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RESEARCH ARTICLE

PHARMACOGNOSY

EVALUATION OF PREFORMULATION AND FORMULATION PARAMETERS OF AN ANTISTRESS HERBAL CAPSULE.

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ABSTRACT

The formulated capsule is a bi-herbal preparation recommended as antistress agent. It contains *Withania somnifera* and *Tinospora cordifolia* in equal proportions. The present work is based on the standardization of individual ingredients and formulation of the capsule with improved formulation parameters. Physicochemical parameters were also checked for individual crude drugs and finished capsules like ash value, extractive value, and loss on drying. Granulation was done by using starch (20 % solution) as binder by wet granulation technique. The preformulation parameters like bulk density, tap density, Carr's index, Hausner's ratio and angle of repose were checked for laboratory granules. The granules were capsulated and the designed formulations were evaluated for content uniformity, weight variation and disintegration time. Quantification for Withaferin-A and Berberine of the finished formulation was done by HPLC method. The designed formulation was in conformity to the properties evaluated for the capsules and further preclinical studies have to be done to test its efficacy.

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KEYWORDS

Withania somnifera, Tinospora cordifolia, Physicochemical tests, Preformulation, Quantification.

INTRODUCTION

There has been an exponential growth in the field of herbal medicine in the last few decades. It is getting popularized in developing as well as in developed countries owing to its natural origin and lesser side effect. In olden times, vaidyas used to treat patients on individual basis, and prepare drug according to the requirement of the patient. But the scenario has changed now; herbal medicines are being manufactured on the large scale in Pharmaceutical units, where manufacturers come across many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of single drugs and formulation, quality control parameters[1].

In bi-herbal Ayurvedic preparations it will be very difficult if we want to estimate each and every ingredient in term of their chemical constituent. But if few major constituents having particular therapeutic action indicated in the labeled can be pinpointed then these constituents should be estimated quantitatively along with the other parameters through which presence of all ingredients can be confirmed. Combined, well coordinated efforts from scientific workers of different disciplines are required for this purpose.

Background of the Invention Ensuring stress free world is a fundamental step in building the health of a nation. Stress refers to inappropriate physiological response to any demand. It covers a wide range of phenomena, from mild irritation to drastic dysfunction that may cause severe health breakdown. Due to lack of modern pharmacopoeial standards laid down and followed for processing of the capsule, the medicine prepared using traditional methods may not have the desired quality and batch to batch consistency. Hence this formulation required standardization of following scientific parameters including organoleptic characters, physicochemical analysis, chromatographic pattern and microbial screening. The work deals with the details of following latest standardization guidelines involving Good Manufacturing Practices (GMP) for preparation of Ayurvedic medicines. The capsule is formulated with improved formulation parameters to act against stress occurring due to various illness and mental behaviours, i.e., to formulate a pharmaceutically modified herbal extract for prolonging the therapeutic efficacy.

MATERIALS AND METHODS

The present invention relates to a new herbal pharmaceutical formulation useful as a antistress agent, processes for its preparation, and quantitative analysis of the individual phytochemicals contained in the formulation.

Plant Material:

The bi-herbal formulation is made up of two plants namely, *Withania somnifera* (Linn.) Dunal, *Tinospora cordifolia* (Willd.) Miers, in equal proportions.

Figure1
Withania somnifera root

Figure 2
Tinospora cordifolia stem

Withania somnifera Dunal is a popular Indian plant, commonly known as Ashwagandha or Indian Ginseng belonging to family Solanaceae[2, 3]. Ashwagandha is widely used in Ayurveda and has long been known for its health benefits. In Ayurvedic medicine there is a class of herbs, including *Withania somnifera*, known as adaptogens or vitalizers[4]. This herb has been extensively evaluated for various pharmacological activities. The roots are found to be a potent anti-stress[5], anxiolytic, and anti-depressant action, anti-convulsant, anti-inflammatory activity[6]. In addition to modulating the immune system, *Withania somnifera* has been found to have activity against the various cancer cell lines. *Withania somnifera* has been reported for its anti-microbial activity against Gram positive bacteria and also for hypoglycemic, diuretic, hypocholesterolemic[7] , hepatoprotective and cardioprotective agent[8].

