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Research Article

DEVELOPMENT AND STANDARDIZATION OF POLYHERBAL FORMULATION

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ABSTRACT

The most important challenges posed by herbal formulations is their evaluation and standardization. Evaluation is necessary to ensure the quality and purity of the herbalproduct. For evaluation of raw materials and poly herbal formulation various parameters are studied as per the World Health Organization's guidelines and the Ayurvedic Pharmacopoeia of India. The present study deals with formulation the polyherbal formulation prepared from hydro-alcoholic (30:70) extracts of *Andrographis paniculata*(Stem and leaves), *Asparagus racemosus*(Root) , *Ipomoea digitata* (Rhizome), *Tinospora cordifolia*(Stem) and *Withania somnifera*(Root). To evaluate of raw materials include physicochemical studies like ash values, extractive values, phytochemical studies and safety profiles which include heavy metal analysis, pesticide residue analysis and microbial load analysis. The Preformulation parameters and parameters for finished product (hard gelatin capsule) include uniformity of weight, disintegration time, moisture content, pH, phytochemical estimation and microbial load assay. The HPTLC finger print profile of finished product was also carried out.

Keywords: Development, Standardization, Polyherbal formulation, WHO guidelines

1. INTRODUCTION

Plants are very useful to mankind. Many of them are used exclusively for medicinal purposes. According to the World Health Organization (WHO), "a medicinal plant is a plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." Such plants are in great demand by pharmaceutical companies for their active ingredients [1, 2].

Diabetes mellitus (DM) is a chronic complication of derangement of protein, carbohydrate and lipid metabolism characterized by increased blood glucose level resulting from the defects in insulin secretion, insulin action or both [3]. DM is the worldwide problem to leading micro vascular and macrovascular complications [4]. DM is a chronic complication that affected an estimated 135 million people in 1995, 285 million people worldwide in 2010 and data reached that approx 500 million people in 2025 mainly in rural and poor population throughout the world [5].

In traditional systems of medicine, many plants have been documented to be useful for the treatment of various systemic disorders. Many of the traditional/indigenous systems of medicine are effective but they suffer from lack of complete standardization which is one of the important challenges posed by the traditional systems of medicine. The concept of polyhedral formulation is well documented in the ancient literature. Compared to the single herb, the polyherbal formulation has better and extended therapeutic potential. Hence, the present study was planned to formulate and standardize a polyherbal formulation using plants having known anti diabetic polantial.

2. MATERIALS AND METHODS

2.1. Collection of the plant and Authentication

The selected plant materials *viz Andrographis paniculata* (Stem and leaves), *Asparagus racemosus* (Root) ,*Ipomoea* digitata(Rhizome), *Tinospora cordifolia* (Stem), *Withania somnifera* (Root).were procured from the authentic suppliers and further authenticated by Dr. P.Jayaraman, Director, Plant Anatomy and Research Centre, Tambaram, Chennai. (Specimen numbers PARC/2015/3049 for *Andrographis paniculata,* PARC/2015/3053 for *Asparagus racemosus,* PARC/2015/3050 for *Ipomoia* digitata, PARC/2015/3052 for *Tinospora cordifolia,* PARC/2015/3051 for *Withania somnifera).*

2.2. Standardization of raw material

The Organoleptic evaluation and determination of Foreign organic matter of raw materials were carried out as per the Ayurvedic Pharmacopoeia of India [6].

2.3. Physicochemical studies

The ash values, extractive values and loss on drying were performed according to the official methods prescribed in Ayurvedic Pharmacopoeia of India [6].

2.4. Preliminary phyto-chemical screening

One gram of each of the extracts of *Andrographis paniculata* (Stem and leaves), *Asparagus racemosus* (Root) ,*Ipomoea digitata* (Rhizome), *Tinospora cordifolia* (Stem),

Withania somnifera was dissolved in 100 ml of its own mother solvent to obtain a stock of concentration 1% w/v and tested for the presence of alkaloids, flavanoid, tannins, saponin, glycosides, terpenoids, steroid, carbohydrate and phenolic compounds, and saponins [7].

