

FORMULATION, STANDARDIZATION AND QUANTIFICATION OF WITHAFERIN

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Abstract: In This research we have used herbs extract for more potential the product value; The formula we have developed for aphrodisiac purpose. This research includes standardization, development, evaluation, and quantification of a Withaferin-A drug. We have standardized chromatogram of *Withania somnifera* extract and Developed Capsule extract with standard Withaferin-A chromatogram. In the overlain spectrum of standard Withaferin-A, extract of *Withania somnifera* and capsule product, there is an exact match. We Find out the quantitative estimation of Withaferin-A in aphrodisiac products followed by a quantitative evaluation of Withaferin-A. These results were confirmed with the chromatograms of HPTLC fingerprinting.

Materials: A mixture of well researched Ashwagandha was taken and standardized with Standard Withaferin-A 95%, this standard was purchased from Sigma Aldrich Company.

Project content and method^{4, 5, 11, 12} All of which were extracted, which are quite evident in increasing the intercourse of sex. The identification of the extract with its testing, raw material evaluation, drug percentages were determined therein by HPTLC chromatograph and UV spectrometer, and some phytochemical processing of Withaferin-A 95%, which is extracted from raw materials extract. The chromatogram of the extract, HPTLC chromatogram of the finished product (capsule), was matched to the standard chromatogram. A heavy metal detection and quantization instrument, ICP-MS, was used. Along with raw material and finished product evaluation, a microbiological study was also done. Ultimately, my project's capsule was designed to increase the strength of mental, physical, immune-boosting, intellectual power, and sexual intercourse. The entire work of this project is done by Baidyanath Ayurvedic Company branch, Siddhayu Ayurvedic Research Foundation Pvt. Ltd. and completed in a well-stabilized lab located at Nagpur.

Key words: *Withania somnifera*, Ashwagandha, Sexual booster, Immuno booster, Standardization, Withaferin A, Chromatogram

Background: Keeping in mind the modern era, the task of creating a more immuno-boosting capsule was taken up. I know the importance of Ayurveda. Polluting the environment, preservatives in eating, porn sites, promoting sex in public, starting to collapse physical development, before full physical and sexual development, In such a situation, my contribution is important for society, so the project to create a capsule that enhances mental strength, physical strength, as well as sexual intercourse, because people are afraid to use synthetic medicines for sexual stimulation. In this way, people believe in the perfect Ayurvedic herbs, which have been going on for millions of years. The Ayurvedic medicine system is the oldest and most reliable medical system in the whole world. As a result, tree plants that increase sexual power, sexual intercourse, immunity busters, and are more prevalent were chosen. I had started the work of making a capsule, making his formula, which had plant extract for it, more stimulation of sexual desire, and to increase the strength in a short time, and had to create a capsule without any side effects.

METHODS AND EXPERIMENTATION: PHYSICO-CHEMICAL VALUATION^{4, 5, 11, 12}

Withania somnifera raw extract was evaluated as per the USFDA guidelines for raw material evaluation. Specifications for different parameters were developed for each herbal extract. The parameters and procedures considered for the purpose were as per WHO guidelines, Ayurvedic Pharmacopoeia of India (2000), Indian Pharmacopoeia (1996), and Quality Control Manual for Ayurvedic, Siddha, and Unani Medicine. The herbal extracts were evaluated on the basis of their organoleptic, physical, and phytochemical properties.

Organoleptic Properties^{25, 26, 29} *Withania somnifera* raw materials are evaluated for their state, colour, and odour in order to characterize their organoleptic properties.

Ash values:

Total Ash Value^{15, 17} A tarred silica crucibles was weighed. gm of accurately weighed extract was taken in the crucible and incinerated at a temperature not exceeding 450 °C until it was free from carbon. The crucible was then cooled in a desiccator and weighed. The total ash was calculated by subtracting the weight of the crucible with ash of drug after ignition from the weight of the crucible with drug powder before ignition. The percentage of total ash was calculated with reference to air-dried drug.

