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Analysis of Quality Control Parameters of Commercially available Triphala Churna - A Renowned Polyherbal Formulation of India

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Abstract: ***In recent times, the global preference has shifted from allopathy medicines to herbal supplements for various healthcare needs. India, being a pioneer country of Aayurveda has gifted numerous herbal medicines to the world, that are extremely effective without any side effects. Triphala is one such polyherbal medicine that has worldwide reach as a laxative. Considering broad public consumption of Triphala, it is very much essential to ensure its safety, efficiency and efficacy. In this study, various commercially available Triphala churna samples were screened on the bases of organoleptic, physico-chemical and phytochemical parameters suggested by World Health Organization. Along with these traditional approaches, the present study also includes a modern approach of screening the churna samples with respect to a biochemical marker compound by High Performance Liquid Chromatography (HPLC). The findings of the study indicate a remarkable variation in therapeutically important phytoconstituents among various commercial brands of Triphala. The study emphasizes on strict standardization and quality control guidelines for all the herbal formulations such as Triphala so that maximum people from all around the world can avail their benefits without any reluctance regarding their safety.***

Keywords: Triphala, aayurveda, polyherbal formulation, quality control, HPLC

1. Introduction

Herbal formulations have been gaining acceptance worldwide as therapeutic agents to target various diseases. According to the world health organization, 80% of the world population relies on the herbal remedies for their various healthcare needs (1). India can avail many promising commercial opportunities in the global market. However, there are numerous reports of adulteration in these herbal formulations questioning the authenticity of aayurvedic medicines. The active principles of the ingredient plant species of polyherbal formulations are also subject to variation according to different seasons, environmental conditions, age of the plant etc. Hence, Standardization is an essential factor for polyherbal formulation in order to assess their quality, safety and efficacy. The process of evaluating the quality and purity of crude drugs by means of various parameters like morphological, microscopically, physical, chemical and biological observation is called standardization (2).

The present study focuses on standardization of a popular polyherbal formulation : Triphala. Triphala is one of the most popular polyherbal formulations to be used worldwide. It has various health benefits. This formulation is a very effective laxative used all over the world for the maintenance of a healthy digestive system without showing any side effects. Triphala is 'Sarvadoshaharini'. It balances vaat, pitta, and kapha. According to Ayurveda if these three doshas are balanced, the person is healthy. Triphala is made up of three species i.e. *Terminalia bellerica*, *Terminalia chebula*, and *Phyllanthus emblica*. Considering the worldwide popularity of Triphala churna, it is essential to assess the quality of commercially available Triphala churna samples and ensure its safety, quality and efficacy for public consumption.

The World Health Organization emphasizes the significance of using both qualitative and quantitative biomarker approaches for polyherbal formulation quality control. Gallic acid is employed in this study as a biochemical marker to assess the quality of seven Triphala churna samples that were purchased from Surat, India's local marketplaces. Gallic acid biomarker is estimated both quantitatively and qualitatively using high performance liquid chromatography (HPLC). Plant phenol gallic acid (3,4,5-trihydroxybenzoic acid) is found naturally both free and as a component of the tannin molecule. In addition to its cytotoxicity against cancer cells, gallic acid has anti-tumor, analgesic, hepatoprotective, anti-mutagenic, and anti-inflammatory properties. This study provides qualitative and quantitative essay of the biochemical marker Gallic acid in the commercially available Triphala churna samples with the help of High Performance Liquid Chromatography. Moreover the standard Physiochemical and Phytochemical parameters, suggested by World Health Organization were also evaluated for these Triphala Churna samples to assess their safety, efficiency and efficacy.

2. Literature Survey

Triphala was subjected to phytochemical study (3). According to the study, the tannin content of Terminalia bellirica, Terminalia chebula, and Phyllanthus emblica was estimated to be 21%, 32%, and 28%, respectively. It was discovered that all three fruits have a considerable amount of antioxidant activity. According to the findings of Palav et al., total phenol

contents in herbal formulation raw materials, including triphala, can be utilized for standardization (4)(Palav & D'Mello, 2006). The primary barrier to the standardization of Ayurvedic drugs is their biological source. A marker study of the popular Indian traditional medicine Triphala was conducted (5).

Simulataneous quantitation of gallic acid from fruits of *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz was done. Gallic acid, widely occurring phenolic compound was selected as a bioactive marker due to its easy availability, common presence in these fruits and as antiobesity property. the concept of marker-based standardization of Triphala was done. A simple, sensitive and reliable high performance thin layer chromato-graphic method has been established for simultaneous quantification of gallic acid(6). RP-HPLC method for analysis of Triphala curna was reported for analysis of Triphala Churna using gallic acid, chebulagic acid and chebulinic acid as markers (7). It was reported that the technique is quick and sensitive. The procedure has been validated with provided data. Later on, this technique was refined (8). In triphala churna, ellagic and gallic acid were identified and estimated. To prove the method's selectivity, linearity, accuracy, precision, and robustness, validation was carried out. It was discovered that the suggested RP-HPLC method is straightforward, accurate, and exact; it may be applied to formulation and raw material quality control. High performance thin layer chromatography was used to quantitatively estimate the amounts of gallic and ascorbic acid in commercially available Triphala churna samples (9).

With exceptional resolution, accuracy, and recovery, the approach identified the seven distinct marker compounds in the Haritaki churna.Gallic acid, methyl gallate, ethyl gallate, ellagic acid, chebulagic acid, chebulinic acid, and penta-O-galloyl-β-Dglucose were reported. The hydrolyzable tannins from Triphala churna were quantified by HPTLC densitometric analysis (10). This study has established a straightforward method for the simultaneous quantification of penta-O-galloyl-β-d-glucose (PGG), tetra-O-galloyl-β-d-glucose (TGG), chebulinic acid, chebulagic acid, and gallic acid from various Triphala churna samples using high performance thin-layer chromatography (HPTLC).

For the simultaneous measurement of rutin and gallic acid in the hydroalcoholic extract of Triphala churna, a UV spectrophotometry method was created and validated (7). The ICH requirements were followed in the validation of this method, which can be used for routine analysis of gallic acid and rutin in Triphala churna hydroalcoholic extract. The Triphala formulation was standardized by Bahuguna et al. Investigations were conducted on the organoleptic characteristics, physical attributes, and other physico-chemical attributes such moisture content, ash values, and extractive values. Studies on the heavy metal concentration were also conducted to determine the efficacy, safety, and purity of this polyherbal formulation (11). The quality of commercially sold samples of triphala powder is evaluated (12). Additional morphological and microscopic features of the triphala powder samples revealed the adulteration of the components' powdered endocarp. Among the samples of well-known brands, a notable variance in therapeutically significant phytoconstituents was also noted. The results of this study point to the need for strict quality control procedures to be developed for herbal formulations in order to maximize the advantages of these age-old remedies. In accordance with ICH requirements, Triphala underwent a brief accelerated stability study at 400C/75%RH (13). Throughout the course of the investigation, the concentrations of these active ingredients were tracked and were discovered to be stable: total tannins: 17.16 to 23.49%w/w, and gallic acid: 3.72 to 5.24 %w/w. It was also discovered that the HPTLC chromatographic fingerprint complied with ICH regulations. In a second investigation, the powdered Triphala extracts were screened in five different solvents—water, acetone, chloroform, methanol, and ethanol—for photochemical components. Ten substances, including carbohydrates, tannins, steroids, terpenoids, alkaloids, flavanoids, cardiac glycosides, saponins, coumarins, and others, were found in the preliminary phytochemical investigation (14). Triphala extracts in aqueous and methanolic forms were subjected to phytochemical analysis and in-vitro biochemical characterization (15).

