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Modern Analytical Standardisation of the Classical Ayurvedic Formulation Shimhanda Guggul

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ABSTRACT:
Simhananda Guggulu is a traditional Ayurvedic polyherbal formulation widely utilized for the treatment of chronic inflammatory illnesses, especially Amavata (rheumatoid arthritis) and other vata-kapha dominated ailments. Notwithstanding its extensive clinical application, thorough scientific standardization of this formulation is still lacking. This study sought to create a comprehensive analytical standardization profile for Simhananda Guggulu and its various dose forms in accordance with contemporary pharmaceutical and regulatory standards. The formulation was developed internally as kwatha and solid dosage forms (gutika/tablets) in accordance with traditional Ayurvedic methods. Standardization was conducted via a systematic assessment of organoleptic properties, physicochemical parameters (pH, total ash, acid-insoluble ash, water- and alcohol-soluble extractives, moisture content), tablet quality attributes (hardness, friability, weight variation, and disintegration time), microbial load, and heavy metal contamination, adhering to WHO and Ministry of AYUSH guidelines. Standardization based on markers was accomplished with a proven High-Performance Thin-Layer Chromatography (HPTLC) technique, with gallic acid designated as the phytochemical marker. The established HPTLC method exhibited excellent linearity (200–1200 ng/spot), high precision (RSD <1%), satisfactory accuracy (81.80–118.97% recovery), and sufficient sensitivity, with detection and quantification limits of 45.1 ng/spot and 137.8 ng/spot, respectively. The gallic acid concentration in several formulations varied from 0.54% to 0.63% w/w. All physicochemical, microbiological, and heavy metal characteristics were within acceptable limits, affirming the safety and quality of the developed formulations. The study develops a thorough and reproducible analytical methodology for the standardization of Simhananda Guggulu. The results affirm its uniformity across batches, authenticity, and adherence to regulations, hence improving its acceptance in evidence-based Ayurvedic and integrative healthcare systems.

INTRODUCTION:

Many polyherbal formulations are used in Ayurveda, the traditional medical system that has been practiced in India for more than 5,000 years, to treat a variety of chronic illnesses. Among these, Guggul-based formulations are prominently used for their anti-inflammatory, lipid-lowering, and rejuvenative properties [1]. *Shimhanda Guggul* is a classical Ayurvedic preparation mentioned in traditional texts, primarily indicated for managing joint disorders, musculoskeletal inflammation, and certain neurological conditions. The formulation comprises a synergistic blend of Commiphora mukul (Guggul) resin and several other botanicals that contribute to its therapeutic efficacy [2].

Despite its widespread traditional use, there is a growing demand for scientific validation and standardisation of such classical formulations to ensure consistency, efficacy, and safety. Modern pharmaceutical and analytical sciences offer a robust platform to evaluate and establish quality parameters using validated techniques such as High-Performance Mass spectrometry, Fourier-Transform Infrared Spectroscopy (FTIR), High-Performance Liquid Chromatography (HPLC), and Thin Layer Chromatography (HPTLC) [3,4].

These methods help in chemical fingerprinting and quantification of bioactive constituents such as guggulsterones, which are key therapeutic markers in Guggul formulations [5]. The World Health Organization (WHO) and Ministry of AYUSH, Government of India, have issued comprehensive guidelines for the standardisation and quality control of herbal formulations, including parameters like organoleptic properties, physicochemical profiling, microbial load, heavy metal content, and aflatoxins [6,7]. Integrating these approaches in the standardisation of *Shimhanda Guggul* is crucial for ensuring batch-to-batch uniformity and enhancing its acceptance in evidence-based integrative medicine. This study aims to undertake a comprehensive analytical standardisation of *Shimhanda Guggul* using contemporary methods, aligning with regulatory requirements and scientific expectations.

