Research Article

Development, Standardization and Evaluation of a Polyherbal Syrup

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ABSTRACT

The present study is an effort to develop a polyherbal formulation to treat urolithiasis and standardization of this formulation. The plants chosen were *Aerva lanata, Astercantha longifolia, Cucumis sativus, Cumimum cyminum, Hemidesmus indicus, Lagenaria siceraria, Tribulus trerrestris* which have either a folklore claim of being useful in urolithiasis or which have diuretic properties. The raw materials were collected, authenticated and standardized as per WHO guidelines. The syrup formulation using the aqueous extracts of the various plants was chosen for ease of consumption. Several trial batches were formulated by varying the sweetener and flavouring agents used. Of these, the batch with the most pleasant taste and flavour was chosen. This batch was evaluated and standardized for various evaluation parameters. An accelerated stability study of this formulation was also carried out. The developed formulation complies with the standards and further *in vitro* and *in vivo* studies have to be carried out to test and prove its efficacy.

Keywords: Polyherbal formulation; Urolithiasis; Standardization; WHO guidelines; Accelerated stability studies.

INTRODUCTION

Most of the traditional systems of medicine are effective but they lack proper standardization. Standardization is an important step for the establishment of a consistent biological activity, a consistent chemical profile or simply a quality assurance program for production and manufacturing of herbal formulations. WHO has given specific guidelines, for the assessment of safety, efficacy and quality of herbal medicines as a prerequisite for global harmonization which are of almost importance.

A polyherbal syrup was therefore developed using dried powder decoction of various herbs such as *Aerva lanata, Astercantha longifolia, Cucumis sativus, Cumimum cyminum, Hemidesmus indicus, Lagenaria siceraria* and *Tribulus terrestris* for the treatment of urolithiasis by exploiting the knowledge of traditional system of medicine[1].

The present study includes raw materials standardization for its identity, quality and development of polyherbal syrup and standardization of the developed formulation and accelerated stability studies.

MATERIALS AND METHODS

The basic formula used for preparing the syrup is given in Table 1.

Collection and authentication

Plant materials were collected from authenticated herbal suppliers and their genuine was checked and confirmed by comparing with the standard. The raw materials were primarily identified by the Ayurvedic parameters such as Varna (color), Gandha (odour), Ruchi (taste), Aakruti (shape) and Parimana (size)[2]. The relevant plant parts of the seven plants chosen for the polyherbal formulation were separately shade dried and powdered.

Monographic Analysis of Herbs

The herbs were evaluated for loss on drying, ash value and extractive value to confirm their standard specifications according to the Ayurvedic Pharmacopeia of India[3].

Phytochemical Screening for Raw Materials

The detection of the active principles in medicinal plants plays a strategic role in the phytochemical investigation as well as for linking the phytochemical to its pharmacological actions. Identification of phytochemical constituents present in individual raw materials was done qualitatively and quantitatively by using various chemicals tests [4].

Analysis of Heavy Metals for Raw Materials

The heavy metals were analysed both qualitatively and quantitatively as per the Ayurvedic pharmacopoeia of India [3].

Microbial Load Analysis

For the safe use of the plant drugs, microbial load was tested for all raw materials which include Total aerobic count, Total yeast and moulds count, absence of *Escherichia coli, Salmonellae, Clostridia* and *Shigella* as per WHO guidelines [5].

Development of Polyherbal Syrup

Method of preparation of decoction

The seven dried raw materials were coarsely powdered. The dried powder was mixed with 4000 ml (4 Lit) of water and the mixture was boiled until the total volume become

one fourth of the original volume. The mixture was cooled and filtered. Filtrate was taken to prepare final herbal syrup[6,7].

Method of preparation of simple syrup

350 gm of sugar was weighed and added to purified water and heated until it dissolved with occasional stirring. Sufficient boiling water was added to produce 1000 ml.

All trial batches were prepared as 1 liter quantity by varying the concentration of sweetener and flavouring agents details are given in Table 2. In addition to the raw materials as given in Table 1 the other additives that have been used for preparation of the trial batches are given in this table 2.

