Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 5| Issue 2|APR-JUN|2015|167-178

Research Article
Biological Sciences

ASSESSMENT OF STANDARDIZATION AND QUALITY CONTROL PARAMETERS OF PATENTED POLYHERBAL FORMULATIONS

Ananth Kumar Kammalla[1], Kasthuri Bai Narayanan[1], Mohan Kumar Ramasamy[1], Agarwal Aruna[2], Dubey Govind Prasad[3], Ilango Kaliappan[1]*

[1] Interdisciplinary School of Indian System of Medicine, SRM University, Kattankulathur-603203, Tamil Nadu, INDIA.

[2] National Facility for Tribal & Herbal Medicine, Institute of Medical sciences, Banaras Hindu University, Varanasi, INDIA.

[3] Faculty of Ayurveda, Institute of Medical sciences, Banaras Hindu University, Varanasi, INDIA.

*Corresponding Author Email: ilangok67@gmail.com

ABSTRACT

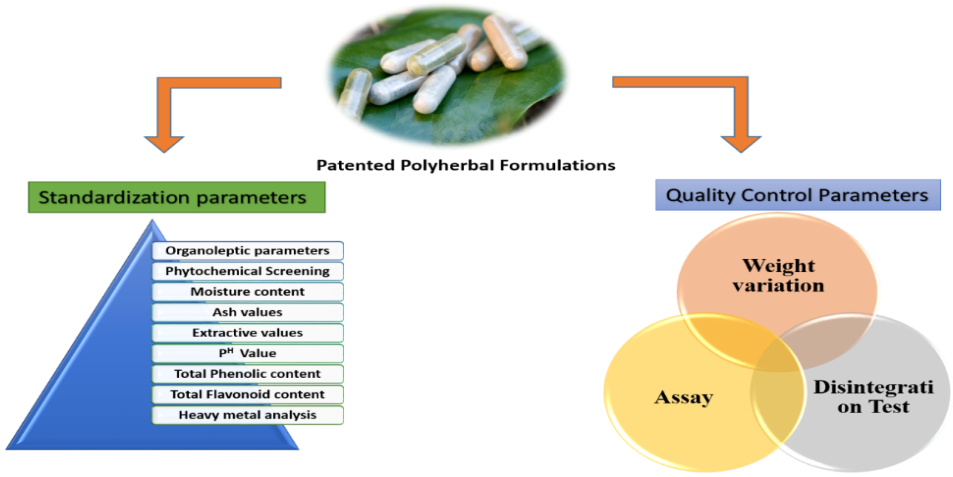
Background: *A novel US and European patented polyherbal formulations used to treat Type II Diabetes (D-formulation) and Neurological Disorders (N-formulation) was investigated in this study. Standardization of polyherbal formulations is essential in order to assess the quality of drugs.* ***Aim****: The present study was performed to evaluate Physiochemically by testifying it on classical tests along with modern scientific techniques.* ***Materials and Methods****: In order to standardize and to lay down the standard operating procedures (SOPs) and pharmacopoeial standards, the formulation was prepared in three batches at laboratory scale. It was subjected to analysis for physicochemical parameters, phytochemical studies, heavy metals and quality control parameters according to the ayurvedic texts and WHO guidelines.* ***Results and Conclusion****: Total ash values for D and N formulation were 1.9 ± 0.01 and 2.1 ± 0.56 %w/w. Moisture content of D and N formulation were 0.4 and 0.6%w/w. Total Phenolic and Flavonoid content of the D and N formulation found to be 0.197±0.0002, 0.048±0.005 for D-formulation and 0.163±0.004,0.04±0.009 for N-formulation. The limits obtained from the different physicochemical parameters of the individual herbal extracts and the polyherbal formulations could be used as reference standard for standardization of the polyherbal formulations in a quality control laboratory. Heavy metal analysis was done and concluded that levels of heavy metals fall within the permissible range and can be preferred to consume by mankind for various medicinal purpose.*

KEY WORDS

Polyherbal formulations, Standardization, Heavy metal analysis, Quality control, Flavonoid and phenolic content.

Available Online through
www.ijpbs.com (or) www.ijpbsonline.com
IJPBS |Volume 5| Issue 2 |APR-JUN|2015|167-178

Graphical Abstract



INTRODUCTION

Recently, there has been a shift in the universal trend of medicine selection from synthetic to herbal medicine, which we can say "Return to Nature." [1]Herbal medicines are in great demand in the developed and developing countries for primary health care because of their wide range of biological activities, higher safety margins and cost effective. [2] One of the major problems faced by the herbal industry is the unavailability of rigid quality control profiles for herbal materials and their formulations. Standardization of herbal formulations is essential in order to assess quality of drugs. The quality assessment of herbal formulations is of paramount importance in order to justify their acceptability in modern system of medicine. [3] The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy.[4]