2.5. Fluorescence analysis

The crude drug was studied for any fluorescence under ordinary light and UV light. Samples were studied as such, before and after treating with 50% HCl and 50% NaOH and the results were tabulated [8].

2.6. Safety profile studies

The safety profile parameters like heavy metal analysis, pesticide residual analysis and microbial load analysis were studied according to the official methods prescribed in Ayurvedic Pharmacopoeia of India.

2.6.1. Quantitative estimation of heavy metals

Quantitative estimation of heavy metals was done for the detection of arsenic, lead, cadmium and mercury as per the Ayurvedic pharmacopoeial procedures [9].

2.6.2. Quantitative estimation of pesticide residues

Quantitative estimation of pesticide were done for the detection of Organochlorine compound, Oragnophosphorous compound and Carbamates as per the Ayurvedic pharmacopoeial procedures [10].

2.6.3. Microbial load analysis

For the safe use of raw material for the polyherbal capsules, microbial count was done and it was checked whether the total aerobic viable count, yeasts and molds were within the prescribed limits and the microorganisms, *Escherichia coli,*Salmonellae, *Pseudomonas Staphylococcus* and *Shigella* were checked [11].

2.7. Preparation of Extract

The selected plants materials were shade dried and stored in an airtight container, each drug coarsely powdered and extracted with hydro-alcoholic (30:70) by hot percolation method using soxhlet apparatus. The prepared hydro-alcohol extracts were concentrated under vacuum using rotary vacuum evaporator at 40°C temperature (removal of alcohol). The concentrated extracts were freeze dried at -20°C. The powders were stored in an airtight container and kept in the desiccator until further use.

2.8. Formula of Polyherbal formulation

The polyherbal formulation (capsules) contained the hydro-alcoholic extracts of *Andrographis paniculata, Asparagus racemosus, Ipomoea digitata*, *Tinospora cordifolia, Withania somnifera* in the ratio of 1:1:1:1:1.

2.9. Preparation of formulation by wet granulation method

The formulation preparation began with trials by adding a different ratio of binders and selecting the quantity of lubricants and preservatives, and finally the procedure was optimized. *Andrographis paniculata, Asparagus racemosus, Ipomoea digitata*, *Tinospora cordifolia, Withania somnifera* extracts were powdered (sieve 40), and mixed in the ratio of 1:1:1:1:1 and taken for the preparation of capsules by wet granulation technique using 5% starch paste as a binder. The wet mass was passed through sieve number 22 to obtain granules. The granules were dried at 45°C in a tray [12].

2.10. Pre formulation studies.

Preformulation parameters such as bulk density, tap density, Compressibility index, Hausner's ratio, and angle of repose were determined for the prepared polyherbal granules and the best trial batch were taken for capsule filling and further studies [13, 14].

2.11. Standardization of polyherbal formulation (hard gelatin capsule)

***Capsule evaluation*:** The polyherbal capsules were evaluated for their description, average weight, weight variation, moisture content, disintegration time, pH and microbial load and compared with Indian pharmacopoeial standards[15]. and also preliminary screening of phyto constituents, quantitative estimation of phyto-constituent were carried out.

Average weight: Twenty capsules were individually weighed and the average weight of the capsule was calculated.

***Weight variation*:** The individual weights of the each capsule should be within the limits of 90% and 110% of the average weight.

***Moisture content*:** Moisture content was determined by using automatic Karl Fischer titration apparatus.

***Disintegration time*:** Disintegration test was performed using the digital microprocessor based disintegration test apparatus (Electro lab, Mumbai, India). One capsule was introduced into each tube and a disc was added to each tube. The assembly was suspended in water in a 1000 ml beaker. The volume of water at its highest point was at least 25 mm below the surface of the water and at its lowest point was at least 25 mm above the bottom of the beaker. The apparatus was operated and maintained at a temperature of 37 ± 2°C.

pH value: pH of 1% solution was determined by using a digital pH meter.