Tinospora cordifolia (Willd.) Miers (Menispermaceae)[9], distributed in tropical and subtropical India up to 1200m elevation. The stem shows the presence of terpenoids, alkaloids, lignan, carbohydrates, bitters, steroids and glycosides[10,11]. The drug has shown to have anti – cancer[12], anti-inflammatory[13], analgesic, CNS depressant[14], cardio tonic activities[15], anti–oxidant, antipyretic, hepatoprotective, diuretic, anti-stress, antihyperglycemic[16], anti- tuberculotic[17], hypolipidemic. It is also used in constipation, diabetes, fevers, skin diseases, vomiting and vaginal discharges.

Authentication:

The identities of the plants were confirmed by Dr.P.Jayaraman, Botanist, PARC, Chennai. A Voucher specimen (No.PARC/2010/656, PARC/2010/657) has been deposited in the institute. The fresh plant material collected was thoroughly cleaned and air-dried. It was then homogenized to fine powder and stored in air-tight bottles for further studies.

Physicochemical Studies*:*

Physicochemical parameters of the dried roots of *Withania somnifera* and stem of *Tinospora cordifolia* were determined as per guidelines of WHO. Total ash value, loss on drying, water soluble ash, acid insoluble ash, heavy metals, alcohol soluble extractive value and water soluble extractive value were determined[18].

Microbial Screening:

For the safe use of the plant drug, microbial screening[19] was done for individual raw materials and checked whether total aerobic count, total yeast and mould count are within the prescribed WHO limits.

Preparation of Formulation

Withania somnifera root and *Tinospora cordifolia* stem were finely powdered (# 40), and taken for preparation of capsules by wet granulation technique[20] using starch (20 %) solution as binder. The wet mass was passed through # 30 to obtain granules. The granules were dried at 45° C in tray dryer. The granules of # 30/60 size were lubricated with 1 % magnesium stearate. Diluents and preservatives[21] were added and filled in capsules coloured yellow - grey, size '0'in capsule filling machine. The capsules were evaluated for weight variation, content uniformity and disintegration time. Average weight (20 capsules) of capsule was 500 mg.

Preformulation and Formulation Study

The granules were analysed for preformulation parameters[22] like bulk density, tap density, Carr's index, Hausner's ratio and angle of repose. Also weight variations, content uniformity and disintegration time of finished product were performed.

Microbial Screening

For the safe use of the plant drug, microbial count was done and checked whether total aerobic count, total yeast and mould count are within the prescribed WHO limits. The capsule was subjected to microbial screening to ensure that the product is free from microorganisms

Quantification

The total alkaloid content and total terpenoid content of the finished formulation was estimated [23] and the results are tabulated. In view of the potential therapeutic importance of the drug, and considering the degree of adulteration/substitution of the raw materials, a simple reverse phase HPLC method was used in order to quantify berberine[24] and withaferin-A[25] since these are the markers of the plants *Tinospora cordifolia and Withania somnifera* respectively.

Solvents used were of HPLC grade (E. Merck). Test solutions were filtered through 0.20 µm nylon-6, 6 membrane before injection. All analyses were run in triplicate and averaged. The standard berberine and withaferin-A used were purchased from Fluka Chemicals, Switzerland and Natural Remedies, India respectively. Satisfactory retention times and good resolution of both markers were achieved using Phenomenex – 5u (Gemini) C-18 column eluted with acetonitrile-water (50:50 v/v) at a flow rate of 0.5 ml/min. Injection volume was 20µl. Detection wavelength is 215nm.

RESULT AND DISCUSSION

Withania somnifera root, *Tinospora cordifolia* stem powder were studied individually for its characteristics.

PHYSICOCHEMICAL STANDARDS

Physico-chemical parameters of powdered *Withania somnifera* root and *Tinospora cordifolia* stem like total ash, water soluble ash, acid insoluble ash, water soluble extractive, ethanol soluble extractive and moisture content are shown in Table 1.

.

Table 1
Physicochemical standards

S.No	Physicochemical parameters	Withania somnifera root	Tinospora cordifolia stem
1.	Total Ash	8.4	7.5
2.	Acid Insoluble ash	1.147	1.16
3.	Water soluble extractive value	11.63	12.05
4.	Alcohol soluble extractive value	9.88	7.27
5.	Loss on drying	7.28	2.31

Table No.2
Determination of Heavy Metals [26]

S.No.	Heavy metal	Withania somnifera root	Tinospora cordifolia stem
1.	Arsenic	Nil	Nil
2.	Lead	Less than 5ppm	Less than 5ppm
3.	Cadmium	Nil	Nil
4.	Mercury	Nil	Nil

Although, there was minor presence of some heavy metals but the sample did not exceed the limit given according to the WHO guidelines. Therefore, the samples investigated were free from heavy metal contamination.

