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## Standardization of Harithaki (*Terminalia chebula* Retz.) and Trivrit (*Operculina turpethum* L.) churna: Two important drugs used for purgation in Ayurveda

**Nidhin PS, Yaligar MG, Arun Raj GR, Koppala Narayana Sunil Kumar, Ravi M**

**Abstract**

Standardization of Ayurvedic formulations is most important for the establishment of its biological activity, its chemical profile and its quality assurance in production and manufacturing of herbal drugs. As such the standardization is a burning topic in Ayurvedic drug manufacture today. Most of the pharmaceutical industries are using substitute drugs instead of authentic drugs. So to prepare best quality drugs it is necessary to authenticate raw drugs. Keeping the current trend in mind, Harithaki (*Terminalia chebula* Retz.) powder and Trivrit (*Operculina turpethum* L.) powder were subjected for standardization procedures. From the current study, genuinity indicating parameters for both Harithaki churna (powder) and Trivrit churna were derived.

**Keywords:** Harithaki, *Terminalia chebula* Retz., Trivrit, *Operculina turpethum* L.

**Introduction**

Approximately 85-90% of the world's population consumes traditional herbal medicines for their health care needs as per World Health Organization (WHO) [1-2]. WHO considers phytotherapy in its health program because these herbal drugs are safe, cost effective and most significantly people have faith in them [3]. WHO has even evolved guidelines for the validation of plant based drugs [4]. Standardization of Ayurvedic formulations is most important for the establishment of its biological activity, its chemical profile and its quality assurance in production and manufacturing of herbal drugs. As the usage of herbal medicines has increased, issues regarding their quality, safety, and efficacy have raised up [5]. As such the standardization is a burning topic in Ayurvedic drug manufacture today. Since Ayurvedic medicines come under the purview of Drugs and Cosmetics Act, there is increased general awareness about the necessity for developing standards for the purpose of quality control by the manufacturers as well as by the Drug control Authorities and for quality assurance to the public [6]. At present, the quantity of raw material is not sufficient in the market. Most of the pharmaceutical industries are using substitute drugs instead of authentic drugs. So to prepare best quality drugs it is necessary to authenticate raw drugs. Keeping the current trend in mind, Harithaki (*Terminalia chebula* Retz.) [7-10] powder and Trivrit (*Operculina turpethum* L.) powder were subjected for standardize procedures. From the current study, genuinity indicating parameters for both Harithaki [11] churna (powder) and Trivrit churna [12] were derived.

**Materials and Methods**

Phytochemical tests like tests for alkaloids, steroids, saponins, tannins, flavonoids, phenol, coumarins, triterpenoids, carboxylic acid, resin, quinine and HPTLC were carried out as per the WHO guidelines [13], Ayurvedic Pharmacopoeia [14] and Indian Pharmacopoeia [15].

**Plant Material**

Harithaki (*Terminalia chebula* Retz.) [16] powder (Batch no: 140088) Trivrit (*Operculina turpethum* L.) powder (Batch no: 120301) were collected from SDM pharmacy, Udupi, Karnataka state, India

**Methodology**

The studies were done at SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka state, India as per standard procedure.

**1. HPTLC:** one gram of powdered samples were dissolved in 10 ml ethanol and kept for cold percolation for 24h and filtered. Five and ten  $\mu$ l of the above samples were applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate: Formic acid (5:4: 0.1). The developed plates were visualized in UV 254, 366 and then derivatised with vanillin sulphuric acid reagent and scanned under UV 254 and 366 nm.  $R_f$ , colour of the spots and densitometric scan were recorded.

### Preliminary phytochemical tests

#### Tests for alkaloids

**a. Dragendorff's test:** To a few mg of extract dissolved in alcohol, a few drops of acetic acid and Dragendorff's reagent were added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.

**b. Wagners's test:** To a few mg of extract dissolved in acetic acid, a few drops of Wagner's reagent was added. A reddish brown precipitate formed indicates the presence of alkaloids.

