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Quality Control Evaluation of Swarasa Bhavita Guduchi (Tinospora Cordifolia Willd.) Churna

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ABSTRACT

Introduction: Samskara (transform), a fundamental Ayurvedic concept, involves Bhavana (soaking). Applying Bhavana of Swarasa (drug juice) to powdered drugs enhances efficacy and alters intrinsic attributes. In ChurnaKalpana (powder form of drug), like Guduchi Churna, a strategic approach is crucial for dose reduction. Despite its significance, no published information exists on the analytical profile of SwarasaBhavitaGuduchiChurna prepared by soaking method. **Aim:** To assess the quality control parameters for SwarasaBhavitaGuduchiChurna. **Materials and Methods:** Three batches of SwarasaBhavitaGuduchiChurna were prepared and their characteristics were documented. Organoleptic parameters, physicochemical parameters, qualitative tests for functional groups, quantitative tests for total alkaloids, high-performance thin-layer chromatography (HPTLC) profiles and heavy metal analyses were conducted. **Results:**Average increase yield percentage and time duration of three batches in SwarasaBhavitaGuduchiChurnawere 26.4 % and seven days respectively. Physicochemical parameters revealed pH 6.99, Loss on Drying 9.54%, Total Ash 3.79%, Acid insoluble Ash1.05%, Water Soluble Extractive 22.48% and AlcoholSoluble Extractive 3.82%. HPTLC analysis displayed six peaks under 366 nm. The percentage of berberine and total alkaloid content was 0.016% and 0.26%,respectively. No heavy metals were detected in thesample. **Conclusion:** The present observation can be considered as standard for quality control parameter.

KEYWORDS: Swarasa, Churna, quality control parameter, Tinosporacordifoliawilld.

I. INTRODUCATION

Samskarais a fundamental concept of Ayurvedic pharmaceutics, encompassing techniques for herbs, metals and minerals. Shodhana (purification), Jarana (open pan frying) and Marana (incineration) are most common processing's of single metallic and mineral drugs. Conversely, Samskara employed to augment the therapeutic efficacy of individual herbs. It is transformation of the inherent attributes of a substance which leads to the addition of new properties or qualitative improvement. It carries the qualities and action of liquid media with powdered drugs to be soaked; thus, it presumably regulates the quality level by change in potency or addition of new properties.

Bhavana(soaking) applied to the powder of an herb using Swarasa from the same herb, as per research, yields a substantial boost in therapeutic potential. The increased therapeutic potential can be accessed by various analytical testing's. Its uses are explained as quicker, augmented action with possible reduction in the required therapeutic dose of the drug under process. ChurnaKalpana (powdered drug) where the dose is large, SwarasaBhavitaChurna proves helpful in reducing the dose.According to analytical point of view, Bhavana results in changing the chemical profile ofBhavita material.Which have been studied on Nishamalaki Churna found positively enhancing the drug action. However such change need to be studied on analytical ground to know its actual extent as well as to know the change occurred in final product.

International Journal of Pharmaceutical Research and Applications
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Guduchi (Tinosporacordifoliawilld.) is one of the well-known medicinal herb which have many medicinal properties such as anti-inflammatory, anti-diabetic, ant arthritic, antioxidant, anti-stress, antileprotic, antimalarial, hepatoprotective, antiallergic and immunomodulatory activities. It is an important drug and is used in form of different preparations like Swarasa, Churna, Ghana and many more.The analytical techniques have always been mentioned in classical texts to understand the quality of the end product. However, qualitative and quantitative analysis of drugs by using the modern techniques and instruments of the science is also the need of time. Till date no published information available on analytical profile ofSwarasaBhavitaGuduchiChurna (SBGC) prepared by soaking method. Considering this, an effort has been made todevelop analytical profile of it.

II. MATERIAL AND METHODS

Procurement of raw material and its authentication

The fresh samples of Guduchi stem were procured from Junagadh, Gujarat in the month of March 2022 by adopting Good Collection Practices guidelines. Identification and authentication of the Guduchi stem was done at Pharmacognocy laboratory of Upgraded Department of Dyavyaguna, Government Ayurved College Vadodara, Gujarat.