In order to have a good coordination among the quality of raw herb material, in process materials and in final product, it has become essential to develop reliable, specific quality control methods using combination of classical and modern instrumental method of analysis.[5]Standardization is an essential factor for poly herbal formulation in order to assess the quality of the drugs based on the concentration of their active principle.[6] World Health Organization (WHO) provided some guidelines about standardization parameters for herbal formulations.[7]

In the present study we have taken two patented polyherbal formulations, The US patented polyherbal formulation consists of *Salacia oblonga, Salacia roxbhurghii*, *Garcinia indica* and *Lagestroemia parviflora* was developed for prevention and management of Type II Diabetes and its vascular complications associated with diabetes mellitus and An European patent polyherbal formulation for the management of cardiovascular and neurological disorders consists of leaves of *Bacopa monnieri* (Scrophuliariaceae), fruits of *Hipphophae rhamnoides* (Elaganaceae) and bulbs of *Dioscorea bulbifera* (Dioscoraceae) developed and obtained by Dubey *et al*[8,9]has been investigated to standardize the polyherbal formulation in accordance to WHO norms and standard laboratory procedures. Individual plant extracts and formulation was investigated for their organoleptic characters, physicochemical parameters, phytochemical parameters and Heavy metal analysis. The research out comings of the standardization can be used for evaluating the quality and purity of the formulations.

MATERIALS AND METHODS

Plant extracts and polyherbal formulations

The details of the polyherbal formulations were given in **Table 1**.

All the plant materials are supplied by Bhaijnath pharmaceuticals, Himachal Pradesh and Venkateswara Food Industries, Chindwara. Plant materials are authenticated by Dr.P.Jayaraman, Director, Plant Anatomy Research Centre, Tambaram, TamilNadu.The authenticated plant materials were subjected to preparation of the hydro alcoholic extracts. All the ingredients (**Table 1**) were collected, dried and powdered separately, passed through the 45# sieve and then mixed together in specified proportions in geometrical manner to get uniform mixture. The formulation composition was formulated in to capsule as per the patent information of the effective doses by the M/s Varanasi Bio Research Pvt Ltd. Varanasi, India. Three batches of the capsule bottles has been received from the M/s Varanasi Bio Research Pvt Ltd.

Evaluation of Organoleptic evaluation

Organoleptic evaluation refers to evaluation of individual drugs and formulations by color, odor, taste, texture, etc. The organoleptic characters of the samples were carried out based on the method as described by Wallis. For determining the odor of an innocuous material, small portion of the sample was placed in the beaker of suitable size, and examined by slow and repeated inhalation of the air over the material. If no distinct odor was perceptible, the sample was crushed between the thumb and index finger, between the palms of the hands, using gentle pressure or if the material was known to be dangerous, by other suitable means such as pouring a small quantity of boiling water onto the crushed sample placed in a beaker. First, the strength of the odor was determined (none, weak, distinct, strong) and then the odor sensation (aromatic, fruity, musty, mouldy, rancid, etc.) was studied. Taste was distinctively classified as aromatic, pungent, sweet, sour, astringent, mucilaginous, or bitter.

Preliminary phytochemical screening of hydro alcoholic extracts of individual plant extracts

Standard screening test of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins and anthraquinones using standard procedures.[10]

i. Test for alkaloids

a) **Preliminary test**: A 100 mg of an alcoholic extract was dissolved in dilute hydrochloric acid. Solution was clarified by filtration. Filtrate was tested with Dragendroff's and Mayer's reagents. The treated solutions were observed for any precipitation.

b) **Confirmatory test**: Five grams of the alcoholic extract was treated with 40% calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then extracted twice with 10 ml portions of chloroform. Chloroform extracts were combined and concentrated *in vacuo*to about 5 ml. Chloroform extract was then spotted on thin layer plates. Solvent system (n-hexane-ethyl acetate, 4:1) was used to develop chromatograms and detected by spraying the chromatograms with freshly prepared Dragendroff's spray reagent. An orange or dark colored spots against a pale yellow background was confirmatory evidence for presence of alkaloids.

ii. Test for steroidal compounds

a) **Salkowski's test**: 0.5 g of the alcoholic extract was dissolved in 2 ml chloroform in a test tube. Concentrated sulfuric acid was carefully added on the wall of the test tube to form a lower layer. A reddish brown colour at the interface indicated the presence of a steroid ring (i.e. the aglycone portion of the glycoside).

b) **Lieberman's test**: 0.5 g of the alcoholic extract was dissolved in 2 ml of acetic anhydride and cooled well in an ice-bath. Concentrated sulfuric acid was then carefully added. A colour change from purple to blue to green indicated the presence of a steroid nucleus i.e. aglycone portion of' the cardiac glycosides.

