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# Phytochemical Screening and HPTLC Finger Printing Analysis of *Roscea procera* (Kakoli) and *Lilium polyphyllum* (Kshirkakoli)

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**ABSTRACT**

Plant and plant based products are the basis of many of the modern pharmaceuticals we use today for various ailments. Astavarga group have a long history of use in traditional system of indigenous medicine. In present study, the powdered plant material was extracted using different solvents. Phytochemical screening and HPTLC fingerprinting analysis were carried out by using CAMAG LINOMAT 5 instrument. The result revealed that ethanol and chloroform extract of *Roscea procera* (Kakoli) and, ethyl acetate and chloroform extract of *Lilium polyphyllum* (Kshirkakoli) showed appreciable amount of secondary metabolites as compared to another solvent system. The HPTLC fingerprinting analysis was carried out for alkaloid, flavonoids and glycoside group of Kakoli extracts and for saponin and steroid groups of Kshirkakoli. The result scientifically validates the use of both plants in traditional medicine and it can be used to treat various disorders. Further studies are going on these plants in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds.

## INTRODUCTION

Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on human body[1]. Plants which are rich in a wide variety of secondary metabolites, such as alkaloids, flavonoids, steroids, tannins etc. have been found to have several biological properties. In recent years, advancements in chromatographic and spectral fingerprints have played an important role in the quality control of complex herbal medicines. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence, the modern methods describing the identification and quantification of active constituents in the plant material may be useful for standardization of herbals and its formulations. Also, the World Health Organization has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards with reasonable accuracy in a short time[2].

In present study *Roscea procera* (Kakoli) and *Lilium polyphyllum* (Kshirkakoli) plants which have been described under "Astavarga" group is selected. Astavarga drugs suffer a lot of confusion in Ayurvedic literature in accordance with the identification and authentification. The astavarga group contains Jivaka, Rishibhak, Mahameda, Meda, Kakoli, Kshirkakoli, Riddhi and Vriddhi. Kakoli and kshirkakoli come under "Brhneeya" (the drug which promote the formation of mansadhatu that is flesh formation) in Dhanvantari Nighantu, Charaka Samhita, Sushruta Samhita and Astanghridaya[3-6].

Kakoli (*Roscea procera* wall.) is an ancient Indian medicinal plant belonging to family Zingiberaceae. Tubers of kakoli are found to contain alkaloid, glycoside, flavonoid, tannin, saponins and active phenolic compounds. The modern screening methods revealed its principal pharmacological activities like antidiabetic, immunomodulator, spermopiotic, fever, burning and phthisis[6-10].

*Lilium polyphyllum* (D. Don.) commonly known as kshirkakoli or white lilly (family Liliaceae) is extensively used in many indigenous preparations. Kshirkakoli is reported to contain sugar, alkaloid, flavonoids, steroids. Medicinally, bulb of species has been used for diuretic, antipyretic, tonic[7, 11-14].

The present study aims to analyze the HPTLC fingerprinting profile of bioactive compounds present in different extracts obtained by successive extraction of kakoli and kshirkakoli respectively.

## MATERIALS AND METHODS

### Plant materials

Marketed rhizomes of Kakoli and bulb of Kshirkakoli plants were collected locally, authenticated by Dr. Dongarwar, Department of Botany R.T.M. Nagpur University, Campus, Nagpur. A voucher specimen has been deposited in the Herbarium of Department of Botany, with collection no 9480 and 9481.

### Extraction

The dried, coarsely powdered rhizomes of Kakoli and Kshirkakoili plants (500 g each) were extracted with petroleum ether (55-60˚C), ethyl acetate (50-55˚C), chloroform (50-55˚C), acetone (55-60˚C) and ethanol (60-65˚C) successively by soxhlation. The dried marc after ethanol extraction was cold macerated to obtain hydroalcoholic extract. The extracts were evaporated to dryness in oven (45˚C.).

### Instruments and chemicals used

For HPTLC fingerprinting CAMAG "Linomat 5" HPTLC system made up of Linomat 5 sample applicator, a twin trough plate development chamber. TLC Scanner "Scanner-170422" S/N 170422 and Win CATS 1.4.6 software were used. HPTLC plates 10.0 cm X 10.0 cm with 0.2 mm layers of silica gel 60 $F_{254}$ (E. Merck, Mumbai, India) pre-washed with methanol was used. All other solvents used for experimental work were of analytical grade.

### Preliminary phytochemical screening of extracts[15,16]

The solution of extracts of test materials of each plant was prepared and subjected to screen out the presence of various bioactive phytoconstituents.

### HPTLC fingerprinting analysis[16,17]

For HPTLC fingerprinting analysis, test solutions (ethanol, chloroform extract of kakoli and ethyl acetate, chloroform extract of kshirkakoli in respective solvents) were loaded as 8.0 mm band length in the 10.0 cm x 10.0 cm silica gel $60F_{254}$ TLC plate using Linomat sample

applicator respectively. The sample loaded plates was kept in TLC twin trough developing chamber (after saturated with solvent vapor with respective mobile phases). The plates were developed in respective mobile phase up to 80.0 mm. The developed plates were dried by hot air to evaporate solvents from them. The plates were scanned by TLC Scanner "Scanner-170422" S/N 170422 programmed through WINCATS 1.4.6 software. Images were captured at UV 254 nm and UV 366 nm. The peak table, peak display and peak densitogram were noted (Rf vs. AU). For optimization, the analysis was carried out by using three separate concentrations of 5 µL, 10 µL and 15 µL of each extract were performed separately and separate track was maintained for each concentration with separate peak development for each extract.

**Analysis details**

**Alkaloids-**

Mobile phase: toluene - ethyl acetate - diethylamine (7:2:1)

Spray reagent- Dragendorff's reagent.

**Glycosides-**

Mobile phase: ethyl acetate – methanol - water (10:1.4:1)

Spray reagent- Alcoholic potassium hydroxide solution.

**Flavanoids-**

Mobile phase: ethyl acetate – formic acid – glacial acetic acid - water (10:0.5:0.5:1.3)

Spray reagent- Anisaldehyde sulphuric acid reagent.

**Saponins-**

Mobile phase: chloroform – acetic acid – methanol – water (6.4:3.2:1.2:0.8)

Spray reagent- Anisaldehyde sulphuric acid reagent.

**Steroids-**

Mobile phase: n-butanol – methanol – water (3:1:1)

Spray reagent- Anisaldehyde sulphuric acid reagent.

## RESULTS

### Phytochemical screening of extracts

The phytochemical screening has been conducted for each extract obtained after successive solvent extraction. In the screening, ethanol and chloroform extract of kakoli showed the presence of maximum constituents like carbohydrate, flavonoids, alkaloids, phenolics, protein, phytosterols and glycosides, while ethyl acetate and chloroform extracts of kshirkakoli showed the prominent results for sugar, steroids and saponins.

### HPTLC fingerprinting profile

HPTLC profile of plant extracts was generated in solvent systems of different polarities in order to ascertain the total number of chemical moieties, which will also help in method of isolation and characterization of bioactive compounds. Chromatograms of each extract of kakoli and kshirkakoli are given in figure 1-4.

The HPTLC fingerprinting of ethanol and chloroform extracts of kakoli revealed the presence of group of alkaloids, glycosides and flavonoids in it. For alkaloid, the ethanol extract of kakoli showed 9 spots in 5 µL concentration, 7 spots in 10 µL concentration and 6 spots in 15 µL concentration (Figure 5a, 5b, 5c) while chloroform extract of kakoli showed 5 spots in 5 µL, 10 µL and 15 µL concentration (Figure 6a, 6b, 6c), Rf values are given in Table 1. For glycosides, the ethanol extract of kakoli showed 7 spots in 5 µL, 10 µL concentration and 9 spots in 15 µL concentration and (Figure7a, 7b, 7c) while chloroform extract of kakoli showed 3 spots in 5 µL concentration, 4 spots in 10 µL concentration and 7 spots in15 µL concentration (Figure 8a, 8b, 8c), Rf values are given in Table 2. For Flavonoids, the ethanol extract of kakoli showed 6 spots in 5 µL concentration, 7 spots in 10 µL concentration and 8 spots in 15 µL concentration and (Figure 9a, 9b, 9c) while chloroform extract of kakoli showed 9 spots in 5 µL, 10 µL and 15 µL concentration. (Figure 10a, 10b, 10c), Rf values are given in Table 3.