Preparation of drug

All the samples of SBGCwere prepared in pharmaceutical laboratory of Upgraded Department of Rasashastra and BhaishajyaKalpana, Vadodara, Gujarat.A total of 3 batches of SBGC were prepared. Initially 500 g fresh Guduchi stems were taken and cleaned well with water for 3 times. Then they were converted into small pieces (avg. size of 1 inch) with the help of cutter.After that the pieces of Guduchi stem were made into paste form in mixer grinder by adding double water. Then paste was squeeze with the help of cotton cloth and green colored liquid (GuduchiSwarasa) was obtained. GuduchiChurna was prepared as per the reference of SharangadharaSamhita. Initially 30 Kgfresh Guduchistem were taken and washed with water for 3 times. Guduchi stem was chopped into small pieces of uniform size(avg. size of 1 inch).Chopped pieces were placed in a tray and dried under sun light.After proper drying, grinding process was carried out in pulverizer. Then it was sieved through no. 80 sieve.After that it was stored into airtight glass container. SBGC was prepared as per the reference of BhaishajyaRatnavali. Initially 1000 ml GuduchiChurna was taken in S.S.tray and spread it.1000mlGuduchiSwarasawas added in GuduchiChurna and mixed well. Then it was dried in sunlight for 24 hour. After drying, it was crushed in a mixer grinder.Then it was weighed and again spread in S.S. tray and added GuduchiSwarasa. This whole process was repeated for six times. After complete drying it was collected as SwarasaBhavitaGuduchiChurna. The average increased yield percentage and time duration for the preparation were 26.4 % and 7 days respectively.

Analytical study

Fresh Guduchi stem, GuduchiChurna and SBGC were analyzed by employing various analytical parameters. Organoleptic parameters, physicochemical parameters, qualitative tests for various functional groups, quantitative estimations of total alkaloids, HPTLC profile under 366nm ultraviolet light and tests for presence of certain heavy metals were also carried out.

Organoleptic parameters

The organoleptic parameters of SBGC are evaluating the qualities of preparation by color, texture, taste, odor and appearance through sense organs and it is providing the idea about the quality of formulations without using chemical tests. The final product was made of fine powder form. Results of organoleptic parameters are mentioned in Table no. 3.

Physicochemical parameters

Different physicochemical parameters of fresh Guduchi stem, GuduchiChurna and SBGC were carried out on all the three batches using standard API methods including pH value, Loss on Drying at 110°C, Ash value, Acid insoluble Ash, Specific gravity at 40°C, Total solid content, Water Soluble Extractives and Alcohol Soluble Extractives at Quality control Laboratory of Upgraded Department of Rasashastra and BhaishajyaKalpana, Vadodara, Gujarat. Results of Physicochemical parameters are mentioned in Table no.4-5.

Test for various functional groups

The techniques employed to isolate active substance are termed as extraction method. Crude extracts obtained from such processes can be qualitatively tested to ascertain the presence of

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different types of components. Qualitative tests are used to detect the presence of functional groups and quantitative test for alkaloids used to calculate the percentage of total alkaloids content, which plays very important role in the expression of biological activity. Results of qualitative test for functional groups and quantitative test for alkaloids are mentioned in Table no.6-7.

HPTLC Profile

HPTLC is a sophisticated and automated form of TLC. HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time with HPTLC, the analysis can be viewed using different wavelengths of light thereby providing a more complete picture of the plant than is typically observed with more specific types of analyses. HPTLC was carried out at Vasu Research Centre, Division of Vasu Healthcare PVT. LTD. Vadodara, Gujarat.

Principle of HPTLC

Principle remains the same as of TLC i.e. adsorption. One or more compounds are spotted in a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flows through because of capillary action. The component with more affinity towards stationary phase travels faster. Thus the components are separated on a thin layer chromatographic plate based on the affinity of the components towards the stationary phase.

Instrument specification:

Table No. 1. Instrument specification for HPTLC

Sample application device	**4 spotter - Linomat 5**
Company	**CAMAG® Switzerland**
Name of software	Vision CATS
Scanner	HPTLC scanner 4
Photo documentation	UV cabinet
Run	Twin through chamber
Object support	For objects up to 20 x 20 cm
Stage drive	Stepping motor 3200 steps/rotation, 8 steps = 0.1 mm Band length 0 (spot) - 190 mm in steps of 0.1 mm Speed approx. 10 mm/s.
Dosage syringe drive	Stepping motor 1600 steps/rotation 100 nL = 120 steps with 100 µL syringe 100 nL = 24 steps with 500 µL syringe
Sample dosage syringe	Selectable 100 µL or 500 µL
Memory	10 methods, backup min. 10 years
LCD display	2 lines of 16 characters ea.
Power connection	85–250 V~ 47–63 Hz 30 VA.
Dimensions (W x D x H)	360 x 510 x 410 mm
Weight	12.5 kg

Step involved in HPTLC were selection of chromatographic layer, sample and standard preparation, layer pre-washing, layer pre-conditioning, application of sample and standard, chromatographic development, detection of spots, scanning and documentation of chromatic plate.