Problem Definition: For the benefit of public health, pharmaceutical companies should all adhere to the same set of quality control requirements. In order to verify the current standards of safety and efficacy of Triphala churna circulating in the market, this study screens seven commercially available samples collected from Surat, Gujarat, India. It also streamlines the standardization process and includes all the major standardization parameters suggested by the World Health Organization.

3. Materials and Methods

Procurement of Samples: Triphala churna samples from seven reputed pharmacies were procured from the local market of Surat, Gujarat, India

Organoleptic Evaluation: All the organoleptic properties viz. color, odour, taste, and texture of the drug to touch were performed as per standard procedure and noted down

Physico-Chemical Evaluation:

Determination of total ash: The total ash content was determined by the protocol mentioned hearewith (12). Two grams of air-dried material were placed in a crucible that had already been lit, weighed, and burned at 500–600 ˚C until the matter turned white, signifying the lack of carbon. After being allowed to cool in a desiccator, the crucible was weighed with a weighing machine. The total ash content was calculated using the following formula:

Total ash (%) = (final weight of crucible containing ash–initial weight of ignited crucible) ×100

Determination of acid insoluble ash content: Acid insoluble ash content was determined by the protocol followed by Sharma et al. (2015)(12).To the crucible containing the total ash, 15 ml of hydrochloric acid was added, covered with a watch-glass and made to boil gently for 5 minutes. A watch-glass with vapours was rinsed with 5 ml of hot distilled water and this water was added to the crucible. Insoluble matter was

collected on an ash less filter paper and washed with hot water until the filtrate became neutral. Filter-paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in suitable desiccators for 30 minutes and then weighed. The acid insoluble content was determined using the following formula:

$$\text{Insoluble Ash (\%)} = \frac{Weight\ of\ acid\ insoluble\ residue}{Weight\ of\ the\ sample\ taken} \times 100$$

Measurement of total moisture (12): 2-gram powder of triphala was taken in a previously dried and tarred flat weighing bottle and kept in an oven 105˚C. Samples were dried again and again until the weight of the sample in two consecutive weighing did not differ by more than 5mg and then weight this sample using a way machine and recorded. The moisture content was calculated using the following formula:

$$MC = \frac{M2-M3}{M3-M1} \times 100$$

Where MC= Moisture content, M1 is mass of empty container, M2 is mass of container plus sample, M3 is mass of dried sample plus container

Measurement of pH: The pH of all the seven commercially available Triphala sample was measured by using previously calibrated pH meter and recorded. 5% solution was prepared for each sample with distilled water and shaken for an hour before the pH was recorded.

Qualitative Phytochemical analysis:
All seven Triphala churna samples were prepared for extraction and the extracts were evaluated for the presence of major phytochemicals such as Flavonoids, Tannins, Alkaloids, Glycosides, Terpenoids, Steroids, Phenolic Compounds, and Saponins. (2).

Extract preparation: 1 gm powder of Triphala Churna were extracted with 100 ml distilled water by heating at 70-80˚C for 1 hr separately. Extract was filtered and volume adjusted to 100 ml in volumetric flask

Quantitative analysis of Phytochemicals:

Extract preparation: 1 gm powder of Triphala Churna were extracted with 100 ml distilled water by heating at 70-80˚C for 1 hr separately. Extract was filtered and volume adjusted to 100 ml in volumetric flask

All the seven sample extracts were further assessed for the following phytochemical parameters:

Estimation of total phenols (16)**:**

Preparation standard stock solution: 100 µg/ml Gallic acid standard stock solution was prepared by 10mg Gallic acid dissolved in methanol and makeup volume up to 100 ml with methanol in volumetric flask.

Procedure: 1ml of the sample solution was transferred into 25 ml volumetric flask. 0.5, 0.75, 1.0, 1.25, 1.5, and 2.0 ml of the standard stock solution were put into a 25 ml volumetric flask, yielding concentrations of 2, 3, 4, 5, 6, and 8 µg/ml, respectively. Each volumetric flask was filled with 10 ml of water and 1.5 ml of Folin-Ciocalteu reagent (Folin ciocalteu reagent: distilled water (1: 2)). After letting the mixture sit for five minutes, four milliliters of a 20% sodium carbonate solution were added. Distilled water was added to the volume to make it to 25 ml. After 30 minutes, the blue color absorbance of these combinations was measured at 765 nm. The calibration curve of gallic acid was used to determine the percentage of total phenols.

Estimation of Tannin (17) **:**
Total Tannin content was estimated by the redox titration protocol.. 10 ml of extract from stock solution was transferred into 500 ml conicalflask.10 ml indigo carmine was added in flask as indicator then volume was adjusted up to 300 ml. Solution was heated at 70˚C for 20min. Solution was titrated with 0.1 N $KMnO_4$(3.16g $KMnO_4$ per 1000 ml of Distilled water). End point was characterized by change color of solution from colorless to pink. Factor: 1 ml of 0.1 N $KMnO_4$ = 0.004157 g. of total tannin calculated as tannic acid.

Estimation of Ascorbic acid (22):
Ascorbic acid was estimated by the protocol given by Sadasivam, S. and Balasubramanian, T. (1987)

Preparation of stock standard solution: 100 mg ascorbic acid was dissolved in 100 ml of 4% oxalic acid solution in a standard flask.

Preparation of working standard: 10 ml of the stock solution was diluted to 100 ml with 4% oxalic acid. The concentration of working standard was 100 g/ml.

Procedure: 5 ml of working standard solution pipetted out in 100 ml conical flask. 10 ml of 4 % oxalic acid was added and titrated against the dye (V1 ml) (Dye preparation: 42 mg sodium bicarbonate was dissolved into a small volume of distilled water. 52 mg 2, 6-dichloro phenol indophenols was dissolved in it and made up to 200 ml with distilled water). End point was the appearance at pink colour which persists for a 30 seconds. The amount of the dye consumed was equivalent to the amount of ascorbic acid. The sample (2 g) was extracted in 4 % oxalic acid and made the volume 100 ml and centrifuged. Pipetted out 5 ml of supernatant, 10 ml of 4% oxalic was added and titrated against the dye (V2 ml). The % of Vitamin C was calculated by the formula mentioned as bellow.

$$\text{Amount of Ascorbic acid (mg/100g)} = \frac{0.5\ mg}{V1} \times \frac{V2}{5\ ml} \times \frac{100\ ml}{Weight\ of\ the\ sample\ taken} \times 100$$

Estimation of antioxidant activity (18) : Antioxidant activity was performed by phosphomolybdate method mentioned by Saeed et al. (2012). A 0.1-ml aliquot of the sample solution was shaken with 1 mL of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tubes were covered and incubated in a water bath at 95°C for 90 min. After the samples were cooled, the absorbance of the mixture was measured at 765 nm. Ascorbic

acid was used as standard. The antioxidant capacity was estimated using the following formula:

$$\text{Total antioxidant activity (\%)} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Biochemical marker analysis:

Gallic acid was selected as a marker bioactive compound to evaluate the quality of Triphala churna. It's a common compound found in all three plant species of Triphala. Hence, it can be utilized to assess the authenticity of Triphala churna samples. All the samples were extracted and subjected to HPLC for qualitative and quantitative estimation of Gallic acid. The method for the same was as follows:

1) Chromatographic system: High Performance Liquid Chromatographic system using Empower 3 software.

