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STANDARDIZATION OF YAKRIT PLIHANTAK CHURNA: AN AYURVEDIC POLYHERBAL FORMULATION

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ABSTRACT

Most of the traditional systems of medicine are effective but they lack of standardization. So there is need to develop standardization technique. Central Council for Research in Ayurveda has given preliminary guidelines for standardization the conventionally used formulations. 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 Yakrita Plihantak Churan a polyherbal Ayurvedic formulation used as hepatoprotective. Samples were collected from local market and were subjected standardized on the basis of organoleptic properties, physical characteristics, and physic-chemical evaluation. Microscopic characterization was compared with authentic ingredients as a reference. It was observed that commercial sample from market matched exactly with that of authentic standards after performing the standardization. The set parameters were found to be sufficient to evaluate the studied churn can be used as reference standard for the quality control/assurance purpose.

INTRODUCTION: Traditional system of medicine recommends various crude drugs for the treatment of hepatic disorder. The management of the liver disease is still challenge to modern medicine. No drug has been developed in modern system of medicine which may stimulate the liver function, protect it from damage or help in generation of hepatic cells. The only available drugs for the treatment for liver disorders are corticosteroids and immunosuppressive agents. But they are having many side effects.

Some of the important hepatoprotective polyherbal formulations are Liv-52, Livol, Arogyvarthini, Stimuliv, Terfroliv forte, Livit, Livomyn, and Adliv forte,[1] etc. Yakrit Plihantak Churna (Yakrit means liver and Phila means spleen) is an herbal mixture of rare herbs to improve liver function.

It helps the liver in clearing away the toxins, regernates the liver cells and prevents liver failure. It is also useful in liver cirrhosis, jaundice, liver damage due to alcohol, toxins and infection. The herb *Picrorhiza kurrao* increase the bile productin and exerction,where as *Solanum nigrum,Eclipta alba* and *Andrographis paniculata* improve liver function.

Boerrhavia diffusa and *Phyllanthus niruri* regenerate liver cells and useful in liver enlargement and inflammation. *Tephrosia purpurea* and *Cichorium intybus* are immunodialator and corrects the liver metabolism. The combination of all eight herbs is a wonderful remedy for all liver problems. It contains excellent herbs for gall bladder clearing and clear gall stones naturally.

Standardization of herbal formulations is an essential factor in order to assess the quality, purity, safety and efficacy of drugs based on the concentration of their active principles. It is very important to establish a system of standardization for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous.

Plant material when used in bulk quantity may vary in its chemical content and therefore, in its therapeutic effect very according to different batches of collection e.g. collection in different seasons and/or collection from sites with different environmental surroundings or geographical locations. The increasing demand of the population and the chronic shortage of authentic raw materials have made it incumbent, so there should be some sort of uniformity in the manufacture of herbal or Ayurvedic medicines so as to ensure quality control and quality assurance [2]. 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].

In the present research work, an attempt was made to standardize Yakrit Plihantak Churna a polyherbal formulation containing of eight herbs (**Table 1**).

TABLE 1: INGREDIENTS OF YAKRIT PLIHANTAK CHURNA

Botanical name	Common Name	English Name	Family	Quantity
Phyllanthus niruri	Bhumi Amla	Feather Foll	Euphorbiaceae	40 gm
Picrorhiza kurrao	Katuki	Picrorhiza	Scrophulariaceae	20 gm
Solanum indicum	Makoy	BlackNight Shade	Solanaceae	20 gm
Boerrhavia diffusa	Punarnava	Hogweed	Nyctaginaceae	20 gm
Andrographis paniculata	Kalmegh	King of Bitters	Acanthaceae	20 gm
Cichorium intybus	Kaasni	Chicory	Asteraceae	20 gm
Tephrosia purpurea	Sharpunkha	Purple Tephrosia	Fabaceae	20 gm
Eclipta alba	Bhringraj	Eclipta	Asteraceae	20 gm

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, microbial contamination and heavy metals analysis were carried out as per the WHO guide lines [3], Ayurvedic Pharmacopoeia [17] and Indian Pharmacopoeia [6]. Preliminary phytochemical tests were performed as per the standard methods.