Preparation of test Solutions

Take 0.1g of sample in a test-tube and dilute it with1ml of hexane and mix well. Use the upper hexane solution thus obtained for HPTLC fingerprinting.

Table No. 2. Chromatographic conditions of HPTLC

Application Mode	CAMAG Linomat5–Applicator
Filtering System	What man Filter paperno.1

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 28-36 www.ijprajournal.com ISSN: 2456-4494

Stationery Phase	MERCK-TLC/HPTLCSilicagel60F254on Aluminum sheets
Application(Yaxis) Start position	10 mm
Development End position	80mmfromplatebase
Sample Application Volume	5.0µl ofeach
Distance Between Tracks	16 mm
Development Mode	CAMAGTLCT win Through Chamber
Chamber Saturation Time	30 minutes
Mobile Phase(MP)	Petroleumether: Di-ethylether:Aceticacid (9:1:0.1v/v)
Pre-chromatographic derivatization	After sample spotting pre-chromatographic derivatization done with 5% Alcoholic KOH (2.0 µl) followed by heating the plate for 10minutes on TLC Plate Heater Preheated at 100± 5^0C
Visualization	@254nm, @366nm (after derivatization) and @540 nm (after derivatization)
Spray reagent	5% Sulphuric acid in Methanol
Devatization mode	CAMAG-Dip tank about 1minute
Drying Mode, Temp. &Time	TLC Plate Heater preheated at 100±5^0C for 3Minutes

Preparation of Sprayreagent [5%Sulphuricacidin Methanolreagent]: 5 ml Sulphuric acid is cautiously mixed with 100 ml Methanol.

Tests for Heavy metals

Heavy metal analyses were carried out by ICP-OES Inductive Coupled Plasma Optical Emission Spectrometer (Make: Perkin Elmer Model: Optima 3300 RL) at Vasu Research Centre, Division of Vasu Healthcare PVT. LTD.Vadodara. Reagents used in this preparation were deionized water(resistivity > 18.2 M ohm cm), Metal stock solution 100 mg/L (Multi std. CPA Ltd. Bulgeria1000 mg/L Multi std. VHG Lab. USA), Hydrochloric acid (37% GR, Merck), Nitric acid (69%GR, Merck).

III. OBSERVATIONS AND RESULTS

Organoleptic character observed in the fresh Guduchi stem, GuduchiSwarasa, Guduchi Churna and SBGC. The most important analysis is organoleptic character because the palatability of a formulation is highly dependent on these characteristic.

Table No. 3: Organoleptic characteristic of different sample of Guduchi

Sr. No.	Ingredients	Color	Texture	Taste	Odor	Appearance
1	Fresh Guduchistem	Creamish brown	Soft, Slimy	Bitter	No specific	Solid
2	Guduchi Swarasa	Green	Soft, Slimy	Bitter	Characteristic	Liquid

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 28-36 www.ijprajournal.com ISSN: 2456-4494

3	Guduchi Churna	Creamish yellow	Smooth	Bitter	Characteristic	Powder
4.	SBGC	Creamish yellow	Smooth	Bitter	Characteristic	Powder

Data of table no.3 shows the variation in the color of fresh Guduchistem, Guduchi Swarasa and SBGC. Physicochemical parameters are useful in set standards for a crude drug as these parameters are generally constant for a plant.

