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Research Article

**FORMULATION, DEVELOPMENT AND MICROBIOLOGICAL  
STUDY OF APHRODISIAC CAPSULE, AND IDENTIFICATION  
AND DETERMINATION OF GALLIC ACID IN *EMBELICA  
OFFICINALE* BY HPTLC****Yogesh B. Bawne<sup>1</sup>, Sharda L. Deore<sup>2</sup>, Bhushan K. Shrikhande<sup>3</sup>, Sharad S. Dhurde<sup>4</sup>,  
Unmesh Keshwar<sup>5</sup>**<sup>1</sup>Shri Chhatrapati Shahu Maharaj Shikshan Sanstha Institute Of Pharmacy Maregaon-445303,<sup>2</sup>Associate Professor and Head of Pharmacognosy Department at Govt. Pharmacy College Amravati-444602, <sup>3</sup>Director of Siddhayu Ayurvedic Research and Foundation Pvt. Lit Nagpur, 440024, <sup>4</sup>Manager of Siddhayu Ayurvedic Research and Foundation Pvt. Lit Nagpur. – 440024,<sup>5</sup>QC Head of Siddhayu Ayurvedic Research and Foundation Pvt. Lit Nagpur-440024**Abstract:**

Research has been proposed. The polyherbal formula, only herbal Extract is taken to developed to efficient sexual activity and increase the sexual intercourse, Hydrolysable tannins convert into Gallic acid, Induce erectile dysfunction and antioxidant activity; it is more efficient with Sildenafil and also with Aphrodisiac natural compound. A polyherbal formula was developed which has mostly aphrodisiac activity and antioxidant activity. Raw materials evaluation and specifications were confirmed before development. The specified tannin assay of Awala extract was found to be 27.08% and the total tannins in the Aphrodisiac herbal capsule was found to be 8.31%. 0.67% and 0.93% Gallic acid was calculated in Awala extract and finished capsule. The percentage of saponin is calculated in the finished capsule at about 16.23%. For standardization and identification of gallic acid, the standard Gallic acid was ordered from Sigma Aldrich Company which was 97% pure. The chromatograph of Gallic Acid from Extract, Chromatogram of Gallic acid from Capsule and Chromatogram of Standard Gallic acid were an exact match in the overlaying spectrum of chromatographic conditions. Stability study of aphrodisiac product maintained at Accelerated Conditions (: 40 °C ± 2 °C/ 75 Rh ± 5 °C).

**Materials & Instruments:** Camag HPTLC system (Anchrom, Switzerland) consisting of Camag Linomat-V, Camag twin trough Chamber with stainless steel lids for chromatographic development, Camag TLC Scanner- III equipped with cats 4 software for scanning and documentation, and Camag UV cabinet. UV Spectrophotometer. PH meter, Toshcon instruments Pvt Ltd Haridwar. Horizontal laminar Air Flow, kirloskar India Ltd Mumbai. Electronic weighing balance, Mettler Toledo, AG 135, Tokyo. Muffle furnace, Tempo Instruments and Equipments (p) Ltd, Mumbai. Sonicator, Bieora Enterprises, Mumbai. Incubator, Elite Scientific instruments company, Bombay. **Chemicals and Media:** All chemicals were of analytical grade or HPLC grade. The raw extracts were obtained from Phyto Life Sciences P. Ltd. Ahmadabad, Gujarat, India. Markers were used for analytical method development and quantitative analysis. They were purchased from supplier Sigma Life Sciences Pvt, Ltd. by Siddhayu Ayurvedic Research Foundation Pvt. Ltd. Bahadura. Nagpur.

**Conclusion:** As per results and Observation, we concluded that all polyherbal formulations should contain natural awala or awala extract. And doctors may write additional vitamin c tablets along with other medication to increase the effectiveness of their medication. Overlining the spectra of three chromatograms was the same at every point, so we concluded and confirmed that standardization of Gallic acid has been successful. During the stability study, the parameters and specifications of aphrodisiac capsules were not changed. Those changed but they were within limits, and that all the activities and efficacy of natural organic compounds were still active.

**Keywords:** Gallic acid, Ellagic acid, Embillica officinale, Goose barry, Awala, Antioxidant, sexual Dysfunction, HPTLC.

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**INTRODUCTION:****Polyherbal preparations [06] [07] [29]:**

Polyherbal formulations as mentioned in classical texts of Ayurveda are used by a number of pharmaceutical companies. The polyherbal preparation as a number of ingredients may vary from 2 to 25 or more.