- Column: PURITAS^{TM} EXIMUS C18, 5 µm, 100A
- Detection wavelength: 270 nm wavelength Using Waters 2487 Dual λ Absorbance detector
- column temp: 25 ºC,
- Injection volume: 20 µl.
- Flow rate : 1.50 ml/min for 45 minutes

2) Chromatographic Conditions:

- Solution A (Buffer): In 900 ml of HPLC grade water, 0.136 g of anhydrous potassium dihydrogen orthophosphate (KH2PO4) was dissolved. The volume is made upto 1000 mL with distilled water. The solution is then filtered through 0.45 m membrane followed by the degassing in a sonicator for 3 minutes.
- Solution B: Acetonitrile.

The concentration gradient of mobile phase was according to the Table 1.

Table 1: Mobile phase composition using Gradient method

Time (min)	Buffer concentration (%)	Acetonitrile concentration (%)
0.01	95	5
18	80	20
25	65	35
28	65	35
35	80	20
40	95	5
45	95	5

1) Standard preparation: For the standard Gallic acid solution, 0.1 mg/ml concentration was prepared. 10 mg of Gallic acid was weighed in to 100 ml volumetric flask and dissolved in 50 ml of hot water by sonication, cooled down and made up to 100 ml with water. To prepare a standard curve for quantitative analysis different Gallic acid standard concentrations were prepared i.e. 0.02 mg/ml, 0.04 mg/ml, 0.06 mg/ml, 0.08 mg/ml. Peak areas for each concentration were measured at 270 nm wavelength and standard curve was prepared.
2) Triphala sample preparation: 0.250 g churna samples of all the seven pharmacies were collected in a 100 ml volumetric flask, dissolved in 50ml of hot water and sonicated for 10 minutes. The samples were cooled down and the volume is made upto 100 with distilled water. The samples were filtered through 0.45 microns (PES filter papers only) membrane filter papers. 20 microlitlre samples were injected along with the Gallic acid standard solution and the chromatograms were recorded at 270 nm.

4. Results and Discussion

All the seven commercially available Triphala churna samples, collected from different regions of Gujarat, India were successfully evaluated on the bases of their major organoleptic, physicochemical and biochemical parameters. All the parameters reported in Table 2, show significant variation among different market samples.

Table 2: Organoleptic evaluation of commercially available Triphala churna samples

No	Properties	TCS1	TCS2	TCS3	TCS 4	TCS 5	TCS 6	TCS 7
1	Appearance	Powder	Powder	Powder	Powder	Powder	Powder	Powder
2	Colour	Yellowish brown	Yellowish brown	Light brown	Light brown	Light brown	Yellowish brown	Brown
3	Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	characteristic
4	Taste	Salty and sour	Salty and sour	Bitter	Salty and sour	Salty and sour	Bitter	Bitter
5	Texture	Fine powder	Fine powder	Fine powder	Fine powder	Fine powder	Fine powder	Moderately fine powder

Physicochemical evaluation:

During the present investigation, the total ash content varied between 2.67% to 8.45%. Whereas insoluble ash content was observed in the range of 0.5% to 7.6%. The pH values in all the seven Triphala samples were found to be around 3. Collected samples of Triphala powder were analysed for their moisture content percentage. The samples showed variation from 4.16% to 8.5% in their values. The values of all the parameters are mentioned in Table 3 and graphically represented in Figure 1.

Table 3: Physicochemical evaluation of commercially available Triphala churna samples TCS1 to TCS7

Sample Name	Total ash content (%)	Acid insoluble ash (%)	pH	% Moisture content
TCS1	3.56	0.66	3.45	5.25
TCS2	8.45	1.55	3.16	3.90
TCS3	2.67	0.25	3.78	7.67
TCS4	7.34	0.80	3.00	8.50
TCS5	6.34	0.53	3.24	4.16
TCS6	4.50	0.74	3.90	3.45
TCS7	4.41	0.62	3.88	5.32

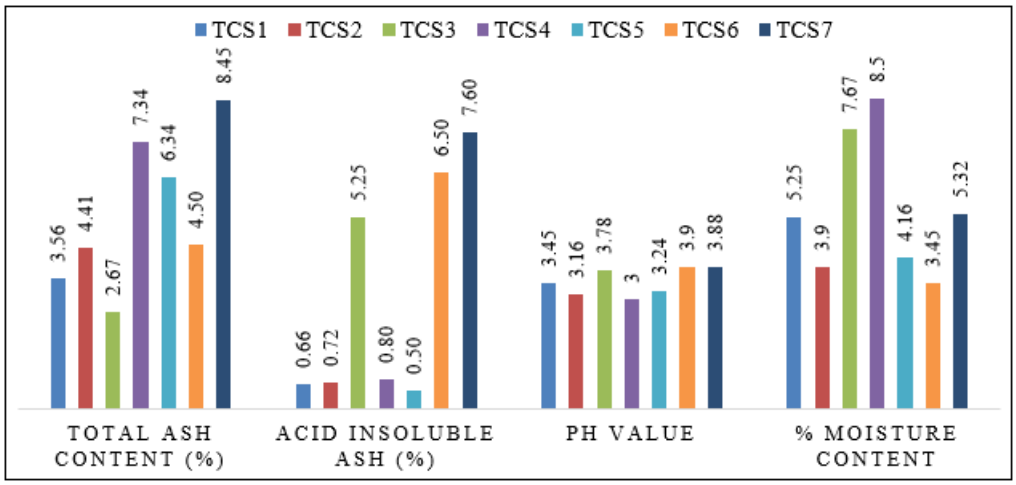

Figure 1: Comparative analysis of variation among physicochemical parameters of commercially available triphala churna samples)

Qualitative phytochemical analysis: All the seven samples were evaluated for the presence of major phytochemicals like, tannins, Phenols, flavonoids, steroids, alkaloids, terpenoids, saponin, glycosides. The results are recorded in table 4.

Table 4: Qualitative evaluation of major phyto constituents in commercially available Triphala churna samples

Sr.no	Phyto constituents	Name of test	TCS1	TCS2	TCS3	TCS4	TCS5	TCS6	TCS7
1	Alkaloids	Mayer's test	+	+	+	+	+	+	+
2	Flavonoids	Lead acetate	+	+	+	+	+	+	+
3	Saponin	Foam test	+	+	+	+	+	+	+
4	Tanins	Ferric chloride test	+	+	+	+	+	+	+
5	Terpenoids	Copper acetate test	-	-	-	-	-	-	-
6	Phenols	Ferric chloride test	+	+	+	+	+	+	+
7	Steroids	Salkowski's test		+	+	+	+	+	+
8	Glycosides	Keller Killani's test	-	-	-	-	-	-	-

Quantitative phytochemical analysis: The level of major therapeutically important phytoconstituents vary significantly among various brands of Triphala churna. Tannins, Ascorbic acid and antioxidant activity in Triphala powder of different brands. The concentration of total phenolics was found between 20.45% and 60.20%. The level of Tannins were found between 10.70% to 40.20%. The ascorbic acid content was found between 0.53% to 1.72%. Antioxidant activity was found high in all the seven samples. The variation in the values is mentioned in Table 5 and graphically represented shown in Figure 2.

