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Original Article

PHYTOCHEMICAL SCREENING AND STANDARDIZATION OF POLYHERBAL FORMULATION: MAHARISHI AMRIT KALASH 5

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ABSTRACT

Objective: The main aim of the study was to standardize the polyherbal formulation on the basis of organoleptic characters, phytochemical analysis, physicochemical parameters and fluorescence analysis.

Methods: All the above tests were performed based on WHO norms.

Results: Organoleptic characters revealed that formulation was light brown in color, characteristic odor, bitter in taste and moderately fine texture. Physicochemical parameters resulted in water soluble extractive (35.8 ± 0.35), alcohol soluble extractive (38.6 ± 0.24), total ash (9.25 ± 0.12), acid insoluble ash (1.94 ± 0.23), water soluble ash (6.5 ± 0.18), pH (7.49 ± 0.02), crude fat (0.3 ± 0.1), LOD at 105^{0}C (7.2 ± 0.6) and moisture content (6.2 ± 0.8). Phytochemical analysis shows the presence of alkaloids, tannins, flavonoids, steroids, terpenoids, etc. Fluorescence analysis of formulation was studied using different chemical reagents.

Conclusion: The in-house formulation was prepared and screened for various standardization parameters as per ayurvedic pharmacopoeial standards.

Keywords: MAK-5, Polyherbal, Organoleptic, Phytoconstituents, Standardization, Physicochemical.

INTRODUCTION

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 [1]. One of the major problems faced by the herbal industry is the unavailability of rigid quality control profiles for herbal materials and their formulations. 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 [2]. It has become extremely important to make an effort towards standardization of plant to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies [3]. The present study aims to standardize polyherbal formulation having anti cancer activity.

Maharishi Amrit Kalash (MAK) belongs to a group of herbal formulations called "Rasayanas". MAK enhances immunity, optimizes physiological balance (homeostasis), counters the degenerative effects of ageing and promotes health and longevity. MAK enhances immunity and prevents free radical generation [4]. Maharishi Amrit Kalash is presented in two forms: a paste called MAK-4 made up of thirty-eight herbs lipophilised in cow ghee and a hydrophilic tablet known as MAK-5 composed of thirteen herbs. The present study was taken up to standardize MAK-5.

MATERIALS AND METHODS

Plant material

Polyherbal formulation consists of 13 ingredients mentioned in Table 1. All these plant parts were procured from the local market of Mumbai, Pune and nearby forest areas and were authenticated by Blatter herbarium, Mumbai and Sunrise agro services, Pune depending on the availability of plants.

Preparation of polyherbal formulation

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.

Standardization parameters

The various standardization parameters studied were organoleptic properties, physicochemical investigations, preliminary phytochemical analysis, determination of moisture content, determination of pH, determination of crude fat and fluorescence analysis.

Organoleptic evaluation

The organoleptic characters of the formulations were evaluated based on the method described by Siddique et al. [5]. Organoleptic evaluation refers to the evaluation of formulation by color, odor, taste, etc.

Physicochemical investigations

Preliminary phytochemical tests were performed as per the standard methods. Physicochemical parameters like total ash, water soluble ash, acid insoluble ash, water and alcohol soluble extractive values, loss on drying at 105^{0}C, etc were carried out as per the WHO guidelines [6].

Determination of pH

1% solution of polyherbal formulation was prepared in distilled water and pH was determined using standard simple glass electrode pH meter.

Preparation of extract

The methanolic extract of polyherbal formulation was prepared using soxhlet extraction. 10.0 g of dried and powdered formulation was extracted using 200 ml methanol until the solvent becomes colorless. The extract was filtered and used for testing various phytoconstituents present.

Preliminary phytochemical analysis

Preliminary qualitative phytochemical analysis of methanolic extract of polyherbal formulation was carried out by employing standard conventional protocols [7, 8].

Determination of moisture content

Moisture content was determined by loss on drying (LOD) at 105^{0}C method [9] and Karl Fischer method. 1.0 g of weighed quantity of drug was taken in a pre-weighed crucible and kept in an oven at

105^{0}C. The crucible was cooled in dessicator 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 crude fat

2.0 g of moisture free polyherbal formulation with petroleum ether in soxhlet extractor, for 6 h till a drop taken from the drippings left no greasy stain on the filter paper.

The residual petroleum ether was filtered and filtrate was evaporated in a pre-weighed beaker. Increase in weight of beaker gave the crude fat [10].

RESULTS AND DISCUSSION

Polyherbal formulation was prepared using 13 ingredients as mentioned in Table 1 and passed through 45 # sieve to get uniform mixture. It was subjected to various standardization parameters. Organoleptic characters revealed that formulation was light brown in color, have a characteristic odor, bitter taste and moderately fine texture (Table 2).