Herbal aphrodisiac: An aphrodisiac is defined as any food or drug that arouses the sexual instinct, induces veneral desire and increases pleasure and performance. Aphrodisiacs can be classified by their mode of action into three types: Those that increase libido, potency, or sexual pleasure. Various substances of animal and plant origin have been used in folk medicines of different cultures to energize, vitalize and improve sexual function, and physical performance in men medicine. Out of these, very few have been identified pharmacologically. <sup>[01][02] [05]</sup>

**Evaluation of the Herbal Aphrodisiac Product (Capsule) [06] [07]:**

Physicochemical Evaluation:

- **Organoleptic evaluation:** Shape, size (diameter and thickness), weight (size and weight determines the density), score (or groove), Imprinting, Color are a few important parameters that need to be evaluated for capsules.
- **Weight variation:** To know content uniformity, weight variation is an important parameter. 80 mg or less = 10 %; More than 80 mg or less than 250 mg = 7.5 %; 250 mg or more = 5 %
- **Disintegration studies:** disintegration is the first step for a drug to become bioavailable. The tablet must first disintegrate and discharge the drug into the body fluids.
- **Heavy metal detection:** Trace elements and heavy metals analysis is used as a standardization

tool of the plant material. As per WHO, the limit for lead is 10 mg/kg and for Cadmium it is 0.3 mg/kg. There are several laboratory methods that determine heavy metal concentrations with accuracy down to the ppm range or below: atomic absorption spectroscopy (AAS), colorimetry, inductively coupled plasma atomic emission spectroscopy (ICP-AES), polarography, selective ion electrodes, X-ray fluorescence, energy dispersive analysis via X-rays (EDAX), and electron microprobe analysis.

- **Microbial study:** Plant materials normally carry a great number of bacteria and moulds often originating in soil. Current practices of harvesting, handling and production may cause additional contamination and microbial growth. In addition, the presence of aflatoxin in plant material can be hazardous to health if absorbed in very small amounts.
- For contamination of crude plant material intended for further processing.
  - E. Coli -  $10^4$  per gram.
  - Mould propagules -  $10^5$  per gram.
- For plant material that are used as topical dosage form.
  - Aerobic bacteria max  $10^7$  per gram.
  - Yeasts or mould max.  $10^4$  per gram.
  - E. Coli max.  $10^2$  per gram.
- Other enterobacteria max  $10^4$  per gram.
  - Salmonella - none.
- For plant material use for internal purpose
  - Aerobic bacteria  $10^5$  per gram.
  - Yeasts or mould max.  $10^3$  per gram.
  - E. Coli max.  $10^2$  per gram.
  - Other enterobacteria max  $10^3$  per gram.
  - Salmonella - none.

**Table 1: Medias for various bacterias: [09]**

Bacteria	Media	Observation
<i>Enterobacteriaceae</i> and other Gram-negative bacteria	Violet red bile agar with glucose and lactose	Red or reddish in color
<i>Escherichia coli</i>	Maconkey broth	Reddish brown precipitation
<i>Salmonella Species</i>	Deoxycholate citrate agar	Well developed, colorless
<i>Pseudomonas aeruginosa</i>	Soyabean- casein digest medium	Green fluorescence
<i>Pseudomonas aeruginosa</i>	Baird-Parker agar	Black colonies

- Prepare the selected media and transfer to the petri plate, apply the herbal sample and allow incubation for 24-48 hrs. Then observe and compare colonies.

**Table 2: Standard Gallic Acid**

Sr. no	Compound	Purity	Supplier
1.	Gallic acid	97%	Sigma Aldrich

**Formulation Profile [15] [20] [24] [25]:**

There are a number of polyherbal medicinal preparations available on the market. Siddhayu Ayurvedic Research Foundation Pvt. Ltd. Bahadura ,Nagpur has also developed many polyherbal preparations like sundarikalp, shankhapushpi, turmeric HRx capsule, BLS Ltd. During the interaction with BLS Ltd., we came across different formulations that have been developed by them. One of them is the herbal aphrodisiac capsule. Herbal aphrodisiac capsules have been taken for standardization and validation.

Ingredients of Herbal Aphrodisiac Capsule: Each herbal capsule contains Dry Extract of

1. Aswagandha (*Withania somnifera*)
2. Gokharu (*Terrestris tribulus linn*)<sup>[18]</sup>
3. Pippali (*Pipper longum*)<sup>[19]</sup>
4. Vidarikand Root (*Pueraria tuberosa*)<sup>[21]</sup>
5. Awala Fruit (*Embelica officinale*)
6. Safed musli(*Asparagus adscendens* )
7. Semal Musli ( *Bombax mulabaricum*)
8. Shilajit (*Asphaltum*)
9. Kaunch (beej) (*Mucuna pruriens*)
10. Excipients q.s.

***Emblica officinalis*. (Amalaki)<sup>[10]</sup>****Biological source:**

The drug consists of fresh and dried fruit of *Emblica officinalis* Gaertn. (Syn. *Phyllanthus emblica* Linn) of the family Euphorbiaceae.

**Synonyms:**

- English: Embelic Myrobalan
- Hindi: Mala.
- Marathi: Anvala, Avala.

**Chemical Constituents:**

Ascorbic acid, Gallic acid, chebulinic acid, chebulagic acid, ellagic acid, 3-ethyl Gallic acid, corilagens. Isostrictiniin, terchebin, emblicanin-A and B, punigluconin, pedunculagin, 1-0-galloyl-,6-D-glucose. 3, 6-di~o-galloyl-D-glucose, quercetins.

