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Physico-Chemical and Preliminary Phytochemical Analysis of Amrabeejadi Lepa A Polyherbal Formulation

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Abstract: An Ayurvedic medicine Amrabeejadi lepa is a polyherbal formulation containing Amra beeja majja (*Mangifera indica*) and Haritaki (*Terminalia Chubela*). This formulation is effective in the management of daruna darunaka roga (Severe dandruff). The values obtained after the analysis of physico-chemical properties were found to be significant and within the limits. The preliminary phytochemical analysis study revealed that individual drug of Amrabeejadi lepa has shown the presence of organic elements viz., carbohydrates, reducing sugar, mucilage, tannins, alkaloids, steroids, flavonoids and inorganic elements like sodium, iron, phosphate, sulphate, chloride, carbonates and nitrites qualitatively.

Keywords: - Amra beejadi lepa, Polyherbal formulation, Physico-chemical and Phytochemical analysis.

Introduction

India has rich cultural heritage of traditional medicines which chiefly comprised the two widely flourishing system of treatments i.e. Ayurveda and Unani systems since ancient times. The development of these traditional systems of medicine with the perspective of safety, efficacy and quality will help not only traditional heritage but also rationalize the use of natural products and health care. For herbal Formulations, one must has profound knowledge of important herbs found in India which are widely used in Ayurvedic formulations. Hence, there is need to explore the medicinally important plants; this can be achieved only when herbal products are screened by using sophisticated modern techniques. Amrabeejadi lepa churna is a herbal formulation consisting of two herbal ingredients 1.Amrabeeja (*Mangifera indica*), 2.Haritaki (*Terminalia Chubela*). It is used in the conditions like darunaka (dandruff).^[1]

There is scope to explore hidden, not highlighted, highly efficacious formulations mentioned in the classical Ayurvedic texts by analyzing them and establishing their results. The physico-chemical and preliminary phyto-chemical properties help to understand their various uses in different diseases. With the same aim this study has been undertaken.

Materials and method

Collection and Procurement of Materials:

Amrabeejadi lepa churna consists of 2 ingredients, viz., Amrabeeja (*Mangifera indica*), and Haritaki (*Terminalia chubela*). Amra beeja majja was collected from the local garden of Nanded, Maharashtra. Haritaki was procured from GMP certified K.L.E.S Ayurvedic Pharmacy Belgaum.

Authentication of raw drugs

Amra beeja majja and Haritaki phala majja authentication were carried out in AYUSH approved drug testing laboratory, (CRL), KLEU Shri BMK Ayurved Mahavidhyalaya by the expert in this field.

Preparation of churna^[2]

The Amrabeejadi lepa churna was prepared by adopting standards of Churna Kalpana as per the specifications under the supervision of experts. Here Amra beeja majja and Haritaki were used in the ratio of 1:1.

Physico-chemical studies^[3]

Physico-chemical studies carried out were Loss on drying at 105 ° C, Total ash, Acid insoluble ash, Water soluble ash, Alcohol and Water soluble extractives & pH. Results showed below in tables.

Table 1: Organoleptic properties of Amrabeejadi lepa churna.

Appearance	Colour	Odour	Taste	Touch
Churna	Yellowish Brown	Characteristic Haritaki smell	Bitter	Soft

Table 2: Physico chemical characteristics of Amrabeejadi lepa churna.

Sl.No	Parameter	Percentage mean
1	Moisture content	12.8%
2	Ash content (w/w %)	2%
3	Acid insoluble ash (w/w %)	1%
4	Water soluble ash (w/w %)	0.03%
5	Alcohol soluble extractive (w/w %)	43.2%
6	Water soluble extractive (w/w %)	39.2%
7	p ^H	3

Determination of physical characteristics of powder formulation^[4,5]

Physical characteristics like bulk density, tap density, angle of repose, Hausner ratio and Carr's index were determined