Table 1: Composition of polyherbal formulation (MAK-5)

S. No.	Sanskrit name	Plant name	Family	Part used	Quantity per 500mg
1	Ashwagandha	Withania somnifera	Solanaceae	Root	90 mg
2	Yashtimadhu	Glycyrrhiza glabra	Fabaceae	Root	90 mg
3	Vidarikandha	Ipomoea digitata	Convolvulaceae	Tuberous root	90 mg
4	Safed musali	Asparagus adscendens	Asparagaceae	Tuberous root	90 mg
5	Amalaki	Emblica officinalis	Phyllanthaceae	Fruit rind	20 mg
6	Giloy	Tinospora cordifolia	Menispermaceae	Stem	20 mg
7	Shatavari	Asparagus racemosus	Asparagaceae	Tuberous root	20 mg
8	Nirgundi	Vitex trifolia	Lamiaceae	Leaf	20 mg
9	Shankhpuspi	Convolvulus pluricaulis	Convolvulaceae	Whole plant	20 mg
10	Vriddharuk	Argyreia speciosa	Convolvulaceae	Root	10 mg
11	Kali musali	Curculigo orchioides	Hypoxidaceae	Tuberous root	10 mg
12	Karir	Capparis aphylla	Capparidaceae	Bark	10 mg
13	Babul	Acacia Arabica	Fabaceae	Bark	10 mg

Table 2: Organoleptic properties of polyherbal formulation

Appearance	Colour	Odor	Taste	Texture	Particle size
Powder	Light brown	Characteristic	Bitter	Moderately fine	45# size

Physicochemical parameters resulted in water soluble extractive value (35.8±0.35), alcohol soluble extractive value (38.6±0.24), total ash content (9.25±0.12), acid insoluble ash content (1.94±0.23), water soluble ash content (6.5±0.18), pH 1% aqueous solution (7.49±0.02), crude fat (0.3±0.1), loss on drying at 105^{0}C (7.2±0.6) and moisture content using Karl Fischer technique (6.2±0.8) (Table 3). Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards. The less value of moisture content could prevent bacterial, fungal or yeast growth [11].

Table 3: Physicochemical parameters of polyherbal formulation

S. No.	Parameters	% mean (n=3)± SD
1	Water soluble extractive (w/w %)	35.8 ± 0.35
2	Alcohol soluble extractive (w/w %)	38.6 ± 0.24
3	Total ash content (w/w %)	9.25 ± 0.12
4	Acid insoluble ash content (w/w %)	1.94 ± 0.23
5	Water soluble ash content (w/w %)	6.5 ± 0.18
6	pH	7.49 ± 0.02
7	Crude fat	0.3 ± 0.1
8	LOD at 105^{0}C	7.2 ± 0.6
9	Moisture content by Karl Fischer method	6.2 ± 0.8

All parameters are mentioned in % except for pH

Table 4: Fluorescence analysis of polyherbal formulation

S. No.	Powdered drug	Visible/ day light	Ultra violet light
1	Powder as such	Light brown	No fluorescence
2	Powder +$FeCl_3$	Dark green	Dark brown
3	Powder + conc. HCl	Orange yellow	Fluorescent yellow
4	Powder + 10% HNO_3	Orange	Green
5	Powder + 10%$K_2Cr_2O_7$	Orange	Green
6	Powder + 1M NaOH	Brownish yellow	Green
7	Powder + conc. HNO_3	Orange yellow	Fluorescent yellow
8	Powder + conc. H_2SO_4	Orange yellow	Fluorescent yellow
9	Powder + 5% H_2O_2	Yellow	Fluorescent green
10	Powder + CCl_4	Light brown	Dark brown
11	Powder + methanol	Brownish yellow	Greenish yellow
12	Powder + CH_3COOH	Brownish yellow	Green
13	Powder + NH_3	Yellow	Fluorescent green

Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. 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 [12, 13]. The results of fluorescent studies of the powdered formulation using different chemical reagents were studied and mentioned in Table 4. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. 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 [12, 13]. The results of fluorescent studies of the powdered formulation using different chemical reagents were studied and mentioned in Table 4.

As seen in Table 5, the preliminary phytochemical screening of methanolic extracts indicated the presence of alkaloids, flavonoids, steroids, tannins, proteins, terpenoids, triterpenoids, carbohydrates, reducing sugar, cardiac glycosides and mucilage and gums; and does not indicate the presence of anthraquinones.

These constituents may be possibly responsible for the biological activities of polyherbal formulation.

Table 5: Phytochemical screening results of the formulation

S. No.	Phytoconstituent	Name of the test	Result
1	Alkaloids	Dragendroff test	+
		Mayers test	+
		Hagers test	+
		Wagners test	+
2	Tannins	Ferric chloride test	+++
		Lead acetate test	+++
3	Steroids	Liebermann-Burchard test	++
4	Saponins	Froth formation test	++
5	Flavonoids	Shinoda test	+++
		NaOH test	+++
6	Terpenoids	Salkowski test	+++
7	Cardiac glycosides	Keller Killiani's test	+++
8	Protiens	Biuret test	+++
9	Tri terpenoids	Liebermann-Burchard test	+++
		Salkowski test	+++
10	Carbohydrates	Molischs test	+++
11	Reducing sugars	Fehlings test	+++
12	Anthraquinones	Borntragers test	-
13	Mucilage and gums	Alcoholic ppt. test	+

+++: intense; ++: moderate; +: slight; -: absent

CONCLUSION

The present work was taken up in the view to standardize the polyherbal formulation in accordance to WHO norms and standard laboratory procedures. Formulation was investigated for their organoleptic characters, physicochemical parameters, fluorescence analysis and phytochemical parameters. The research outcomings of the standardization can be used for evaluating the quality and purity of the formulations.

CONFLICT OF INTERESTS

Declared None

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