Table No. 4: Physicochemical parameters of GuduchiSwarasa

Sr. No.	Parameters	Average results of each Bhavana		
		Batch 1	Batch 2	Batch 3
1	pH	6.61	6.60	6.98
2	Specific gravity (%)	1.006	1.006	1.001
3	Total solid content (%)	2.90	2.52	2.58

Table No. 5: Physicochemical parameters of different samples of Guduchi

Sr. No.	Parameters	Fresh Guduchi stem	Guduchi Churna	Average results of SBGC		
				Batch 1	Batch 2	Batch 3
1	pH	5.03	7.52	6.20	6.25	6.85
2	Loss on Drying (% w/w)	75	08	11	12	11.56
3	Ash value (% w/w)	1.66	04	04	4.12	04
4	Acid insoluble Ash (% w/w)	1.07	1.26	0.50	0.58	0.40
5	Water Soluble Extractive (%w/w)	7.12	20	23	23.45	23.40
6	Alcohol Soluble Extractive (%w/w)	15.00	3.2	04	4.10	04

Table No. 6: Results of qualitative test for various functional groups

Sr.No.	Tests for	Fresh Guduchi stem	Guduchi Churna	SBGC
1.	Alkaloids	+Ve	+Ve	+Ve
2.	Glycosides	+Ve	+Ve	+Ve
3.	Flavanoids	+Ve	+Ve	-Ve
4.	Tannin	+Ve	+Ve	+Ve
5.	Steroid	+Ve	+Ve	+Ve
6.	Terpanoids	+Ve	+Ve	-Ve
7.	Saponin	+Ve	+Ve	+Ve
8.	Carbohydrate	+Ve	+Ve	-Ve
9.	Protein	-Ve	-Ve	-Ve
10.	Starch	-Ve	-Ve	-Ve

+Ve =present, -Ve=absent

Table No.6 shows the results of the qualitative test carried out for various functional groups. Presence of major active constituents of raw drugs into the finish product suggests the extraction of this functional group in the formulation.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 28-36 www.ijprajournal.com ISSN: 2456-4494

Table No. 7: Total alkaloid content (%) in different samples of Guduchi

Name of Sample	Total alkaloid content (%)
Fresh Guduchi stem	0.57
Guduchi Churna	0.99
SBGC	0.26

The presence of alkaloids in the sample was detecting using quantitative testing. They are insoluble sparingly in water but pass readily into solution on treatment with dilute acids with formation of soluble salts. From aqueous solution of their salts the free alkaloids are precipitated by basification (e.g. by alkali carbonates). In organic solvents such as ether or chloroform, alkaloids themselves dissolve freely, but their salts only sparingly. The % of total alkaloids content was 0.57, 0.99, 0.26in fresh Guduchi stem, Guduchi Churna and SBGC respectively as shown in Table no. 7.

Table No. 8: Rf values of different samples of Guduchi under 366 nm

Sample	Under 366 nm		Sample	Under 366 nm		Sample	Under 366 nm	
	No. of spot	R_f value		No. of spot	R_f value		No. of spot	R_f value
Guduchi stem	6	0.22	Guduchi Churna	1	0.34	SBGC	5	0.18
		0.31						0.31
		0.58						0.37
		0.66						0.42
		0.77						0.66
		0.80						

HPTLC study of fresh Guduchi stem, GuduchiChurna and SBGC were carried out in comparison.At 366 nm 6 prominent spots bearing R_f value 0.22. 0.31, 0.58, 0.66, 0.77, 0.80 in track of fresh Guduchi stem,1 prominent spots bearing R_f value 0.34 in track of GuduchiChurna while 5 prominent spots bearing R_f value 0.18, 0.31, 0.37, 0.42, 0.66 could be seen from the table No. 8. HPTLC photo-document and chromatogram shown in figure no.1, 2, 3.

Table No. 9: Percentage content of berberine estimated using HPTLC

Sr. No.	Sample	Berberine (% w/w)
1.	FreshGuduchi stem	0.074
2.	GuduchiChurna	0.012
3.	SBGC	0.016

In HPTLC peak areas and absorption spectra were recorded and the amount ofberberine was calculated using its calibration curve.

Table No. 10:Heavymetal analysis of finish products

Sr.No.	Heavy Metal	SBGC	Permissible Limits as per API
1.	Lead	1.150 ppm	NMT10ppm
2.	Cadmium	0.033 ppm	NMT0.3ppm
3.	Arsenic	0.883 ppm	NMT3ppm
4.	Mercury	0.477ppm	NMT1ppm

The heavy metal content in the analysis falls within acceptable limits as per the results of the heavy metal test.

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IV. DISCUSSION

Raw drug was procured in month of March 2022. AcharyaYadavjiTrikamji has mentioned to use 'Angusthapramana' (thumb size) of Guduchi stem, accordingly thumb sized or medium size stem diameter (1.6-2.0 cm) was selected for study. It was authenticated and analyzed before processing because good quality products mainly depend on genuine raw materials.