HPTLC profiling of ethyl acetate and chloroform extract of kshirkakoli revealed the presence of saponins and steroids in it. For saponins, the ethyl acetate extract of kshirkakoli showed 5 spots in 5 µL, 7 spots in 10 µL and 15 µL concentration (Figure 11a, 11b, 11c) while chloroform extract of kshirkakoli showed 9 spots in 5 µL concentration, 13 spots in 10 µL

and 15 µL concentration (Figure 12a, 12b, 12c), Rf values are given in Table 4. For steroids, the ethyl acetate extract of kshirkakoli showed 7 spots in 5 µL, 10 µL and 15 µL concentration (Figure 13a, 13b, 13c) while chloroform extract of kshirkakoli showed 7 spots in 5 µL concentration, 9 spots in 10 µL and 15 µL concentration (Figure 14a, 14b, 14c), Rf values are given in Table 5.

**Table 1. Peak table with Rf value, height and area of Kakoli extracts for Alkaloid group. EEK: ethanolic extract of Kakoli; CHEK: chloroform extract of Kakoli.**

| Sr. No | Extracts | Peak | 5 µL | | | 10 µL | | | 15 µL | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rf | Height of peak | Area of peak | Rf | Height of peak | Area of peak | Rf | Height of peak | Area of peak |
| 1 | EEK | 1 | 0.20 | 8.2 | 915.6 | 0.19 | 16.3 | 1272.1 | 0.20 | 20.5 | 1355.5 |
| | | 2 | 0.28 | 19.5 | 745.0 | 0.28 | 37.1 | 1558.3 | 0.28 | 50.7 | 1909.0 |
| | | 3 | 0.34 | 29.1 | 938.5 | 0.51 | 231.5 | 16994.6 | 0.50 | 309.3 | 24293.7 |
| | | 4 | 0.53 | 134.2 | 10105.1 | 0.57 | 241.1 | 11659.3 | 0.56 | 325.2 | 14984.5 |
| | | 5 | 0.59 | 131.2 | 7105.1 | 0.73 | 298.5 | 42067.0 | 0.88 | 1.4 | 79485.0 |
| | | 6 | 0.67 | 152.3 | 8217.3 | 0.88 | 3.0 | 15589.3 | 0.99 | 1.1 | 217.7 |
| | | 7 | 0.76 | 130.3 | 14117.0 | 1.00 | 1.2 | 239.0 | | | |
| | | 8 | 0.88 | 10.8 | 7212.4 | | | | | | |
| | | 9 | 1.0 | 2.6 | 433.7 | | | | | | |
| 2 | CHEK | 1 | 0.19 | 9.4 | 385.0 | 0.20 | 8.7 | 457.2 | 0.19 | 11.1 | 505.8 |
| | | 2 | 0.28 | 22.3 | 861.1 | 0.28 | 33.1 | 1212.4 | 0.28 | 43.6 | 1963.0 |
| | | 3 | 0.57 | 160.4 | 16859.9 | 0.56 | 253.8 | 29095.6 | 0.55 | 331.7 | 35654.4 |
| | | 4 | 0.87 | 2.3 | 35584.9 | 0.87 | 1.1 | 57080.0 | 0.85 | 2.0 | 70648.5 |
| | | 5 | 0.99 | 0.6 | 213.8 | 0.99 | 0.0 | 232.6 | 0.98 | 0.0 | 233.6 |



**Table 2. Peak table with Rf value, height and area of Kakoli extracts for Glycoside group. EEK: ethanolic extract of Kakoli; CHEK: chloroform extract of Kakoli.**

| Sr. No | Extracts | Peak | 5 µL | | | 10 µL | | | 15 µL | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rf | Height of peak | Area of peak | Rf | Height of peak | Area of peak | Rf | Height of peak | Area of peak |
| 1 | EEK | 1 | 0.12 | 16.8 | 5730.7 | 0.12 | 31.8 | 9428.4 | 0.12 | 47.1 | 12648.2 |
| | | 2 | 0.34 | 47.5 | 3155.6 | 0.43 | 134.2 | 20005.6 | 0.28 | 66.5 | 2861.5 |
| | | 3 | 0.43 | 79.4 | 9831.8 | 0.46 | 115.1 | 3280.0 | 0.43 | 179.8 | 26347.7 |
| | | 4 | 0.46 | 67.0 | 1819.0 | 0.64 | 42.7 | 9903.2 | 0.46 | 155.0 | 4592.3 |
| | | 5 | 0.64 | 22.5 | 5731.4 | 0.70 | 33.1 | 2099.4 | 0.49 | 162.1 | 4105.8 |
| | | 6 | 0.70 | 16.7 | 1079.3 | 0.76 | 25.4 | 1567.8 | 0.63 | 64.8 | 13335.1 |
| | | 7 | 0.85 | 5.9 | 744.0 | 0.90 | 0.2 | 1604.8 | 0.70 | 49.9 | 3021.1 |
| | | 8 | | | | | | | 0.76 | 37.4 | 2528.8 |
| | | 9 | | | | | | | 0.90 | 0.6 | 2357.6 |
| 2 | CHEK | 1 | 0.12 | 48.1 | 12377.0 | 0.12 | 87.3 | 18578.5 | 0.11 | 122.5 | 22310.0 |
| | | 2 | 0.42 | 117.5 | 15323.8 | 0.17 | 74.3 | 3556.5 | 0.19 | 99.6 | 6821.5 |
| | | 3 | 0.76 | 18.2 | 12497.8 | 0.42 | 184.9 | 22287.5 | 0.28 | 123.6 | 7310.7 |
| | | 4 | | | | 0.89 | 1.0 | 24204.8 | 0.42 | 227.5 | 21360.9 |
| | | 5 | | | | | | | 0.46 | 239.3 | 7306.8 |
| | | 6 | | | | | | | 0.49 | 247.9 | 5990.5 |
| | | 7 | | | | | | | 0.90 | 0.2 | 33372.8 |