2.11.1. Preliminary phyto-chemical screening

One gram of polyherbal formulation was dissolved in 100 ml of its own mother solvent to obtain a stock of concentration 1% w/v and tested for the presence of alkaloids, flavanoid, tannins, saponin, glycosides, terpenoids, steroid, carbohydrate and phenolic compounds, and saponins [7].

2.11.2. Fluorescence analysis

The crude drug was studied for any color changes under ordinary light and UV light. Samples were studied as such, after treating with 50% HCl and 50% NaOH and the results were tabulated [8].

2.11.3. Quantitative estimation of phytoconstituents

Total Alkaloids, Phenolics compounds, Flavonoid and Tannin Content were estimated in the polyherbal formulation [16-19].

2.11.4. Microbial load analysis

For the safe use of raw material for the polyherbal capsules, microbial count was done and it was checked whether the total aerobic viable count, yeasts and molds were within the prescribed limits and the microorganisms, *Escherichia coli,* Salmonellae, *Pseudomonas Staphylococcus* and *Shigella* were checked.

2.11.5. High-performance thin layer chromatography (HPTLC) fingerprint analysis

Randomly few capsules were selected. They were opened and the contents were collected. The capsule content was dissolved in a minimum volume of mobile phase and used for the fingerprint analysis. Twenty milligram either individual herbal extract or polyherbal formulation was reconstituted in 1 ml of ethanol and filtered through a 0.45 µm membrane filter. The filtrate was used for the HPTLC analysis. The solvent system Toluene:Ethylacetate:Methanol:Formicacid (6:3:1:0.2) was used as mobile phase for the development of chromatogram [20, 21].

3. RESULTS AND DISCUSSION

The most important part of any formulation is standardization which ensures the quality, safety and reproducibility. It involves the complete process of bio-prospection right from the collection of raw materials to development of finished product. In the present study, standardized polyherbal mixture was formulated in hard gelatin capsule to replace the traditional liquid dosage form.

Polyherbal formulation composed of five ingredients, belonging to different families, different morphological plant parts and different phytoconstituents.

3.1. Foreign Organic Matter

Foreign organic matter for the raw materials was done with samples taken from the suppliers. The results obtained and the standard values are given in table 1.

Table1: Foreign organic matter of samples

Plant Name	Observation	Acceptable Limits (w/w %)
Andrographis paniculata	0.62±0.32	NMT 2
Asparagus racemosus	0.65±0.37	NMT 2
Ipomoea digitata	0.85±0.58	NMT 2
Tinospora cordifolia	0.76±0.42	NMT 2
Withania somnifera	0.51±0.21	NMT 2

The value are expressed as mean ± SD, (n=3); NMT-Not more than

3.2. Organoleptic Characters

Organoleptic characters for the raw materials were done with samples taken from the suppliers. The results obtained and the standard values are given table in 2.

Table 2: Organoleptic characters of selected plants

Plant Name	Nature	Colour	Odour	Taste
Andrographis paniculata	Coarse powder	Green	Characteristic	Bitter
Asparagus racemosus	Coarse powder	Pale yellow	Aromatic	Bitter
Ipomoea digitata	Coarse powder	White	Characteristic	Characteristic
Tinospora cordifolia	Coarse powder	Light brown	Aromatic	Bitter
Withania somnifera	Coarse powder	Light yellow	Characteristic	Bitter

3.3. Physicochemical Parameters

Various physicochemical parameters were calculated for the herbal drugs used in the polyherbal formulation. Table 3 depicts the report of various physicochemical parameters.