Major Therapeutic Claims: Bleeding disorders, jaundice, gastritis and anti-diabetes.

**Uses:** Hepatotoxicity, Anti-inflammatory, Antioxidant.

**Assay of Total tannin in Awala extract, [23] [25]:**

Take 1.0 g extracted powder in a 100 ml volume flask and 50 ml of hot water with constant shaking

.When powder goes completely in solution, make up the volume with distilled water up to 100 ml .Filter

**Table 3: Results of Total tannin in Awla extract**

Total tannins	Materials	Sample weight	Blank reading	Sample reading	Percentage found
	Awla Extract	0.09965 g	0.9 ml	7.4 ml	27.08 %

**Table 4: Raw material evaluation observation: [06][07][09]**

Raw material evaluation and specification of <i>Embelica officinale</i> Dry extract			
Name of Drug: <i>Embelica officinale</i> Dry extract			
Name of Supplier: Phyto Life Sciences Ahmadabad Gujarat			
Sr. no	Parameters	Passing Limits	Observation
01	Organoleptic properties		
	State	Fine powder	Complies
	Colour	Grayish to brown coloured	Complies
	Odour and taste	Characteristic odour	Complies
02	Phytochemical characterization		
	Identification/ Group detection:		
	Ferric chloride test	Positive for saponine	Complies
	PH (1 % Aqueous solution)	3-6	3.89
	Total Ash	NMT 20 %	3.63 %
	Water soluble extractive (WSE)	NLT 80%	87.0 %
	Loss on drying(LOD)	NMT 8.0 %	4.12 %
	Assay (Gallic acid)	NLT 20 %	21.67 %
03	Heavy metals		
	Arsenic	NMT 3 ppm	0.50 ppm
	Lead	NMT 10 ppm	0.98 ppm
	Mercury	NMT 1 ppm	Not detected
	Cadmium	NMT 0.3ppm	Not detected
04	Microbiological study		
	Total Aerobic microbial count	< 10 <sup>5</sup> cfu/g	346 cfu/g
	Total Yeast/ Mould	< 10 <sup>3</sup> cfu/g	29 cfu/g
	<i>Escherichia .coli</i>	Absent	Complies
	<i>Salmonella sp</i>	Absent	Complies
	<i>Staphylococcus aureous</i>	Absent	Complies
	<i>Pseudomonas aeruginosa</i>	Absent	Complies

**Development of Aphrodisiac Product (Veg Capsule) [13] [14]:**

Gokharu, Vidarikand, safed musli and Semal musli plants are well known aphrodisiacs. But in the presence of Gallic acid (Awala), the potency of sexual activity enhances. Gallic acid is a strong antioxidant and adaptogenic. Studies have proved that Fulvic acid is responsible for some degree of erectile dysfunction and enhances sexual behaviour.

Withaferin-A is helpful in strengthening the immunity of a person and enhancing sexual behaviour, Piperine enhances the bioavailability of the chemical compound. Doses of powder of extracts from nine selected plant materials were chosen based on previous literature. Excipients were mixed with accurately weighed extracted powder, sieved and evaluated for powder characteristics. Based on results, final batch B3 was selected.

**Table 5: Composition of three trial batches of Aphrodisiac capsules:**

Sr no	Ingredients	Each batch contains 350 capsule's materials		
		B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>
1	Ashwagandha extract	20 g	25 g	30 g
2	Gokhru extract	20 g	25 g	30 g
3	Pippali extract	20 g	25 g	30 g
4	Vidarikand extract	20 g	25 g	30 g
5	Awla extract	20 g	25 g	30 g
6	Safed musli extract	20 g	25 g	30 g
7	Semal musli extract	15 gm	17 g	20 g
8	Shilajit extract	10 mg	13 g	15 g
9	Kaunch beej Powder	15 mg	17 g	20 gm
10	DCP	4.550g	4.550g	4.550g
11	Talk	3.3g	3.3g	3.3g
12	Stearic acid	1.73g	1.73g	1.73g
13	Aerosile	1.70g	1.70g	1.70g
14	Sodium methyl paraben	0.2599g	0.2599g	0.2599g
15	Sodium propyl paraben	0.100g	0.100g	0.100g

**Assay of Total tannin in Awala extract and Aphrodisiac capsule: [22]:**

Take 1.0 g extract powder in a 100 ml volume flask and 50 ml hot water with constant shaking .When powder goes completely in solution, make up the volume with distilled water up to 100 ml .Filter

**Table 6: Results of Total tannin in Awala extract and Aphrodisiac capsule**

Total tannins	Materials	Sample weight	Blank reading	Sample reading	Percentage found
	Awala Extract	0.09965 g	0.9 ml	7.4 ml	27.08 %
	aphrodisiac capsule	0.0999 g	0.9 ml	2.9 ml	8.31 %