Table 5: Evaluation of total phenolics, Tannins, Ascorbic acid, Antioxidant activity levels in commercially available Triphala churna samples

Sr no.	Sample Name	Total Phenol (%)	Tannis (%)	Ascorbic acid (%)	Antioxidant activity (%)
1	TCS1	32.87	28.25	0.99	98.48
2	TCS2	20.45	40.20	0.69	95.63
3	TCS3	57.80	18.94	1.08	95.42
4	TCS4	60.20	36.20	0.80	97.54
5	TCS5	27.15	20.68	1.46	97.31
6	TCS6	42.39	23.55	1.72	96.72
7	TCS7	35.55	10.70	0.53	94.23

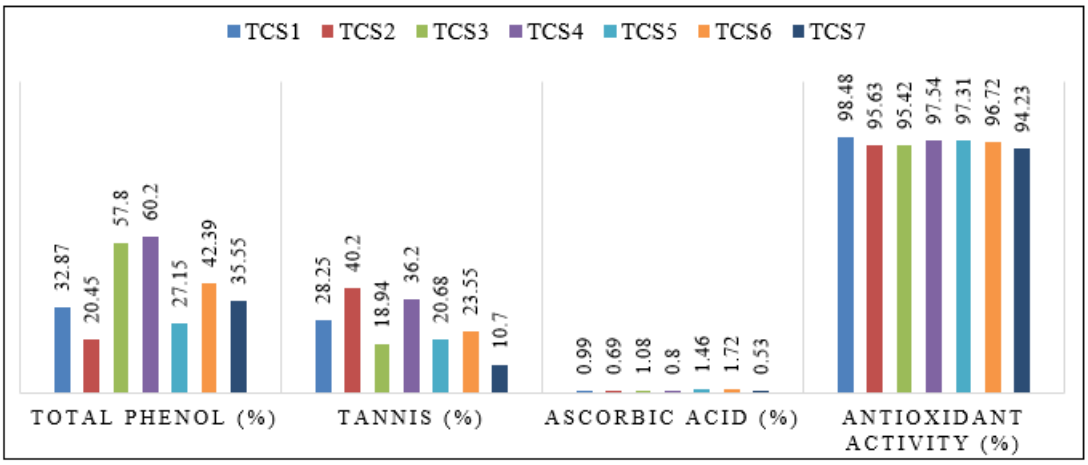

Figure 2: Comparative analysis of variation in quantitative parameters in commercially available Triphala Churna samples.**)**

Quantitative estimation of Biochemical marker Gallic acid by High Performance Liquid Chromatography: In the current study quantitative estimation of specific biologically active Gallic acid component was successfully conducted in the seven commercially available Triphala churna samples for their quality control purpose. The concentration of Gallic acid is calculated by using calibration curve of standard Gallic acid. Standard gallic acid solution of 0.1 mg/ml was diluted to dilutions 0.02 mg/ml, 0.04 mg/ml, 0.06 mg/ml and 0.08 mg/ml and these solutions were analysed to study linearity. Area count for each concentration and the graph showing linearity are presented in table 6 and Figure 3 respectively. Figure 4 indicates the autoscaled chromatogram of Gallic acid standard of 0.1 mg/ml. A chromatogram of standard Gallic acid solution (0.1 mg/ml) is shown in figure 4.

Table 6: Standard area counts for different concentrations of Gallic acid

Sr no.	Concentration of Gallic acid (mg/ml)	Area count
1	0.02	919707
2	0.04	1827733
3	0.06	2708368
4	0.08	3663683

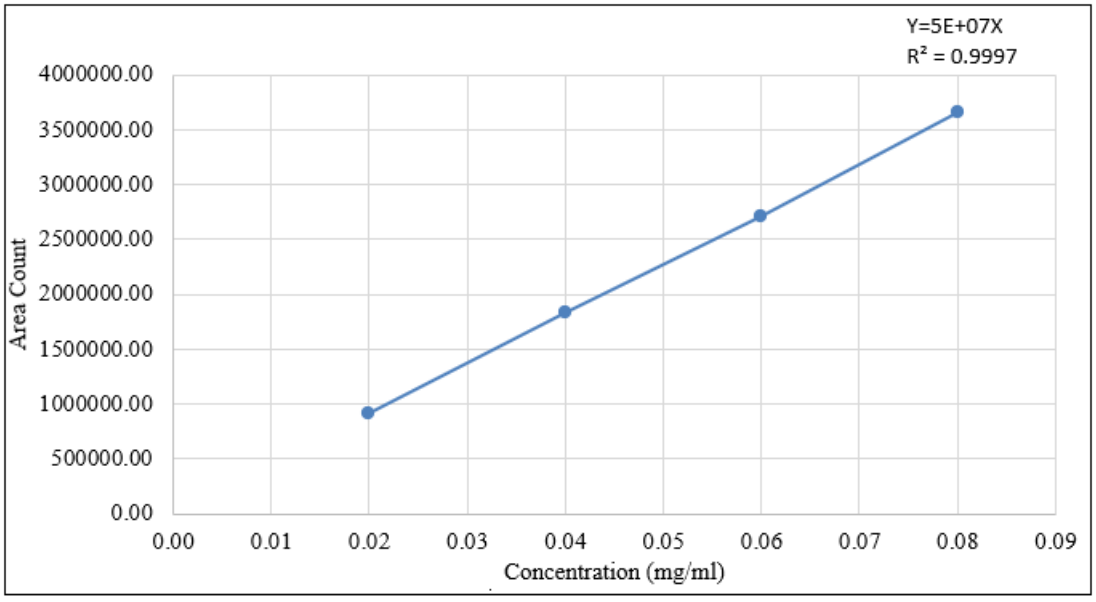

Figure 3: Standard curve of Gallic acid concentration vs Area**)**

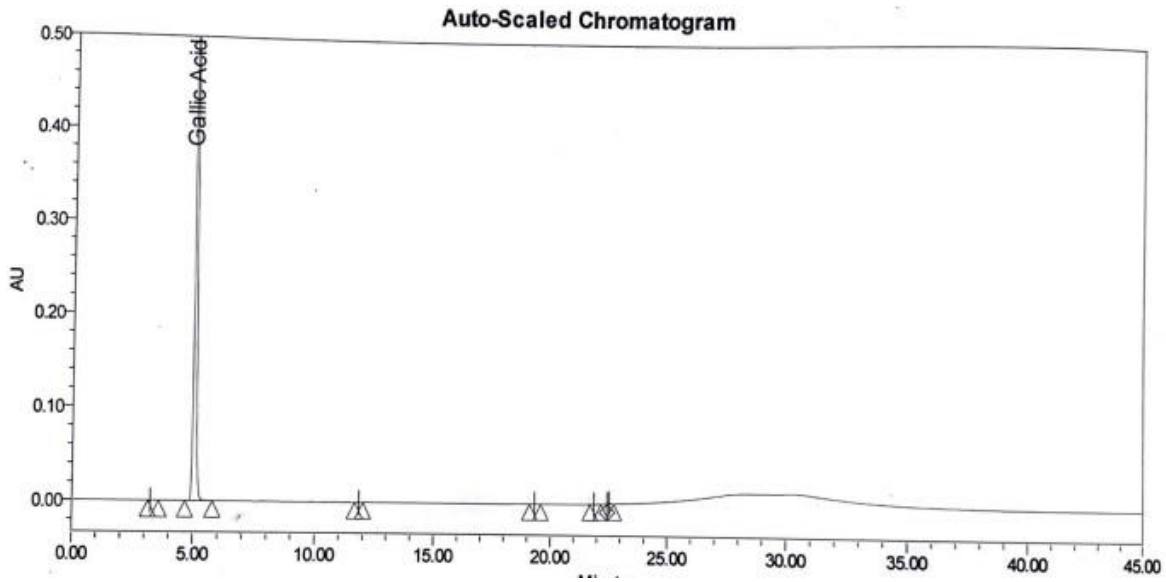

Figure 4: Chromatogram of Gallic acid standard with known 0.1 mg/ml concentartion