Table 1: Composition of Polyherbal Formulation

Biological Name	Family	Part Used	Quantity/liter
Aerva lanata	Amaranthaceae	Whole plant	80 g
Astercantha longifolia	Acanthaceae	Seeds	80 g
Cucumis sativus	Cucurbitaceae	Seeds	80 g
Cumimum cyminum	Umbelliferae	Fruits	50 g
Hemidesmus indicus	Asclepiadaceae	Roots	50 g
Lagenaria siceraria	Cucurbitaceae	Climbers	80 g
Tribulus terrestris	Zygopyllaceae	Fruits	80 g

Table 2: Composition of Trial Batches

Name of the Ingredients	Trial- 1	Trial -2	Trial -3	Trial – 4	Trial - 5	Trial - 6
Methyl parabeen sodium	2 g	2 g	2 g	2 g	2 g	2 g
Propyl parabeen sodium	1 g	1 g	1 g	1 g	1 g	1 g
Sodium benzoate	2 g	2 g	2 g	2 g	2 g	2 g
Sodium chloride	1 g	1 g	1 g	1 g	1 g	1 g
Bronopol	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g
Saccharin sodium	**1 g**	**1 g**	**1 g**	**1 g**	**1 g**	**1 g**
Sugar	**666 g**	**300 g**	**-**	**350 g**	**350 g**	**350 g**
Sorbitol	**-**	**-**	**2 % Sorbitol**	**2% sorbitol**	**-**	**-**
Sweet orange flavor	**-**	**-**	**5 ml**	**5 ml**	**5 ml**	**-**
Mixed fruit flavor	**-**	**-**	**2.5 ml**	**2.5 ml**	**2.5 ml**	**-**
	Pineapple (0.4%)	**Rosewhite (0.8%)**	**-**	**-**		**Tonowin (0.8%)**

Preparation of the Polyherbal syrup

One part of decoction was mixed with five parts of simple syrup (1:5). Required quantity of methyl paraben sodium, propyl paraben sodium, sodium chloride, sodium benzoate, bronopol, saccharin sodium, sweet orange and mixed fruit was added to the above mixture. Solubility was checked by observing the clarity of solution visually. The final herbal syrup was then subjected to evaluation of production quality as per official standards.

Evaluation of polyherbal syrup

The chosen batch of the Polyherbal syrup after scaling up was evaluated for physical constants, phytochemical screening, heavy metals and microbial load analysis and HPTLC fingerprinting. The polyherbal syrup was evaluated for physical appearance (colour, odour, taste), pH, total solids, specific gravity, and viscosity.

1. Evaluation of Physical Constants

Determination of pH: The pH of polyherbal syrup was determined by using pH meter. The pH meter was calibrated using distilled water, buffer (at pH 4 and 9) till constant readings were obtained.

Determination of total solids: The term 'total solids' is applied to the residue obtained when the prescribed amount of the preparation is dried to constant weight.

Determination of specific gravity: Pycnometer was used to determine the specific gravity at 25°C. It was determined dividing the weight of sample (expressed in gm) by the weight of water (in ml).

Determination of viscosity: Ostwald viscometer was used to determine the viscosity of polyherbal syrup. The method was followed as per the standard procedure[8].

2. *Phytochemical screening*

The phytochemical screening was done using standard procedure[4].

3. *Quantitative Estimation of Phytoconstituents*

The quantitative estimation for the following phytoconstituents: Phenols[9], Flavanoids[10], Alkaloids[11], Tannins[12], Sugar[13] was also carried out in the Polyherbal syrup.

4. *Quantitative Estimation of Heavy Metals*

Analysis of heavy metals in the syrup was quantified by ICP-OES method[14].

5. *Microbial Load Analysis*

Microbial load was tested for the Polyherbal syrup which includes Total yeast and moulds count, absence of *Escherichia coli*, *Salmonellae*, *Clostridia* and *Shigella* as per WHO guidelines [5].

6. *HPTLC finger-printing of the Polyherbal syrup*

HPTLC is High Performance Thin Layer Chromatography or High Pressure Thin Layer Chromatography. This is a sophisticated advancement of Thin Layer Chromatography (TLC). HPTLC is one of the most versatile chromatographic methods. It has several advantages like better resolution, faster development of spots and also easy detection and quantification of separated compounds. The time required for the demonstration of most of the characteristic constituents of a sample standards are very quick and short. The fingerprint obtained is suitable for monitoring the identity and purity of drugs and for detecting adulteration and substitution in the sample.