Plant material: The samples were collected from physician and manufacturers of Gurukul Pharmacy, Pvt. Ltd. (Formulation code CC1) which is being used as a liver protective Ayurvedic drug [18]. For in house preparation, the ingredients were purchased from local raw material traders, which were authenticated by Pharmacopoeia Laboratory of Indian Medicine, Ghaziabad, India. (Formulation code CC2) and used as control. Polyherbal formulation consists of eight ingredients (Table 1).

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 analysis, preliminary phytochemical analysis, fluorescence analysis, Heavy metal analysis, microbial evaluation, 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 [4, 5]: The organoleptic characters 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 (**Table 2**).

TABLE 2: BOTANICAL PARAMETERS OF VARIOUS FORMULATIONS OF YAKRIT PLIHANTAK CHURNA

Organoleptic Characters	Formulation code	
	CC1	CC2
Color	Yellow Brown	Brown
Odor	Pungent	Pungent
Taste	Bitter	Bitter

Physico-chemical investigations [6, 7]: Physico-chemical investigations of formulations were carried out were the determination of extractive values and ash values (**Table 3**).

Determination of pH [8]: 1% solution of Polyherbal formulation was prepared in distilled water and pH was determined using pH meter Orion digital pH meter (**Table 3**).

TABLE 3: PHYSICAL AND CHEMICAL EVALUATION OF SAMPLES OF YAKRIT PLIHANTAK CHURNA

Parameters	Formulation code	
	CC1	CC2
Total ash value (%w/w)	11.987 ± 0.19	13.483 ± 0.07
Acid insoluble ash value (%w/w)	0.9 40 ± 0.01	0.967 ± 0.03
Water soluble ash value (%w/w)	1.982 ± 0.26	1.340 ± 0.21
Alcohol soluble extractive value (%w/w)	25.539 ± 0.170	24.703 ± 0.080
Water soluble extractive value (%w/w)	24.510 ± 0.100	24.733 ± 0.154
Methanol soluble extractive value (%w/w)	15.21±0.12	14.3±0.35
Chloroform soluble extractive value (%w/w)	9.05±0.45	10.19±0.26
Ethyl acetate soluble extractive value (%w/w)	19.08±0.28	18.58±0.65
Petroleum ether soluble extractive value (%w/w)	11.08±0.58	11.84±0.23
Loss on drying at 105^0(%w/w)	6.867 ± 0.06	6.667 ± 0.06
pH 1 % solution (%w/v)	5.85 ± 0.03	5.77 ± 0.03
pH 10% Solution (%w/v)	5.3 7 ± 0.06	4.83 ± 0.06
Crude fiber (gm)	0.27 ± 0.08	0.13 ± 0.04
Foreign organic matter	Nil	Nil

Note: Values are expressed as Mean ± SEM (n = 3)

Fluorescence analysis [4]: Fluorescence characters of powdered plant material with different chemical reagents were determined under ordinary and ultraviolet blight. 1mg 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 (**Table 4**).

TABLE 4: FLUORESCENCE ANALYSIS OF SAMPLES OF YAKRIT PLIHANTAK CHURN

Parameters	Formulation code			
	CC1	CC1	CC2	CC2
Powdered drug	Visible/day light	Ultraviolet light	Visible/day light	Ultra violet light
Powder as such	Dark brown	Dark brown	Dark brown	Dark brown
Powder + $FeCl_3$	Dark grey	Greyish yellow	Dark grey	Dark grey
Powder + conc. HCl	Orange yellow	Greyish yellow	Orange yellow	Greyish yellow
Powder + 10% HNO_3	Orange	Yellow	Orange	Yellow
Powder + 10% $K_2Cr_2O_7$	Yellow	Green	Yellow	Green
Powder + 1 M NaOH	Brownish yellow	Green	Brownish yellow	Green
Powder + $AgNO_3$	Buff brown	Light brown	Buff brown	Light brown
Powder + conc. HNO_3	Orange yellow	Fluorescent yellow	Orange yellow	Yellow
Powder + conc. H_2SO_4	Orange	Fluorescent green	Orange	Fluorescent green
Powder + Br_2 water	Brown	Light brown	Brown	Brown
Powder + 5% H_2O_2	Brown	Greyish green	Brown	Green
Powder + CCl_4	Brown	Dark brown	Brown	Brown
Powder + Methanol	Blackish brown	Greenish yellow	Blackish brown	Greenish yellow
Powder+CH_3COOH	Orange brown	Dark green	Orange brown	Dark green
Powder + Xylene	Grey	Orange green	Dark green	Orange green
Powder + NH_3	Yellowish brown	Yellowish green	Yellowish brown	Yellowish green
Powder + I_2	Blackish brown	Grey	Blackish brown	Grey