**Percent of Gallic acid in Awala extract:**

$$\begin{aligned}
 &= \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard concentration} \times \text{Purity} \times 100}{\text{sample concentration} \times 100} \\
 &= \frac{3251.8}{9237.1} \times \frac{1}{50.5} \times \frac{97}{100} \times 100 \\
 &= \frac{3154}{4664} = 0.67 \%
 \end{aligned}$$

**Percent of Gallic acid in Aphrodisiac capsule:**

$$\begin{aligned}
 &= \frac{\text{Sample area } X}{\text{Standard area}} \times \frac{\text{Standard concentration } X}{\text{sample concentration}} \times \frac{\text{Purity } X}{100} \times 100 \\
 &= \frac{11084.4}{9237.1} \times \frac{1}{124.84} \times \frac{97}{100} \times 100 \\
 &= \frac{1075}{1153} = 0.93 \%
 \end{aligned}$$

**Assay of saponins for Aphrodisiac capsule:**

Weigh accurately 1.5 to 2 gm of the material in a beaker. Add 50 ml of petroleum ether (500' 30°) and gently heat to 40°C in bath water for 5 minutes with regular shaking. Filter the petroleum ether and repeat the operation with a further 2x 50 ml of petroleum ether. Discard petroleum ether and preserve the marc. Extract the marc obtained in the previous test with 4 X 60 ml of methanol with mild heating. Filter the methanol layer into another beaker. Concentrate the combined methanol layer to about 25 ml. add 150 ml of dry acetone to precipitate the saponins. Filter the saponins through a tarred glass crucible or through a paper filter and dry at 100°C for constant weight.

**Calculation:**

Weight of sample = 2.0339 g

Weight of filter paper (A) = 1.3650 g

Weight of filter paper + residue (B) = 1.6953

A-B = 0.3303 g

$$\begin{aligned}
 \text{Percentage of total saponins} &= \frac{\text{Weight of residue} \times 100}{\text{Weight of sample taken}} \\
 &= \frac{0.3303 \times 100}{2.0339} = 16.2397 \%
 \end{aligned}$$

**Study of Gallic acid by HPTLC Chromatographic condition: [16]****Preparation of reference standard solution:**

Accurately weighed 1 mg, in a 100 ml volumetric flask and dissolved in 10ml methanol, Sonicate, filtered and made up volume up to 10 ml methanol.

**Preparation of sample (Raw extract) solution:**

About 0.1010 gm accurately weighed, added 20 ml of diethyl ether reflux the Residue further for three times more, cool and filtered and combine the filtrate and evaporated up to dryness residue dissolved in 9 ml methanol, Sonicate, filtered and volume made up 10 ml.

**Preparation of a sample solution of Aphrodisiac capsule content:**

10 capsules were taken and homogeneous capsule powder was made. Accurately weighed 0.3121 g of fine powder and treated with 50 ml diethyl ether and reflux in a water bath for 15 minutes, cool and filtered, evaporate diethyl ether .reflux the Residue further with methanol for three times more, cool and filtered .combine the filtrate and evaporated up to dryness. Then the residue is

dissolved in 5 ml of methanol, sonicate, filtered and volume made up to 10 ml of methanol.

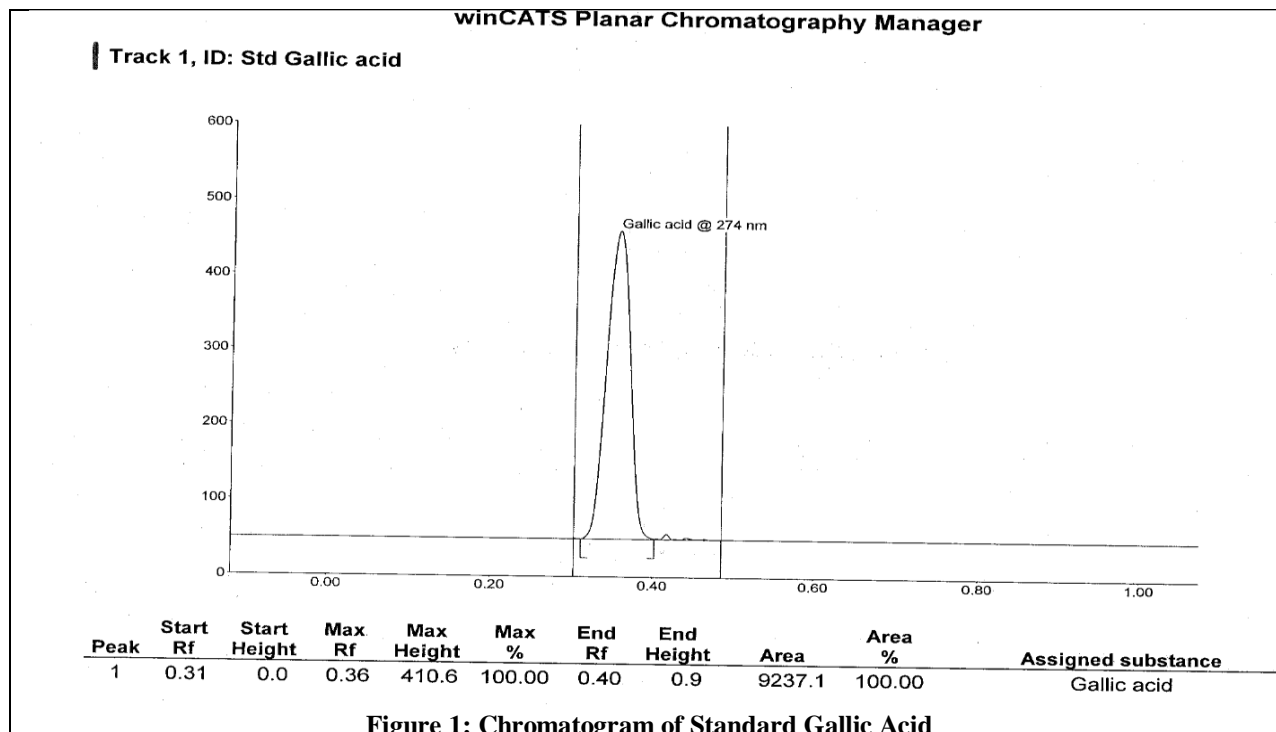
Selection of mobile phase: The most usable mobile phase is (Toluene: Ethyl acetate: Formic acid) in (5:4:1) ratio was selected among all mobile phases, was prepared during identification of Protodioscin by HPTLC method