**Table 3. Peak table with Rf value, height and area of Kakoli extracts for Flavonoids group.**

| Sr. No | Extracts | Peak | 5 µL | | | 10 µL | | | 15 µL | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rf | Height of peak | Area of peak | Rf | Height of peak | Area of peak | Rf | Height of peak | Area of peak |
| 1 | EEK | 1 | 0.36 | 57.4 | 3849.5 | 0.17 | 13.4 | 277.0 | 0.22 | 56.7 | 2187.4 |
| | | 2 | 0.44 | 89.5 | 4151.2 | 0.43 | 125.6 | 13759.4 | 0.31 | 120.2 | 4536.4 |
| | | 3 | 0.57 | 46.4 | 16247.4 | 0.56 | 86.3 | 24774.9 | 0.42 | 137.9 | 14679.2 |
| | | 4 | 0.67 | 42.2 | 4340.5 | 0.65 | 83.5 | 8393.9 | 0.54 | 193.1 | 33148.1 |
| | | 5 | 0.71 | 25.7 | 1088.1 | 0.70 | 50.3 | 2307.7 | 0.64 | 221.2 | 21112.2 |
| | | 6 | 0.85 | 7.3 | 1718.4 | 0.85 | 10.5 | 3410.7 | 0.69 | 141.0 | 6786.8 |
| | | 7 | | | | 0.93 | 0.1 | 273.4 | 0.85 | 34.2 | 10253.4 |
| | | 8 | | | | | | | 0.92 | 0.0 | 1076.0 |
| 2 | CHEK | 1 | 0.18 | 30.7 | 984.1 | 0.18 | 41.9 | 1629.9 | 0.19 | 48.3 | 2269.7 |
| | | 2 | 0.23 | 58.1 | 1716.9 | 0.23 | 66.9 | 1994.0 | 0.23 | 68.8 | 1988.3 |
| | | 3 | 0.32 | 90.2 | 4833.2 | 0.32 | 96.8 | 5349.2 | 0.31 | 93.8 | 5297.7 |
| | | 4 | 0.39 | 119.5 | 6366.0 | 0.39 | 137.7 | 7091.2 | 0.38 | 138.7 | 6959.2 |
| | | 5 | 0.43 | 102.3 | 2934.1 | 0.42 | 96.4 | 3192.1 | 0.42 | 84.1 | 3230.9 |
| | | 6 | 0.54 | 87.8 | 16692.1 | 0.53 | 127.6 | 19109.4 | 0.53 | 151.3 | 19076.4 |
| | | 7 | 0.64 | 91.6 | 9560.2 | 0.63 | 161.7 | 14644.8 | 0.62 | 223.4 | 18049.9 |
| | | 8 | 0.69 | 68.6 | 3798.4 | 0.69 | 127.9 | 7323.5 | 0.75 | 108.0 | 17217.7 |
| | | 9 | 0.75 | 32.4 | 2415.4 | 0.90 | 0.2 | 8389.5 | 0.89 | 0.4 | 4485.9 |

EEK: ethanolic extract of Kakoli; CHEK: chloroform extract of Kakoli.

**Table 4. Peak table with Rf value, height and area of Kshirkakoli extracts for Saponins. EAEKS: ethyl acetate extract of Kshirkakoli; CHEKS: chloroform extract of Kshirkakoli.**

| Sr. No | Extracts | Peak | 5 µL | | | 10 µL | | | 15 µL | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rf | Height of peak | Area of peak | Rf | Height of peak | Area of peak | Rf | Height of peak | Area of peak |
| 1 | EAEKS | 1 | 0.45 | 8.4 | 595.4 | 0.34 | 3.3 | 470.3 | 0.33 | 4.8 | 643.2 |
| | | 2 | 0.50 | 13.7 | 1452.8 | 0.39 | 13.5 | 342.6 | 0.39 | 13.8 | 488.7 |
| | | 3 | 0.56 | 5.4 | 3105.6 | 0.43 | 15.7 | 1070.7 | 0.43 | 22.8 | 1432.7 |
| | | 4 | 0.67 | 4.2 | 2782.5 | 0.48 | 25.8 | 2467.9 | 0.47 | 35.5 | 3150.4 |
| | | 5 | 0.95 | 0.0 | 1194.0 | 0.54 | 10.8 | 5246.0 | 0.54 | 15.4 | 6738.3 |
| | | 6 | | | | 0.65 | 8.6 | 4859.8 | 0.64 | 12.4 | 6366.4 |
| | | 7 | | | | 0.96 | 0.0 | 1868.2 | 0.95 | 0.1 | 2411.7 |
| 2 | CHEKS | 1 | 0.26 | 13.3 | 574.2 | 0.20 | 12.0 | 580.6 | 0.20 | 14.4 | 692.2 |
| | | 2 | 0.32 | 23.8 | 965.0 | 0.25 | 20.9 | 900.7 | 0.25 | 25.7 | 1066.9 |
| | | 3 | 0.43 | 114.8 | 3906.2 | 0.31 | 40.0 | 1486.7 | 0.31 | 48.1 | 1743.8 |
| | | 4 | 0.47 | 172.7 | 8367.1 | 0.35 | 36.0 | 1128.5 | 0.34 | 39.1 | 1287.3 |
| | | 5 | 0.55 | 52.8 | 20462.7 | 0.42 | 149.3 | 5194.1 | 0.42 | 159.1 | 5530.4 |
| | | 6 | 0.66 | 26.7 | 26956.7 | 0.47 | 208.5 | 10154.9 | 0.47 | 221.6 | 10939.6 |
| | | 7 | 0.73 | 16.0 | 1548.5 | 0.49 | 521.6 | 7997.2 | 0.49 | 444.0 | 8211.1 |
| | | 8 | 0.85 | 24.3 | 2490.3 | 0.55 | 75.7 | 12984.4 | 0.55 | 85.1 | 12646.2 |
| | | 9 | 0.96 | 0.0 | 1757.1 | 0.64 | 40.7 | 31464.7 | 0.65 | 47.5 | 32883.5 |
| | | 10 | | | | 0.72 | 26.5 | 2577.1 | 0.72 | 33.5 | 2826.5 |
| | | 11 | | | | 0.85 | 35.2 | 3687.6 | 0.85 | 41.6 | 4102.3 |
| | | 12 | | | | 0.89 | 40.4 | 1223.3 | 0.91 | 84.8 | 2111.7 |
| | | 13 | | | | 0.96 | 0.0 | 2508.2 | 0.96 | 0.0 | 1270.7 |

**Table 5. Peak table with Rf value, height and area of Kshirkakoli extracts for Steroids. EAEKS: ethyl acetate extract of Kshirkakoli; CHEKS: chloroform extract of Kshirkakoli.**

| Sr. No | Extracts | Peak | 5 µL | | | 10 µL | | | 15 µL | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rf | Height of peak | Area of peak | Rf | Height of peak | Area of peak | Rf | Height of peak | Area of peak |
| 1 | EAEKS | 1 | 0.05 | 0.0 | 221.3 | 0.06 | 0.1 | 374.6 | 0.06 | 0.7 | 422.2 |
| | | 2 | 0.14 | 52.4 | 5150.8 | 0.17 | 74.0 | 10117.0 | 0.19 | 81.0 | 14118.6 |
| | | 3 | 0.21 | 7.5 | 1683.9 | 0.24 | 6.5 | 2377.9 | 0.26 | 12.0 | 2759.8 |
| | | 4 | 0.38 | 15.9 | 5961.6 | 0.38 | 31.9 | 10849.2 | 0.38 | 39.3 | 13697.6 |
| | | 5 | 0.44 | 2.2 | 379.4 | 0.43 | 3.5 | 670.2 | 0.43 | 1.8 | 792.8 |
| | | 6 | 0.55 | 6.1 | 693.4 | 0.55 | 10.7 | 1218.7 | 0.54 | 11.8 | 1395.6 |
| | | 7 | 0.89 | 0.1 | 2145.6 | 0.88 | 0.4 | 3656.7 | 0.87 | 1.5 | 5117.1 |
| 2 | CHEKS | 1 | 0.09 | 508.7 | 36196.0 | 0.04 | 92.9 | 7468.3 | 0.03 | 19.3 | 1133.4 |
| | | 2 | 0.18 | 252.3 | 29366.3 | 0.18 | 34.2 | 20981.4 | 0.17 | 33.9 | 22715.1 |
| | | 3 | 0.36 | 1.8 | 24500.0 | 0.23 | 18.2 | 1525.3 | 0.22 | 0.3 | 1327.3 |
| | | 4 | 0.47 | 24.6 | 1475.7 | 0.35 | 0.1 | 5920.8 | 0.35 | 0.1 | 5387.8 |
| | | 5 | 0.57 | 0.4 | 1819.3 | 0.40 | 7.1 | 236.8 | 0.39 | 12.2 | 265.5 |
| | | 6 | 0.75 | 23.3 | 2126.2 | 0.47 | 28.1 | 1837.5 | 0.46 | 30.2 | 1806.2 |
| | | 7 | 0.89 | 0.0 | 1314.4 | 0.55 | 1.5 | 2392.9 | 0.54 | 0.2 | 2480.1 |
| | | 8 | | | | 0.75 | 40.6 | 4134.8 | 0.74 | 58.2 | 5240.2 |
| | | 9 | | | | 0.88 | 0.6 | 2185.8 | 0.88 | 0.0 | 2879.2 |

