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Research Article

PHYTOCHEMICAL SCREENING AND STANDARDIZATION OF POLYHERBAL FORMULATION FOR DYSLIPIDEMIA

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ABSTRACT

Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles, physical and chemical standards. This article reports on standardization of a Polyherbal ayurvedic formulation used as anti-hyperlipidemic. Specific morphological parts of the plants are used in the Polyherbal formulation. Polyherbal formulation has been standardized on the basis of organoleptic properties, physical characteristics, and physico-chemical properties.

Key words: Standardization, Polyherbal, Anti-hyperlipidemic, Morphological, Organoleptic, Physico-chemical.

INTRODUCTION

Hyperlipidemia is an elevation of lipids (fats) in the bloodstream. These lipids include cholesterol, cholesterol esters (compounds), phospholipids and triglycerides. They're transported in the blood as part of large molecules called lipoproteins. Hyperlipidemia or hyperlipoproteinemia is the condition of abnormally elevated levels of any or all lipids and/or lipoproteins in the blood[1]. The quality assessment of herbal formulations is of paramount importance in order to justify their acceptability in modern system of medicine. One of the major problems faced by the herbal industry is the unavailability of rigid quality control profiles for herbal materials and their formulations. Regulatory bodies have laid down the standardization procedures and specifications for ayurvedic preparations. In India, the department of AYUSH, Government of India, launched a central scheme to develop a standard operating procedures for the manufacturing process to develop pharmacopeial standards for ayurvedic preparations[2]. The subject of herbal drug standardization is massively wide and deep. There is so much to know and so many seemingly contradictory theories on the subject of herbal medicines and their relationship with human physiology and mental function. India can emerge as the major country and play the lead role in production of standardized, therapeutically effective ayurvedic formulations. India needs to explore the medicinally important plants. This can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization. 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[3]. It has become extremely important to make an effort towards standardization of the plant to be used as medicine.The process of standardization can be achieved by stepwise pharmacognostic studies[4].

MATERIAL AND METHODS

Physico-chemical studies like total ash, water soluble ash, acid insoluble ash, water and alcohol soluble extract, loss on drying at 105°C and successive extractive values by soxhlet extraction method were carried out as per the WHO guide lines[5]. Preliminary phytochemical tests were performed as per the standard methods[6].

Plant material

Polyherbal formulation consists of 12 ingredients, viz., *Picorrhiza kurroa , Emblica officinalis , Syzygium cumini , Trachyspermum ammi , Musa paradisiacal , Terminalia arjuna , Pistacia vera , Zingiber officinale , Allium cepa , Aloe vera , Eugenia caryophyllus , Avena sativa* . All these plant parts were procured from the local market of Dehradun, Uttrakhand, India, and were authenticated by Botanical Survey of India (BSI), Dehradun (UK).

Preparation of polyherbal formulation

All the ingredients (Table-1) were collected, dried and powdered separately, passed through 100 # sieve and then mixed together in specified proportions in a geometrical manner to get uniform mixture.

Standardization parameters

The various standardization parameters studied were Organoleptic properties, Physico-chemical investigations, determination of pH, Fluorescence analysis, Preliminary Phytochemical analysis, determination of moisture content, swelling factor, determination of viscosity, surface tension and density, determination of crude fat and determination of physical characteristics of powder formulation

Organoleptic evaluation

The organoleptic characters[7] of the samples were evaluated based on the method described by Siddiqui *et al*. Organoleptic evaluation refers to evaluation of the formulation by color, odor, taste and texture etc.

Physico-chemical investigations

Physico-chemical investigations of formulations were carried out were the determination of extractive values and ash values [8].

Determination of pH

1% solution of Polyherbal formulation was prepared in distilled water and pH was determined using pH meter SYSTRONICS DIGITAL pH METER, MK VI.

Fluorescence analysis

Fluorescence characters of powdered plant material with different chemical reagents were determined under ordinary and ultraviolet light[9].1 mg of the Polyherbal sample was taken in a glass slide and treated with various reagents for the presence of their fluorescence characters under ultra-violet lamp.

Preliminary Phytochemical analysis

Preliminary qualitative phytochemical analysis of all the extracts was carried out by employing standard conventional protocols [10- 12].

