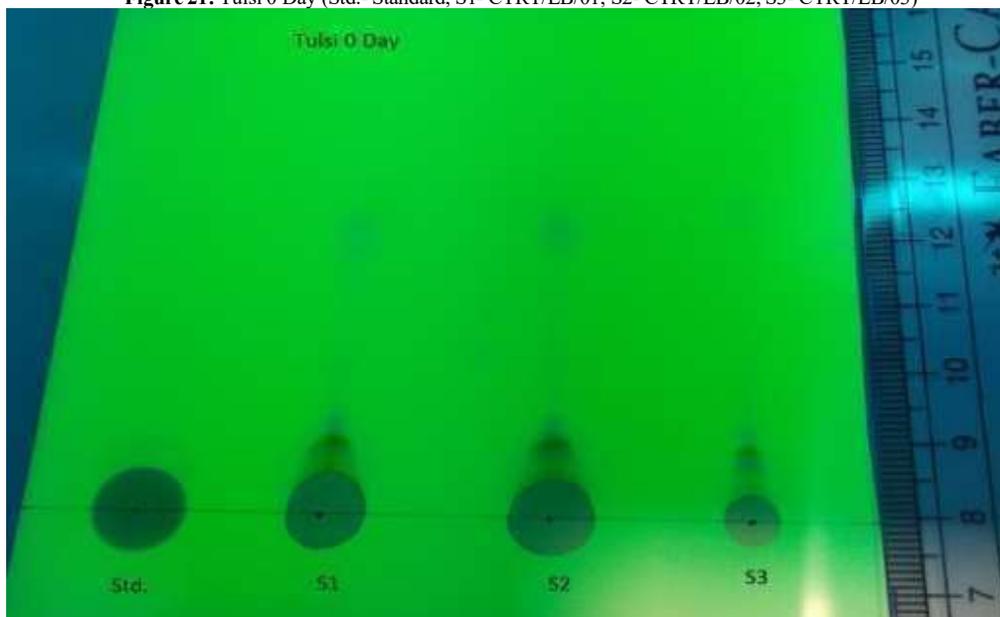


Figure 22: Tulsi ACC 3 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)



Figure 23: Tulsi ACC 6 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

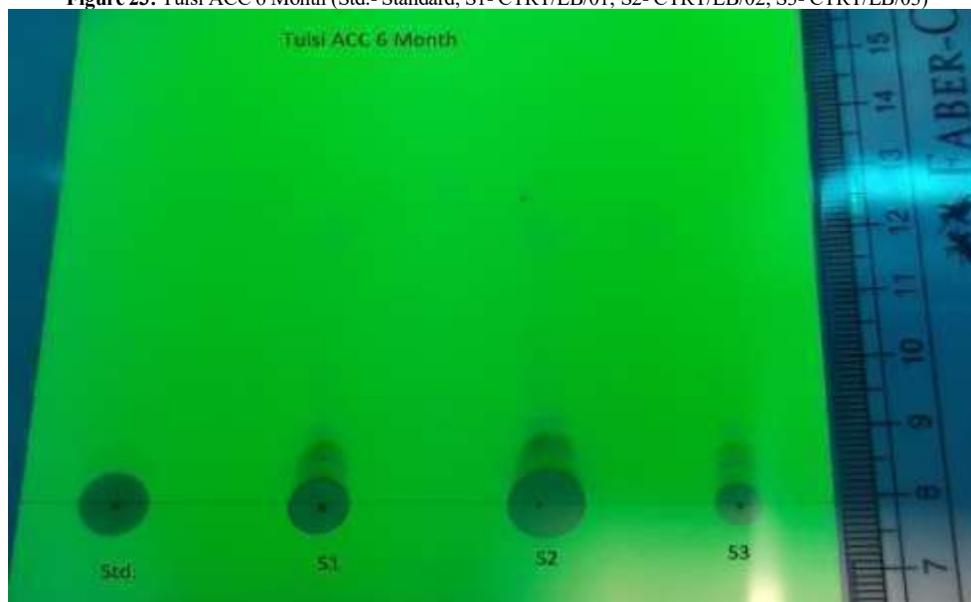
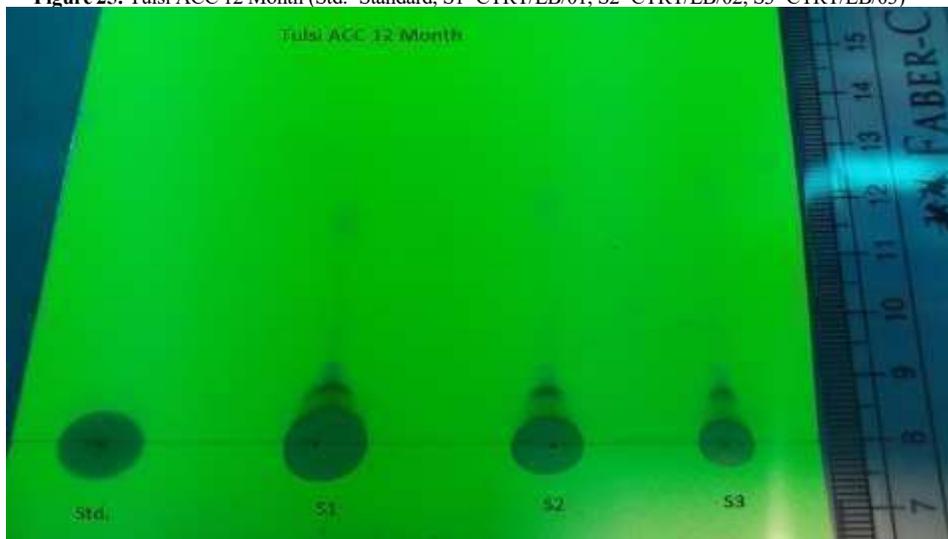


Figure 24: Tulsi ACC 9 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)



**Figure 25:** Tulsi ACC 12 Month (Std.- Standard, S1- CTRT/LB/01, S2- CTRT/LB/02, S3- CTRT/LB/03)

## DISCUSSION AND CONCLUSION

From the above study, it is concluded that the product (every three consecutive batches) is physically & chemically stable throughout the study period & it contains Alkaloids, Tannins, natural steroids, Natural Steroids & Terpenoids, which can be primarily represented by TLC study & further confirmation was done by Phytochemical Screening. And also, it is established by performing the same experiments in three repeated batches & obtaining the same sort of results, that this method is easy & reproducible for the standardisation of a poly-herbal formulation. Furthermore, a study is needed to confirm the obtained results.

## ACKNOWLEDGEMENT

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## Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this research.

## Informed consent statement

This study did not involve any animal or human participants, and therefore, informed consent was not required.

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