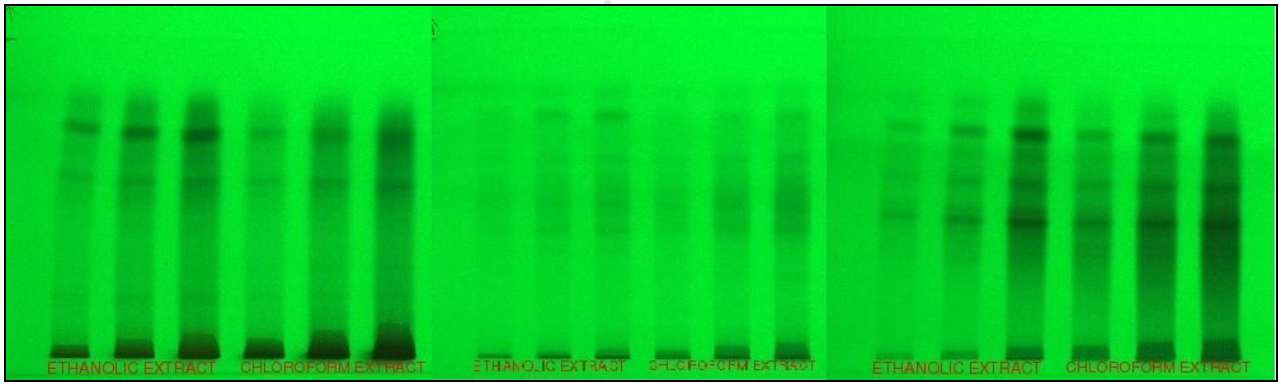


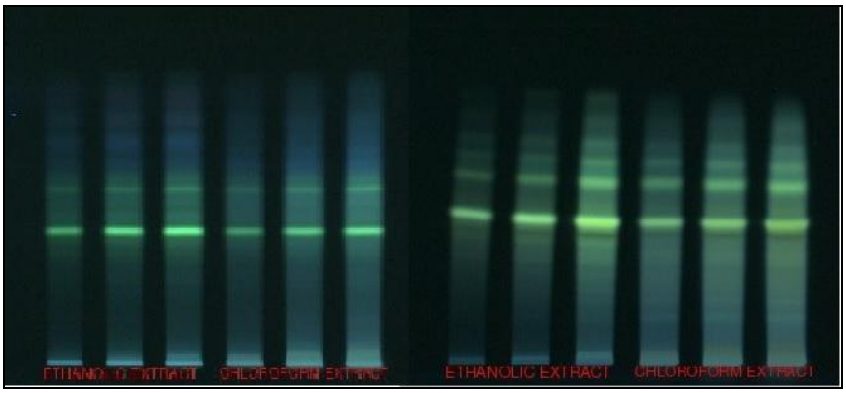

**Figure 1.** Chromatogram of different solvent extracts of Kakoli at 254 nm.

Track 1, 2 and 3- 5 µL, 10 µL and 15 µL of ethanol extract of Kakoli; Track 4, 5 and 6- 5 µL, 10 µL and 15 µL of chloroform extract of Kakoli for alkaloids.

Track 7, 8 and 9- 5 µL, 10 µL and 15 µL of ethanol extract of Kakoli; Track 10, 11 and 12- 5 µL,

10 µL and 15 µL of chloroform extract of Kakoli for glycosides.

Track 13, 14 and 15- 5 µL, 10 µL and 15 µL of ethanol extract of Kakoli; Track 16, 17 and 18- 5 µL, 10 µL and 15 µL of chloroform extract of Kakoli for flavonoids.



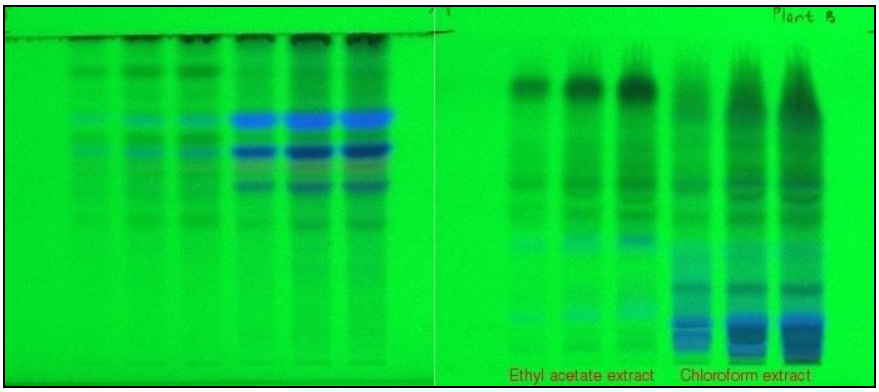

1 2 3 4 5 6 7 8 9 10 11 12

**Figure 2.** Chromatogram of different solvent extracts of Kakoli at 366 nm.

Track 1, 2 and 3- 5 µL, 10 µL and 15 µL of ethanol extract of Kakoli; Track 4, 5 and 6- 5 µL, 10 µL and 15 µL of chloroform extract of Kakoli for glycosides.

Track 7, 8 and 9- 5 µL, 10 µL and 15 µL of ethanol extract of Kakoli; Track 10, 11 and 12- 5 µL,

10 µL and 15 µL of chloroform extract of Kakoli for flavonoids.




1 2 3 4 5 6 7 8 9 10 11 12

**Figure 3.** Chromatogram of different solvent extracts of Kshirkakoli at 254 nm.

Track 1, 2 and 3- 5 µL, 10 µL and 15 µL of ethyl acetate extract of Kshirkakoli; Track 4, 5 and 6- 5 µL, 10 µL and 15 µL of chloroform extract of Kshirkakoli for saponins.

Track 7, 8 and 9- 5 µL, 10 µL and 15 µL of ethyl acetate extract of Kshirkakoli; Track 10, 11 and 12- 5 µL, 10 µL and 15 µL of chloroform extract of Kshirkakoli for steroids.

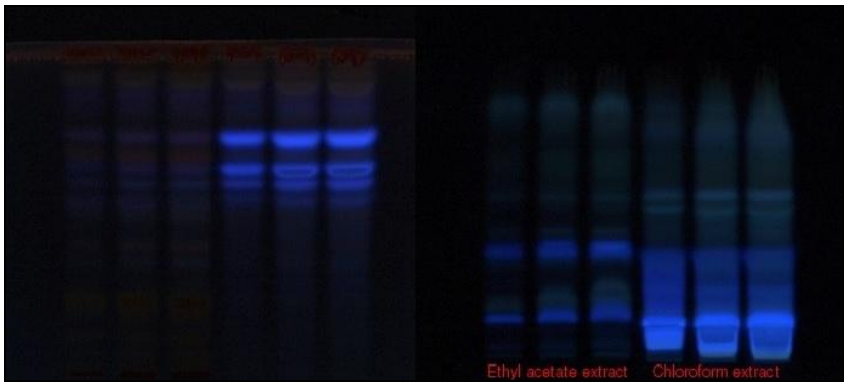



**Figure 4.** Chromatogram of different solvent extracts of Kshirkakoli at 366 nm.
Track 1, 2 and 3- 5 µL, 10 µL and 15 µL of ethyl acetate extract of Kshirkakoli; Track 4, 5 and 6- 5 µL, 10 µL and 15 µL of chloroform extract of Kshirkakoli for saponins.
Track 7, 8 and 9- 5 µL, 10 µL and 15 µL of ethyl acetate extract of Kshirkakoli; Track 10, 11 and 12- 5 µL, 10 µL and 15 µL of chloroform extract of Kshirkakoli for steroids.