Determination of moisture content and swelling factor

Moisture content was determined by loss on drying (LOD) method[13]. 3 gm of the weighed quantity of the drug was taken and kept in oven

at 105 ° C till a constant weight was obtained. Amount of moisture present in the sample was calculated as reference to the air dried drug[13]. Swelling factor is estimated for the amount of mucilage present in the drug. The technique has been accepted as an official method for evaluation by various pharmacopoeias. One gram of the Polyherbal was taken and kept for 24 hours in a graduated, stoppered cylinder, in contact with the water up to the mark of 20 ml. After 24 hours the increase in volume was noted[14].

Determination of viscosity, surface tension and density

Density, surface tension and viscosity of the 1% aqueous Polyherbal formulation was estimated[15] (Table 6).

Determination of crude fat

2 g of moisture free Polyherbal with petroleum ether in soxhlet extractor, for 6 h till a drop taken from the drippings left no greasy stain on the filter paper. After boiling with petroleum ether [16], the residual petroleum ether was filtered and filtrate was evaporated in a pre weighed beaker. Increase in weight of beaker gave the crude fat[17].

Determination of physical characteristics of powder formulation

Physical characteristics like bulk density, tap density, Haussner ratio, and Carr's index were determined for different formulations [18, 19].The term bulk density refers to packing of particles or granules. The volume of packing can be determined in an apparatus consisting of graduated cylinder mounted on mechanical tapping device (jolting volumeter) that has a specially cut rotating can. Hundred grams of weighed formulation powder was taken and carefully added to cylinder with the aid of a funnel. The initial volume was noted and sample was then tapped until no further reduction in volume was noted. The initial volume gave the bulk density value and after tapping the volume reduced, it gives the value of tapped density. Haussner ratio is related to interparticle friction and as such can be used to predict the powder flow properties. Carr's index is a method of measuring the powder flow from bulk density [20].

RESULTS AND DISCUSSION

Polyherbal formulation was subjected to various analytical techniques. Botanical parameters revealed that dark brown in color, with a pungent odor, bitter taste, and fine texture (Table 2).

Table 1: Composition of polyherbal formulation (PHF -1)

S. no.	Sanskrit Name	Plant Name	Family	Part used	Strength
1.	Katuka	*Picorrhiza kurroa*	Scrophulariaceae	Roots	10 %
2.	Amalaki	*Emblica officinalis*	Euphorbiaceae	Fruits	3 %
3.	Jambu	*Syzygium cumini*	Myrtaceae	Seeds	5%
4.	Dipyaka	*Trachyspermum ammi*	Umbelliferae	Fruits	10%
5.	Kadali	*Musa paradisiaca*	Musaceae	Stem	8 %
6.	Arjuna	*Terminalia arjuna*	Combretaceae	Stem bark	4 %
7.	Mukulaka	*Pistacia vera*	Anacardiaceae	Fruits	8 %
8.	Shunthi	*Zingiber officinale*	Zingiberaceae	Rhizomes	4 %
9.	Palandu	*Allium cepa*	Liliaceae	Bulbs	3 %
10.	Kumari	*Aloe vera*	Liliaceae	Juice	2 %
11.	Lavangaha	*Eugenia caryophyllus*	Myrtaceae	Buds	3 %
12.	Yuvika	*Avena sativa*	Graminae.	Seeds	40 %

Table 2: Organoleptic properties of polyherbal formulation

Appearance	Color	Odor	Taste	Texture	Particle size
Powder	Dark brown	Pungent	Bitter	Fine	100#

Results of quantitative analysis for Total ash (10.33 ± 0.11), Acid insoluble ash (3 ± 0.27) , Water soluble ash (7.28 ± 0.39), Alcohol soluble extractives (48 ± 0.23), Water soluble extractive (20 ± 0.21), Hexane soluble extractive (18 ± 0.34), Chloroform soluble extractive (8 ± 0.33), PET soluble extractive (12 ± 0.24), Ethyl acetate soluble extractive (0.44) ,Particle size (100 mesh), Loss on drying at 105º C was found to be (6.5 ± 0.8) , pH (1% aq. Soln.),Crude fat were calculated and results were shown (Table-3). Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards[16]. Percent weight loss on drying or moisture content was found to be 6.5% w/w. The less value of moisture content could prevent bacterial, fungal or yeast growth[21].

The results of fluorescent studies of the powdered plant material using different chemical reagents were studied and a given in (Table-4). Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material[22]. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of Pharmacognostical evaluation[23].