iii. Test for phenolic compounds

a) To 2 ml of filtered solution of the aqueous macerate of the plant material, 3 drops of a freshly prepared mixture of 1 ml of 1% ferric chloride and 1 ml of potassium ferro cyanide was added to detect phenolic compounds. Formation of bluish-green color was taken as positive.

b) The dried alcoholic extract (100 mg) was dissolved in water. Few crystals of ferric sulfate were added to the mixture. Formation of dark-violet color indicated the presence of phenolic compounds.

iv. Flavonoids

a) **Test for free flavonoids**: Five milliliters of ethyl acetate was added to a solution of 0.5 g of the

extract in water. The mixture was shaken, allowed to settle and inspected for the production of yellow colour in the organic layer which is taken as positive for free flavonoids.

b) **Lead acetate test**: To a solution of 0.5 g of the extract in water about 1 ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavonoids.

v. Test for saponins

Froth test: 0.5 g of the alcoholic extract was dissolved in 10 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. The test tube was allowed to stand in a vertical position and observed over a 30 minute period of time. If a "honey comb" froth above the surface of liquid persists after 30 min. the sample is suspected to contain saponins.

vi. Test for tannins

a) **Ferric chloride test:** A portion of the alcoholic extract was dissolved in water. The solution was clarified by filtration. 10% ferric chloride solution was added to the clear filtrate. This was observed for a change in colour to bluish black.

b) **Formaldehyde test**: To a solution of about 0.5 g of the extract in 5ml water, 3 drops of formaldehyde and 6 drops of dilute hydrochloric acid were added. The resulting mixture was heated to boiling for 1 minute and cooled. The precipitate formed (if any) was washed with hot water, warm alcohol and warm 5% potassium hydroxide successively. A bulky precipitate, which leaves a colored residue after washing, indicated the presence of phlobatannins.

c) **Test for Phlobatannins**: Deposition of a red precipitate when an aqueous extract of the plant part was boiled with 1% aqueous hydrochloric acid was taken as an evidence for the presence of phlobatannins.

vii. Test for Anthraquinones

a) Test for free anthraquinones (Borntrager's test)

The hydro-alcoholic extract of the plant material (equivalent to 100 mg) was shaken vigorously with 10 ml of benzene, filtered and 5 ml of 10% ammonia solution added to the filtrate. Shake the mixture and the presence of a pink, red or violet color in the ammonia (lower) phase indicated the presence of free anthraquinones.

b) Test for O-anthraquinone glycosides (Modified Borntrager's test)

For combined anthraquinones, 5 g of the plant extract was boiled with 10 ml 5% sulphuric acid for 1 hour and filtered while hot. The filtrate was shaken with 5 ml benzene; the benzene layer separated and half its own volume of 10% ammonia solution added. The formation of a pink, red or violet color in the ammonia phase (lower layer) indicated the presence of anthraquinone derivatives in the extract.

viii. Test for Carbohydrates

The extracts were treated with 3 ml of alpha naphthol in alcohol and Conc.Sulphuric acid was carefully added to side of the test tubes. Formation of a violet ring at the junction of two liquids indicates presence of carbohydrates.

- **Fehling`s Test:** To the sample Fehling`s solution A and B was added and heated for two minutes. Appearance of reddish brown color indicates presence of reducing sugars.
- **Benedict's Test:** To the sample benedict's solution was added and heated, appearance of reddish orange precipitate indicates presence of reducing sugars.
- **Barfoed`s Test:** The sample were treated with Barfoed`s reagent and heated, appearance of reddish orange precipitate indicates presence of reducing sugars.

ix. Test for Proteins

- **Biuret`s Test**: To the extracts copper sulphate solution followed by sodium hydroxide solution, a violet color precipitates indicates presence of proteins.
- **Million's Test:** To the extracts million's reagent was added, appearance of pink color indicates presence of proteins.

x. Test for Gums and Mucilage

The extracts were treated with 25 ml absolute alcohol and then the solution was filtered. The filtrate was examined for its swelling properties.

xi. Test for Glycosides

A pinch of the extract were dissolved in glacial acetic acid and few drops of ferric chloride solution was added followed by the addition of Conc.Sulphuric acid, formation of red ring at the junction of the two liquids indicates presence of glycosides.

xii. Test for Terpenes

The extracts were treated with tin and thionyl chloride, appearance of pink color indicates presence of terpenes.

Determination of moisture content

Moisture content was determined by loss on drying (LOD) at 105°C by Karl Fischer method[10]. 1.0 g of weighed quantity of drug was taken in a pre-weighed crucible and kept in an oven at 105°C. The crucible was cooled in desiccator and weight was taken. Procedure was repeated till a constant weight was obtained. The loss of weight was calculated as the amount of moisture content in mg per g of air dried material. Weighed quantity of drug was also subjected to Karl Fischer titration to determine the moisture content present in the prepared drug.