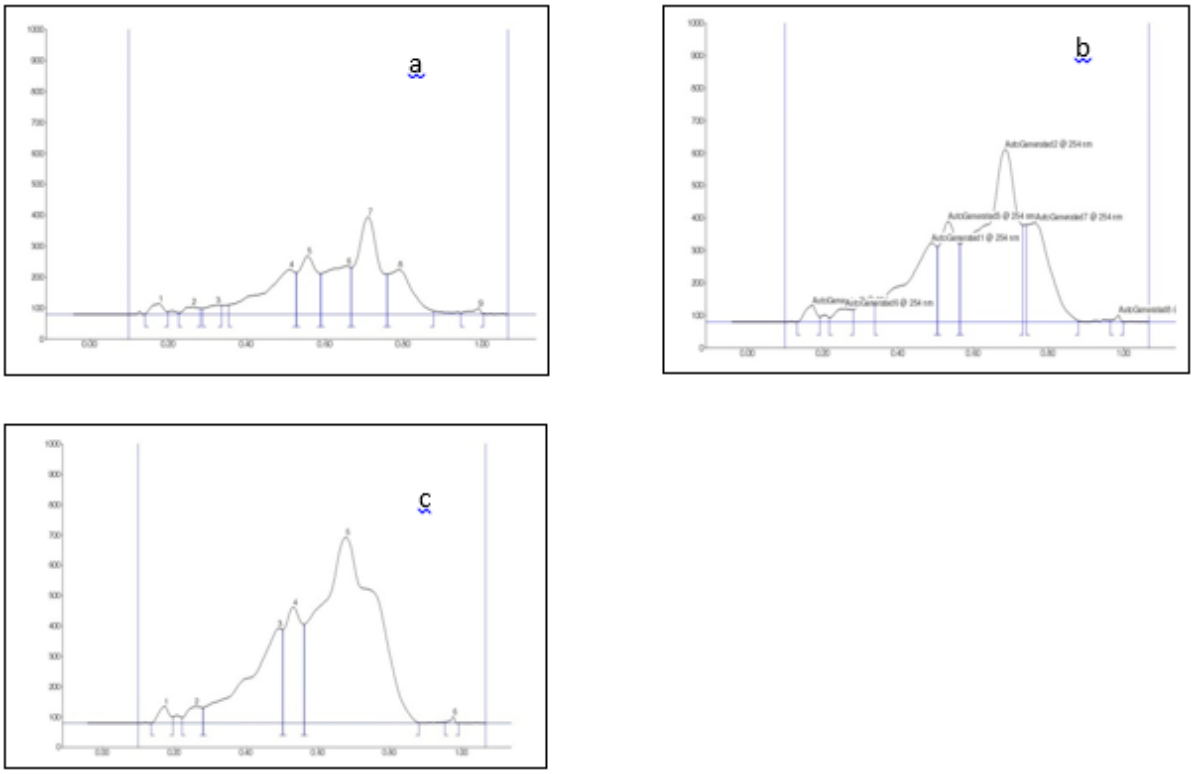



**Figure 5.** Densitogram display for alkaloids a) ethanol extract of kakoli (5 µL) at 254 nm; b) ethanol extract of kakoli (10 µL) at 254 nm; c) ethanol extract of kakoli (15 µL) at 254 nm; Mobile phase- toluene: ethyl acetate: diethylamine (7:2:1).

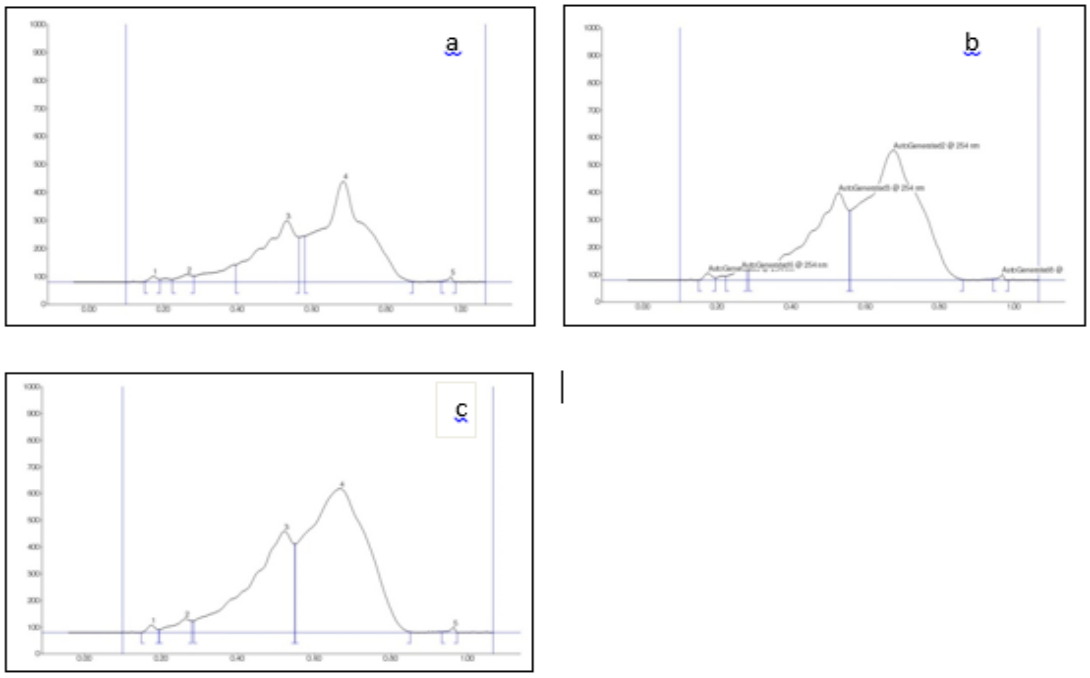



**Figure 6.** Densitogram display for alkaloids a) chloroform extract of kakoli (5 µL) at 254 nm; b) chloroform extract of kakoli (10 µL) at 254 nm; c) chloroform extract of kakoli (15 µL) at 254 nm;

Mobile phase- toluene: ethyl acetate: diethylamine (7:2:1).

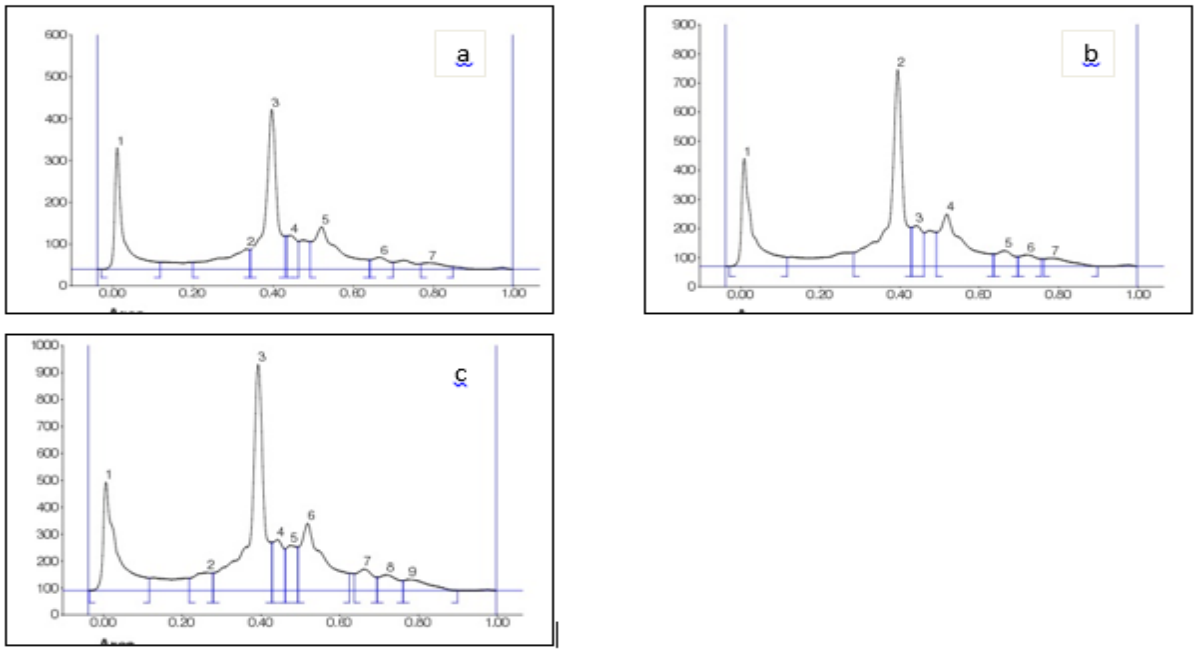



**Figure 7.** Densitogram display for Glycosides a) Ethanol extract of kakoli (5 µL) at 254 nm; b) Ethanol extract of kakoli (10 µL) at 254 nm; c) Ethanol extract of kakoli (15 µL) at 254 nm;

Mobile phase- ethyl acetate: methanol: water (10: 1.4: 1).