Simhananda Guggulu:

Simhananda Guggul is a well-known classical polyherbal formulation in Ayurveda, traditionally indicated for the management of chronic inflammatory and joint-related disorders. This formulation is documented in several authoritative texts, including the *Chakradatta*, *Bhaishajya Ratnavali*, and *Yogaratnakara*, where it is prescribed for conditions such as Amavata (rheumatoid arthritis), Vatarakta (gout), and other vata-kapha predominant ailments [8,9].

The term *Simhananda* signifies potency and rapid action, symbolizing the efficacy of this formulation in eliminating deeply embedded pathologies associated with toxins (*ama*) and inflammatory processes. The principal component, Guggul (*Commiphora mukul*), possesses well-established anti-inflammatory, lipid-lowering, and detoxifying actions. Other important constituents include Triphala (a combination of *Emblica officinalis*, *Terminalia chebula*, and *Terminalia bellirica*), Trikatu (*Piper longum*, *Piper nigrum*, *Zingiber officinale*), Haridra (*Curcuma longa*), and Shuddha Gandhaka (purified sulfur), among others, which act synergistically to amplify its therapeutic potential [10].

From a pharmacological perspective, the formulation works through multiple pathways. Guggulsterones—the bioactive compounds in Guggul—are known to inhibit inflammatory mediators such as NF-κB, COX-2, and TNF-α, thus providing relief in chronic arthritis and other autoimmune conditions [11,12]. Ingredients like Triphala promote gut detoxification and metabolic balance, while Trikatu improves digestion and enhances the bioavailability of other drugs [13]. This aligns with the classical Ayurvedic concept of *srotoshodhana* (channel purification) and *agni deepana* (enhancing digestive fire), crucial in the treatment of Amavata.

Despite its long-standing use, Simhananda Guggul requires scientific validation and analytical standardisation to ensure batch-to-batch consistency and safety. Application of modern tools such as HPTLC, FTIR, and HPLC for chemical profiling can support quality assurance and foster broader acceptance in integrative and evidence-based medicine systems.

METHODOLOGY:

Material:

Yucca businesses provided the standard gallic acid. The chemical agents and reagents used in the experiment were analytical grade and were acquired from Rankem and S. D. Fine Chemicals, India. Merck supplied the silica gel

60F HPTLC pre-coated plates.

General Method of Preparation"
Procedure for the formulation of Simahanda Guggul Kwatha"
The fruits were blend in the powder form. The powder was then extracted by utilizing the extraction vessel. The whole process was carried out at fixed temperature condition. Reduction was done until the quantity was reduced to 1/4th of initial quantity given in table 1and 3. The Kwath was filtered using filter cloth.

Preparation of pills"
Weighed the specified quantity of coarsely ground herbs. In iron vessels, the amount of water was added, and it was cooked until it was reduced to half. Add the leftover herb to the strained mixture. In order to achieve gudapaka (half solid) consistency, it is cooked further while being constantly stirred and pounded. filtered the cooking process. The remaining herbs were added to the above-filtered preparation after being finely pulverized. After thoroughly mixing the entire amount, it is transferred to containers that have been greased with ghee. After that, the pills are rolled.

Preparation of tablets:
Weighed the given quantity of ingredients in coarse powdered form. Heat the ingredients in vessels to produced dump mass. Press the mass under tablet compression machine to produced desired size tablets.

Formula of formulation:
Formulation and preparation of Simhananda kalpa

Table 1: Formula for Simhananda kwatha

Sr. No.	Name of Ingredients	Quantity Taken
1.	Haritaki	33 gm
2.	Bibhitaki	33 gm
3.	Amalaki	33 gm
4.	Shuddha Guggulu	33 gm

Formulation and preparation of Simhananda guggul gutikas

Table 2: Formula for Simhananda guggul gutikas

Sr. No.	Name of Ingredients	Quantity Taken
1	Haritaki	33 gm
2	Bibhitaki	33 gm
3	Amalaki	33 gm
4	Shuddha Guggulu	33 gm
5	Shuddha Gandhak	33 gm
6	Eranda Taila (Castor Oil) - for Bhavana	Q.S. (Sufficient)