**c. Mayer's test:** To a few mg of extract dissolved in acetic acid, a few drops of Mayer's reagent was added. A dull white precipitate formed indicates the presence of alkaloids.

**b. Hager's test:** To a few mg of extract dissolved in acetic acid, 3 ml of Hager's reagent was added, the formation of yellow precipitate indicates the presence of alkaloids.

#### Tests for carbohydrates

**a. Molisch's test:** To the extract, 1 ml of  $\alpha$ -naphthol solution and conc. sulphuric acid were added along the sides of test tube. Violet colour formed at the junction of the two liquids indicates the presence of carbohydrates.

**b. Fehling's test:** A few mg of extract was mixed with equal quantities of Fehling's solution A and B. The mixture was warmed on a water bath. The formation of a brick red precipitate indicates the presence of carbohydrates.

**c. Benedict's test:** To 5 ml of Benedict's reagent, a few mg of extract was added, and boiled for two minutes and cooled. Formation of a red precipitate indicates the presence of carbohydrates.

#### Test for steroids

**a. Liebermann-Burchard test:** To the extract dissolved in chloroform, 1 ml of acetic acid and 1 ml of acetic anhydride

were added, then heated on a water bath and cooled. Few drops of conc. Sulphuric acid were added along the sides of the test tube. Appearance of bluish green colour indicates the presence of steroids.

**b. Salkowski test:** The extract was dissolved in chloroform and equal volume of conc. Sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.

**Test for saponins:** To a few mg of extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponin.

**Test for tannins:** To the extract, a few drops of dilute solution of ferric chloride was added, formation of dark blue colour shows the presence of tannins.

**Test for flavonoids: Shinoda's test:** To the extract in alcohol, a few magnesium turnings and few drops of conc. hydrochloric acid were added and heated on a water bath. Formation of red to pink colour indicates the presence of flavonoids.

**Test for phenol:** To the extract in alcohol, added two drops of alcoholic ferric chloride. Formation of blue to blue black indicates the presence of phenol.

**Test for coumarins:** To the extract in alcohol, a few drops of 2 N sodium hydroxide solution was added. Dark yellow colour formation indicates the presence of coumarins.

**Test for triterpenoids:** The extract was warmed with tin bits and few drops of thionyl chloride. Formation of pink colour indicates the presence of triterpenoids.

**Test for carboxylic acid:** Extract dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.

**Test for resin:** Few mg of the sample was mixed with water and acetone. Turbidity indicates the presence of resin.

**Test for quinine:** A few mg of alcohol extract was treated with 0.5% of sodium hydroxide. Deep coloration like pink, purple or red indicates the presence of quinine.

### Results

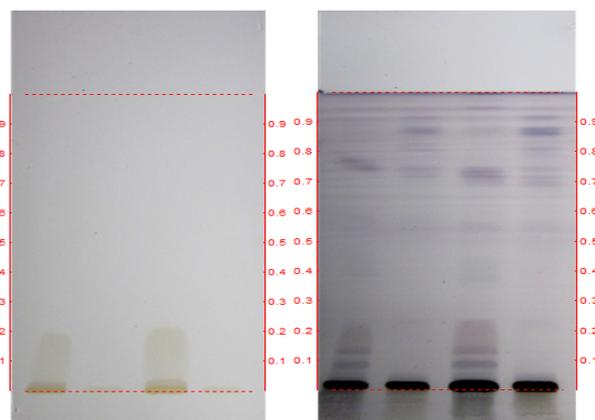
**Table 1:** Results of preliminary phytochemical tests