The term bulk density refers to the method used to indicate packing of particles or granules. The equation for determining bulk density (D_b) is $D_b = M/V_b$ where M is the mass of particles and V_b is the total volume of packing. The volume of packing can be determined in an apparatus consisting of graduated cylinder mounted on mechanical tapping device that has a specially cut rotating can. Particular weight of 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, giving the value of tapped density. Angle of repose has been used as an indirect method quantifying powder flowability, because of its relationship with inter particle cohesion. The fixed funnel and the free standing cone method employs a method that is secured with its tip at a given height (H), above the glass paper that is placed on a flat horizontal surface. Powder or granules were carefully poured through the funnel until the apex of the conical pile just touched the tip of funnel. Thus, with R being the radius of the conical pile $\tan a = H/R$ or $a = \arctan H/R$, where a is the angle of repose. Hausner ratio is related to inter particle friction and as such can be used to predict the powder flow properties. The equation for measuring hausner ratio is D_f / D_b . Where, D_f = Tapped density and D_o = Bulk density.

Carr's index is another indirect method of measuring the powder flow from bulk density. The equation for measuring Carr's index is

$$\text{CARRS INDEX} - I = \left(\frac{D_f - D_b}{D_f} \right) \times 100$$

Where D_f = tapped density, D_b = Bulk density

Table 3: Physical characteristics of the Amrabeejadi lepa churna

Sl.no	Parameter	Amrabeeja churna	Haritaki chuirna	Amrabeejadi lepa churna Mean (n=5) ± SD
1	Angle of repose	50 ⁰	39 ⁰	47 ⁰ ± 0.02
2	Bulk density	0.43	0.58	0.51 ± 0.076 (g/cm ³)
3	Tapped density	0.52	0.83	0.59 ± 0.008 (g/cm ³)
4	Carrs index	17.31	30.12	13.56% ± 3.275
5	Haustners ratio	1.21	1.43	1.16 ± 0.042

Preliminary Phytochemical analysis^[6]

Preliminary qualitative phytochemical analysis of inorganic elements like calcium, magnesium, iron, phosphate etc. and organic elements like Carbohydrates, Flavonoids, Glycosides, Steroids, Alkaloids, tannins were done in alcoholic extract of Amrabeejadi lepa churna. Results showed below in tables.

Table -4:- Showing test for Inorganic components of Amrabeejadi lepa

Sl. No	Test	AB lepa
01	Test for Iron: a)Test soln. + potassium ferrocynide	Present
02	Test for Sodium: a)Test soln. + Potassium pyroanthlollate	Present
03	Test for Calcium: a) Test soln. + Ammonium carbonate	Absent
04	Test for Potassium: a)Test soln.+ Sodium Cobalt Nitrate	Absent
05	Test for Sulphate: a)Test soln.+ lead acetate	Absent
06	Test for Chlorides: a)Test soln.+ lead acetate	Present
07	Test for Magnesium : a)Test soln. +ammonium chloride	Absent
08	Test for Phosphate: a)Test soln. + ammonium molybdate	Present
09	Test for Carbonate: a)Ash + dil. Acid	Present
10	Test for Nitrates: a)Test soln.+ ferrous sulphate + sulphuric acid	Absent

Table 5: Phytochemical screening of Amrabeejadi lepa churna.

Sl	Constituents	Tests	Aqueous	Alcohol
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1	Carbohydrate	Molish's test	Absent	-
		Benedicts test	Present	Present
		Barfoeds test	Present	Present
2	Test for pentose sugar	Bail's orcinol test	Absent	Absent
		Selwinoffs test	Present	Present
3	Test for Hexose sugar			
4	Test for non-reducing polysaccharide	Iodine test	Absent	Absent
		Tannic acid	Absent	Absent
5	Test for mucilage	Swelling test	Present	Absent
6	Test for Proteins	Millons test	Present	Present
		Protein containing sulphur	Present	Present
7	Test for Amino acids	Ninhydrin test	Absent	Absent
		Test for Tryosine	Absent	Absent
		Test for cysteine	Absent	Absent
		FeCl ₃	Present	Present
8	Phenolic compounds and Tannins	Lead acetate Solution	Present	Present
		Acetic acid solution	Present	Present
		Dil. Iodine solution	Present	Present
		Dil. HNO ₃	Present	Present
9	Test for alkaloids	Dil. potassium permanganate	Present	Present
		Dragendroff's test Borntrager's test	Present Absent	Absent Absent
10	Antraquinone glycoside	Modified Borntrager's test	Absent	Absent
11	Saponin Glycosides	Foam test	Absent	Absent
12	Test for coumarin glycoside	Coumarin test	Present	Present
13	Test for steroids	Salkowski test	Present	Present