Determination of Ash values

Total ash

Two grams of grounded air-dried material was accurately weighed in a previously ignited and starred silica crucible. The drug was gradually ignited by raising the temperature to 450°C until it was white. The sample was cooled in a desiccator and weighed. The percentage of total ash was calculated with reference to air-dried drug.

Acid Insoluble ash

The ash was boiled with 25 ml of 2 M hydrochloric acid for 5 minutes, the insoluble matter was collected on an ash less filter paper, washed with hot water, ignited, cooled in a desiccator, and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Water soluble ash

The ash was boiled with 25 ml of water for 5 minutes, the insoluble matter on ash less filter paper collected, washed with hot water, ignited, cooled in a desiccator, and weighed. The weight of the insoluble matter from the weight of the total ash was subtracted; the difference represents the water soluble ash. The percentage of water insoluble ash was calculated with reference to the air-dried drug.

Determination of Extractive values

Water soluble extractives

Five grams of coarsely powdered air-dried drug was macerated with 100 ml of water in closed conical flask for 24 hours, shaken frequently for the first 6 hours and allowed to stand for 18 hours. This was filtered through Whatman filter paper grade no.100. Twenty-five milliliters of the filtrate was evaporated to dryness in petri dish, dried at 105°C, and weighed. Percentage of water soluble extractive with reference to air-dried material was calculated.

Alcohol soluble extractives

Five grams of air-dried and coarsely powdered drug was macerated with 100 ml of 70% ethanol in a closed conical flask for 24 hours, shaken frequently during the first 6 hours, and allowed to stand for 18 hours. This was filtered rapidly taking precaution against loss of ethanol. Twenty-five milliliters of the filtrate was evaporated to dryness in a petri dish, dried at 105°C, and weighed. Percentage of alcohol soluble extractive was calculated with reference to air-dried drug.

Determination of pH of suspension of the drugs

The freshly prepared 1% w/v suspension and 10% w/v suspension in distilled water was determined using simple glass electrode pH meter.

Total phenolic content

Total phenolic content was determined by Folin – Ciocalteu reagent assay.[11] The methanolic sample (1ml) is mixed with 1ml of the 1N Folin-Ciocalteu reagent. After 3 min, 1ml of the 20% sodium carbonate solution is added. After that volume made up to 10 ml of distilled water. The mixed solution is allowed to stand for another 90 min before the absorbance at 765nm. Gallic acid is used as a standard for the calibration curve. The total phenolic content is expressed as µg gallic acid equivalents (GAE) per 1mg of sample (µg/mg).

Total flavonoid content

Total flavonoid content was measured by the method described by the Moreno *et al.*[12]An aliquot of 1 ml of sample methanolic solution added to the 10 ml volumetric flask containing 0.2ml of the 10% aluminium nitrate, 0.2 ml of a 1M potassium acetate solution and make up to 10 ml with methanol. After 40 min at room temperature, the absorbance is measured at 415nm. Quercetin is used as standard.

Heavy Metal Analysis

In the environment, heavy metals pollutants are released from many different anthropogenic sources.[13]Depending upon the geographical sources heavy metals and trace elements may differ which may lead to severe toxicity. Lead and cadmium are

among the most abundant heavy metals and are particularly toxic. Excessive content of these metals in food is associated with number of diseases, especially of the cardiovascular, renal, nervous and skeletal systems.[14-16] These heavy metals are also implicated in carcinogenesis, mutagenesis and teratogenesis. The aim of this study was to determine the concentration of heavy metals in ingredients of the polyherbal formulations and finished product of the formulation and to estimate their contribution to the consumption by mankind.

Determination of Heavy metals by AAS Instrumentation

Weight accuratelyabout2.0gofSampleanddigestitwith25mlof0.5NitricAcid.Mixproperlyandheatonawaterbathfor 15minutes.Filterthesampleandmakeupwith25ml,0.5 MNitricacid.Aspirateblank,standardsandsamplesolutionsseparatelybyusingaboveparameters.

Weight variation test

Filled capsules (n=20) of each were weighed. The weights of the intact capsule and empty capsule shell were taken, in order to determine the variation in weight of the prepared formulation.

Disintegration test

Disintegration time of different formulated capsules (n=20) were determined according to I.P. disintegration test for hard gelatin capsules using a disintegration test apparatus with discs (disintegration media: water and 0.1N HCl solution; disintegration temperature: 37±0.5 °C). The time taken until no material from any of the capsule was left on the mesh was considered as the disintegration time.

Estimation of major bioactive components present in the polyherbal formulation

D - Formulation consists of *Salacia roxbhurghii* (Hippocrateaceae), *Salacia oblonga* (Hippocrateaceae), *Garcinia indica* (Guttiferae),*Lagestroemia parviflora* (Lythraceae). Three chemical makers were selected for the quantification and one was from each medicinal herb used as raw materials, Mangiferin for *Salacia species*,[17] Ellagic acid for *Lagestroemia parviflora*[18]and Hydroxycitric acid for *Garcinia indica*.