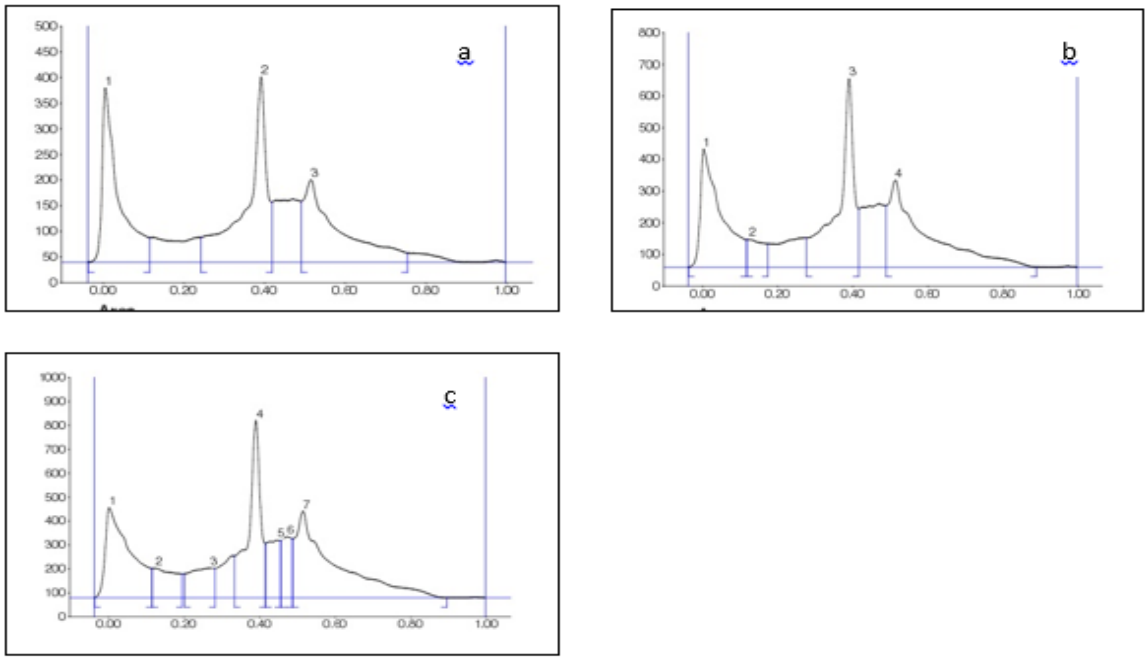



**Figure 8.** Densitogram display for Glycosides a) chloroform extract of kakoli (5 µL) at 254 nm; b) chloroform extract of kakoli (10 µL) at 254 nm; c) chloroform extract of kakoli (15 µL) at 254 nm;

Mobile phase- ethyl acetate:methanol: water (10:1.4: 1).

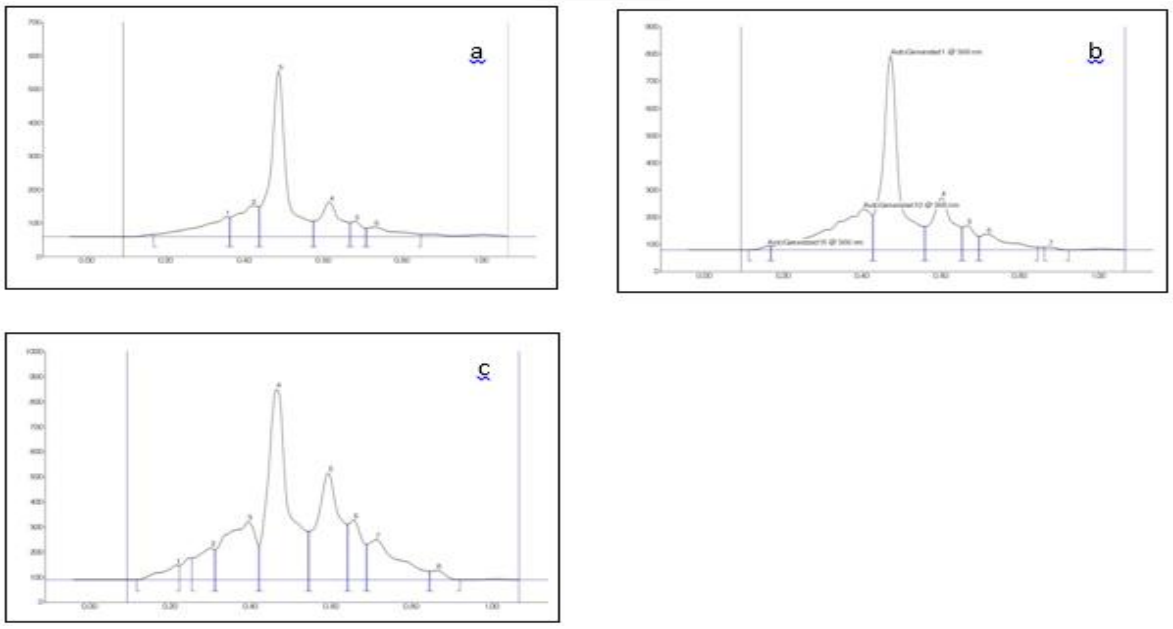



**Figure 9.** Densitogram display for Flavonoids a) ethanol extract of kakoli (5 µL) at 254 nm; b) ethanol extract of kakoli (10 µL) at 254 nm; c) ethanol extract of kakoli (15 µL) at 254 nm;

Mobile phase- ethyl acetate : formic acid : glacial acetic acid : water (10 : 0.5 : 0.5 : 1.3).