Tests	Colour if positive	Harithaki Churna	Trivrit Churna
Alkaloids			
Dragendorff's test	Orange precipitate	Orange Red Solution	Orange Red Solution
Wagners test	Red precipitate	Reddish Brown Colour	Reddish Brown Colour
Mayers test	Dull white precipitate	Light Yellow Colour	Light Yellow Colour
Hagers test	Yellow precipitate	Yellow Colour	Light Yellow Colour
Steroids			
Liebermann- buchard test	Bluish green	Brown Colour	Green Colour
Salkowski test	Bluish red to cherry red	Reddish Brown at junction	Reddish Brown at junction
Carbohydrate			
Molish test	Violet ring	Violet ring	Violet ring
Fehlings test	Brick red precipitate	Brick red precipitate	Brick red precipitate
Benedicts test	Red precipitate	Red precipitate	Blue Colour
Tannin			

With FeCl <sub>3</sub>	Dark blue or green or brown	Brown colour solution	Light Green
Flavanoids			
Shinoda's test	Red to pink	Light Yellow colour	Pink Colour
Saponins			
With NaHCO <sub>3</sub>	Stable froth	No Froth formed	No Froth formed
Triterpenoids			
Tin and thionyl chloride test	Pink	Yellow precipitate	Brown Colour
Coumarins			
With 2 N NaOH	Yellow	Yellow precipitate	Yellow precipitate
Phenols			
With alcoholic ferric chloride	Blue to blue black, brown	Brown colour solution	Light Green Colour
Carboxylic acid			
With water and NaHCO <sub>3</sub>	Brisk effervescence	No brisk effervescence	No brisk effervescence
Resin			
With aqueous acetone	Turbidity	No Turbidity	No Turbidity
Quinone			
5% NaOH	Pink/purple/red	Yellow precipitate	Brown Red

**Table 2:** Summary

Test	Harithaki churna	Trivrit churna
Alkaloid	-	-
Carbohydrate	+	+
Carboxylic acid	-	-
Coumarins	+	+
Flavanoids	-	+
Phenol	+	-
Quinone	-	-
Resins	-	-
Steroid	+	+
Saponins	-	-
Tannin	+	+
Terpenoid	-	-



At 540 nm

After post derivatisation

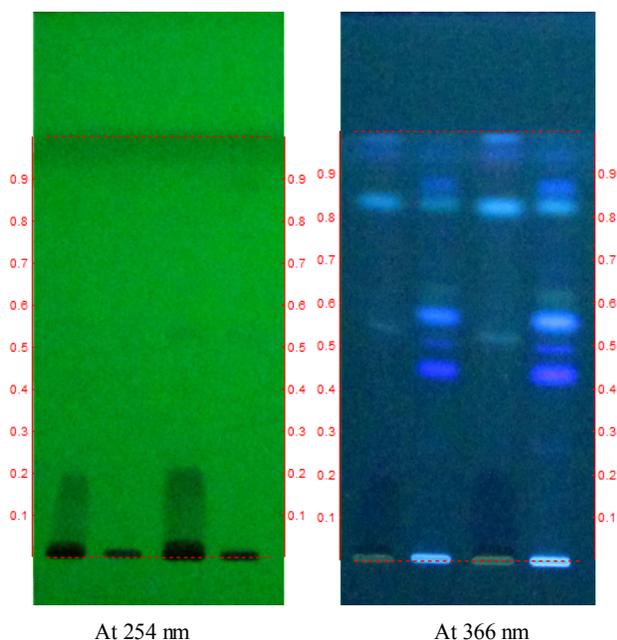
track 1- Harithaki churna – 4 µl  
 track 2- Trivrit churna – 4 µl  
 track 3- Harithaki churna – 8 µl  
 track 4- Trivrit churna – 8 µl

**Solvent system: Toluene: Ethyl Acetate: Acetic Acid (5:4:0.1)**

**Table 3:** R<sub>f</sub> values of Harithaki Churna

At 254 nm	At 366 nm	At 540 nm	After post derivatisation
-	-	-	0.05(L Violet)
-	-	-	0.08(Violet)
-	-	-	0.14(Violet)
0.21(Green)	0.21(F Blue)	0.21(L Brown)	0.21(L Violet)
0.53(L Green)	0.33(F L Green)	-	-
-	-	-	0.37(L Violet)
-	-	-	0.44 (L Violet)
-	0.52(F L Green)	-	-
-	-	-	0.55(L Violet)
-	-	-	0.73(Violet)
-	0.83(F Blue)	-	-
-	-	-	0.87(L Violet)
-	0.95(F L Blue)	-	0.95(L Violet)