Selection of plate and adsorbent

Precoated aluminium plates with Silica Gel 60F254 (E. Merck, India) of 10 x 10 cm and 0.2 mm thickness, were used for the detection. The plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography.

Sample solution

Accurately weighed finished product equivalent to 1 g was taken in separate iodine flask. Then 50 ml methanol was added into each flask and refluxed for 1 hour. The solution was filtered and the filtrate was concentrated to 1-2 ml. This solution was used for HPTLC finger-printing.

Application of sample

The automatic device "CAMAG LINOMAT IV" was used to apply 1 band of 6 mm width with 10 µl of the "sample solution".

Development

The plate was developed in CAMAG glass twin-through chamber (10-10 cm) previously saturated with the solvent for 60 min (temperature 25.2°C, relative humidity 40%). Subsequently scanning was done.

Solvent system: Toluene: Methanol: Ethyl acetate: Formic acid (3:0.5:5:1.5)

Detection

The plate was scanned at UV 366 nm and 254 nm using CAMAG TLC Scanner-2 and LINOMAT-IV. R_f value of each compound which were separated on plate and data of peak area of each band were recorded.

7. *Accelerated Stability testing of polyherbal syrup*

The Accelerated Stability study of prepared syrup was carried out for 3 months. The syrup was kept at 40°C ± 2° C / 75% RH ± 5% and syrup was stored in ambered coloured bet bottle. The parameters evaluated every month were pH, total solids, specific gravity and viscosity. The quantitative estimation of phytoconstituents, and microbial load was done at the beginning and at end of the 3 months period[15].

Table 3: Monographic Analysis of Herbs

Plant Name	Loss on Drying	Ash values (% w/w)			Extractive values (% w/v)		
		Total ash	Acid insoluble ash	Sulphated ash	Water soluble extractive	Alcohol soluble extractive	Ether soluble extractive
1	6.01 ± 0.01	12.2 ± 0.59 (NMT 17)	0.6 ± 0.31 (NMT 2)	21.6 ± 1.5	12.1 ± 0.7 (NLT 10)	12 (NLT 2)	3.3 ± 0.9
2	2.41 ± 0.12	14.02 ± 0.22 (NMT15)	2.4 ± 0.13 (NMT 8)	12.36 ± 0.5	Nil	19.7 ± 3.7 (NLT 10)	8.92 ± 0.25
3	3.56 ± 0.01	4.37 ± 0.33 (NMT 6)	0.34 ± 0.07 (NMT 1)	5.71 ± 0.52	13.4 ± 1.8 (NLT 7)	26.9 ± 5.3 (NLT 5)	35.3 ± 3.9
4	6.36 ± 0.53	7.22 ± 0.52 (NMT 8)	0.36 ± 0.04 (NMT 1)	9.0 ± 0.7	22.4 ± 1.6 (NLT 15)	16.7 ± 2.3 (NLT 7)	8.83 ± 0.83
5	2.63 ± 0.32	3.8 ± 0.08 (NMT 4)	0.43 ± 0.09 (NMT 0.5)	12.6 ± 0.8	16.4 ± 2.8 (NLT 13)	22.67 ± 1 (NLT 15)	10.9 ± 1.3
6	6.38 ± 0.14	11.4 ± 0.16 (NMT 12)	0.53 ± 0.05 (NMT 0.6)	25 ± 1.6	29.7 ± 0.7 (NLT 25)	19.2 ± 0.4 (NLT 10)	11.4 ± 1.8
7	3.66 ± 0.09	14.12 ± 0.25 (NMT 15)	1.47 ± 0.08 (NMT 2)	16.6 ± 2.6	18.1 ± 1.9 (NLT 10)	10.6 ± 1 (NLT 6)	7.4 ± 0.6

NMT – Not more than, NLT – Not less than; *1. Aerva lanata*, 2- *Astercantha longifolia*, 3-*Cucumis sativus*, 4- *Cumimum cyminum*, 5- *Hemidesmus indicus*, 6- *Lagenaria siceraria*, 7- *Tribulus terrestris.*