Formulation and preparation of Simhananda guggul Kwatha

Table 3: Formula for Simhananda guggul tablets

Sr. No.	Name of Ingredients	Quantity Taken
1	Haritaki	33 gm
2	Bibhitaki	33 gm
3	Amalaki	33 gm
4	Shuddha Guggulu	33 gm
5	Shuddha Gandhak	33 gm
6	Eranda Taila (Castor Oil) - for Bhavana	Q.S. (Sufficient)

Analysis of formulations:
pH:
Potentiometric analysis was used to estimate the formulation's pH. A digital or analog pH meter, a glass electrode, and a reference electrode are all need for the procedure. One gram of the formulation was mixed with ten milliliters of distilled water to perform the estimate.

Total ash:
Two to three grams of formulation were burned in a tared silica or platinum dish in order to estimate the total amount of ash. The procedure was continued until there was no more carbon in the formulation. A temperature of no more than 450°C is necessary. After cooling, the dish was weighed. The procedure was repeated until the weight did not change. The percentage of total ash was computed using the weight of the air-dried medication as a reference.

Acid insoluble ash:
Dil. HCl of about 25 ml was added in a crucible containing total ash. The undissolved content was filtered through whatman 41 filter. The filtrate was washed using the hot water. Neutralised the filtrate by water washing. The filtrate was then transfer in the crucible using the filter paper. Drying and ignition of residue on hot plate was continue until there is no change in weight. The residue was weighed after cooling for 30 min in desiccator. With a reference to the weight of air-dried drug, acid insoluble ash was calculated.

Water soluble extractives:
Five grams of the sample were macerated for twenty-four hours with around 100 milliliters of water, and then the mixture was shaken for six hours. After eight hours of standing, the mixture was filtered. A tared petri dish was used to dry the filtrate (25 ml). The dried filtrate was weighed after the drying process, which was completed at 105 °C. The percentage of water-soluble extract was computed using the air-dried medication as a reference.

Alcohol soluble extractives:
Ethanol was used as a solvent in place of water to repeat the water-soluble extraction process. After the filtrate was dried at 105°C, it was weighed, and the percentage of alcohol-soluble extract was computed using the air-dried medication as a reference.

Moisture Content (Loss on Drying at 105°C):
Ten grams of air-dried material were weighed and put in a tared plate. The medication was dried in a hot air oven set at 105°C for five hours. weighed the drug's dry sample. Until the difference between two following weigh-ins is less than 0.25%, the process is repeated.

Microbial Contamination:
Total bacterial count:
Ten grams of the powdered samples were weighed and dissolved in one hundred milliliters of sterile nutritional broth. Separately, the broth was incubated for 24 hours at 37°C. Serial dilutions were carried out if the culture demonstrated organism development at the end of the 24-hour incubation period. Nine milliliters of sterile saline were mixed with one milliliter of the sample (plant material plus nutritional broth). Similarly, 1 ml of 106 dilutions were pipetted into each of two sterile nutrient agars, and dilutions were carried up to 1010. In a similar manner, duplicate plates were pipetted with dilutions 107, 108, 109, and 1010. Using a sterile spreader, samples were applied to nutritional agar. The plates were further incubated for five days at 37°C. Both positive and negative controls were used. In terms of microorganisms per gram of plant sample (colony forming units, or cfu per gram of plant sample), the average of the colonies on three plates was calculated.

Yeast and Mould:
Ten grams of the powdered material were dissolved in one hundred milliliters of sterile nutritional broth to create samples with a concentration of 0.1 mg/ml. At 37°C, each falsk was incubated for 24 hours. Serial dilutions were carried out once the organism's growth in culture was noted at the end of the incubation period. The plant material and nutrient broth samples were diluted in sterile saline with concentrations ranging from 106 to 1010. Pipetting 1 milliliter of the 106 dilution into each of two sterile Sabourands chloramphenicol agars was done. In a similar manner, duplicate plates were pipetted with dilutions 107, 108, 109, and 1010. For five days, plates were subsequently incubated at 20–25°C. There were both positive and negative controls. Microorganisms per gram of plant sample colony forming units (cfu per gram of plant sample) were used to express the average number of colonies across three plates.