\*L-Light,F-Fluorescence



At 254 nm

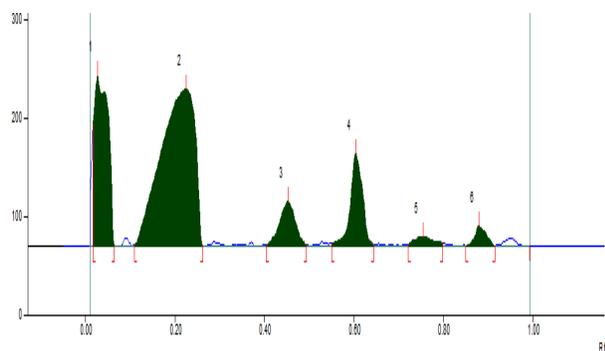
At 366 nm

**Fig 1:** HPTLC photo documentation of ethanol extract of Harithaki churna & Trivrit Churna

**Table 4:** R<sub>f</sub> values of Trivrit Churna

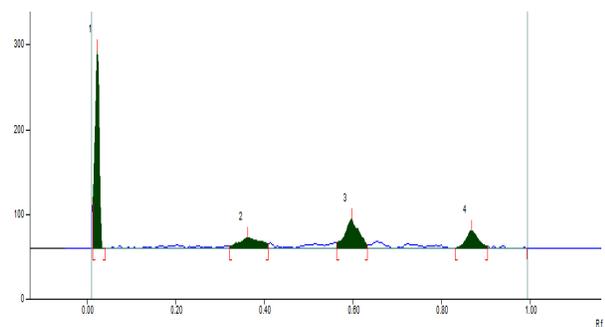
At 254 nm	At 366 nm	At 540 nm	After post derivatisation
-	-	-	0.05(L Violet)
-	-	-	0.09(L Violet)
-	-	-	0.13(L Violet)
-	-	-	0.23(L Violet)
-	0.27(F L Violet)	-	-
-	0.45(F Violet)	-	-
-	0.50(F Violet)	-	-
-	-	-	0.55(L Violet)
-	0.63(F L Green)	-	-
-	-	-	0.72(L Violet)
-	-	-	0.80(L Violet)
-	0.83 (F Blue)	-	-
-	0.87(F Violet)	-	0.87(Violet)
-	-	-	0.91(L Violet)
-	0.94(F L Violet)	-	-

\*L-Light, F-Fluorescence



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	123.7 AU	0.03 Rf	172.3 AU	34.50 %	0.06 Rf	0.2 AU	3875.1 AU	23.75 %
2	0.11 Rf	1.9 AU	0.23 Rf	158.8 AU	31.80 %	0.26 Rf	0.4 AU	8760.0 AU	53.68 %
3	0.40 Rf	1.5 AU	0.45 Rf	44.7 AU	8.96 %	0.50 Rf	1.1 AU	1156.9 AU	7.09 %
4	0.55 Rf	3.0 AU	0.60 Rf	93.6 AU	18.74 %	0.65 Rf	1.5 AU	1848.3 AU	11.33 %
5	0.72 Rf	1.6 AU	0.76 Rf	10.0 AU	2.00 %	0.80 Rf	3.3 AU	309.9 AU	1.90 %
6	0.85 Rf	0.2 AU	0.88 Rf	19.9 AU	3.99 %	0.92 Rf	0.3 AU	367.7 AU	2.25 %