All the seven commercially available Triphala churna samples along with the Triphala component species *Terminalia bellirica*, *Terminalia chebula* and *Phyllanthus emblica* fruit powders were subjected to quantitative estimation of biochemical marker Gallic acid with the help of HPLC. The obtained results of HPLC parameters for each sample are mentioned in Table 7. The chromatograms of all the seven Triphala churna samples TCS1 to TCS7 are shown in Figures 5a, 5b, 5c, 5d, 5e, 5f, 5g respectively. The chromatograms of the fruit powders of *Terminalia bellirica*, *Terminalia chebula* and *Phyllanthus emblica* are shown in Figures 6a, 6b and 6c respectively.

Table 7: HPLC parameters of standard Gallic acid, Triphala churna samples TCS1 to 7, Fruit powders of *T. bellirica*, *T. chebula* and *P. emblica*

Sr no.	Sample Name	Area	Concentration mg/ml	Area (%)	Height	Retention Time
1	Gallic acid(Standard)	4316346	0.1	99.59	662710	5.011
2	TCS1	1660293	0.0364	21.17	274079	5.022
3	TCS2	1859429	0.0408	24.60	304008	5.033
4	TCS3	2563850	0.0563	33.37	416434	5.032
5	TCS4	1756259	0.0385	33.57	284794	5.041
6	TCS5	3148611	0.0691	26.48	511110	5.038
7	TCS6	1635246	0.0359	31.79	263548	5.028
8	TCS7	1984138	0.0435	22.86	319903	5.014
9	*T. bellirica* fruit powder	3057091	0.0671	54.31	451518	5.008
10	*T. chebula* fruit powder	2082355	0.0457	11.63	322785	5.005
11	*P. emblica* fruit powder	2909039	0.0638	35.42	464940	5.007