Table 4: Phytochemical Analysis of Raw Materials

Components	*Aerva Lanata*	*Astercantha Longifolia*	*Cucumis sativus*	*Cumimum cyminum*	*Hemidesmus indicus*	*Lagenaria siceraria*	*Tribulus terrestris*
Alkaloid	+	_	+	_	+	_	+
Flavaoid	+	+	+	+	+	_	_
Tannins	_	+	+	+	+	_	_
Saponins	_	+	+	_	+	+	+
Phenols	_	_	_	+	_	_	+
Sugar	+	_	+	_	+	+	_
Glycosides	+	_	+	_	_	_	_
Terpenoids	+	_	_	_	+	_	+
Protein	+	+	_	+	_	+	+
Resins	_	_	_	_	_	_	_
Steroids	+	_	+	_	+	_	+

+ ive indicates presence, - ive indicates absence.

RESULTS AND DISCUSSION

1. Raw materials analysis:

The results of the monographic analysis of the 7 herbs are given in Table 3.

The Foreign organic matter, Loss on drying, Total ash, Acid insoluble ash, Sulphated ash, Water soluble extractive, Alcohol soluble extractive, Ether soluble extractive values were analysed for all the seven plants raw materials complies with the standard given in Ayurvedic Pharmacopoeia of India.

The phytochemical analysis of the individual herbs was carried out and results are given in Table 4.

From the results it is clear that the herbs are rich in alkaloids, flavonoids, tannins, saponins, phenols, terpenoids etc.

The analysis for total heavy metals in the raw materials (Table 5) indicates that in all the raw materials, the total heavy metal was within the Ayurvedic Pharmacopoeia of India prescribed standard.

Table 5: Analysis of Total Heavy Metals in the Raw Materials

Ingredients	Total Heavy Metal (Std NMT 20 ppm)
Aerva lanata	Complies
Astercantha longifolia	Complies
Cucumis sativus	Complies
Cumimum cyminum	Complies
Hemidesmus indicus	Complies
Lagenaria siceraria	Complies
Tribulus terresris	Complies

The microbial load analysis in the raw materials were carried out and the results are recorded and detailed in Table 6. The results show that there is no microbial load in all the raw materials making them safe for use.

Table 6: Microbial Load Analysis for Raw Materials

Parameters	Results	Limits As Per WHO
Total Microbial Count	Nil	NMT 1000 cfu/ml
Yeast and Moulds	Nil	NMT 100 cfu/ml
E.Coli	Absent	Should be Absent
Salmonella	Absent	Should be Absent
Shigella	Absent	Should be absent

Cfu – colony forming units

2. Evaluation of trial batches:

The trial batches were evaluated for physical parameters such as pH, total solids, specific gravity, viscosity and taste. Based on the results, the trial batch 5 was chosen as this had the most acceptable flavor and taste.

3. Evaluation of polyherbal syrup:

The evaluation of the final chosen batch of the Polyherbal syrup was performed and the results tabulated in Table 7.

Table 7: Evaluation of Polyherbal Syrup

Parameters	Observed values
pH of Decoction	7 ± 0.02
Total solids of Decoction	25.4 ± 0.23
Specific Gravity of Decoction	1.01 ± 0.01
Viscosity of Decoction	0.01 ± 0.03 poise
pH of Syrup	5.95 ± 0.06
Total solids of Syrup	52 ± 0. 03 %
Specific Gravity of Syrup	1.18 ± 0.02
Viscosity of Syrup	0.09 ± 0.04 poise

The phytochemical evaluation of the syrup shows that all the important phytochemicals that were present in the crude drugs are also intact in the final formulation (Table 8) evaluation.

Table 8: Phytochemical Analysis of Polyherbal Syrup

S. No	Chemical constituents	Results
1	Alkaloids	+
2	Flavonoids	+
3	Glycosides	+
4	Phenols	+
5	Proteins	+
6	Saponins	+
7	Steroids	+
8	Sugars	+
9	Tannins	+
10	Terpenoids	+

The total amount of the important phytochemicals has been quantified and is given in Table 9.