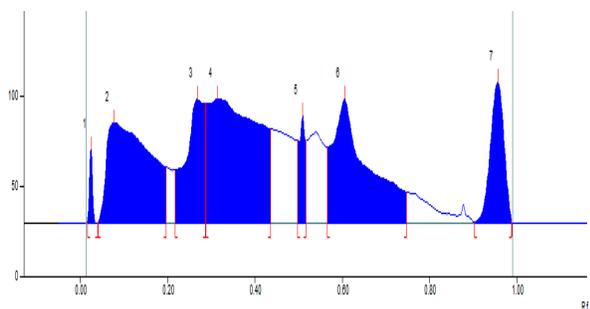
**Fig 2a:** Harithaki Churna at 254 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	53.8 AU	0.02 Rf	233.2 AU	77.67 %	0.04 Rf	0.0 AU	1697.3 AU	51.09 %
2	0.32 Rf	2.5 AU	0.36 Rf	12.9 AU	4.29 %	0.41 Rf	5.0 AU	438.3 AU	13.19 %
3	0.56 Rf	6.9 AU	0.60 Rf	33.8 AU	11.27 %	0.63 Rf	4.1 AU	770.9 AU	23.20 %
4	0.83 Rf	0.3 AU	0.87 Rf	20.3 AU	6.76 %	0.90 Rf	2.6 AU	415.8 AU	12.52 %

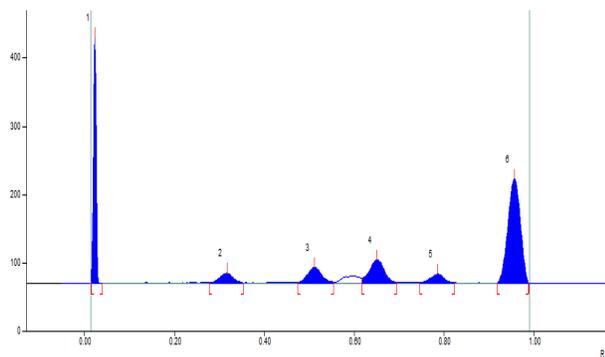
**Fig 2.b:** Trivrit Churna At 254 nm

**Fig 2:** Densitometric scan at 254nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	1.8 AU	0.03 Rf	40.6 AU	9.27 %	0.04 Rf	0.0 AU	209.8 AU	1.14 %
2	0.04 Rf	0.7 AU	0.08 Rf	55.3 AU	12.61 %	0.20 Rf	30.8 AU	3947.3 AU	21.47 %
3	0.22 Rf	29.5 AU	0.27 Rf	68.2 AU	15.56 %	0.29 Rf	66.2 AU	2130.5 AU	11.59 %
4	0.29 Rf	66.1 AU	0.31 Rf	68.3 AU	15.56 %	0.44 Rf	51.9 AU	5649.1 AU	30.73 %
5	0.50 Rf	45.5 AU	0.51 Rf	59.3 AU	13.52 %	0.52 Rf	45.3 AU	645.4 AU	3.51 %
6	0.57 Rf	42.2 AU	0.61 Rf	68.6 AU	15.64 %	0.75 Rf	17.3 AU	4039.5 AU	21.97 %
7	0.90 Rf	0.6 AU	0.96 Rf	78.2 AU	17.83 %	0.99 Rf	1.9 AU	1761.4 AU	9.58 %

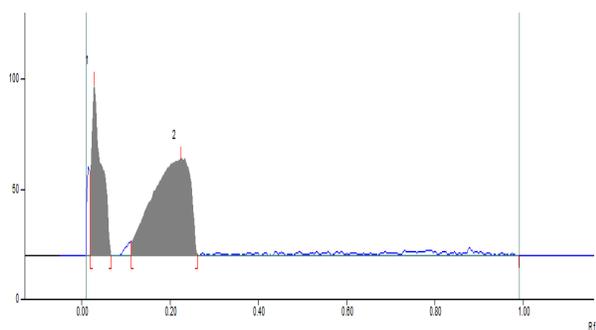
**Fig 3a:** Harithaki Churna at 366 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	1.5 AU	0.02 Rf	360.1 AU	60.07 %	0.04 Rf	0.2 AU	1648.3 AU	24.48 %
2	0.28 Rf	0.9 AU	0.32 Rf	14.8 AU	2.48 %	0.35 Rf	0.5 AU	328.0 AU	4.87 %
3	0.48 Rf	1.4 AU	0.51 Rf	23.6 AU	3.94 %	0.55 Rf	2.1 AU	540.6 AU	8.03 %
4	0.62 Rf	8.3 AU	0.65 Rf	34.2 AU	5.70 %	0.70 Rf	1.9 AU	834.5 AU	12.39 %
5	0.75 Rf	1.1 AU	0.79 Rf	13.6 AU	2.27 %	0.82 Rf	0.8 AU	307.7 AU	4.57 %
6	0.92 Rf	0.4 AU	0.96 Rf	153.2 AU	25.55 %	0.99 Rf	2.1 AU	3073.5 AU	45.65 %