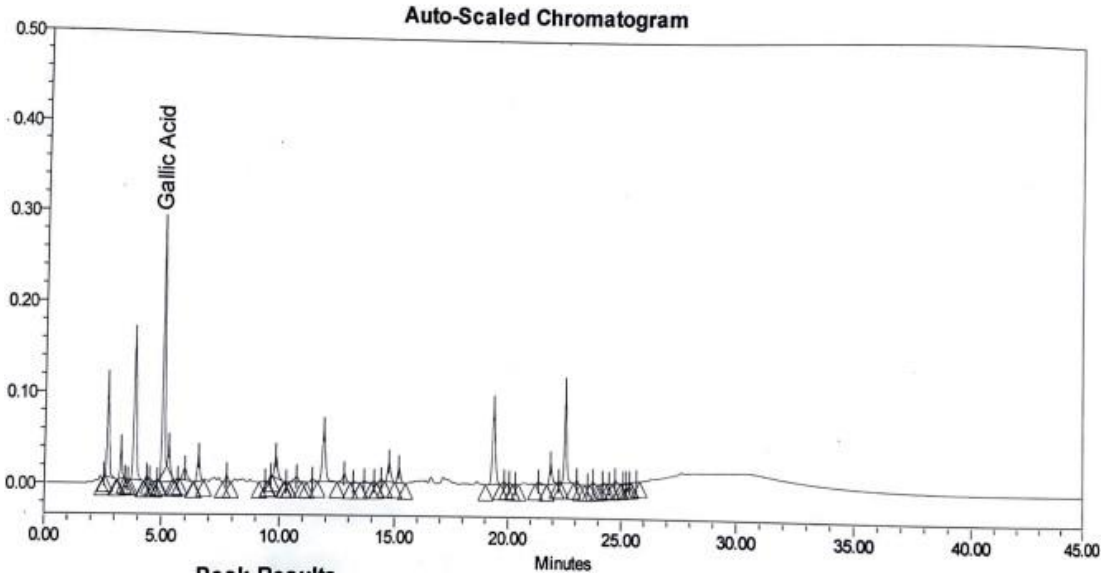

Figure 5 (a): Chromatogram of the Triphala sample TCS1**)**

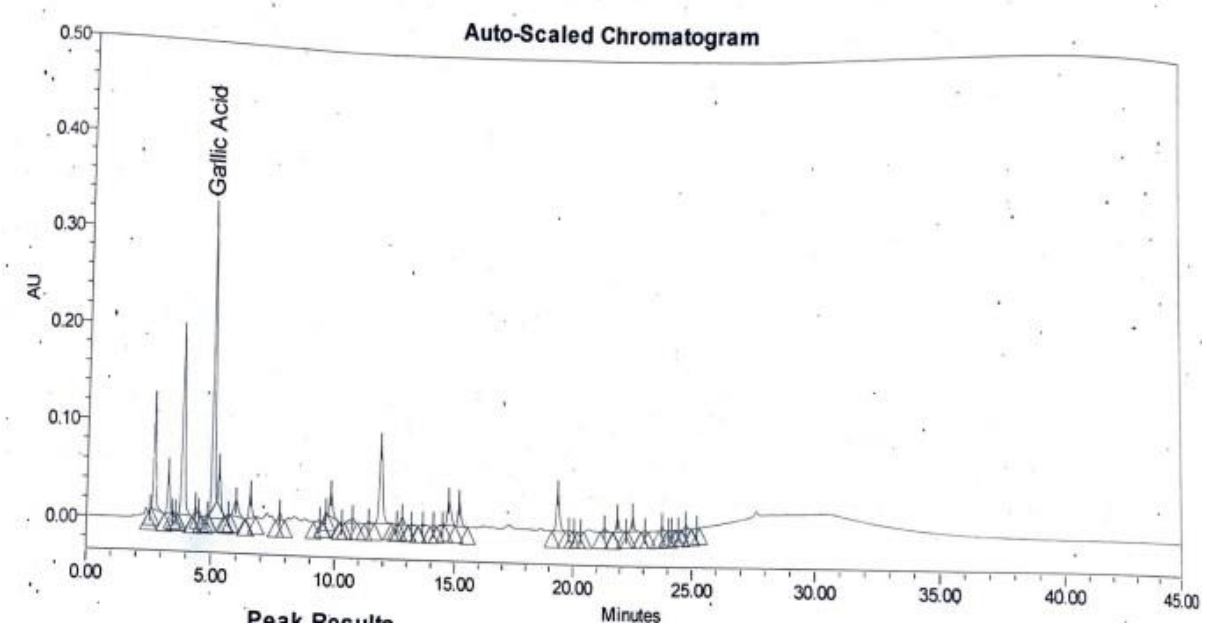

Figure 5 (b): Chromatogram of the Triphala sample TCS2**)**

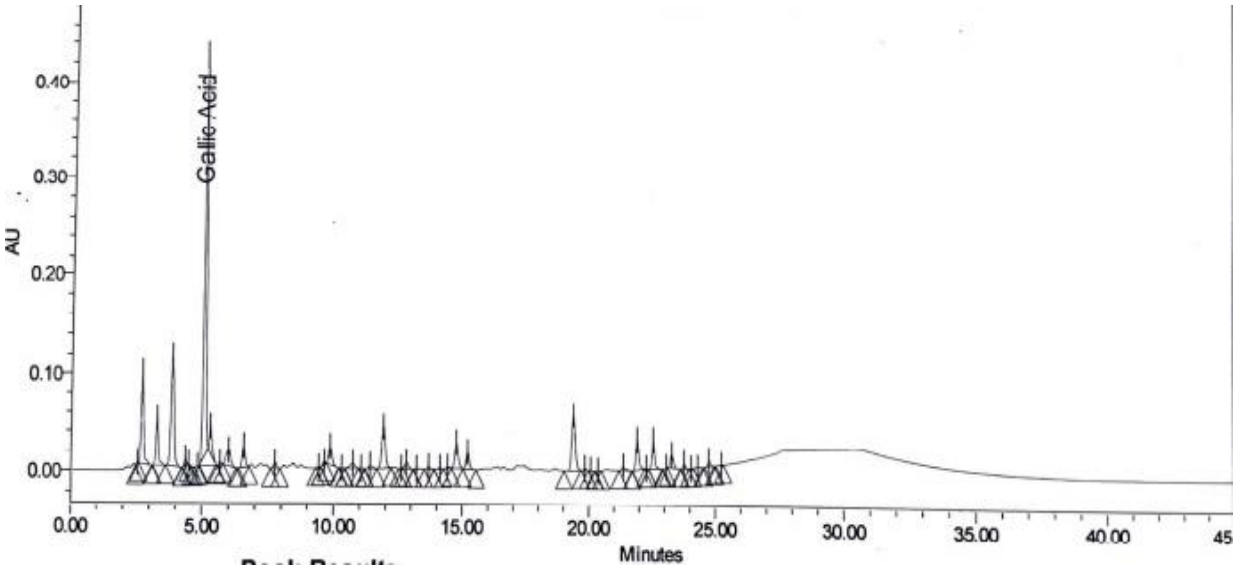

Figure 5 (C): Chromatogram of the Triphala sample TCS3**)**

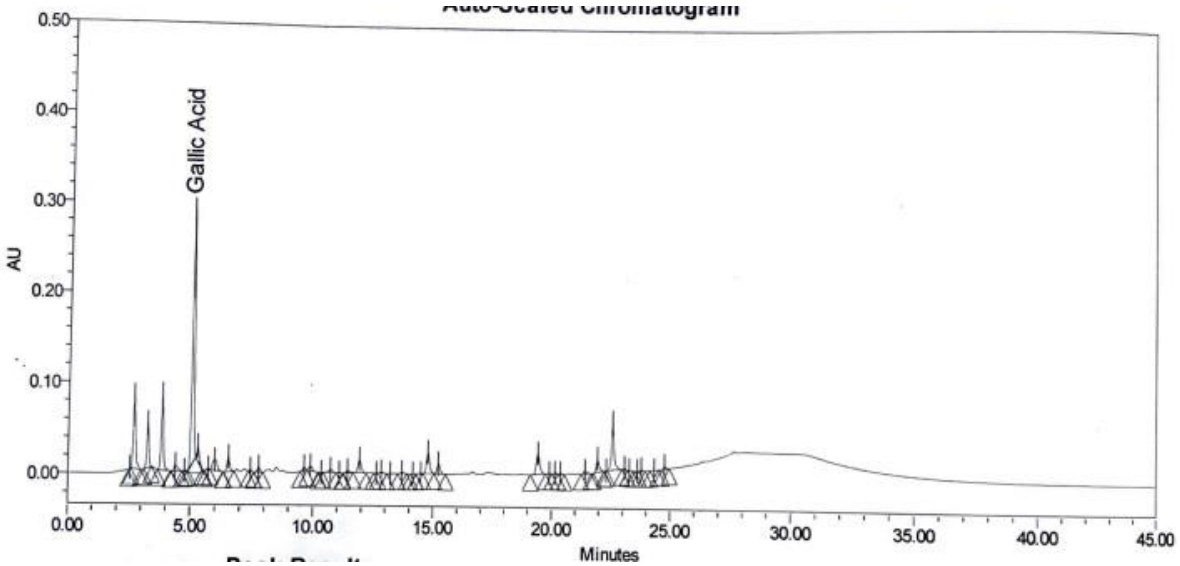

Figure 5 (d): Chromatogram of the Triphala sample TCS4**)**

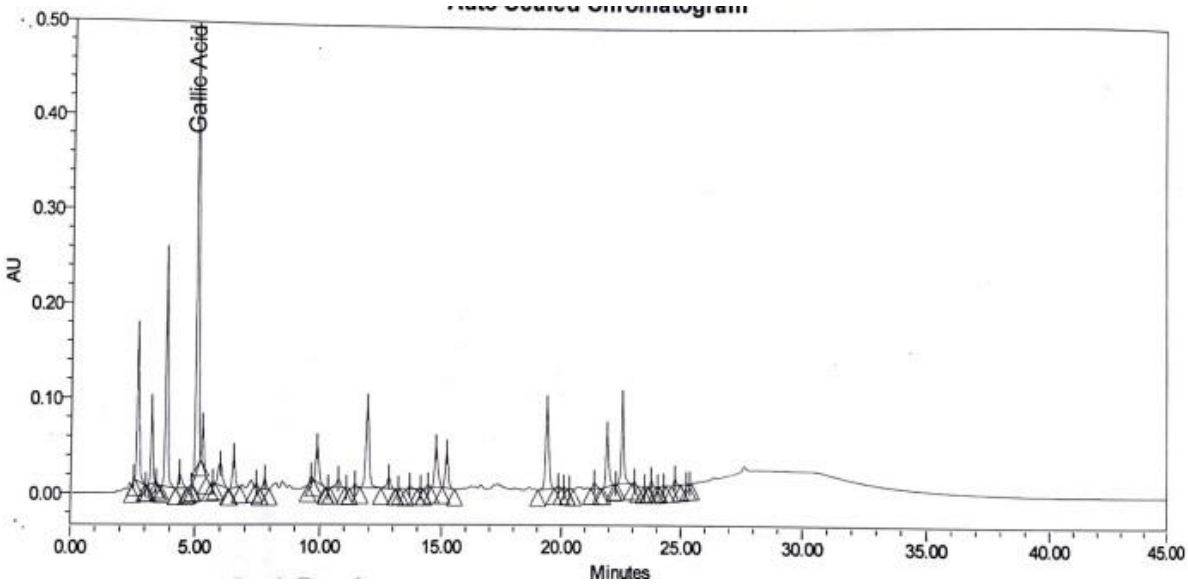

Figure 5 (e): Chromatogram of the Triphala sample TCS5**)**

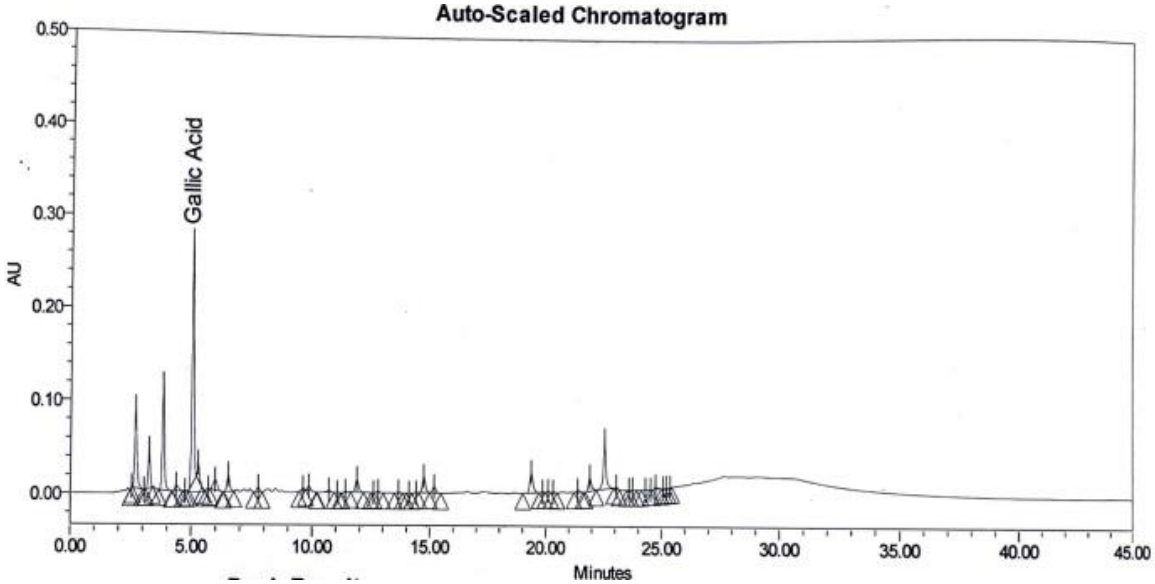

Figure 5 (f): Chromatogram of the Triphala sample TCS6**)**

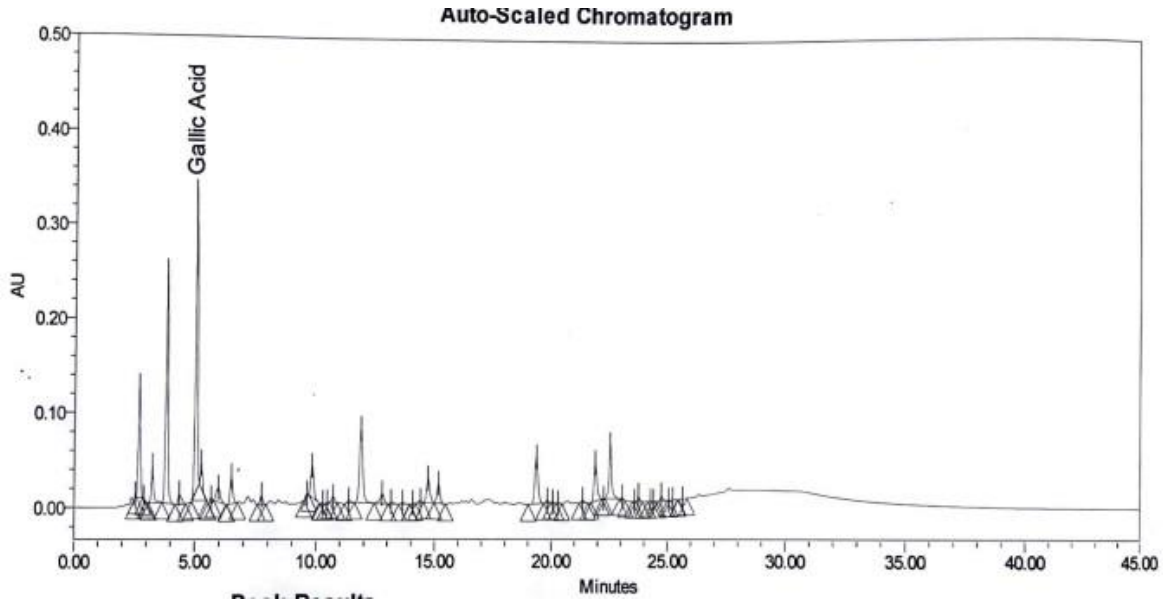

Figure 5 (g): Chromatogram of the Triphala sample TCS7

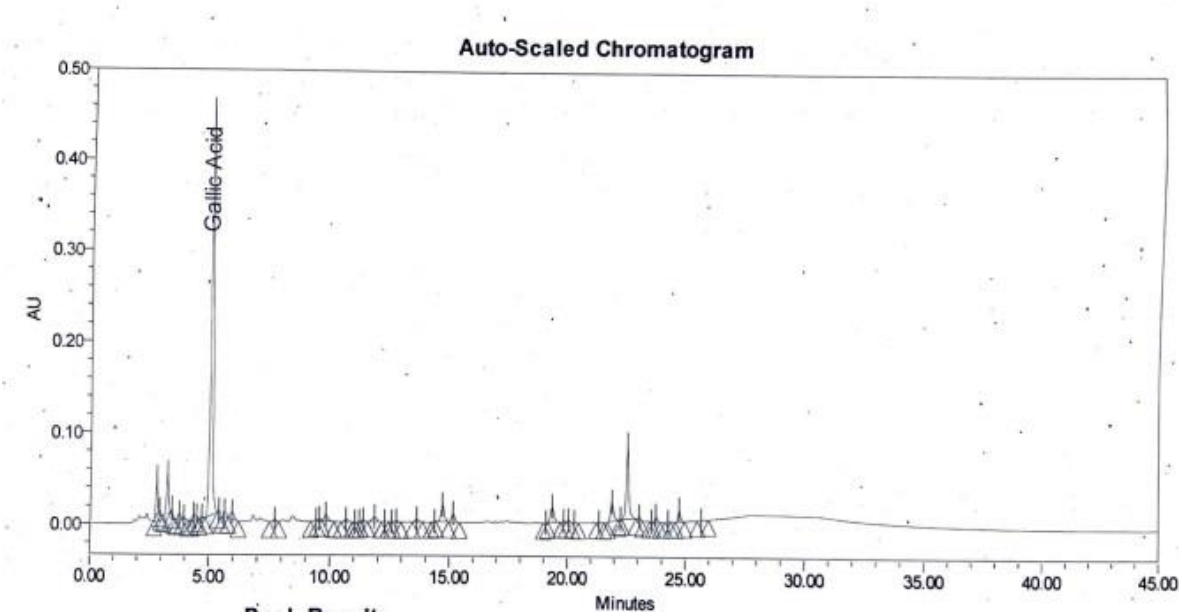

Figure 6 (a): Chromatogram of the *Terminalia bellirica* fruit powder**)**

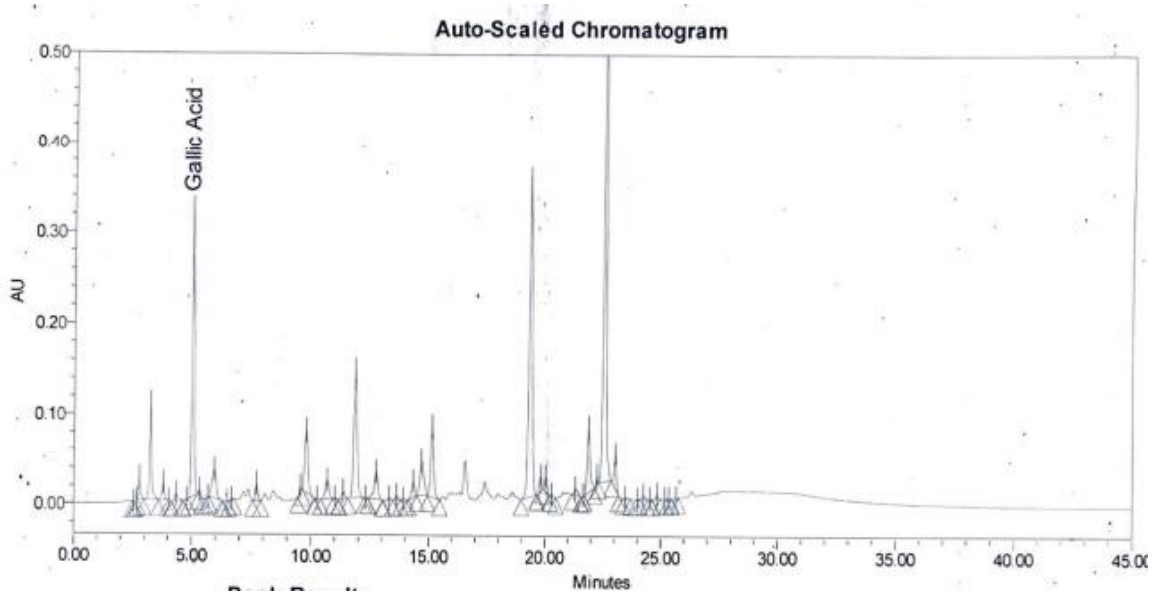

Figure 6 (b): Chromatogram of the *Terminalia chebula* fruit powder**)**

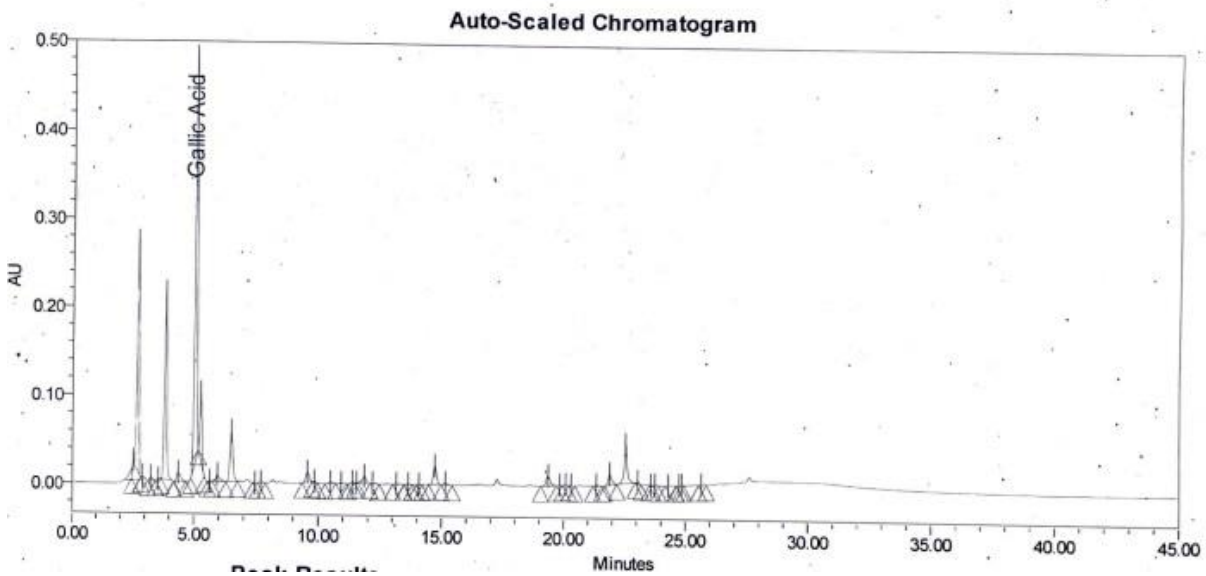

Figure 6: (C) Chromatogram of the *Phyllanthus emblica* fruit powder**)**

During the present investigation a large variation in the morphological features (colour, and texture) of Triphala powder of different brands was observed. Total ash and acid insoluble ash was found within limits, however high value of ash is an indicator of contamination, adulteration, substitution or negligence in the preparation of herbal formulations whereas acid insoluble ash indicates the quantity of sand and