Heavy Metals:
The external testing source looked at the following metals: lead, mercury, arsenic, and cadmium.
Marker based standardization of the formulation using HPTLC:

HPTLC Instrumentation and Method development:
The stationary phase utilized was the precoated silica gel aluminum plate 60F254 (20 cm × 10 cm, 250 µm thick; E. Merck, Darmstadt, Germany, supplied by An¬chrom Technologists, Mumbai). Using a CAMAG microliter syringe, the test solutions were spotted as 6 mm wide bands. Before chromatography, the plates were pre-washed with methanol and activated for five minutes at 60°C. The slit's dimensions were maintained at 5 mm × 0.45 mm, its bandwidth was set at 7 mm, and it used a scanning speed of 10 mm/s per track. The mobile phase was made up of toluene, ethyl acetate, and formic acid (3.5: 5.5: 1) v/v/v for gallic acid. In a twin trough glass chamber that was saturated with the mobile phase, the linear ascending development was conducted. At room temperature (25 ± 2°C), the mobile phase's ideal chamber saturation time was 30 minutes. The chromatogram run measured 80 mm in length. The plate was then let to air dry at ambient temperature. The HPTLC plates' separated bands were scanned between 200 and 400 nm in wavelength. The tungsten lamp, also known as deuterium illumination, served as the radiation source. Gallic acid's highest absorbance was measured at 271 nm.

Preparation of standard solution:
The stock solution of gallic acid was 1 mg/ml and working solution was 100 μg/ml.

Sample preparation:
Simhananda guggul kwatha samples were made by mixing 100g of each sample with 50ml of methanol in a volumetric flask. To achieve a concentration of 50 µg/ml, additional dilutions were conducted. All samples were filtered using a 0.22 µ membrane filter.

Method validation: [14]
In accordance with ICH requirements, the developed HPTLC method was validated [14].

Linearity:
The linearity was examined for a range of concentrations, from 1 to 1000 ng/spot. For the data of the peak regions plotted against the respective concentrations, least-square regression analysis was employed.

Precision studies:
The chosen method's intra- and inter-day precision was assessed. Three triplicates of each gallic acid and piperine concentration were spotted and analyzed under the appropriate chromatographic conditions on the same day for the intra-day study and on two separate days for the inter-day study.

Accuracy studies:
The recovery study approach was used to assess the method's accuracy. 80, 100, and 120 percent of the median concentrations of the standards were added to the samples. The following formula was used to calculate accuracy: [(spiked concentration − mean concentration)/spiked concentration] × 100.

Robustness:
By purposefully altering the mobile phase and chamber saturation time combination to ascertain their impact on the retention factor and quantitative analysis, robustness was estimated.
A 5% change in the methanol level altered the composition of the mobile phase. The chamber's saturation time was extended from 15 to 30 minutes.

LOD and LOQ:
The standard deviation approach was used to estimate the LOD and LOQ. Where S is the slope of the calibration curve and σ is the residual standard deviation of a regression line, the detection limit is equal to 3.3σ/S and the quantitation limit is equal to 10σ/S.

RESULTS AND DISCUSSION:
Simhananda kwatha's organoleptic characteristics, including color, flavor, odor, yield percentage, and drying loss, were assessed and are listed in tables 5 and 8. Ensuring the safety, effectiveness, and purity of medicinal plants is crucial. To ascertain authenticity and purity, contaminants, moisture, ash content, and solvent residues must all be investigated. The degree of "cleanness" is indicated by the ash value, and large values could be the consequence of using incorrect sample handling methods [15].