Preliminary Phytochemical Analysis [9]: Preliminary qualitative phytochemical analysis of all the extracts was carried out on methanolic extract by employing standard conventional protocols (**Table 5**).

TABLE 5: PHYTOCHEMICAL SCREENING OF YAKRIT PLIHANTAK CHURNA

Parameters	Formulation code	
	CC1	CC2
Alkaloids	–	–
Glycosides	+	+
Tannins	+	+
Flavonoid	+	+
Steroids	–	–
Carbohydrates	+	+

Note: + indicates presence – indicates absence

Determination of Moisture Content and Swelling Factor [10]: Moisture content was determined by loss on drying (LOD) method. 5gm of the weighted 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 (Table 3).

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.

Determination of Physical Characteristics of Powder Formulation [11, 12]: Physical characteristics like bulk density, tap density, Hausner's ratio, and Carr's index were determined for different formulations 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. 100 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.

Hausner's ratio is related to inter particle 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 (**Table 6**).

TABLE 6: PHYSICAL CHARACTERSTICS OF SAMPLES OF YAKRIT PLIHANTAK CHURNA

Parameters	Formulation code	
	CC1	CC2
True density (1% soln)	0.564 ± 0.010	0.946 ± 0.010
Bulk density(gm/ml)	0.392 ± 0.018	0.488 ± 0.019
Porosity %	88.1 ± 0.012	91.0 ± 0.026
Angle of Repose	42° ± 0.24	41° ± 0.31
Viscosity (1% soln)	1.02cp	1.05cp
Surface tension(1% soln)	49.5	50.2
Fluff density(gm/ml)	0.392 ± 0.010	0.488 ± 0.014
Tapped density(gm/ml)	0.587 ± 0.007	0.647 ± 0.001
Hausner's ratio	1.371 ± 0.005	1.328 ± 0.006
Carr's compressibility index	19.25	19.95

Determination of Crude Fat [10]: 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, the residual petroleum ether was filtered and filtrate was evaporated in a pre weighed beaker. Increase in weight of beaker gave the crude fat.

Heavy Metal Analysis [11]: (**Table 7**)

Preparation of Samples by Acid Digestion Method: Accurately weighed 2 g of sample was taken in Kjeldahl flask. Acid mixture of HNO_3: $HClO_4$ (4:1) was added in the flask and heated continuously till the solution is colorless. The sample was then transferred in a 25 ml volumetric flask and the volume was made-up with distilled water.

Reagent blank was synchronously prepared according to the above procedure. The standards of Lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) were prepared as per the protocol in the manual and the calibration curve was developed for each of them.

Detection: Then samples were analyzed for the presence of Pb, Cd, As and Hg using Atomic Absorbance Spectrophotometer (AAS) 6300 (by SHIMADZU).

TABLE 7: HEAVY METAL ANALYSIS OF MARKETED AND IN-HOUSE FORMULATIONS OF YAKRIT PLIHANTAK CHURNA

Heavy Metals	Formulation code	
	CC1	CC2
Arsenic	–	–
Lead	–	–
Mercury	–	–
Copper	–	–

Microbial Analysis [6]: Microbial analysis was carried for determination of microbial contamination as per procedures of Indian pharmacopoeia [6] 2010 and WHO Guideline [3]. The test included total bacterial count, total yeast and mould count, Identification of specified organisms such as *Escherichia coli, Salmonella sp., Staphylococcus aureus* and *Pseudomonas aeruginosa* (**Table 8**).