Table No.3
Microbial Screening of Powder

S.No.	Microorganism	Withania somnifera root	Tinospora cordifolia stem
1.	Total bacterial count	5 cfu/gm	5 cfu/gm
2.	Yeast and moulds	Nil	Nil
3.	*Escheria coli*	Negative	Negative
4.	Salmonella	Negative	Negative
5.	S.aureus	Negative	Negative

As per the WHO standards, the plant material is free from microbial load and safe for human consumption.

Preformulation study:

Preformulation parameters like bulk density, tap density, Carr's index, Hausner's ratio and angle of repose were obtained for the laboratory granules. The granules showed excellent flow property.

Table No. 4
Preformulation parameters:

S.No	Parameters	Result
1.	Bulk density	0.437
2.	Tap density	0.516
3.	Carr's index	15.31
4.	Hausner's ratio	1.18
5.	Angle of repose	34.27°

As per the standards, the flow property of the blend to be filled in the capsules was in good range and was confirmed by the above parameters.

Formulation development

The bi-herbal capsule (Figure 3) contains *Withania somnifera* root, *Tinospora cordifolia* stem 250mg each. Three trials were taken. The final batch was tested for all physicochemical parameters, batch to batch consistency and kept for stability studies to assess the shelf life of the product.

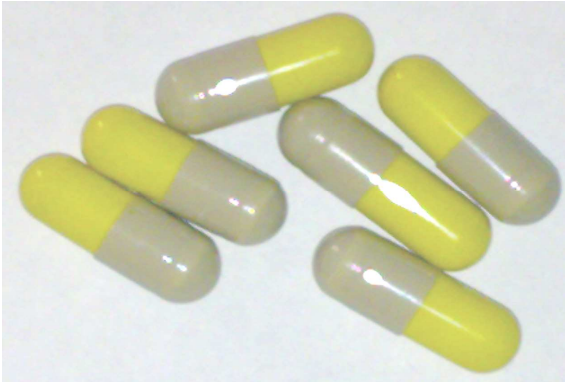

Figure 3
Formulated Antistress capsule

Evaluation of filled capsules:

All other evaluation parameters like weight variation, uniformity of weight and disintegration time of finished capsules were performed. The results shown in Table 5.

Table No. 5
Capsule evaluation

S.No	Parameters	Result
1.	Weight variation	Within limits
2.	Content uniformity	Approx. 495mg.
3.	Disintegration time	6 mins 28 secs.
4.	Loss on drying	4.32%
5.	Total ash	6.4%

Microbial screening of finished product

Table No. 6
Microbial screening

S.No.	Micro-organism	Batch I	Batch II	Batch III
1.	Total bacterial count	5cfu/gm	5cfu/gm	5cfu/gm
2.	Total yeast and moulds	Nil	Nil	Nil
3.	Salmonella and E.coli	Negative	Negative	Negative
4.	Aflatoxin	Nil	Nil	Nil
5.	Pesticides	NF	NF	NF

Quantification:

The total terpenoid content was found to be 0.301mg/100g of sample and the total alkaloid content was found to be 0.2998mg/100gm of the sample.

A sharp and symmetric peak for Berberine and Withaferin-A was obtained, with good baseline resolution and minimal tailing, thus facilitating the accurate measurement of peak area. Both the standards have the R_f value: Standard Withaferin-A -15.377, Berberine -11.053 and the sample has both corresponding peaks at 15.413 and 11.077 respectively. This shows that the final product contains both the constituents as a mark of identification. The concentration of Berberine [B] and withaferin-A [W] in the antistress capsule was found to be 0.349% and 0.027% (w/w), respectively. Typical HPLC chromatograms of Sample and Standards are shown in figures.