Acid-insoluble ash value^{15, 18} The ash obtained in the total ash method was boiled with 25 ml of 2 M hydrochloric acid for 5 min. Insoluble matter was collected on ash-free filter paper, washed with hot water, and then ignited in a muffle furnace at a temperature not exceeding 450 °C, and heating continued until the crucible's constant weight was reached. The crucible is then taken out of the furnace, cooled in desiccators, and weighed. The percentage of acid insoluble ash is calculated with reference to air dried material.

Extractive value:

Water Soluble Extractive¹⁸: Exactly 5 g of drug was weighed and macerated with 50 ml of chloroform water in a closed flask for 24 hours, shaken frequently during the first 6 hours and allowed to stand for 18 hours. 25 ml of the filtrate was transferred to a tarred porcelain dish. The solvent was evaporated and dried at 105 °C in an oven, cooled in a desiccator, and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug.

Alcohol Soluble Extractive¹⁸: For exactly 2.5g of drug, it was weighed and macerated with 90 % alcohol in a closed flask for 24 hours, shaken frequently during the first 6 hours and allowed to stand for 18 hours. 25 ml of the filtrate was transferred to a tarred porcelain dish. The solvent was evaporated and dried at 105°C in an oven, cooled in desiccators, and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.

Loss on drying¹⁶: In a previously dried weighing bottle, 1 gm of drug was placed. The sample was dried in an oven at 105°C until a constant weight, and then the bottle was cooled in desiccators and weighed. The percentage of weight loss was calculated with reference to the air dried drug.

Determination of pH^{3, 26}: 1% solution of samples was prepared and pH was measured with the help of a digital pH meter.

Heavy metal detection^{24, 25} (see pg. No 50)

Microbial study²¹: Plant materials normally carry a great number of bacteria and molds, 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 a very small amount.

- For contamination of crude plant material intended for further processing.
 - E. Coli -10⁴ per gram.
 - Mould propagates - 10⁵ per gram.
- For plant material that are used as topical dosage form.
 - Aerobic bacteria max 10⁷ per gram.
 - Yeasts or mould max. 10⁴ per gram.
 - E. Coli max. 10² per gram.
- Other enterobacteria max 10⁴ per gram.
 - Salmonella - none.
- For plant material use for internal purpose Aerobic bacteria 10⁵per gram.
 - Yeasts or mould max. 10³ per gram.
 - E. Coli max. 10 per gram.
 - Other enterobacteria max 10³per gram.
 - Salmonella - none.

Medias for various bacterias

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 chosen media and transfer to a petri plate, then add the herbal sample and incubate for 24-48 hours. Then observe and compare the colonies.

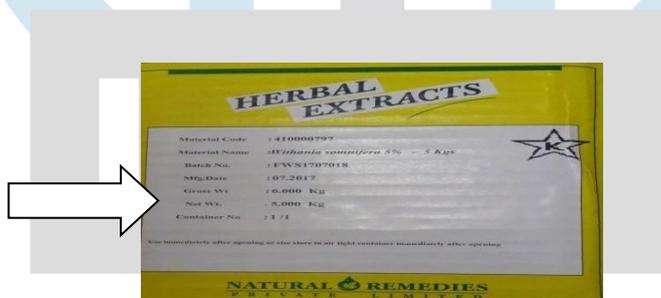
RESULTS OF RAW MATERIAL STANDARDISATION^{4, 5, 11, 12}

Table 5.3: Raw material evaluation and specification of <i>Withania somnifera</i>			
Name of Drug: <i>Withania somnifera</i> Dry extract			
Name of Supplier: Natural remedies Pvt. Ltd.			
Sr. no	Parameters	Passing Limits	Observation
01	Organoleptic properties		
	State	Fine powder	Complies
	Colour	Brown coloured	Complies