silica in the formulations (19). The pH and moisture content of the samples are also two important factors that are reported to influence the fungal diversity in nature (20). During the present investigation, the low pH strongly suggests the possibility of fungal growth in the samples. The study also revealed the large variation in concentration of phytochemicals among the marketed Triphala powder. Geographical factors may also affect the variation in the diversity of phytoconstituents. It was also suggested that this variation may be due to diversity of geographical factors (soil type, sunlight, temperature and precipitation) and post growth factors (harvesting, storage, transportation and manufacturing process) associated with the ingredients of medicinal formulations (21). In the present study, gallic acid, a common constituent of all the three component species of Triphala churna was used as a biomarker compound for standardization. The Gallic acid was quantified using High Performance Liquid Chromatography to assess the quality, Efficiency and efficacy of the market samples of Triphala churna. The present extraction method and solvent system parameters were found successful in obtaining the biochemical fingerprinting of the polyherbal formulation, that can be used to validate the levels of phytoconstituents in the commercially available Triphala churna samples. The retention time of Gallic acid was found to be around 5.0 minutes in all the samples as well as in the fruit powders of *Terminalia bellirica*, *Terminalia chebula* and *Phyllanthus emblica.* Among all the samples, TCS5 and TCS 6 was found to have the highest and lowest concentration of Gallic acid respectively. Out of the three componant species of Triphala churna, *Terminalia bellirica* was found to be having maximum concentration of Gallic acid.

5. Conclusion

A large variation in concentration of therapeutically important phytochemicals may result in alteration in efficacy, thereby shatter the beliefs of common people in Ayurveda. The lack of uniformity and standard composition found in present study in commercially available Triphala formulations may pose a serious risk for consumers health and can give a serious jolt to Indian herbal industry. Therefore, it is crucial to formulate stringent quality control guidelines for herbal formulations so that maximum benefits can be obtained from these traditional formulations.

Conflict of Interest statement: The authors declare that they have no conflict of interest.

6. Future Scopes

The study can be improved by employing more advanced analytical technniques for the development of complete metabolite fingerprinting of prominent polyherbal formulations such as Triphala. To ensure the safety of such herbal formulations, more sophisticated and accurate molecular standardization techniques such as DNA barcoding, DNA fingerprinting should be employed.

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