The organoleptic characters, which correspond to the PanchagyanedriyaPariksha (perception by five sense organs) of Ayurveda, were performed at three stages of preparation (for fresh Guduchi stem, GuduchiSwarasa and SBGC), because these parameters can change at different stages [Table no.3]. Data shows the variation in the color of fresh Guduchi stem and SBGC. Due to absence of Terpanoids color of SBGC was changed to creamishyellow. Terpanoids are the most abundant group of compound in Guduchi these compounds are involved in giving plants their colors. Pigments are the responsible for natural spectacular color of plants. Red-yellow betalains, green chlorophylls, red-purple anthocyanin and yellow-orange carotenoids are the most common pigments found in vegetables and fruits. Apart from their coloring properties of Guduchi exhibit potential health-promoting functions.

Observations of physicochemical data are tabulated in table no.4-5. Here, mean pH of GuduchiSwarasa (5.03) and different batches of SBGC (6.99) showed significant difference, which indicate few chemical changes were occurring on this phase between solvent and solute, which leads the change in pH. Loss on Drying value of fresh Guduchi stem was 75.00 % w/w. It is because of high moisture content in green state. Average value of Loss on Drying was 9.54 % w/w in SBGC. In conjunction with a suitable temperature moisture will lead to the activation of enzymes and given suitable condition to the proliferation of living organism. Hence, moisture contents may affect the quality of the drug. Although the weight loss in the samples is principally due to water, small amount of other volatile materials will also contribute to the weight loss. Therefore, it must be stored in damp proof polythene covers or glass containers. The average ash values (% w/w) of fresh Guduchi stem and SBGC was 1.66 and 3.79, respectively. The Total ash figure is important as it indicates to some extent the amount of care taken in the preparation of the drug. The Total ash usually consist mainly carbonates, phosphates, silicates and silica. Lower value of ash in prepared drug indicates presence of very fewer inorganic materials in it. Also the value of Water Soluble Extract (% w/w) of SBGC (22.48 %) was more than fresh Guduchi stem (7.12 %) indicating role of BhavanaSamskara (transform) in extraction. There is much difference in Alcohol Soluble Extractive value (% w/w) of fresh Guduchi stem (15 %) and SBGC (3.82 %) sample indicating again role of Bhavana process in changing the alcohol soluble extractive values.

Qualitative tests were done to detect the presence of functional groups and presence of major active constituents of raw drugs into the finished product suggests the extraction of these moieties in the formulation. The methanolic extract of the sample was used for analysis [Table no.6]. The study reveals the absence of Flavonoids and Terpanoidsin SBGC. Because both are very heat sensitive so due to long exposure of sun light Flavonoids and Terpanoids were destroyed. It can be anticipated that, presence of alkaloids as functional group might be responsible in the expression of biological activity of the formulation. Total alkaloid contents are shown in Table no.7.

Chromatographic study (HPTLC) was carried out under 366 nm UV to establish the fingerprinting profile. It showed phyto-components with Rf values 0.18, 0.31, 0.37, 0.42 and 0.66 in SBGC sample, which may be responsible for expression of its pharmacological and clinical actions.

The heavy metal content of SBGC was carried out to finally fulfill and establish the quality standard at finished product level. No heavy metals were detected [Tables no.10].

V. CONCLUSION

The investigation demonstrates adherence to adequate quality control protocols in the development of a novel Ayurvedic preparation. The analysis encompassed fundamental analytical elements of the sample formulation, including examination of phytochemical parameters and HPTLC fingerprinting. These standards serve to establish a comprehensive set of diagnostic criteria, ensuring the identity and authenticity of the formulation. The HPTLC profile generated in this study represents a refined and standardized tool for verifying the genuineness and authenticity of SwarasaBhavitaGuduchiChurnaprepared by soaking method. The present observation can be considered as standard for further studies.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 28-36 www.ijprajournal.com ISSN: 2456-4494