Tables 6 and 9 summarize the evaluation of the several Simhananda guggul kalpa gutikas/tablet parameters. It was discovered that the Simhananda guggul tablet has a 0.5% gallic acid output. The formulations of Simhananda guggul tablets ranged in hardness from 3–4 kg/cm2 to 2-3 kg/cm2. Simhananda guggul tablet friability was determined to be 0.43% and 0.49%, respectively. The weight variation test is passed by both formulations. After 60 minutes, both pills had broken down.

Table 7 shows the quantitative estimation of physicochemical constants for Simhananda Kwatha, including pH, total ash content, acid insoluble ash, water soluble extractive, alcohol soluble extractive, and moisture content. Significant variance was found in physicochemical values such as pH, total ash content, acid insoluble ash, water soluble extractive, alcohol soluble extractive, and moisture content, according to the literature review [16–18]. Both of the formulations have an acidic pH. Simhananda Kwatha's percentage is greater. Additionally, compared to Simhananda guggul kwatha, Simhananda has a higher percentage of moisture content and an extract that is soluble in water and alcohol. In Triphala Kwatha, the percentage of acid-insoluble ash is lower. In accordance with WHO recommendations, the microbiological count was determined [19]. Since the levels of microbial contamination in both produced kwatha were found to be within acceptable bounds, it was determined that neither preparation had any microbial contamination.

The WHO reports that heavy elements like lead, mercury, and cadmium were examined together with arsenic. Medicinal plant materials may contain heavy metals and arsenic, which can be brought on by a number of things, including pollution and pesticide residues. Since these substances are dangerous even in trace amounts, they must be removed from herbal treatments. The prepared kwatha were found to pass the heavy metal and arsenic tests.

Evaluation of Simhananda kwatha:

Table 4: Analytical parameters of Simhananda Kwatha

Sr. No.	Parameters	Values
1	Colour	Blackish brown
2	Odour	Characteristic odour
3	Taste	Kashaya, Tikta, Amla
4	Loss on dying	2.25 w/w

Table 5: Evaluation of Simhananda guggul kalpa gutikas/ Tablet/ Pills

Sr. No.	Parameters	Values
1	Hardness (Kg/Cm^2)	3-4
2	Disintegration time	60
3	Pulverized sugar	18%
4	Friability %	0.43
5	Weight variation test	Pass

Table 6: Evaluation of Simhananda kwatha

Sr. No.	Parameters	Actual Values	Obtained Values
1	pH	-	4.20@23^{0}C
2	Total ash	-	4.35%
3	Acid insoluble ash	-	0.22%
4	Water soluble extractives	-	85.26%
5	Alcohol soluble extractives	-	54.28%
6	Moisture Content (Loss on Drying at 105°C)	-	15.85%
7	Microbial Contamination		
8	Total bacterial count	NOT MORE THAN 1000CFU/GM	COMPLIES
9	Yeast and Mould	NOT MORE THAN 100CFU/GM	COMPLIES
10	E. coli	Absent	ABSENT
11	Salmonella	Absent	ABSENT
12	Heavy Metal		
13	Lead	0.3 ppm	PASSES
14	Mercury	0.1 ppm	PASSES
15	Arsenic	1 ppm	PASSES
16	Cadmium	0.2 ppm	PASSES

Evaluation of Simhananda guggul Kwatha

Table 7: Analytial parameters of Simhananda guggul kwatha

Sr. No.	Parameters	Values

1	Colour	Blackish brown
2	Odour	Characteristic odour
3	Taste	Kashaya, Amla
4	Loss on Drying	2.20 w/w

Table 8: Evaluation of Simhananda guggul tablets/ pills

Sr. No.	Parameters	Values
1	Hardness (Kg/Cm^2)	2-3
2	Disintegration time	60
3	Friability %	0.49
4	Weight variation test	Pass