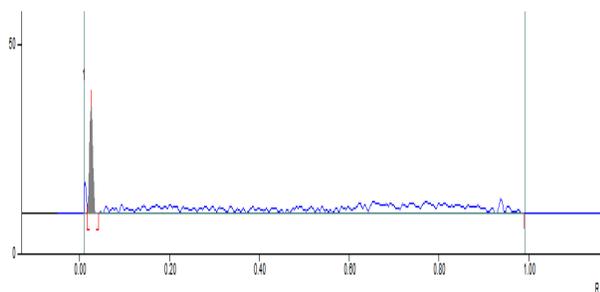
Fig 3b: Trivrit Churna at 366 nm

Fig 3: Densitometric scan At 366 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	37.9 AU	0.03 Rf	77.5 AU	63.93 %	0.07 Rf	0.0 AU	1245.2 AU	31.55 %
2	0.11 Rf	6.2 AU	0.23 Rf	43.7 AU	36.07 %	0.26 Rf	0.3 AU	2701.2 AU	68.45 %

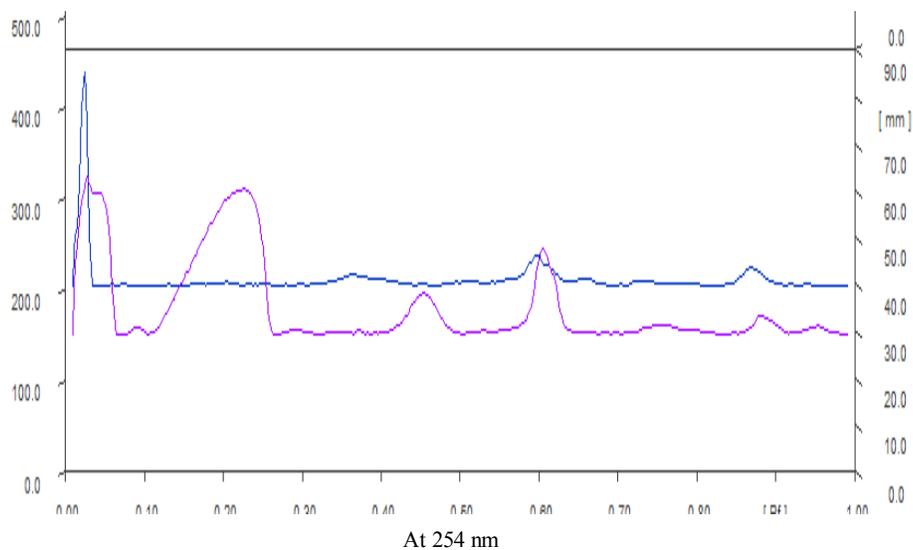
Fig 4a: Harithaki Churna at 540 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	1.7 AU	0.03 Rf	25.7 AU	100.00 %	0.04 Rf	0.1 AU	133.5 AU	100.00 %