	Odour and taste	Having characteristic odour and Bitter	Complies
02	Phytochemical characterization		
	Identification/ Group detection:		
i)	Dragendorff's test	Positive for alkaloids	Complies
ii)	Mayer's reagent test	Positive for alkaloids	Complies
iii)	Identification by TLC	Positive for Withaferin-A	Complies
	PH (1 % Aqueous solution)	4-7	5.59
	Total Ash	NMT 15 %	6.05 %
	Acid insoluble ash	NMT 7 %	0.4 %
	Water soluble extractive (WSE)	NLT 80 %	83.80 %
	Loss on drying (LOD)	NMT 5 %	3.26 %
	Assay for Total Withanolides (By Gravimetry)	NLT 5 %	5.73 %
03	Heavy metals		
	Arsenic	NMT 3 ppm	0.47 ppm
	Lead	NMT 10 ppm	1.09 ppm
	Mercury	NMT 1 ppm	Not detected
	Cadmium	NMT 0.3ppm	Not detected
04	Microbiological study		
	Total Aerobic microbial count	< 10 ⁵ cfu/g	426 cfu/g
	Total Yeast/ Mould	< 10 ³ cfu/g	23 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

Raw material profile

1. *Withania somnifera* ^{6, 7,8,11}



Dry extract of Ashwagandha

Biological Source: The drug consists of the dried *Withania somnifera* of the family Solanaceae. An erect, evergreen, tortentose-branched, 60 to 150 cm tall shrub found as a weed and in cultivation in India's plains, primarily in Madhya Pradesh and neighbouring districts of Rajasthan, as well as in arid, warmer zones and sub-Himalayan tracts ascending to 1200 m altitude.

Synonyms:

- Bengali: Ashvagandha
- English: Winter cherry
- Hindi: Asgandh, Asgand, Asagandha
- Marathi: Asgund, Asvagandhi, Asagandha, AsandhaPunj. Asgandh

Chemical Constituents Major:

Withaferin-A, withanolides I-VII, withanolides A, B ,and D, 17-hydroxy-27—deoxy Withaferin A, withanone.

Major Therapeutic Claims: Immunomodulatory, strength promoting, adoptogenic, Parkinson's disease, nervous disorders , aphrodisiac, fracture, healing, general weakness, cooling, diuretic, tonic, aphrodisiac, calculus affections, urinary discharges, and impotence, cardiac stimulant, antimicrobial, antibacterial, antitumor, general debility, ageing, stress-induced disorders, rheumatic disorders, anxiety, neurosis , and ulcers.

Composition of three trial batches of Aphrodisiac capsules

Sr. No	Ingredients	Each batch contains 350 capsule's materials		
		B ₁	B ₂	B ₃
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 Withaferin-A in *Withania somnifera* extract and Aphrodisiac capsule^{24, 25}

Preparation of reference standard solution: Accurately weighed 1 mg, in a 100 ml volumetric flask and dissolved in 5 ml methanol, Sonicate, filtered and made up the volume up to 10 ml methanol.

Preparation of sample (Raw extract) solution: About 0.5106 gm accurately weighed, added 50 ml methanol reflux the Residue further with Chloroform for three times more, cooler, and filtered. Combine the filtrate and evaporated up to dryness residue dissolved in 10 ml methanol, Sonicate, filtered and volume made up 20 ml.

Preparation of sample solution of Aphrodisiac capsule content: 10 Capsules were taken and homogeneous capsule powder was made. Accurately weighed 2.5297 g of fine powder and treated with 50 ml pet. Ether and reflux on a water bath for 15 minutes, cool and filter, and discard the pet Ether. Reflux the Residue further with Chloroform for three times more, cool, and filter. Combine the filtrate and evaporated up to dryness. Then the residue is dissolved in 5 ml methanol, Sonicate, filtered, and volume made up to 10 ml with methanol.