**Qualitative estimation:**

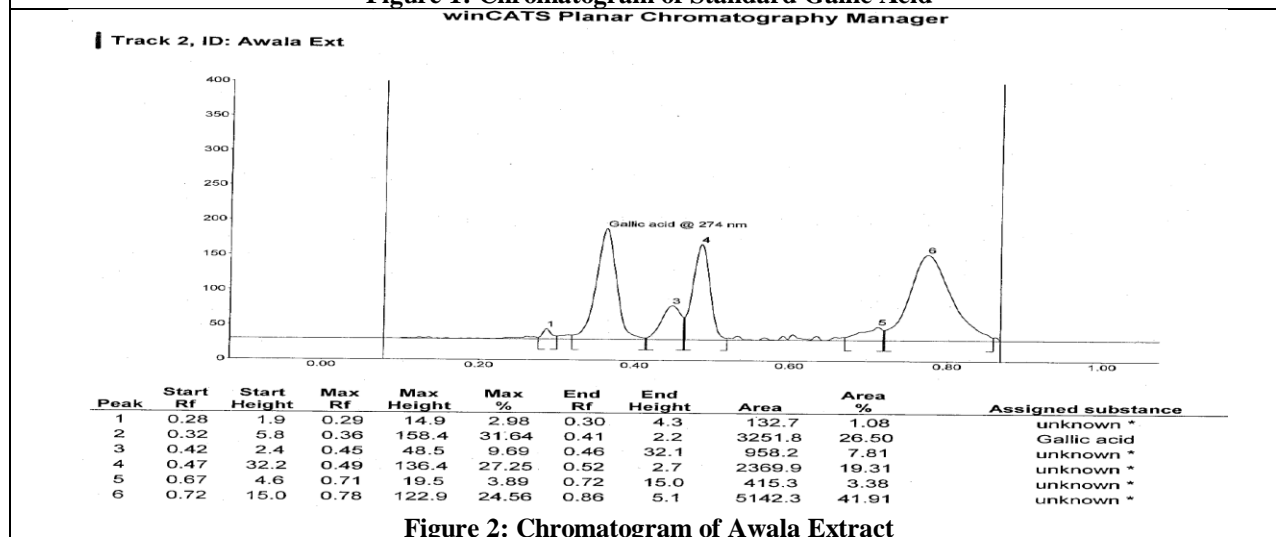
Apply Standard Gallic acid (1µg/µl), Embelica officinale extract (50.5 µg/µl), Aphrodisiac capsule (124.84 µg/µl ), were applied as (10µl,10µl,10µl/spot), per spot respectively on TLC plate, developed with mobile phase and scan at 274 nm. Peak areas corresponding to Rf values 0.31, 0.32, 0.34 were found for Gallic acid in the chromatogram of standard Gallic acid, Awla extract, capsule, and their overlay spectra were shown in the figure.

Chromatographic conditions: The chromatographic conditions were established by trial and error method. After stabilizing the condition, it was kept constant during experimentation.