N - Formulation consists of leaves of *Bacopa monnieri* (Scrophuliariaceae), fruits of *Hipphophae rhamnoides* (Elaganaceae) and bulbs of *Dioscorea bulbifera* (Dioscoraceae). Quercetin and Rutin major flavonoids present in all the ingredients and considered major bioactive components in the formulation. These markers are quantified by the validated chromatographic techniques. The values of drug peak area was used for quantitative determination.

RESULTS AND DISCUSSION

The subject of herbal drug standardization is a massively wide and deep. There is so much to know and so much seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental functions

For the purpose of research work on standardization of herbal formulation, a profound knowledge of the important herbs widely used in Ayurvedic formulations is of utmost importance

In the present study polyherbal and their composition of individual extracts was subjected to physiochemical, phytochemical and analytical techniques. Botanical parameters revealed that D-formulations light brown in colour, with characteristic odor and bitter taste in powdered form. N-formulation was dark green in colour, with characteristic odor and bitter taste in powdered form. The organoleptic characters of the individual plant extract and polyherbal formulations was given in **Table 2**.

The results of the phytochemical analysis of the plant extracts and polyherbal formulations reveals the presence of various phytochemical constituents which represents the medicinal use of its traditional claim. The preliminary phytochemical studies of the plant extracts and formulations were given in the **Table 3**.

Table 1 Details of patented polyherbal formulations

S.No	Polyherbal formulations	Formulation contains the extract of	Effective Dosage (mg per capsule)	Active components	Patent Details
1	Herbal formulation for prevention and management of Type II Diabetes mellitus and vascular complications associated with Diabetes mellitus	*Salacia oblonga* *Salacia roxbhurghii* *Garcinia indica* *Lagestroemia parviflora*	162.5 112.5 87.5 112.5	Mangiferin Ellagic acid Hydroxycitric acid	US Patented 8337911B2 Dec 25, 2012 [8] D-Formulation
2	Herbal formulation for management of cardiovascular and neurologic disorders	*Bacopa monnieri* *Dioscorea bulbifera* *Hippophae rhamnoides*	225 125 125	Bacoside Diosgenin Quercetin and Rutin	EP1569666B1 Dec 13,2011 [9] N-Formulation

Table 2. Physio-chemical parameters of the polyherbal formulations and its composition

S.No	Plant name	Appearance	Colour	Taste	Odour	Total ash (%w/w)	Water soluble ash (%w/w)	Acid insoluble ash (%w/w)	Moisture content (%w/w)	pH values	TPC (m.e.w Gallic acid)	TFC (m.e.w Quercetin)
1	*Salacia oblonga*	Powder	Brownish	Bitter	Characterstic	7.4±2.31	3.16	0.39	5.0	4.56	0.323 ±0.011	0.075±0.031
2	*Salacia roxbhurghii*	Powder	Brownish	Bitter	Characterstic	2.9±0.61	1.13	0.27	3.0	5.32	0.274 ±0.023	0.051±0.018
3	*Garcinia indica*	Powder	White	Sour	Pleasant	3.1±1.32	2.14	0.36	6.0	4.12	0.154 ±0.001	0.051±0.022
4	*Lagestroemia parviflora*	Powder	Green	Sweet	Characterstic	7.6±3.12	4.38	0.39	4.0	6.37	1.232 ±0.017	0.095±0.004
5	D-Polyherbal formulation	Powder	Light brown	Bitter	Characterstic	1.9±0.01	0..5	0.11	0.4	7.01	0.197 ±0.002	0.048±0.005
6	*Bacopa monnieri*	Powder	Green	Bitter	Aromatic	12.0±2.87	4.38	1.66	12.3	4.61	1.222 ±0.016	0.151±0.013
7	*Dioscorea bulbifera*	Powder	Dark brown	Bitter	Characterstic	3.0±1.31	1.9	0.76	10.0	6.41	0.805 ±0.012	0.093±0.054
8	*Hipphophae rhamnoides*	Powder	Yellow	Sweet	Aromatic	3.9±0.51	1.54	0.27	6.0	5.13	0.215 ±0.010	0.057±0.021
9	N-Polyherbal formulation	Powder	Dark green	Bitter	Characterstic	2.1±0.56	1.14	0.15	0.6	6.94	0.163 ±0.004	0.044±0.009

Table 3 Preliminary phytochemical studies of the plant extracts and polyherbal formulations