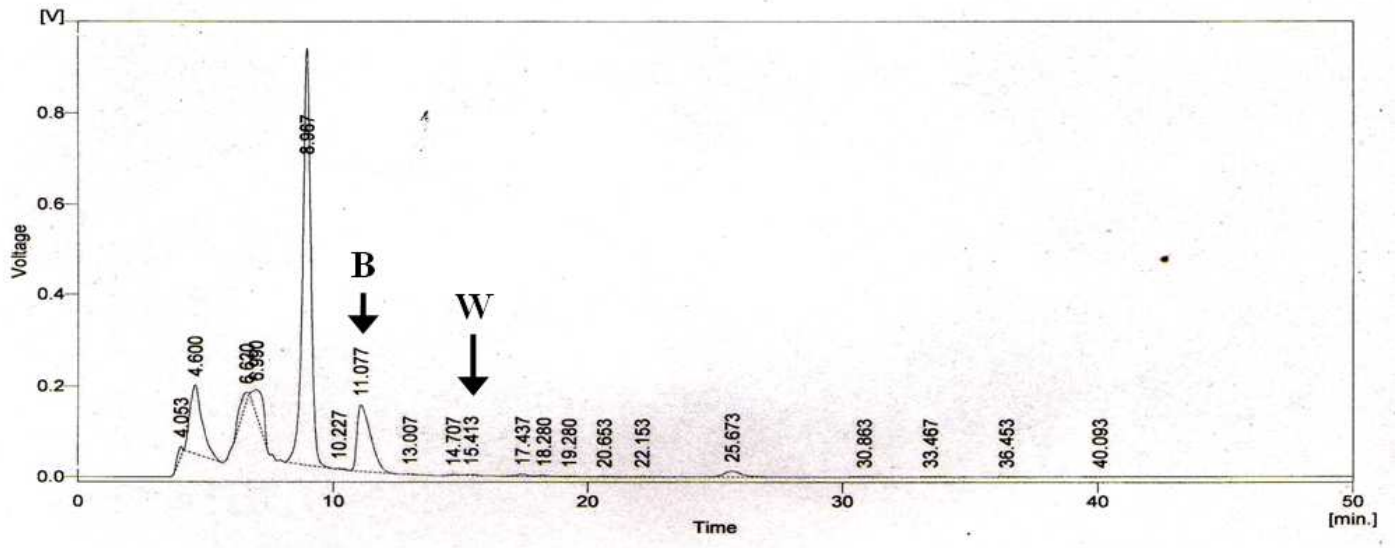

Figure 4 : HPLC chromatogram of sample.

HPLC chromatogram of sample.

Table No.7

Result table for capsule

	Reten.Time [min]	Area [mVs]	Height [mV]	Area [%]	Height [%]	W05 [min]
1.	4.053	312.146	20.755	0.9	1.6	0.18
2.	4.600	5104.812	150.844	14.2	11.3	0.50
3.	6.620	824.935	16.795	2.3	1.3	0.22
4.	6.990	2006.955	56.290	5.6	4.2	0.55
5.	8.967	20940.688	915.084	58.4	68.4	0.33
6.	10.227	48.599	1.861	0.1	0.1	0.36
7.	**11.077**	**5387.990**	**145.386**	**15.0**	**10.9**	**0.58**
8.	13.007	43.131	1.782	0.1	0.1	0.36
9.	14.707	43.165	1.908	0.1	0.1	0.39
10.	**15.413**	**73.443**	**2.980**	**0.2**	**0.2**	**0.39**
11.	17.437	146.594	4.843	0.4	0.4	0.46
12.	18.280	58.544	2.053	0.2	0.2	0.46
13.	19.280	11.065	0.299	3.086e-02	2.233e-02	0.52
14.	20.653	25.058	0.918	0.1	0.1	0.44
15.	22.153	35.022	0.726	0.1	0.1	0.63
16.	25.673	674.070	13.293	1.9	1.0	0.72
17.	30.863	44.291	0.584	0.1	4.369e-02	1.27
18.	33.467	43.824	0.634	0.1	4.739e-02	1.10
19.	36.453	15.370	0.298	4.287e-02	2.226e-02	0.84
20.	40.093	9.686	0.199	2.702e-02	1.490e-02	0.81
	Total	35849.389	1337.533	100.0	100.0	