Table 9: Quantitative Estimation of Phytoconstituents in Polyherbal Syrup

S.No	Phytoconstituents	Results
1	Alkaloids	0.56 µg/mg
2	Flavonoids	63 µg/mg
3	Phenols	11 µg/mg
4	Tannins	19 µg/mg
5	Sugars	320 µg/mg

The heavy metal analysis of the Polyherbal syrup (Table 10) indicates that all the heavy metals are will within acceptable limits.

The HPTLC chromatogram of the syrup shows 4 intense peaks. This indicates the syrup consist of 4 different phytoconstituents. These phytoconstituents may be responsible for antiurolithiatic activity. The HPTLC analysis indicates presence of 4 peaks (figure 1).

Table 10: Quantitative Analysis of Formulation

Heavy metals	Observation in ppm	Standard limits
Arsenic	0.041 ppm	5 ppm
Cadmium	0.014 ppm	0.3 ppm
Lead	0.108 ppm	10 ppm
Iron	0.723 ppm	10 ppm
Mercury	Not detected	0.5 ppm

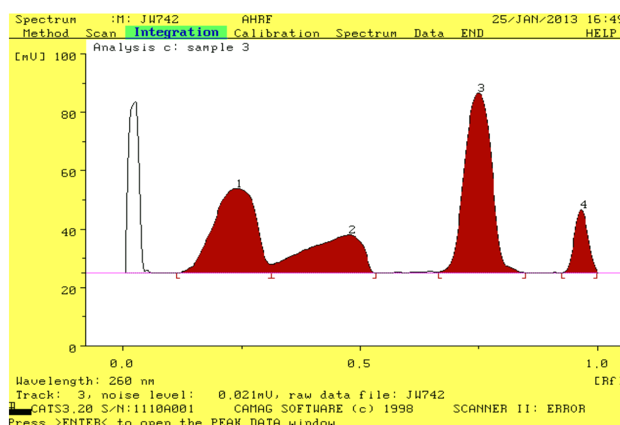

Figure 1: HPTLC Analysis of a Polyherbal Syrup

4. Accelerated stability study:

1. *Evaluation of Physical parameters of Syrup*

The results of the physical parameter evaluation are given in Table 11. It can be seen that there is very little alteration in the parameters even at the end of the 3 months period.

2. *Quantitative Estimation of Phytoconstituents*

The quantitative estimation of the phytoconstituents (Table 12) shows that there is no variation in the quantities even after the 3rd month.

3. *Microbial Load Analysis*

The microbial load limit was also unaltered at the end of three months (Table 13).

Table 11: Evaluation of Physical parameters

S.No	Parameters	Initial study	First month	Second month	Third month
1.	pH	5.95 ±0.06	5.94 ± 0.05	5.92± 0.02	5.94 ±0.04
2.	Total solids (% w/v)	52±0.52	52±0.34	52 ±0.23	50 ±0.14
3.	Specific gravity	1.18±0.01	1.18±0.03	1.18±0.06	1.18±0.02
4.	Viscosity (Poise)	0.09±0.02	0.09±0.02	0.09±0.04	0.097±0.08

Table 12 : Quantitative Estimation of Phytoconstituents

S. No	Parameters	Initial study	Third month
1.	Alkaloids	0.56 µg/mg	0.56 µg/mg
2.	Flavonoids	63 µg/mg	60 µg/mg
3.	Phenols	11 µg/mg	10 µg/mg
4.	Tannins	19 µg/mg	17 µg/mg
5.	Sugars	320 µg/mg	320 µg/mg

Table 13: Microbial Load Analysis

Parameter	Initial study	Third month	Limits as per WHO
Total Microbial Count	Nil	Nil	NMT 1000 cfu/ml
Yeast and Moulds	Nil	Nil	NMT 100 cfu/ml
E.Coli	Absent	Absent	Should be Absent
Salmonella	Absent	Absent	Should be Absent
Shigella	Absent	Absent	Should be Absent

Cfu- colony forming units

CONCLUSION

The Polyherbal syrup consisting of the seven herbs which have folklore claim of being used in urolithiasis and those with diuretic properties was prepared and these were evaluated and standardized. The accelerated stability study (40°C ± 2° C / 75% RH ± 5%) for 3 months indicates that the formulation is stable under these conditions. This Polyherbal syrup needs to be further evaluated for its antiurolithiatic potential in animals and then in human.

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