S.No	Plant constituents and Tests	SO	SR	GI	LP	DF	BM	DB	HR	NF
1	Test for Carbohydrates									
	a.Molisch's test	+	+	+	+	+	+	+	+	+
	b.Fehings's test	+	+	+	+	+	-	+	+	+
	c.Benedict's test	+	+	-	+	+	+	+	+	-
	d.Barfoed's test	-	-	+	+	-	+	+	+	+
	e. Test for Starch	+	+	+	-	+	+	+	-	+
2	Test for Gums and Mucilages									
	a.Alcoholic precipitation	+	-	+	-	+	+	-	-	+
	b.Molisch's test	+	+	+	+	+	+	+	+	+
3	Test for Proteins and Amino acids									
	a.Ninhydrin test	+	-	+	-	+	+	+	-	+
	b.Biuret test	+	+	-	+	+	-	-	+	+
	c.Millon's test	-	+	+	-	+	+	+	+	+
	d.Zanthoproteic test	-	-	+	-	+	+	-	+	+
	e.Tannic acid test	+	+	-	+	+	+	+	-	+
4	Test for Fixed oils and Fats									
	a.Spot test	-	+	-	+	+	+	+	+	+
	b.Saponification test	+	+	+	+	+	+	+	+	+
5	Test for Alkaloids									
	a.Mayer's test	+	+	+	+	+	+	+	+	+
	b.Dragendorff's test	+	+	+	+	+	+	+	+	+
	c.Wagner's test	+	+	+	+	+	+	+	+	+
	d.Hager's test	+	+	+	+	+	+	+	+	+
6	Test for Saponins									
	a.Frothing test	+	+	+	+	+	+	+	+	+
7	Test for Glycosides									
	a.Legal's test	+	+	+	+	+	+	+	+	+
	b.Baljet's test	+	+	+	+	+	+	+	+	+
	c.Borntrager's test	-	+	+	+	+	+	+	-	+
	d.Keller-kiliani test	+	+	+	+	+	+	+	+	+
	e.Terpenoid glycoside test	+	+	-	+	+	+	-	+	+
8	Test for Phytosterols									
	a.Libermann's test	+	+	+	+	+	-	+	+	+
	b.LibermannBurchard test	-	+	-	+	+	+	+	-	+
	c.Salkowski's test	+	+	+	+	+	+	-	+	+
9	Test for Flavonoids									
	a. Ferric chloride test	+	+	+	+	+	+	+	+	+
	b.Alkaline- reagent test	+	+	-	+	-	+	-	+	+
	c.Zinc-hydrochloride	+	+	+	+	+	+	+	+	+
	d.Boric acid test	+	-	+	+	-	+	+	-	+
10	Test for Tannins and Phenolic compounds									
	a. Reaction with copper	+	+	+	+	+	+	+	+	+
	b.Ferric chloride (5%) test	+	-	+	-	+	-	+	+	+
	c.Reaction with Lead	+	+	+	+	+	+	-	+	+

d.Reaction with	-	+	+	-	+	-	+	-	+
e.Reaction with	+	+	+	+	+	+	+	+	+
f.Gelatin test	-	+	-	+	+	-	+	-	+

SO:*Salacia oblonga*, SR:*Salacia roxbhurghii*, GI:*Garcinia indica*, LP:*Lagestroemia parviflora*DB:Diabetic formulation, BM:*Bacopa monnieri*, DB:*Dioscorea bulbifera*, HR:*Hipphophae rhamnoides*, NF:Neuro Formulation '+' is denoted as present , '-' was denoted as absent

Table 4. Heavy metal analysis of plant extracts and polyherbal formulations

Herbal drug	Arsenic (As) (ppm)	Lead (Pb) (ppm)	Mercury (Hg) (ppm)	Cadmium (Cd) (ppm)
Salacia oblonga	0.0056	0.2873	ND	0.0056
Salacia roxbhurghii	0.0046	0.2145	ND	ND
Garcinia indica	ND	0.1456	ND	0.0048
Lagestroemia parviflora	0.0049	0.2648	ND	ND
D-Formulation	0.0054	0.2548	ND	0.0156
Bacopa monnieri	0.0063	0.2720	ND	0.0006
Dioscorea bulbifera	ND	0.1600	ND	0.0140
Hipphophae rhamnoides	0.0096	0.1100	ND	ND
N-Formulation	0.0046	0.1652	ND	0.0198

Table 5. Limits of weight variation test according to standard guidelines

Pharmacoepoeia	Net mass of the capsule contains	Percentage Deviation	Number of capsules
According to I.P.	300mg or more	±7.5	All
According to U.S.P.	300mg or more	±7.5	Minimum 18
		±15	Minimum 2

Table 6. Weight variation of polyherbal formulation capsules

S.NO	N-formulation	%Deviation	D-Formulation	%Deviation
1	507.1	101.42	480.9	96.18
2	493.8	98.76	527	105.4
3	496.2	99.24	506.4	101.28
4	543.8	108.76	504.1	100.82
5	507.6	101.52	516.2	103.24
6	495.8	99.16	504.6	100.92
7	467.1	93.42	509.8	101.96
8	481.5	96.3	491.1	98.22
9	512.6	102.52	494.2	98.84
10	517.5	103.5	499.4	99.88
11	543.8	108.76	527	105.4
12	507.6	101.52	506.4	101.28
13	495.8	99.16	504.1	100.82
14	467.1	93.42	491.1	98.22
15	481.5	96.3	494.2	98.84
16	512.6	102.52	499.4	99.88
17	543.8	108.76	527	105.4
18	507.6	101.52	506.4	101.28
19	495.8	99.16	504.1	100.82
20	467.1	93.42	491.1	98.22