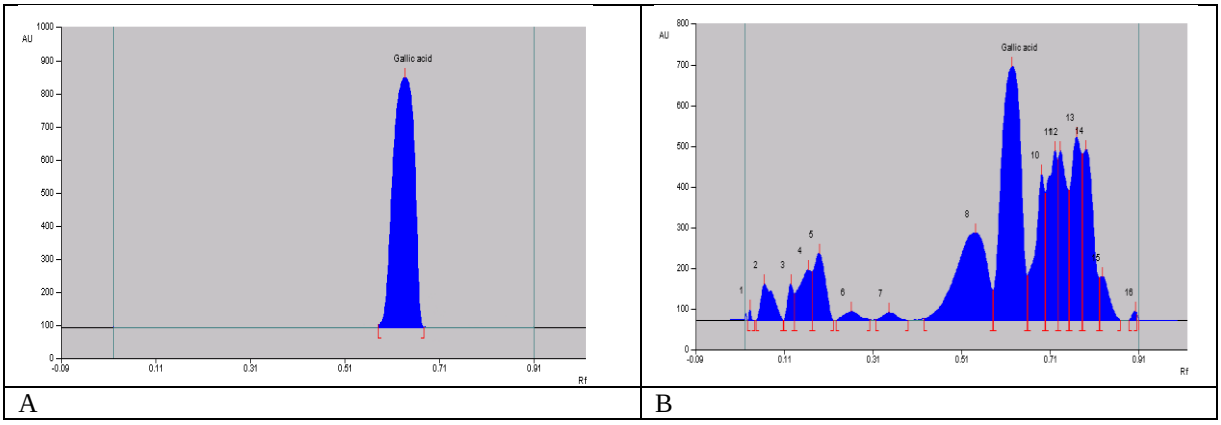
Table 9: Evaluation of Simhananda guggul kwatha

Sr. No.	Parameters	Obtained Values
1	pH	4.18@23^{0}C
2	Total ash	4.15%
3	Acid insoluble ash	0.25%
4	Water soluble extractives	84.60%
5	Alcohol soluble extractives	52.28%
6	Moisture Content (Loss on Drying at 105°C)	14.56%
7	Microbial Contamination	
8	Total bacterial count	COMPLIES
9	Yeast and Mould	COMPLIES
10	E. coli	ABSENT
11	Salmonella	ABSENT
12	Heavy Metal	
13	Lead	PASSES
14	Mercury	PASSES
15	Arsenic	PASSES
16	Cadmium	PASSES

Marker based standardization of sample

Method optimization

Using a CAMAG HPTLC system with a Linomat 5 autosampler, a TLC scanner 3, and WinCATS 1.2.2 software (CAMAG, Muttens, Switzerland), the best HPTLC separation was accomplished on a TLC plate. The solvent solution used for the separation was toluene: ethyl acetate: formic acid (3.5: 5.5: 1 v/v/v) on a silica gel 60 F254 TLC plate. 254 nm was determined to be the detecting wavelength. As shown in figures 2A–G, the standard gallic acid has good separation and resolution thanks to the optimized chromatographic technique (Rf value = 0.62).