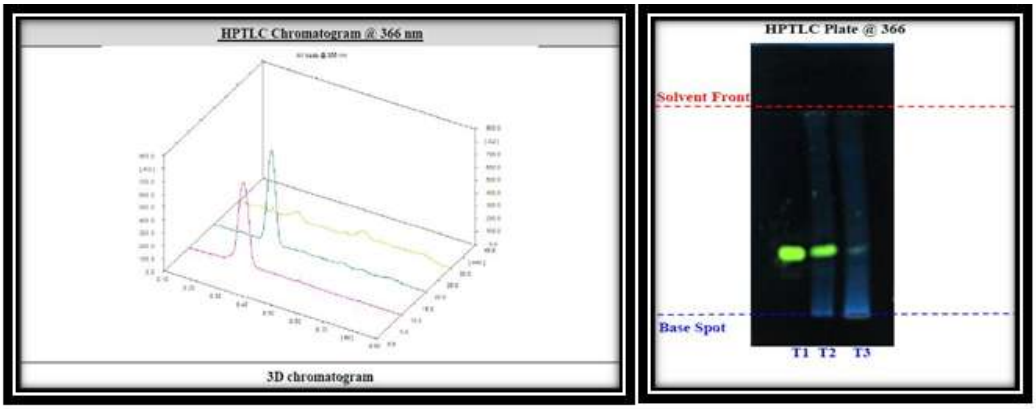

Figure no. 1: HPTLC photo-document and chromatogram of fresh Guduchi stem

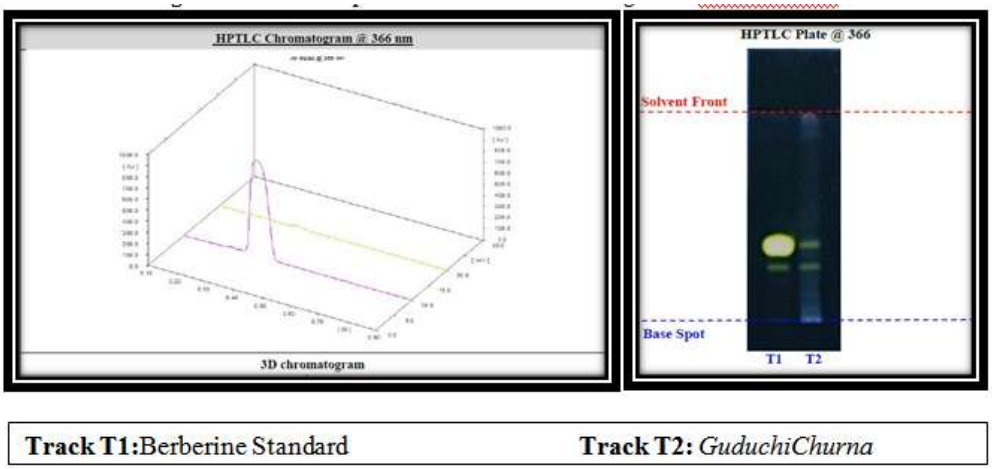

Figure no. 2: HPTLC photo-document and chromatogram of GuduchiChurna

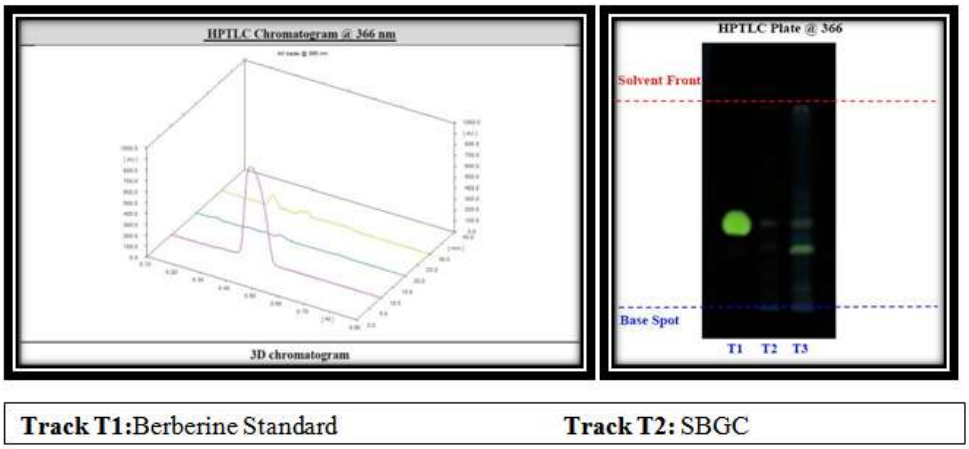

Figure no.3: HPTLC photo-document and chromatogram of SBGC

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