Table 3: Physicochemical parameters

Physicochemical parameters	*Andrographis paniculata*	*Asparagus racemosus*	*Ipomoea digitata*	*Tinospora cordifolia*	*Withania somnifera*
Loss on drying	2.07±0.15	5.78±0.25	5.84±0.36	4.84±0.36	6.23±0.25
Total ash value	6.58±0.28	3.87±0.47	3.72±0.28	9.52±0.76	6.09±0.15
Acid insoluble ash	1.23±0.51	0.38±0.08	0.72±0.07	1.74±0.51	0.34±0.02
water soluble ash	1.57±0.23	2.37±0.22	1.17±0.52	2.34±0.42	1.23±0.32
Sulphated ash	1.25±0.55	7.59±0.87	9.05±0.72	13.16±0.57	10.08±0.25
Water soluble Extractive	21.74±0.26	53.63±.35	19.52±0.71	45.72±0.86	28.76±035
Alcohol soluble extractive	24.5±0.86	31.28±0.58	25.23±0.57	10.75±25	17.23±25
Ether soluble extractive	10.28±0.85	8.35±0.53	23.68±0.48	8.50±0.25	13.25±0.84

The value are expressed as mean ± SD, (n=3); NMT-Not more than

3.4. Phytochemical Analysis

The chemical tests for various Phyto constituents in the raw materials were carried out and the results were recorded and detailed in Table 4.

Table 4: Phytochemical Analyses

Plant Name	Alkaloid	Flavanoid	Tannin	Saponin	Glycoside	Terpenoid	Steroid	Carbohydrate	Phenolics
Andrographis paniculata	+	+	+	-	+	+	+	_	+
Asparagus racemosus	+	+	+	_	+	+	+	-	+
Ipomoea digitata	+	+	-	-	+	-	-	+	-
Tinospora cordifolia	+	_	+	-	+	+	+	+	_
Withania somnifera	+	-	+	+	-	+	-	-	+

3.5. Fluorescence Analysis of Raw Materials

Table 5: Fluorescence Analysis

Sample	Before Treatment			After treating with 50 % HCl			After treating with 50% NaOH		
	Ordinary light	Short UV	Long UV	Ordinary light	Short UV	Long UV	Ordinary light	Short UV	Long UV
A. paniculata	Greenish brown	Green	Green	Green	Greenish brown	Brown	Greenish yellow	Greenish yellow	Dark brown
Asparagus racemosus	Light brown	Brown	Dark brown	Light brown	Brownish green	Dark brown	Light brown	Greenish brown	Green fluorescence
Ipomoea digitata	Dark brown	Light brown	Greenish brown	Brown	Greenish yellow	Dark brown	Light brown	Greenish brown	Green
Tinospora cordifolia	Dull brown	Cream	Brown	Brown	Brownish white	Dark brown	Light brown	Brownish white	Dark brown
Withania somnifera	Greenish brown	Green	Light brown	Brown	Brown	Dark brown	Light brown	Dull brown	Light brown

3.6. Safety Profile Parameters Studies

3.6.1. Heavy metal analysis

Quantitative estimation of heavy metals

Quantitative estimation of heavy metals in the raw materials was carried out and the results were recorded and detailed in Table 6.

Table: 6 Test for heavy metals

Plant name	OBSERVATION (in ppm/ml)			
	Arsenic (NMT 5)	Lead (NMT 10)	Cadmium (NMT 0.3)	Mercury (NMT 0.5)
A. paniculata	0.008	0.081	0.025	0.004
A. racemosus	0.004	0.053	0.005	0.012
I. digitata	0.007	0.089	0.013	0.007
T. cordifolia	0.005	0.076	0.002	0.015
W. somnifera	0.008	0.081	0.025	0.004

3.6.2. Pesticide residual analysis

The powdered mixture of polyherbal was subjected for the detection of pesticide residual analysis after purification by column chromatography and chromatogram is developed by GC-MS technique and detection is done based on the standard retention time of pesticides.

Table: 7 Pesticide residual analysis

Parameters	Observation
Organochlorine compound	Not detected
Oragnophosphorous compound	Not detected
Carbamates	Not detected

3.6.3. Microbial load analysis

Microbial screening for the raw materials was carried out and the results obtained were detailed in Table 8.