A B

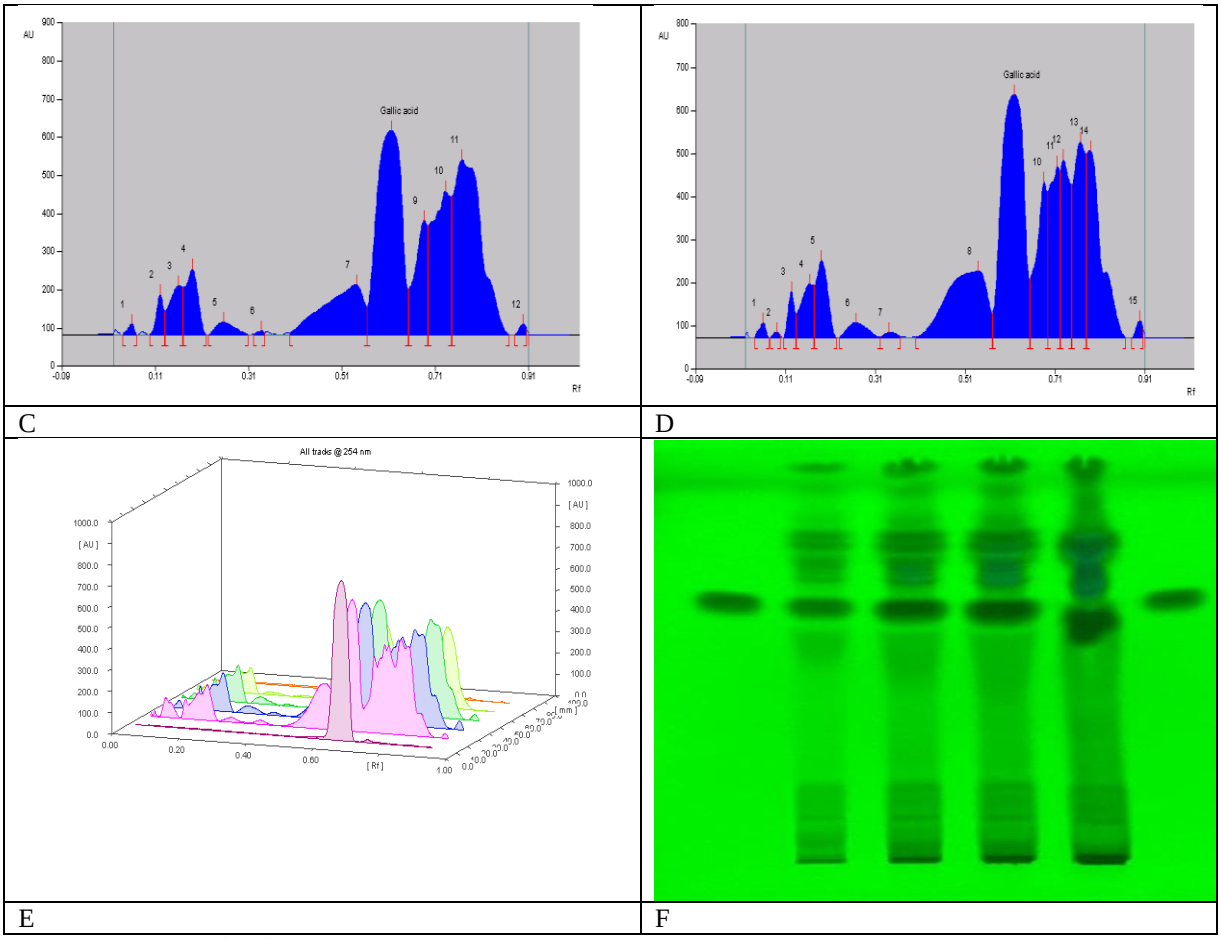


Figure 1: A- HPTLC profile of standard Gallic acid, B- Simhananda Guggul, C- Simhananda Guggul Pills, D- Simhananda Guggul, E- Gallic acid in all track, F- TLC of standard gallic acid and samples

Method validation:

Linearity:

Under the chromatographic conditions mentioned above, a linear relationship between the peak area and the applied concentration was found for gallic acid in the concentration range of 200–1200 ng/spot.

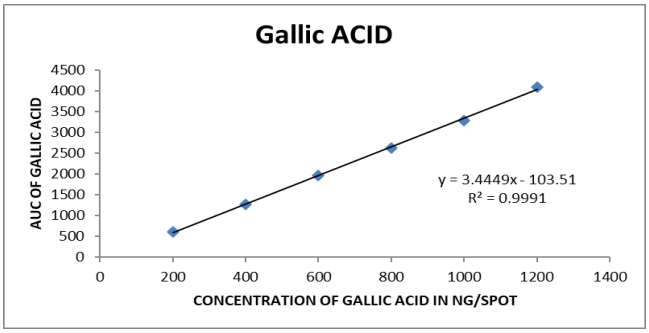


Figure 2: Linearity graph of Standard Gallic acid

Precision:

Precision data on instrumental variation and repeatability (intra-day) were obtained for gallic acid at three different concentration levels. In precision studies, the RSD was less than 1%, indicating great precision.