Selection of mobile phase: The most usable mobile phase is (Chloroform: methanol) in the (9:1) report selected from all moving phases, which was prepared during the HPTLC method Protodioscin identification.

Qualitative evaluation: Apply Standard Withaferin-A (0.112 µg/µl), *Withania somnifera* extract (510.6 µg/µl), and Aphrodisiac capsule (252.97 µg/µl), were applied as (10µl, 10µl, 10µl/spot), per spot respectively on TLC plate, developed with mobile phase and scan at 220 nm. Peak area corresponding to R_f value 0.85 was found for Withaferin-A in the chromatogram of Ashwagandha extract, capsule, and standard Withaferin-A, and their overlay spectra were shown in Figures

Chromatographic Condition: The chromatographic condition was followed by a trial and error method. After considering the confirmative result, established the experimentation

Stationary phase : HPTLC Precoated, silica gel 60, F 254

Thickness : 0.2 mm

Mode of application : Band

Band width : 6 mm

Separation technique : Ascending

Saturation time : 10 min.

Migration distance : 70 mm

Detection : UV- Densitometry scanning

Visualization wavelength : 560 nm

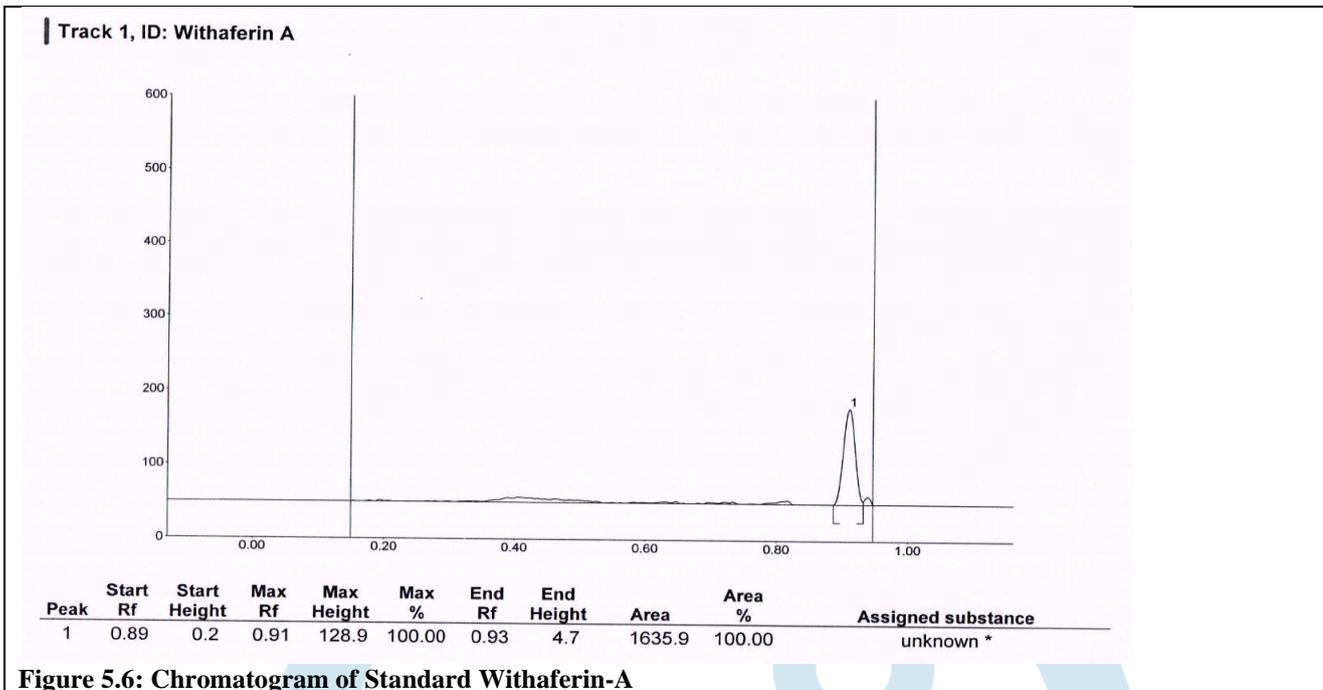


Figure 5.6: Chromatogram of Standard Withaferin-A

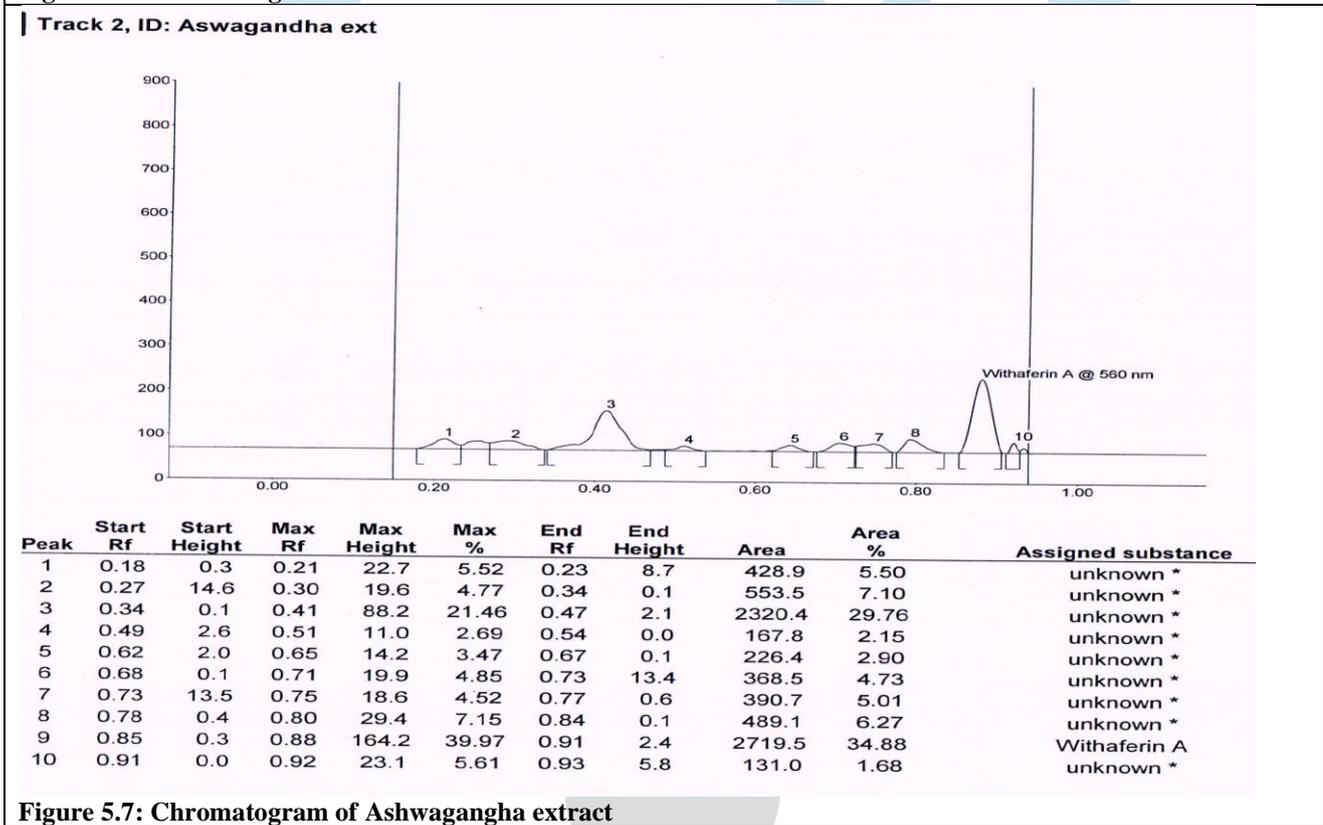


Figure 5.7: Chromatogram of Ashwagandha extract

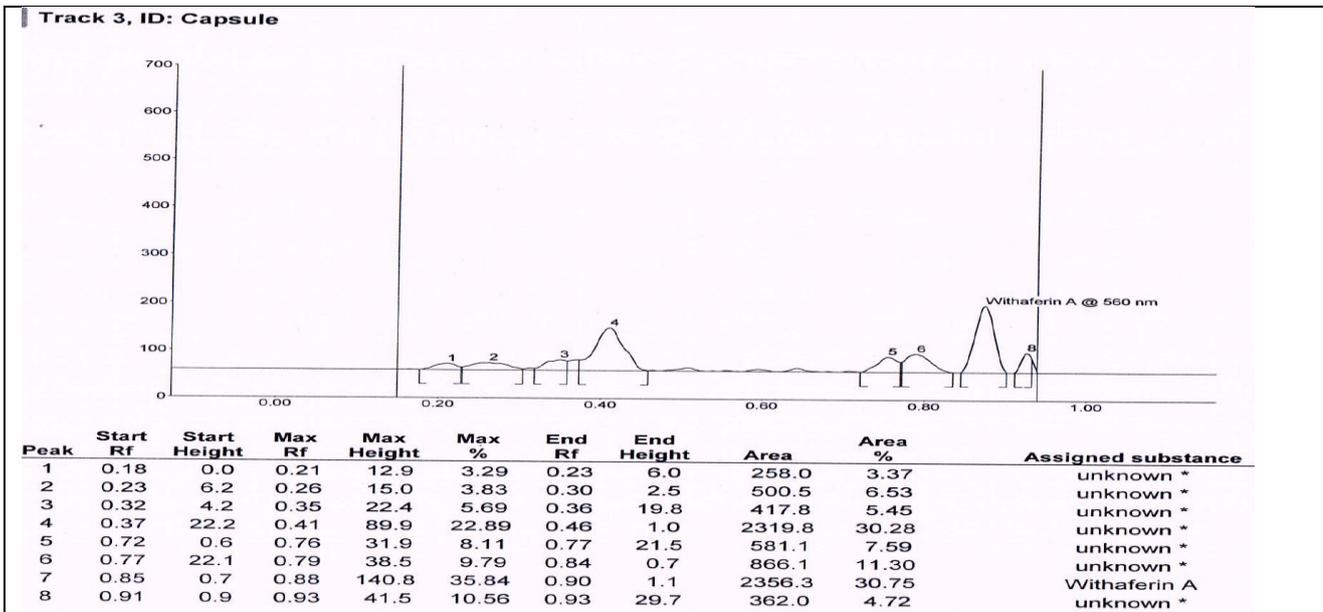


Figure 5.8: Chromatogram of Aphrodisiac capsule

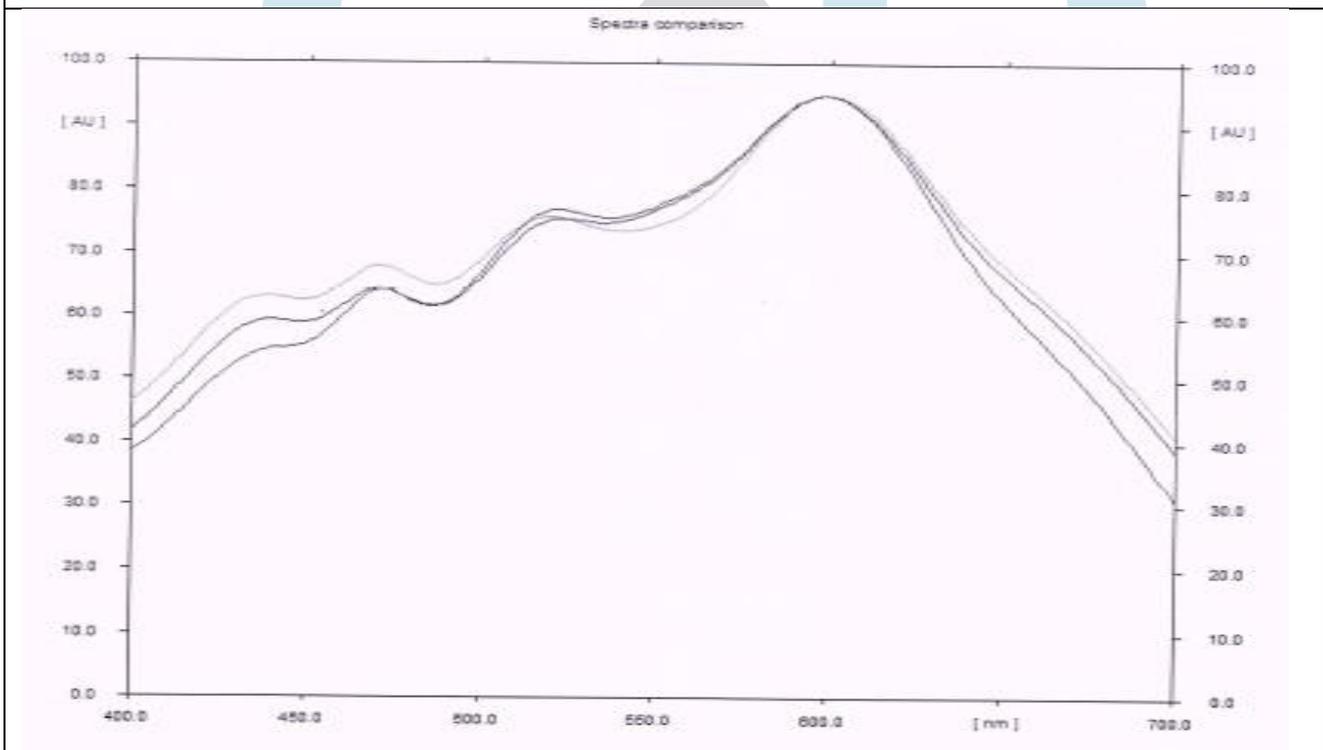