Table 8: Microbial load analyses

Parameters	*A. paniculata*	*A. racemosus*	*I. digitata*	*T. cordifolia*	*W. somnifera*
Total aerobic count (NMT 1000 cfu/g)	NIL	380cfu/g	NIL	650cfu/g	650cfu/g20
Yeast and mould count (NMT 100 cfu/g)	53cfu/g	NIL	58cfu/g	28cfu/g	NIL
E.coli (To be absent)	absent	absent	absent	absent	absent
Salmonella (To be absent)	absent	absent	absent	absent	absent
Pseudomonas (To be absent)	absent	absent	absent	absent	absent
Staphylococcus (To be absent)	absent	absent	absent	absent	absent
Shigella (to be absent)	absent	absent	absent	absent	absent

3.7. Preformulation and Formulation Development Studies

Totally five trials of formulation were carried out using different choices of excipients considering different facets of manufacturing problems as well as quality defects in mind, all the resultant formulations were evaluated for their flow property, uniformity of filling, uniformity of weight, moisture content and disintegration time.

Table 9: Evaluation of trial batches

Parameters	Trial 1	Trial 2	Trial 3	Trial 4
Bulk density (g/cm2)	0.42±0.01	0.38±0.05	0.35±0.04	0.33±0.06
Tapped density (g/cm2)	0.54±0.03	0.61±0.02	0.53±0.03	0.57±0.02
Compressibility index (%w/w/)	26.6±2.08	32±2.64	34.6±2.08	33.28±1.5
Housner's ratio	1.41±0.01	1.43±0.02	1.53±0.04	1.45±0.03
Angle of repose (degrees)	43.03±3.78	37.04±1.0	35.05±1.0	27.6±0.57

Results are reported as Mean± Standard deviation (n=3)

As per the standards, the flow property of the blend to be filled in the capsule should be in good range and was confirmed by the above parameters. Trail batch IV showed excellent flow characters and batch IV was taken for capsule filling.

The trial IV flow properties were Excellent and all parameters were within the Specified limits. So, fourth trial was chosen for further studies.

Table 10: Evaluation of Inprocess Parameters

Parameter	Trial I	Trial II	Trial III	Trial IV
Flow property	Poor flow	Poor flow	Fair	Good
Uniformity of Filling	-	-	Uniform	Uniform
Uniformity of Weight	-	-	Less weight	Uniform
Moisture content	-	-	Within the limit	Within the limit
Disintegration time	-	-	Within the limit	Within the limit

Mean ± Standard Deviation (n=3)

3.8. Standardization of formulation

Capsule evaluation

Description

"light brown " coloured granules packed in "0" size blue capsules. The polyherbal capsules were evaluated for organoleptic characters which include colour, odour, taste and nature.

Table 11: Organoleptic Characters of Capsules

Parameters	Observation
Description	Light brown granule in blue cap and body "0" size capsule
Colour	Light brown granule
Odour	Characteristic odour
Taste	Bitter taste

Table 12: Evaluation of capsules

Parameter	Observation
Average weight	574.31 ±4.5mg
Weight variation	Within I.P. Limit
Moister content(LOD)	2.51±0.1 %w/w
Disintegration time	10.9±0.5(min)
pH(1% aqueous solution)	5.52± 0.68

Result (n=3) are reported as Mean ± Standard deviation

3.9. Preliminary Phytochemical Screening of Capsules

Table 13: Preliminary phytochemical screening

Phytoconstituents	Observation
Alkaloid	Present
Flavanoid	Present
Tannin	Present
Saponin	Present
Glycoside	Present
Terpenoid	Present
Steroid	Present
Carbohydrate	Present
Phenolics	Present

The results established a scientific data which can be used for the identification of the crude drugs.

3.10. Quantitative Estimation of Phytoconstituents

The Polyherbal formulation was found to contain various phytochemical constituents and hence it is desirable to quantify few of them in order to establish a standard to maintain its quality. Among them the estimation of total Alkaloids, phenolics, Flavanoids, and Tannin content in the aqueous extract were decided to be taken as parameters. Samples were

drawn from three random samples of polyherbal capsules and the total alkaloids, phenolics, Flavonoids, and Tannin content present in them were estimated.