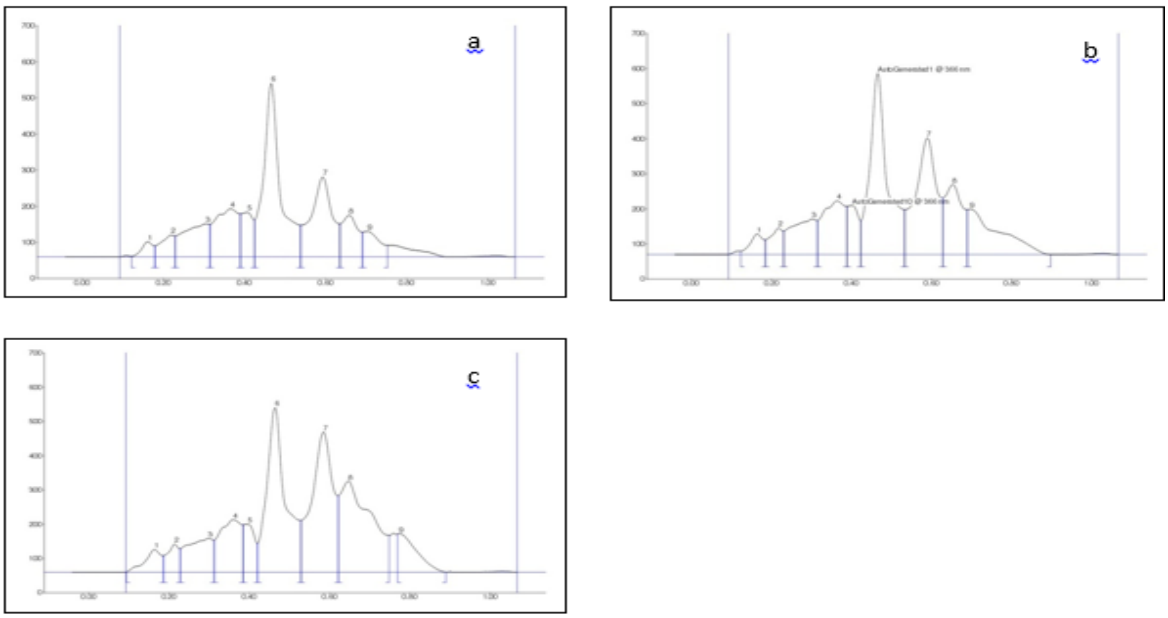



**Figure 10.** Densitogram display for Flavonoids a) chloroform extract of kakoli (5 µL) at 254 nm; b) chloroform extract of kakoli (10 µL) at 254 nm; c) chloroform extract of kakoli (15 µL) at 254 nm;

Mobile phase- ethyl acetate : formic acid : glacial acetic acid : water (10 : 0.5 : 0.5 : 1.3).

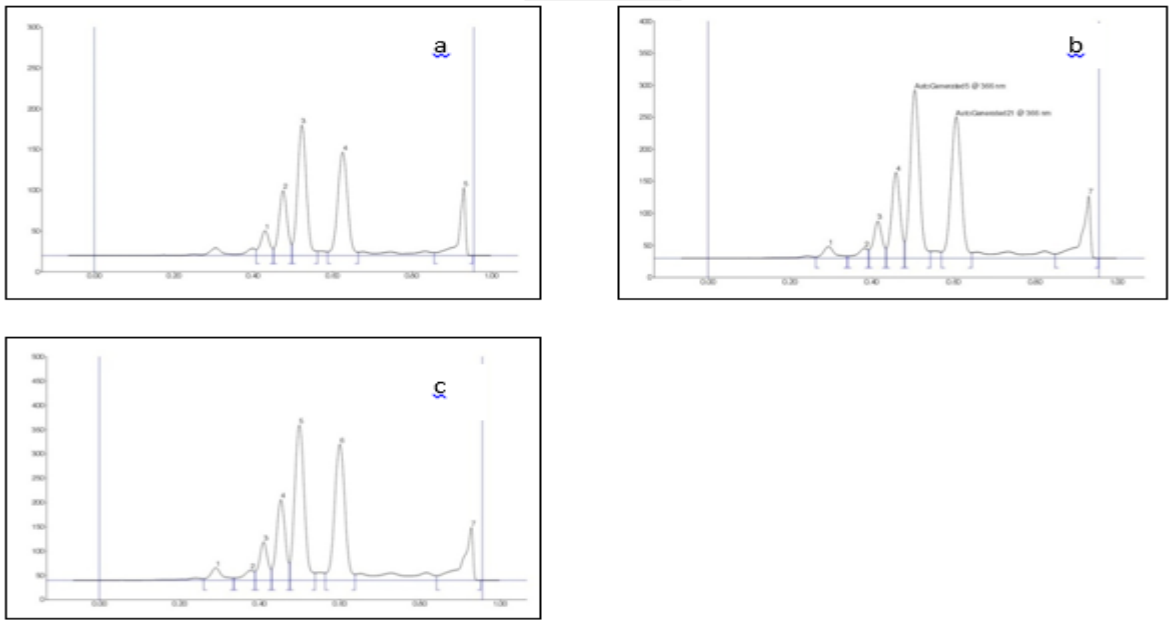



**Figure 11.** Densitogram display for Saponins a) ethyl acetate extract of kshirkakoli (5 µL) at 254 nm;b) ethyl acetate extract of kshirkakoli (10 µL) at 254 nm; c) ethyl acetate extract of kshirkakoli (15 µL) at 254 nm; Mobile phase- Chloroform : Acetic acid : Methanol : Water (6.4 : 3.2 : 1.2 : 0.8).

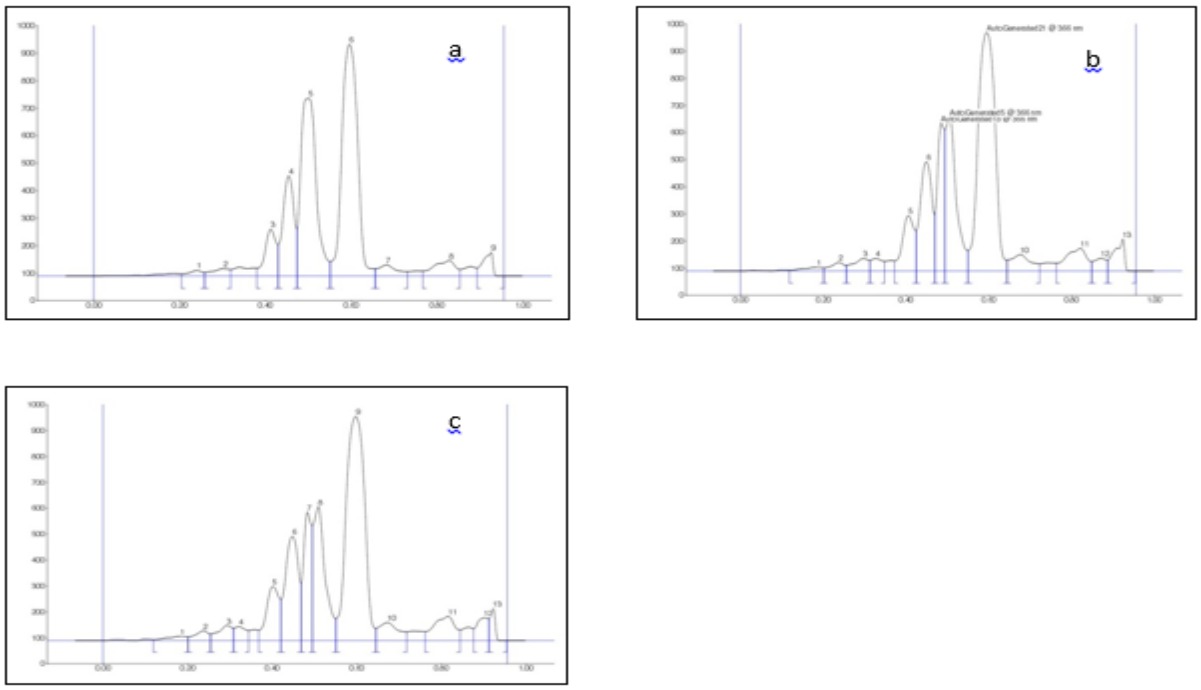



**Figure 12.** Densitogram display for Saponins a) Chloroform extract of kshirkakoli (5 µL) at 254 nm; b) Chloroform extract of kshirkakoli (10 µL) at 254 nm; c) Chloroform extract of kshirkakoli (15 µL) at 254 nm; Mobile phase- Chloroform : Acetic acid : Methanol : Water (6.4 : 3.2 : 1.2 : 0.8).