Table 3: Physio-chemical characteristics of polyherbal formulation

S. no.	Parameter	Percentage mean (n=3) ± SD
1	Water soluble extractive (w/w %)	20 ± 0.21
2	Alcohol soluble extractive (w/w %)	48 ± 0.23
3	Hexane soluble extractive (w/w %)	18 ± 0.34
4	Chloroform soluble extractive (w/w %)	8 ± 0.33
5	PET soluble extractive (w/w %)	12 ± 0.24
6	Ethyl acetate soluble extractive (w/w %)	12 ± 0.44
7	Ash content (w/w %)	10.33 ± 0.11
8	Acid insoluble ash (w/w %)	3 ± 0.27
9	Water Soluble ash (w/w %)	7.28 ± 0.39
10	Particle size	100 # mess size
11	Moisture content	6.5 ± 0.8
12	pH	7 -7.2 ± 0.2
13	Crude fat	0.3 ± 0.1

Table 4: Fluorescence analysis

Powdered drug	Visible/day light	Ultra violet light
Powder as such	Dark brown	brown
Powder + $FeCl_3$	Dark grey	Greyish yellow
Powder + conc. HCl	Orange yellow	Greyish yellow
Powder + 10% HNO_3	Orange	Yellow
Powder + 10% $K_2Cr_2O_7$	Yellow	Green
Powder + 1 M NaOH	Brownish yellow	Green
Powder + $AgNO_3$	Buff brown	Light brown
Powder + conc. HNO_3	Orange yellow	Fluorescent yellow
Powder + conc. H_2SO_4	Orange	Fluorescent green
Powder + Br_2 water	Brown	Light brown
Powder + 5% H_2O_2	Brown	Greyish green
Powder + CCl_4	Brown	Dark brown
Powder + Methanol	Blackish brown	Greenish yellow
Powder + CH_3COOH	Orange brown	Dark green
Powder + Xylene	Grey	Orange green
Powder + NH_3	Yellowish brown	Yellowish green
Powder + I_2	Blackish brown	Grey

The results of preliminary phytochemical analysis of are given in (Table-5). Density, viscosity and surface tension of the Polyherbal formulation (1% aq.) were determined and results were tabulated (Table-6). Physical properties of the Polyherbal formulation, like bulk density, Tap density, Carr's compressibility index, Haussner's ratio, were determined and results were tabulated (Table-7). Swelling factor of the Polyherbal formulation was determined but it does not show appreciable amount of mucilage to be estimated, indicating the presence of very less amount, but the phytochemical screening reveals the presence of mucilage in the Polyherbal formulation.

Table 5: Phytochemical screening

S. no.	Phytoconstituent	Name of the test	Results
1	Carbohydrates	Molischs test	
		Barfoeds test	+ + +
		Benedicts test	
		Fehlings test	
2	Alkaloids	Mayers test	_ _ _
		Hagers test	
		Wagners test	
3	Tannins	Ferric chloride test	+ + +
		Lead acetate test	
		Match stick test	
4	Proteins and amino acids	Heat test	+ + +
		Ninhydrin test	
		Millions test	
		Biuret test	
5	Flavanoids	Zinc chloride test	_ _ _
		Shinoda test	
6	Glycosides	General test	+ +
7	Saponin glycosides	Froth forrmation test	_ _ _
8	Anthraquinones	Borntragers test	+
8	Mucilage and gums	Alcoholic ppt. test	+
		Ruthenium red test	
9	Steroids	Salkowski test	+ +
10.	Volatile oil	Hydrodistillation	+ +
		PET sprit test	
11	Lignin	HCl +Phloroglucinol	+
12	Starch	Iodine test	+ +
13	Inulin	α-napthol and sulphuric acid	+

+ + +: Intense; + + Moderate; +: Slight; - - - : Absent

Table 6: Density, viscosity and surface tension

Parameter	Values
Density (1% soln.)	0.99
Viscosity (1% soln.)	1.01 cp
Surface tension (1% soln.)	58.75

Table 7: Physical properties of polyherbal formulation

Parameters	Values
Bulk density	0.374 gm/ml
Tap density	0.466 gm/ml
Carrs compressibility index	19.95
Haussners ratio	1.249

CONCLUSION

Polyherbal formulation for treartment of hyperlipdemia was formulated by using various anti-lipidemic plant parts in a fixed combination .The prepared formulation was screened for various standardization parameters as per auurvedic pharmacopoeial standards. The research outcomings of the standardization parameters can be used for evaluating the quality and purity of the formulations for the polyherbal phyto formulation.

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