Microbial analysis ^[7]

Microbial analysis was carried out as per procedure of Indian pharmacopoeia 2011. It included the test of presence of pathogens like *Escherichia coli*, *Salmonella ebony*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Results showed below in table.

Table 6: Amrabeejadi lepa churna Microbial limit test

Sl. no	Organism	Limits as per IP	Results
1	<i>Escherichia coli</i>	Absent	Absent
2	<i>Staphylococcus aureus</i>	Absent	Absent
3	<i>Pseudomonas aeruginosa</i> ,	Absent	Absent
4	<i>Salmonella ebony</i>	Absent	Absent

Discussion

Amrabeejadi lepa churna is evaluated. The analysis parameters revealed that churna is yellow brown in color, bitter in taste and soft in touch, with characteristics odor of haritaki. (Table 1)

Physicochemical parameter of amrabeejadi lepa churna is Tabulated (Table 2). Deterioration of churna depends upon the amount of water present in raw material. If water content is high, drug can easily deteriorate due to fungus. The loss on drying at 105⁰ C in Amrabeejadi lepa churna is found to be 12.8%w/w. Total ash value indicate the amount of minerals and earthy material present in raw material. Analytical results show that the ash value of Amrabeejadi lepa churna is 2% w/w. The acid insoluble ash present in plant material is 1% w/w. The water soluble ash present in plant material is 0.03%w/w. Extractive values indicate the amount of chemical constituents of the drug. Water soluble extractive indicates its soluble material. Alcohol soluble extractive values indicate the presence of polar constituents. The physical characteristics (Table 3) show that the flow properties of the powder can be judged from angle of repose. The powder has its angle of repose value of 47⁰. Higher value of this angle is attributed to the stickiness property of Amra beej churna. The bulk density value obtained lie within the acceptable range. $BD = 0.51 \text{ gm/cm}^3$. Tapped density helps in calculating the percentage of compressibility of powder. $TD = 0.59 \text{ gm/cm}^3$. Compressibility index is the % of compressibility of powder blend and is determined by carrs index. As the value % is found to be 13.56%, it indicates good flowability. Hausner's ratio value should be less than 1.25; this value in Amrabeejadi lepa churna is 1.16, which indicates the good flow ability.

The results obtained from phytochemical screening reveals that the constituents like carbohydrates, Reducing sugar, mucilage, Amino

acids, Steroids, tannins Alkaloids etc. are present. (Table -5) Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms. Steroids have been reported to have antibacterial properties. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity. Phenolic and flavonoids compounds act as natural antioxidant. It is found that the Amrabeejadi lepa churna extracts inhibited growth of all bacteria confirming their antibacterial activity. Inorganic constituents of Amrabeejadi lepa churna are determined and presented. (Table-4) It showed presence of Sodium, Iron, Sulphate, Chloride, Carbonate and Nitrates. While Magnesium and Calcium are absent in it. Prepared Amrabeejadi lepa churna is devoid of all microorganisms. (Table-6)

Conclusion:

The good quality of this Polyherbal formulation has become possible by considering various scientific parameters concerning the Quality protocol. Organoleptic character and different densities of Amrabeejadi lepa churna are present in Standard range. Phyto-chemical analysis revealed the presence of tannins, alkaloids, carbohydrates and inorganic element like sodium, chloride etc. which indicates the presence of medicinally important constituents in the formulation. The formulation is free from any microbial load. Hence these values can be considered as the standard parameters of Amra beejadi Lepa churna.

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