Stationary phase	: HPTLC Precoated, silica gel 60, F254 (Merck)	Saturation time	: 20 min.
Thickness	: 0.2 mm	Migration distance	: 70 mm
Mode of application	: Band	Scanning mode	: Absorbance / Reflectance
Band width	: 6 mm	Slit dimension	: 6.0 x 0.45 mm
Separation technique	: Ascending	Scanning wavelength	: <b>274 nm</b>
Temperature	: 25 ± 3°	Detection	: UV
		Densitometric scanning (CAMAG TLC 3 Scan)	



**Figure 1: Chromatogram of Standard Gallic Acid**



**Figure 2: Chromatogram of Awala Extract**

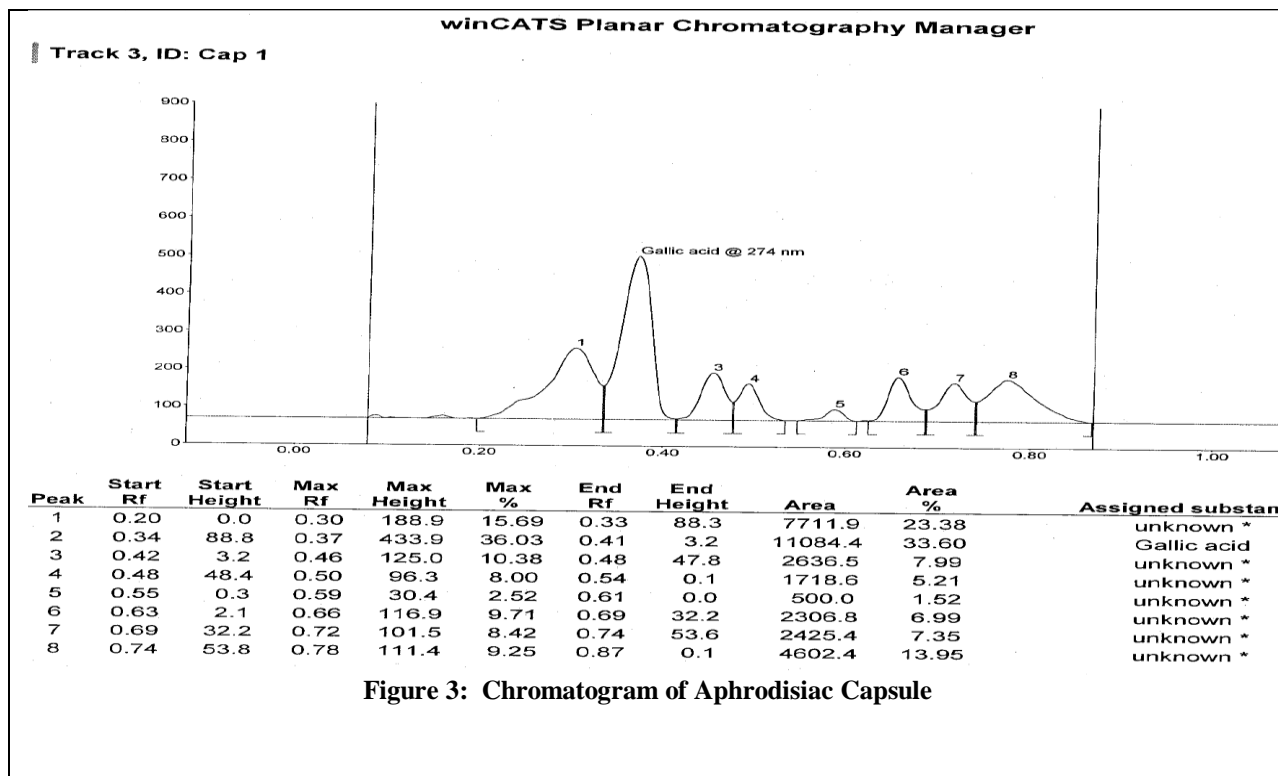


Figure 3: Chromatogram of Aphrodisiac Capsule

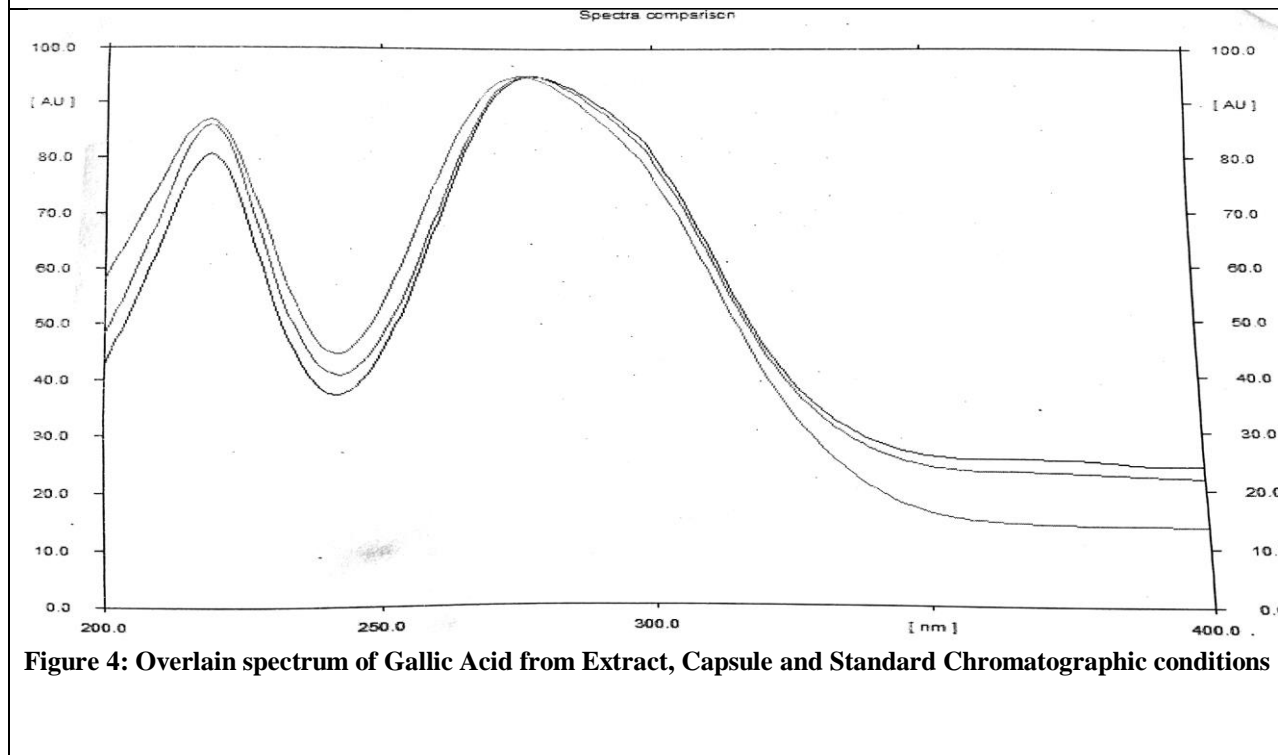


Figure 4: Overlain spectrum of Gallic Acid from Extract, Capsule and Standard Chromatographic conditions



**Table 7: Results of Evaluation of Aphrodisiac Capsule before Stability Test: [17] [28]**

Sr. No.	Parameters	Passing Limits	Test Observation
1	Description	Transparent vege capsule of size "00" filled with grayish brown to brown coloured granular powder.	Complies
2	Identification by TLC		
		Positive for Protodioscin	Complies
		Positive for Withaferin A	Complies
		Positive for Piperine	Complies
		Positive for Gallic acid	Complies
3	Weight variation		
	Weight of 20 Capsules	14.6000 g $\pm$ 7.5 %	14.7085 g
	Average Weight of Capsule	0.7300 g $\pm$ 7.5 %	0.7354 g
	Weight of 20 Capsules content	12.0000 g $\pm$ 7.5 %	12.0566 g
	Average weight of Capsule content	0.6000 g $\pm$ 7.5%	0.60283 g
	Uniformity of weight/ Weight variation	600 mg $\pm$ 7.5 %	Complies
4	Disintegration time	NMT 30 min	8 min
5	Assay		
	Total Saponins <sup>[26]</sup> (By gravimetric)	NLT 10 %	16.23 %
	Fixed oil (By gravimetric)	-	2.46 %