Fig 4b: Trivrit Churna at 540 nm

Fig 4: Densitometric scan At 540 nm



At 254 nm

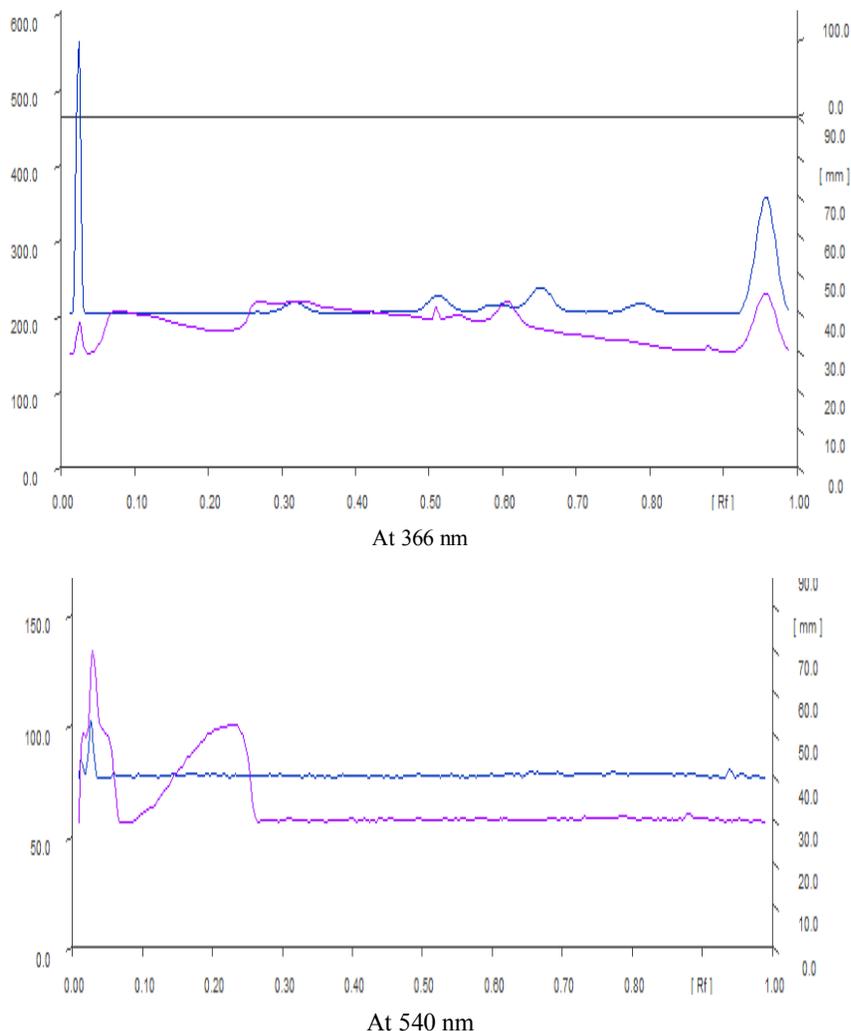


Fig 5: 3-D Display of All the samples

### Discussion

The phytochemical tests carried out serve as preliminary test for the standardization of the formulation. Tests such as tests for alkaloids, steroids, saponins, tannins, flavonoids, phenol, coumarins, triterpenoids, carboxylic acid, resin, quinine, HPTLC, results of TLC photodocumentation, the unique Rf values, densitometric scan and densitogram obtained at different wavelengths can be used as fingerprint to identify both the herbal drugs, Harithaki (*Terminalia chebula* Retz.) powder and Trivrit (*Operculina turpethum* L.) powder.

### Conclusion

Proper Ayurvedic drug standardization requires rational approach and in this regard fundamental aspects of Ayurvedic drug should be preserved. Main obstacle in Ayurvedic drug standardization is the identification of biological source of the drug. The active constituent may vary according to geographical source of the drug and it may not be easy to standardize drug chemically. The parameters used in this work ensure the quality control of raw material, processed powder. The results obtained through this study were quick, reproducible and could be used for routine monitoring of raw material. Both Harithaki (*Terminalia chebula* Retz.) powder and Trivrit (*Operculina turpethum* L.) powder are endowed with various biological properties and hence efforts have been made here to provide scientific data on the same.

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