The estimated amounts of Alkaloids, phenolics, Flavanoids, and Tannins were enumerated in the Table 1.

Table 14: Quantitative estimation of phytoconstituents

Parameter	Observation (% w/w)
Total alkaloid content	1.57±0.58
Total tannin content	0.54±0.15
Total flavonoid content	3.25±0.37
Total phenolic content	1.75±0.21

Result (n=3) are reported as Mean ± Standard deviation

3.11. Fluorescence Analysis of Polyherbal Capsule

Table 15: Fluorescence Analysis of Polyherbal Capsule

Sample	Before treatment			After treating with 50 % HCl			After treating with 50% NaOH		
	Ordinary light	Short UV	Long UV	Ordinary light	Short UV	Long UV	Ordinary light	Short UV	Long UV
Polyherbal formulation	Greenish brown	Green	Green	Green	Greenish brown	Brown	Greenish yellow	Greenish yellow	Dark brown

3.12. Microbial load analysis

Microbial screening is done for the Polyherbal formulation from the fresh packs of the Same Batch and the results obtained were detailed in Table 16.

Table 16: Microbial load analyses

Parameter	Result	Limits as per WHO
Total aerobic count	230cfu/g	NMT 1000 cfu/g
Yeast and Mould	15cfu/g	NMT 100 cfu/g
E.Coli	Absent	Should be absent
Salmonella	Absent	Should be absent
Pseudomonas	Absent	Should be absent
Streptococcus	Absent	Should be absent
Shigella	Absent	Should be absent

From the results, it is shown that the formulation complies with the WHO standards for Microbial load analysis and hence it is safer to be taken internally.

3.13. Chromatographic Finger Print Analysis

Fig. 1: TLC photograph for Sample under 254nm

Mobile phase

Toluene:Ethylacetate:Methanol:Formicacid (6:3:1:0.2)

Stationary phase

Aluminum coated Silica Gel – MerkF254

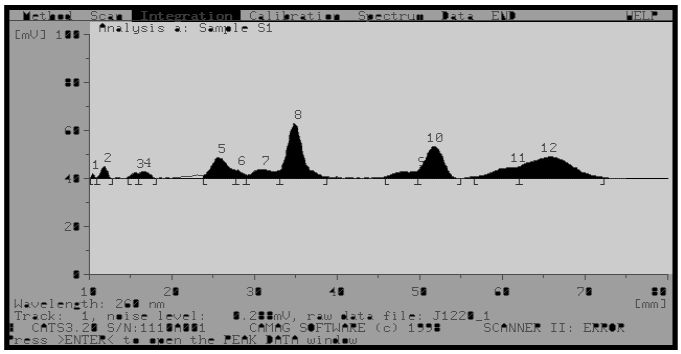

Fig. 2: Chromatographic finger printing analysis of formulation

Table 17: Rf value of the capsules (Chromatographic finger printing)

Rf	Height	Area	λ max
0.10	2.1	8.1	200
0.11	5.0	46.4	200
0.15	2.4	23.1	275
0.16	2.9	41.6	275
0.25	8.6	214.2	223
0.27	3.6	34.0	275
0.31	3.8	121.7	275
0.34	22.9	508.5	200
0.48	3.2	94.4	275
0.51	13.2	345.8	275
0.62	5.0	166.9	275
0.62	9.0	564.5	275

4. CONCLUSION

In this study a Polyherbal capsule was formulated trail batch with the individually standardized raw materials of *Andrographis paniculata*(Stem and leaves), *Asparagus racemosus*(Root), *Ipomoea digitata* (Rhizome), *Tinospora cordifolia*(Stem) and *Withania somnifera*(Root) as per Ayurvedic Pharmacopoeia of India. The T4 was consider to a best batch as it complies with all the pharmacopoeial parameters and was selected for further study.

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