	Total Tannins (By Titration )	- 8.31 %	
	Gallic acid (By HPTLC)	-	0.93 %
10	Heavy Metals		
	Lead	NMT 10.0 ppm	0.49 ppm
	Arsenic	NMT 3.0 ppm	0.10 ppm
	Cadmium	NMT 0.3 ppm	0.002 ppm
	Mercury	NMT 1.0 ppm	0.039 ppm
11	Microbiological study		
	Total microbial plate count	NMT 10 <sup>5</sup> cfu/g	195 cfu/g
	Total Yeast and Mould count	NMT 10 <sup>3</sup> cfu/g	22 cfu/g
	<i>Escherichia .coli</i>	Absent/g	Absent
	<i>Salmonella sp</i>	Absent/g	Absent
	<i>Staphylococcus aureous</i>	Absent/g	Absent
	<i>Pseudomonas aeruginosa</i>	Absent/g	Absent

**Stability Studies: [28]**

**Selection of Batch:** The three samples were selected randomly from each batch, B1, B 2 and B3, as a group of 20 capsules. These samples were packed in the same container and closure system used for packaging.

**Storage conditions:** The stability of capsules was studied by exposing them to elevated conditions of temperature and humidity. Capsules were kept at 40  $\pm$  20 C to 75  $\pm$  5 % RH in a stability chamber.

**Testing frequency:** At the accelerated storage condition, the testing frequency of a minimum of three time points, including the initial and final time points, for a 6 month study is recommended.

Therefore, the required sample was withdrawn for analysis of physical parameters at a time interval of 0, 3 and 6 months and evaluated. Capsules were found slightly stuck to each other but easily separated. Results for stability are shown in Table 9.

**Physical parameters under accelerated stability study:** The evaluation of suspension was done at 3 month intervals at accelerated stability conditions to confirm its stability considering physical parameters. The results are shown in table 9.