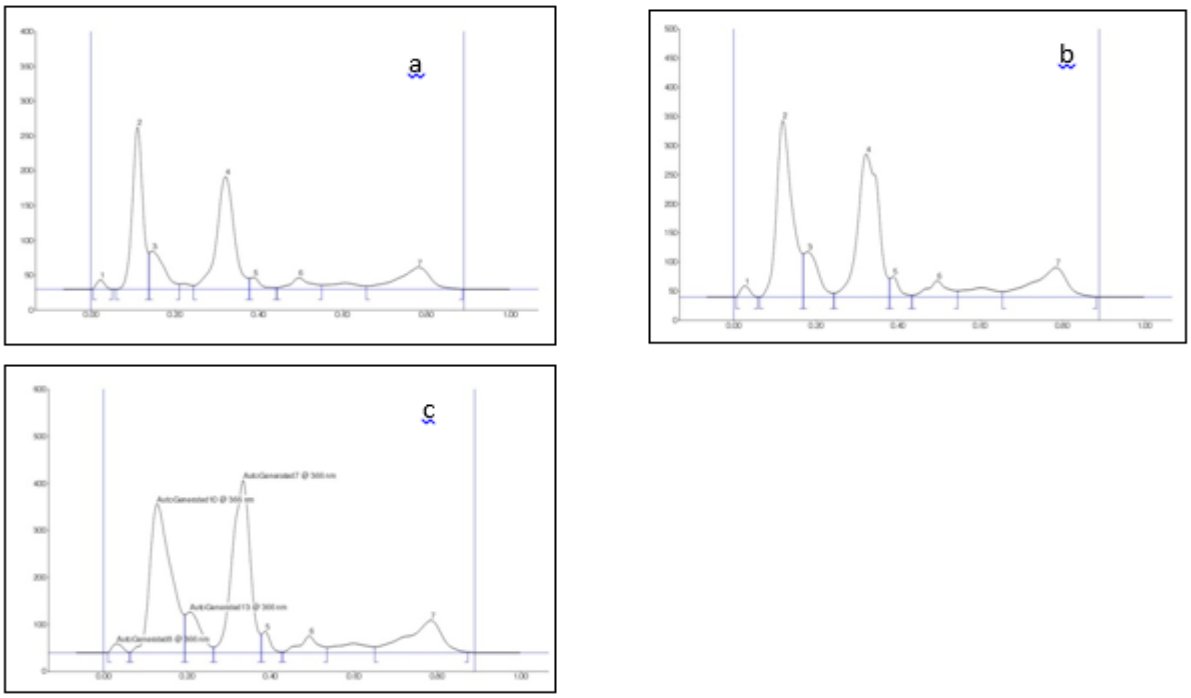



**Figure 13.** Densitogram display for Steroids a) ethyl acetate extract of kshirkakoli (5 µL) at 254 nm; b) ethyl acetate extract of kshirkakoli (10 µL) at 254 nm; c) ethyl acetate extract of kshirkakoli (15 µL) at 254 nm; Mobile phase- N- Butanol : Methanol : Water (3 : 1 : 1)

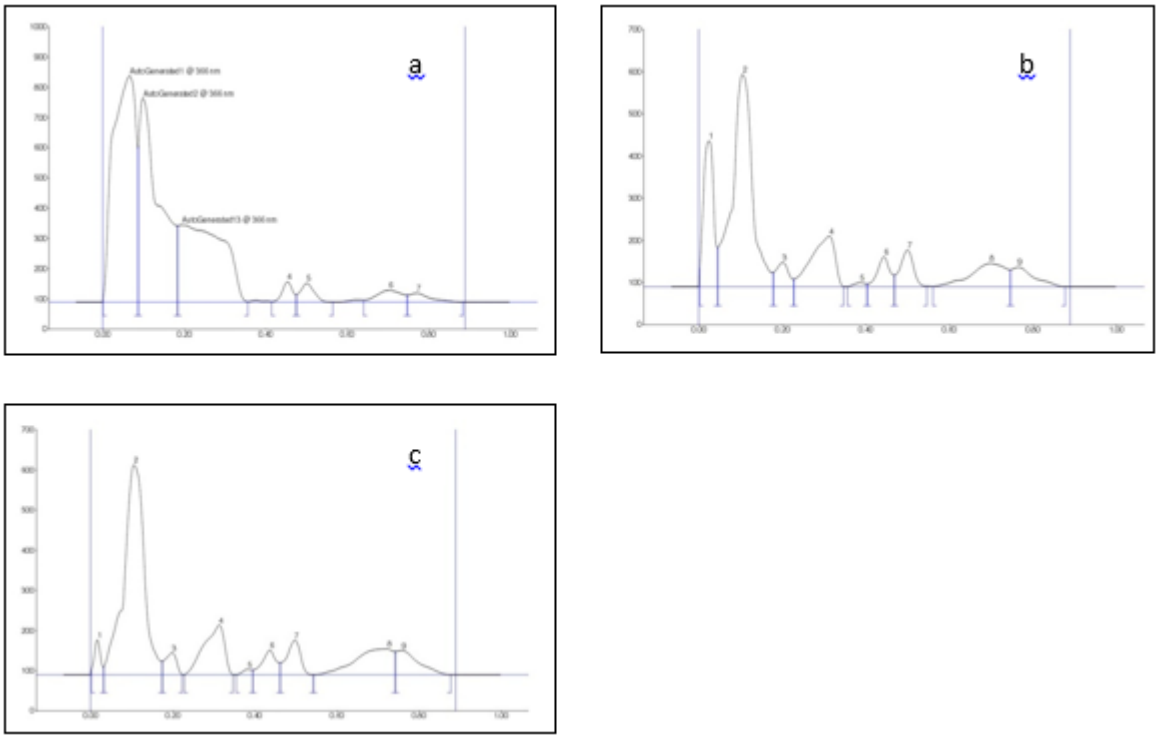



**Figure 14.** Densitogram display for Steroids a) chloroform extract of kshirkakoli (5 µL) at 254 nm; b) chloroform extract of kshirkakoli (10 µL) at 254 nm; c) chloroform extract of kshirkakoli (15 µL) at 254 nm; Mobile phase- N- Butanol : Methanol : Water (3 : 1 : 1).

## DISCUSSION

The phytochemical results of different extracts of the tubers of kakoli and bulbs of kshirkakoli found to contain appreciable amount of primary and secondary metabolites.The HPTLC data for kakoli and kshirkakoli extracts were tabulated in table 1-5. Peak display (chromatogram) and peak densitogram were noted (Figure 1-14). The HPTLC analysis of different extracts of both the plants showed the same or increased number of spots on increasing loading concentration except ethanolic extract of kakoli for alkaloid which showed decrease in number of spots on increasing loading concentration i.e. 9, 7 and 6 spots in 5 µL, 10 µL and 15 µL concentration respectively. The difference in number of spots as per increasing concentration might be due to the concentration of the loading solution and complexity of the solute applied to the layer. This affects the Rf and the resolution which in turn affects the reproducibility of the separation. Application of too much sample tends to overload the chromatographic system, by exceeding the mobile phase capacity or the linear capacity of sorbent. Attraction or repulsion of components of lower concentration by the component of higher concentration can result in change of the Rf values of some

components[18]. The HPTLC results revealed the presence of alkaloid, glycoside and flavonoids in ethanol and chloroform extracts of kakoli while ethyl acetate and chloroform extract of kshirkakoli showed saponins and steroids. Pharmacologically the presence of these phytochemicals explains the use of kakoli and kshirkakoli in ethnomedicine for the management of various ailments. In addition to morphological character, the phytochemical screening along with the HPTLC fingerprinting profile can be use as a tool for estimation of genetic variability in plant population.

## CONCLUSION

Phytochemical screening of ethanol and chloroform extracts of Kakoli and ethyl acetate and chloroform extracts of Kshirkakoli used in this study revealed that these crude extracts contained most of the biologically active phytochemicals. The novel method for HPTLC analysis of these extracts also showed the presence of secondary metabolites such as alkaloids, glycosides, flavonoids, saponins and steroids respectively. The essence of these metabolites is beneficial for maintenance of human health and various other diseases. The plants studied here can be seen as a potential source of useful biomolecules. Further studies are going on these plants in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds.



## ACKNOWLEDGMENT

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