Table 7. Disintegration Test of the Capsules

Formulation	Water	0.1N HCl solution at pH 1.2
D-Formulation	7 min	4 min
N-Formulation	6 min	5 min

Ash Values

Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards. The total ash values of D and N formulations was1.9 %w/w and 2 %w/w was considered to be within the limits as per WHO guidelines. The less values of moisture content could prevent bacterial, fungal or yeast growth. Moisture content of the D and N formulation was found to be 0.4% and 0.6% w/w. The results of the Ash values and Moisture content was given in the **Table 2**.

Extractive values

The extractive values of the individual plants was given in the **Table 5** Alcohol soluble extractive values revealed the presence of polar chemical component present in the plant ingredients. This includes glycosides of steroids, flavonoids, diterpenoids, quinones, coumarins and lignans. Water soluble content was high, which is due to presence of inorganic matters, sugars, carboxylic acids, saponins and tanins. Present in the plant ingredient as well as mineral content. *Bacopa monnieri* of the N-formulation was having the highest value of water soluble extractive value 22.9%w/w and *Lagestroemia parviflora* of the D-formulation having highest alcohol soluble extractive value.

pH values, Total phenolic content and Total flavonoid content of plant extracts and polyherbal formulations

Total phenolic and flavonoid content of the plant extracts represent medical efficacy of the herbals. Phenolic content of D and N formulation was found to be 0.197 and 0.163 mg eqwt of Gallic acid. Flavonoid content of D and N formulation was found to be 0.048 and 0.044 mg eqwt of Quercetin. The results of the Total phenolic content, Total flavonoid content and pH of the 10% aqueous solution of the plant extracts and polyherbal formulations was given in **Table 2**.

Heavy metal analysis

The results analysis of the levels of heavy metal present in the plant extracts and polyherbal formulations was discussed in this section and the concentration of Arsenic, Lead, Mercury and Cadmium in the plant extracts was presented in **Table 4**. The heavy metals analyzed in the herbal extracts and polyherbal formulations are less than the permissible limits. The levels of the heavy metals present in the extracts were expressed as mean of heavy metal concentration (ppm) ± S.D of three replicated.

Weight variation

The weight variation of the polyherbal capsules were within the accepted standard of the Indian Pharmacoepia and United Stated Pharmaceopia which stated that capsule can very up to 7.5% for capsules over 300 mg, and then two capsules should exceed these parameters. All the analyzed capsules had an average weight of N-formulation capsule was 502.28 mg and D-formulation was 504.2 mg the maximum and minimum weight of D-formulation capsules were 467.1 mg and 543.8 and N-formulation capsules were 480.9 mg and 527.84 mg respectively. Thus, none of the analyzed polyherbal formulation capsules were exceeded the acceptable range. The Limits of the weight variation test was given in **Table 5** and the weight variation among the D and N formulation capsules were given in the **Table 6**.

Disintegration studies of polyherbal formulated hard gelatin capsules

Disintegration is a very important step for drug bioavailability and absorption. In order for the capsules to disintegrate efficiently, the active compound much be solubilized and totally disaggregated, consisting only of small particles.[19] The disintegration teat of the capsules showed that rapid disintegration of the granules and the capsules were more efficiently disintegrated in the pH 1.2 solution medium, with total disintegration occurring

by 8 min for both formulated capsules. The disintegration in distilled water were also rapid, but they were also still slower than in the pH 1.2 Hydroalcoholic acid buffer, with total disintegration occurring by 7 and 6 min respectively for both the capsules. All the polyformulated capsules are passed the disintegration test according to I.P. The disintegration values of the capsules were given in Table 7.

Estimation of biomarkers in the polyherbal formulations

The content of major bioactive markers Mangiferin, Hydroxycitric acid and Ellagic acid from D-polyherbal formulation was quantified in the validated HPLC method was reported in our previous literature.[20]The content of Quercetin and Rutin in N-formulation was estimated and found to be 0.09665w/w and 0.0541%w/w by HPLC respectively was mentioned in our previous work.[21]