Figure 5.9: Overlain spectrum of Withaferin-A from Extract, Capsule and Standard Chromatographic conditions

Calculation:

Weight of Aswagandha extract: 0.5106 g →→ in 10 ml methanol
 510600µg →→ in 10,000 µl
 Per µl = 51.06 µg/µl
 51.06µg×10 µl =510.6 µg/ spot

Percent of Withaferin-A in Aswagandha extract

$$= \frac{\text{Sample area} \times \text{Standard concentration} \times \text{Purity} \times 100}{\text{Standard area} \times \text{sample concentration} \times 100}$$

$$= \frac{2719.5}{2719.5} \times \frac{1.12}{1.12} \times \frac{95}{95} \times 100$$

$$= \frac{1635.9}{8352} \times \frac{510.6}{2529.7} \times \frac{100}{100} = 0.3463 \%$$

Percent of Withaferin-A in Aphrodisiac capsule

$$= \frac{\text{Sample area} \times \text{Standard concentration} \times \text{Purity} \times 100}{\text{Standard area} \times \text{sample concentration} \times 100}$$

$$= \frac{2356.3}{1635.9} \times \frac{1.12}{2529.7} \times \frac{95}{100} \times 100$$

$$= \frac{2507}{4138} = 0.6058\%$$

Conclusion: The require objectives for The research has fulfilled, Nowadays, the commercialization of a new synthetic immune booster E.g., Flix immune booster, Hea immunity etc. The resurgence of old herbal remedies that arouse sexual activity and the use of new exotic preparations have coincided with an increase in poisonings associated with the use of these aphrodisiacs.

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