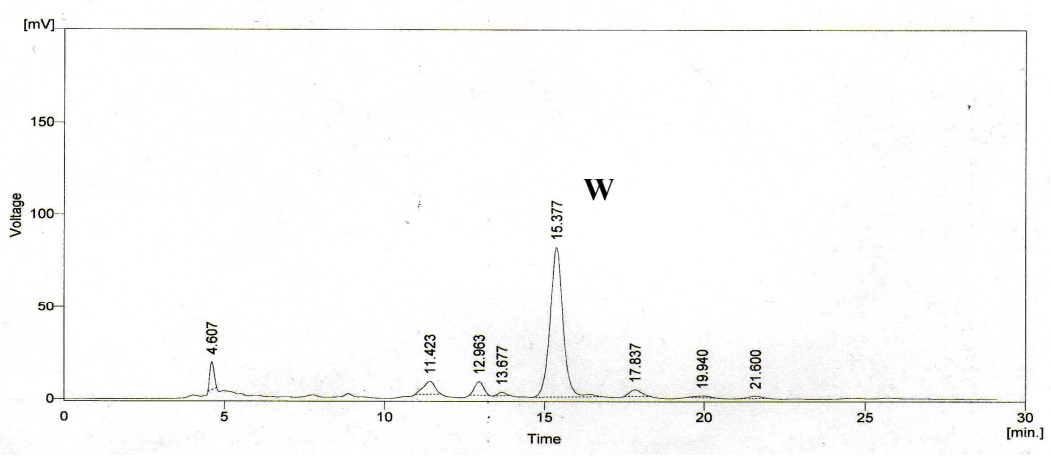

Figure 5
HPLC chromatogram of standard Withaferin A.

Table No.8
Result table for standard Withaferin A.

	Reten.Time [min]	Area [mVs]	Height [mV]	Area [%]	Height [%]	W05 [min]
1.	4.607	137.815	15.079	4.7	12.8	0.16
2.	11.423	174.005	7.051	6.0	6.0	0.40
3.	12.963	139.035	7.327	4.8	6.2	0.32
4.	13.677	29.581	1.764	1.0	1.5	0.29
5.	**15.377**	**2287.807**	**81.290**	**78.2**	**68.7**	**0.41**
6.	17.837	92.777	3.625	3.2	3.1	0.42
7.	19.940	28.373	0.880	1.0	0.7	0.59
8.	21.600	35.058	1.236	1.2	1.0	0.45
	Total	2924.450	118.251	100.0	100.0	

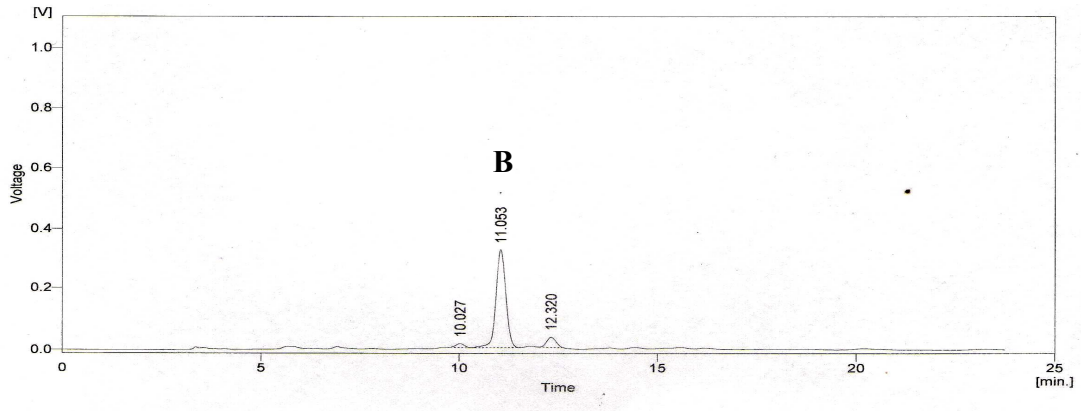

Figure 6
HPLC chromatogram of standard Berberine.

Table No.8
Result table for standard Berberine

	Reten.Time [min]	Area [mVs]	Height [mV]	Area [%]	Height [%]	W05 [min]
1.	10.027	174.937	12.134	2.8	3.3	0.23
2.	**11.053**	**5506.669**	**324.040**	**88.7**	**87.9**	**0.26**
3.	12.320	524.822	32.497	8.5	8.8	0.26
	Total	6206.428	368.672	100.0	100.0	

Thus, the above results show that the raw materials have the prescribed limits in pharmacopeias and are of standard quality and the microbial limits and the heavy metals were in safe limits. The laboratory granules made have the moisture content upto 2.3% thus it is safe for packing and free from microbial load and they were also free from aflatoxin and pesticides. The granules formed were posses the angle of repose below 35 degrees, it has very good flow property and thus ensures correct filling. And the weight variation, microbial limits and heavy metal were in acceptable limits and hence the formulation can be used safely for preclinical and clinical trials.

CONCLUSION

The antistress capsule was formulated using improved formulation parameters and research work can be further extended for justifying its therapeutic efficacy.

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