CONCLUSION

The polyherbal formulations under study were subjected to physiochemical analysis, which is helpful in establishing the standard along with other parameters such as phytochemical analysis. Heavy metal analysis was done and concluded that levels of heavy metals fall within the permissible range and can be preferred to consume by mankind for various medicinal purpose. The ingredients of these polyherbal formulations were effectively extracted by hydro alcoholic and the efficiency of the formulation was demonstrated by the dissolution profile of the major biomarkers from the polyherbal formulations. The marker was best released in acidic and basic medium, based on the average weight and the results of the disintegration test, the developed capsule was within the standards recommended by the Indian Pharmacopeia and WHO standards. Consequently the formulations used to determine and ascertain its quality control. The study is likely to help the QA of polyherbal formulations in Ayurveda and in development of standard parameters.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Science and Technology, Government of India, for providing financial assistance to carry the work and Department of Pharmacology, SRM College of Pharmacy SRM University for providing facilities to carryout animal studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Rasheed N, GuptaV. Standardization of a compound Unani herbal formulation “Qurs-e-Luk” with modern techniques. Pharmacog Res, 2(4): 237-41 (2010).
2. TT T. Herbal Medicine. Indian J Pharm Edu, 32(2):104-6 (1998).
3. Satheesh N, Kumud U, Asha B. Phytochemical screening and standardization of polyherbal formulation for Dyslipidemia. Int J Pharm Pharm Sci, 3(3): 235-8 (2011).
4. Mohd Tariq, Chaudary Shahid Shah, Imtiyaz Shaik, Rahman Khaleequr, Zamam Roohi. Preliminary physiochemical evaluation of Kushtatutia : A Unani Formulation. J Ayur Int Med, 5(3): 148-53 (2014).
5. KK B. Herbal medicine an enigma and challenge to science and direction for new initiatives. J Nat Prod, 19(1): 3-8 (2013).
6. Mohapatra P, Shiwaikara A, Aswatharam H. Standardization of a polyherbal formulation. Pharmacogn Mag, 4(13): 65-9 (2008).
7. World Health Organization. Quality controls Methods for Medicinal Plant Materials, Delhi: AITBS Publisher and Distributors; 2002. pp. 8-70.
8. Dubey GP, Agrawal A, Dubey N, Dubey S, Dubey R, Deborah S. Herbal formulation for the prevention and management of Type-2 Diabetes mellitus and vascular complications associated with diabetes”. USA Patent 8337911B2; 25 Dec 2013.
9. Dubey GP. Herbal preparation for management of cardiovascular and neurologic disorders. European Union, Patent No. EP 1 569 666 B; 2005.
10. Hymete A. Phytochemical investigation of the fruit of *Lagenaria breviflora* Robert. MSc thesis, University of IFE, ILE-IFE, Nigeria; 1986. pp. 54-67.
11. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic, 16(3): 144–53 (1965).
12. Sánchez-Moreno C, Plaza L, Ancos B, Cano MP. Nutritional characterisation of commercial

traditional pasteurised tomato juices: carotenoids, vitamin C and radical-scavenging capacity. Food Chem, 98(4): 749–56 (2006).

13. Celik A, Kartal A, Akdogan A. Kaska Y. Determining the heavy metal pollution in Denizli, Turkey by using *Robinio pseudo-Acacia* L. Environ Int, 31(1): 105-12 (1995).
14. WHO Cadmium environmetal health criteria, Geneva: World Health Organization; 1992. p.134..
15. WHO. Lead environmental health criteria, Geneva: World Health Oraganisation; 1995, p. 165.
16. Steenland K, Boffette P. Lead and cancer in humans:where are we now? Am J Ind Med, 38(3): 295-9, (2000).
17. Yoshikawa M, NN SH, T, MK Y, MH. Polyphenol constituents from *Salacia species*: quantitative analysis of mangiferin with alpha-glucosidase and aldose reductase inhibitory activities.Yakugaku Zasshi 121(5): 371-8 (2001).
18. Hayashi T, Maruyama H, Kasai R, Hattori K, Takasuga S,*et al*. Ellagitannins from *Lagestroemia speciosa* as activators of glucose trasport in fat cells. Planta Med, 68(2): 173-5 (2002).
19. Asira,Taranum,Shamsi Shariq, Zaman Roohi. Development of standard operating procedures of Habbe Shifa : A polyherbal Unani formulation.J Ayur Int Med, 4(3): 147-51 (2013).
20. Kammalla AK, Ramasamy MK, Aruna A, Dubey GP, Kaliappan I. Developement and validation of a RP-HPLC method for the simultaneous determination of Mangiferin. Ellagic acid and Hydroxycitric acid in polyherbal formulation. Phcog J, 6(3): 1-6 (2014).
21. Kammalla AK, Ramasamy MK, Jyothi C, Dubey GP, Agrawal A, Kaliappan I. Comparative pharmacokinetic interactions of Querceitn and Rutin in rats after oral administration of European patented fomulation containing *Hipphophae rhamnoides* and Co-administration of Quercetin and Rutin. Eur J Drug Metab Pharmacokinet. 2014. DOI 10.1007/s13318-014-0206-9

***Corresponding Author:**
ilangok67@gmail.com