**Chemical parameters under accelerated stability study:** The chemical parameters of an Aphrodisiac product were studied by comparing the HPTLC fingerprinting of the initial as well as in sample

stored at different conditions for 0, 3, 6 months. HPTLC finger print for antacid suspension containing Protodioscin and general peaks at initial stages as the

sample stored in different conditions for 0, 3, 6 months are shown in fig.

**Table 8: As ape Stability protocol under ICH guidelines<sup>[14]</sup>**

Study	Storage condition	Minimum time period covered by data at submission
<b>Long term*</b>	30 <sup>0</sup> ± 2 <sup>0</sup> /65 RH ± 5% RH	12 months
<b>Intermediate**</b>	30 <sup>0</sup> ± 2 <sup>0</sup> /65 RH ± 5% RH	6 months
<b>Accelerated</b>	40 <sup>0</sup> ± 2 <sup>0</sup> /75 RH ± 5% RH	6 months

\* 25<sup>0</sup> ± 2<sup>0</sup> /60 RH ± 5% RH or 30<sup>0</sup> ± 2<sup>0</sup> /65 RH ± 5% RH, to be decided by applicant

\*\*If 30<sup>0</sup> ± 2<sup>0</sup> /65 RH ± 5% RH is the long term and no intermediate condition

If a significant change occurs in the chemical stability at 40<sup>0</sup> ± 20 to 75% ± 5% RH for 6 months, the substance is unstable. It is considered unnecessary to continue to test a drug substance for 6 months when a significant change has occurred within the first 3 months.

**Table 9: Results for stability study of Aphrodisiac Capsule: [06] [28]**

Product Name :Aphrodisiac Product (Capsule)					
Batch No: AP -03 Storage condition : 40 0c ± 2 0c/ 75 Rh ± 5 0c					
Pack Size : 15 Capsules Batch Size : 1000 Caps					
Sr. no	Parameters	Passing Limits	'0' Month	'3' Month	'6' Month
1	Description	Brown coloured vege. Caps of size '00' filled with grayish brown to brown coloured granular powder	Complies	Complies	Complies
2	Identification test				
		Positive for Protodioscin	Complies	Complies	Complies
		Positive for Gallic acid	Complies	Complies	Complies
4	Uniformity of weight				
	Weight of 20 Capsules	14.6000 g ± 7.5%	14.7085 g	14.6129g	14.509g
	Average Weight of Capsule	0.730g ± 7.5%	0.7384 g	0.7306g	0.7354g
	Weight of 20 Capsules content	12.0000 g ± 7.5	12.0566 g	12.0480g	12.0465g
	Average weight of 20 Capsule content	0.6000 g ± 7.5	0.60283 g	0.6024g	0.6023g
	Uniformity of weight/ Weight variation	600 mg±7.5	Complies	Complies	Complies
4	Disintegration time	NMT 30 min	8.55 min	9.08 min	9.30 min
5	Assay				
	Total saponin (By gravimetric)	NLT 8 %	16.2001 %	-	-
	Fixed oil (By gravimetric)	-	2.460 %	-	-
	Total tannins (By gravimetric) <sup>[23]</sup>	-	8.31 %	-	-
6	Heavy metals				
	Lead	NMT 10 ppm	Complies	Complies	Complies
	Arsenic	NMT 3 ppm	Complies	Complies	Complies
	Cadmium	NMT 0.3ppm	Complies	Complies	Complies
	Mercury	NMT 1 ppm	Complies	Complies	Complies
7	Microbiological study				

Total microbial plate count	NMT 10 <sup>5</sup> cfu/g	820 cfu/g	825 cfu/g	830 cfu/g
Total Yeast and Mould count	NMT 10 <sup>3</sup> cfu/g	20 cfu/g	30 cfu/g	24 cfu/g
<i>Escherichia .coli</i>	Absent/g	Complies	Complies	Complies
<i>Salmonella sp</i>	Absent/g	Complies	Complies	Complies
<i>Staphylococcus aureous</i>	Absent/g	Complies	Complies	Complies
<i>Pseudomonas aeruginosa</i>	Absent/g	Complies	Complies	Complies

### CONCLUSION:

Awala containing ellagic acid, gallic acid and ascorbic acid are very effective in enhancing drug activity, to enhance absorption, and to enhance sexual activity. So we concluded that all polyherbal formulations should contain natural awala or awala extract. And doctors may write additional vitamin c tablets along with other medication to increase the effectiveness of their medication. The chromatogram of the extract sample and capsule sample exactly matched with the chromatogram of standard Gallic acid. Overlining the spectra of three chromatograms was the same at every point, so we concluded and confirmed that standardization of Gallic acid has been successful. During the stability study, the parameters and specifications of aphrodisiac capsules were not changed. Those changed but they were within limits, and that all the activities and efficacy of natural organic compounds were still active, so we concluded that our formulation was successful.

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