TABLE 8: SCREENING FOR MICRO-ORGANISMS IN MARKETED AND IN-HOUSE FORMULATIONS OF YAKRIT PLIHANTAK CHURNA

Parameters	Formulation Code	
	CC1	CC2
E.coli	–	–
Salmonella typhi	–	–
Pseudomonas aeruginosa	–	–
Staphylococcus aureus	–	–
Total bacterial count	Less than 1000cfu/gm	Less than 1000cfu/gm
Total yeast and mould count	Less than 100cfu/gm	Less than 100cfu/gm

Quantitative Estimation of Tannins [13]: 1 gm of powdered drug was refluxed in 100 ml of 70% aqueous acetone for 2 hours followed by filtration. The filtrate was concentrated and partitioned with solvent ether (3 times) and then with n- butanol previously saturated with water (**table 9**). The n- butanol soluble portion was dried over water bath until constant weight. Total tannin content was calculated by formula:

% w/w total tannin content=

$$\frac{\text{Weight of n- butanol fraction in gm X 100}}{\text{Weight of sample in gm}}$$

Quantitative estimation of Flavonoids [13]: 1gm of powdered drug was boiled in 100 ml methanol for 1 hour followed by filtration. 1ml of filtrate was placed in 10ml volumetric flask. 3ml methanol and 0.3 ml $NaNO_2$ were added in the flask. 3ml of $AlCl_3$ was added after 5min. 2ml of 1M NaOH was added and the net volume was made to 10 ml with methanol and absorbance was measured against a blank at 510nm (**table 9**).

The total flavonoids content was calculated using following equation.

A = 0.01069c - 0.001163

A = absorbance, c = flavonoid content µg/g.

TABLE 9: QUANTITATIVE ESTIMATION OF TANNINS AND FLAVONOIDS IN YAKRIT PLIHANTAK CHURNA

Parameters	Formulation code	
	CC1	CC2
Total Flavonoid content (µg/g)	5.2 ± 0.2	5.5± 0.1
Total Tannin content (%w/w)	5.1 ± 0.1	6.3 ± 0.4

RESULTS AND CONCLUSION: As a part of standardization procedure, both the samples (Formulation code CC1 and CC2) were tested for relevant physical and chemical parameters, and also subjected to microbial screening through quality control measures. Botanical parameters revealed that dark brown in color, with a pungent odor, bitter taste, and fine texture (Table 2).

Results of quantitative analysis for Total ash, Acid insoluble ash, Water soluble ash, Alcohol soluble extractives, Water soluble extractive ,Hexane soluble extractive, Chloroform soluble extractive, Ethyl acetate soluble extractive, Particle size (100 mesh), Loss on drying at 105º C ,pH (1% and 10% 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. Percent weight loss on drying or moisture content was found to be 6.8% w/w. The less value of moisture content could prevent bacterial, fungal or yeast growth.

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. 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.

Phytochemical analysis revealed the presence of glycosides, carbohydrates, saponins, tannins, and flavonoids (Table 5 and 9). 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, Hausner's ratio, were determined and results were tabulated (Table 6).

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. Various microorganisms like *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* contaminate herbal drugs and cause serious health hazards [14].

For detection of such microorganisms, colonies obtained on specific media were subjected to suitable microbial tests along with pure strains to detect their presence or absence. The results obtained (Table 8) revealed the absence of these microorganisms thereby confirming the non toxic nature of the formulations.

Heavy metals may be present in crude drugs through atmospheric pollution and through the soil. Moreover minerals and metals are also used in preparing Ayurvedic formulations. However, heavy metals have been associated with various adverse effects [15] including status epilepticus, fatal infant encephalopathy, hepatotoxicity, congenital paralysis and deafness, and developmental delay. Many case studies have reported serious adverse conditions due to heavy metals in Ayurvedic and other herbal drugs [16].

Hence, heavy metals need to be detected in such preparations. In this study, all the samples tested negative for the presence of heavy metals (Table 7), thereby further confirming the non toxic nature of the preparation. Hence, Yakrit Plihantak Churna is a safe polyherbal formulation and is free from any toxic materials. The results obtained in this study may be considered as tools for assistance to the regulatory authorities, scientific organization and manufacturers for developing standards.

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