Accuracy:

A known quantity of standard was added to the sample, which contained 250 ng of gallic acid. Calculations were

used to determine the percentage ratios between the retrieved and anticipated concentrations. The recoveries fall between 81.80 to 118.97 percent. According to the findings, gallic acid may be accurately measured using the suggested HPTLC approach.

Robustness:
The modifications applied to the mobile phase and chamber saturation time did not result in any variation in the peak shape or retention time of either standard. The separation and resolution of the markings did not change in any way.

LOD and LOQ:
Gallic acid's LOD and LOQ were determined to be 45.1 ng/spot and 137.8 ng/spot, respectively.

Analysis of in-housed formulations of Simhananda guggulu kwatha and their respective solid dosage forms
The new approach produced a well-resolved chromatogram with no changes to the gallic acid peaks.

Table 10: Percentage of gallic acid in formulations

Sr. No.	Formulations	Percent of gallic acid
1	Simhananda Guggul – Kwatha	0.54 ± 0.25
2	Simhananda Guggul – Gutika	0.61 ± 0.14
3	Simhananda Guggul	0.59%±0.75
4	Simhananda Guggul – Churna	0.63%±0.34

CONCLUSION:

Both industrialized and developing nations now use herbal medicines as a powerful kind of treatment. Due to their superior therapeutic value, ayurvedic medications are becoming more and more popular among doctors and patients. The plant sterol guggulsterone is found in guggul resin. In Ayurveda, the resin has been used since ancient times to treat a wide range of conditions, such as obesity, osteoarthritis, bone fractures, inflammation, cardiovascular disease, diabetes, hyperlipidemia, and atherosclerosis. Guggul is never advised to be taken as a single medication in traditional Ayurvedic scriptures. Other herbs should be used in conjunction with it. The paranormal properties of Simhananda Guggul that are responsible for curing many illnesses were examined in this study. Strong antioxidant gallic acid has many uses, including antimutagenic, anti-inflammatory, and anticarcinogenic properties.

The safety and quality of the prepared kwath are provided by the physicochemical evaluation, which includes organoleptic characteristics, % yield, and LOD. Quality control tests, including those for hardness, disintegration time, friability, weight fluctuation, and percentage yield, should be conducted after tablet manufacturing. The disintegration test calculates how long it takes for a batch of pills to break up into smaller pieces under specific circumstances. The physicochemical characteristics of the medicine, such as pH and particle size, influence how well a tablet dosage dissolves. The friability test aids in assessing the tablets' physical strength, which is determined by their breaking force. The key variables that determine weight variance in tablets are the compression machine's tooling, head pressure, machine speed, powder flow characteristics, granulate density, and particle size. According to the results, every parameter in the quality control test was found to be within the Indian Pharmacopoeia's allowed range for monographs.

Gallic acid was chosen as the marker to standardize these formulations, and it was subsequently validated by HPTLC analysis. The Rf value, which served as the foundation for confirmation, was 0.62. To identify the marker compound (gallic acid), the in-house formulations of Simhananda kwatha and their corresponding solid dosage forms were examined. Gallic acid in the prepared formulation was compared as part of the investigation. It was determined that the Simhananda guggul pills are an excellent source of gallic acid since the percentages of gallic acid in the Simhananda kwatha, Simhananda guggul kalpa gutikas, Simhananda tablets, and Simhananda guggulu pills were 0.54%, 0.61%, 0.59%, and 0.63%, respectively. The manufactured formulations' purity and authenticity have been validated by the thorough analytical and pharmacological examination. It was determined that the current study can be applied to routine formulation and raw material analysis.

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