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PHARMACEUTICAL STANDARDIZATION AND CHARACTERIZATION OF AYURVEDIC HERBOMINERAL COMPOUND TRUSHANADI LOHA

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ABSTRACT
This article describes the detailed preparation of Lohabhasma (iron oxide in cinerated iron) and Trushanadi Loha. The metal concentrations present in this compound evaluated by using wavelength dispersive X-ray flourosence (WD-XRF) Spectometry. A total of 19 elements, Na, Mg, Al, Si, P, S, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, As, Cl, Sr and bromine from medicine were characterized. Out of 19 elements listed which are considered to be essential to the life system. 9 are macro-nutrients (Na,,Mg,Al,Si,P,S,K,Ca,Ti) and 5 are defined as micro-nutrients (Mn, Fe, Cu, Zn, Ni). The method was measured by Fast-Vac 34 .The sample was examined by inductively coupled plasma mass-spectrometer (ICP-MS) to estimate the heavy metals and observed that the heavy metals like lead, and Cd were almost within WHO permissible limits. The Compound was analysed for organoleptic tests. The pH of compound was 2.95 and acidic in nature. The compound was negative for carbohydrates and starch & positive to tests of aikaloids, steroids, proteins, Glycosides, tannins, phynolics and Flavonides determined by phytochemical screening. The presence of bioactive compounds (after phytochemical tests) in the drug has been linked to their activities against disease causing microorganisms and also offering the plants to protect themselves against infections by pathogenic microorganisms.
Keywords: Loha bhasma, iron oxide, Trushanadi Loha, phytochemical screening

INTRODUCTION

The pharmaceutical science is closely related with life science. Pharmaceutics is the science of drugs, their discovery, and uses the general aspects of the how and why the drug. It stands in the central place in Bio-medical sciences, with no distinction from biochemistry, physiology, pathology and microbiology. Knowledge of pharmaceutics is an essential element in medical practice and is the basis for the discovery of new medicines. Before going through preparations of any drug, one has to concentrate on all the matters related to that particular drug like, collection of raw drugs, tests about genuineness of it, different process of prevention and purification, special methods of mixing and so on. Thus pharmaceutical standardization of Ayurvedic medicines is necessary to ensure quality, safety and efficacy of drugs. The pharmaceutical science is closely related with life science.

Pharmaceutical standardization of Trushanadiloha

Preparation of lohabhasma

The preparation of the Loha Bhasma (iron oxide) had three stages viz. 1) collection of raw material, 2) Shodana (purification of iron) and 3) Marana (incineration). Mild steel turnings were collected from chemical shops, Hyderabad. Muffle furnace was used for incineration. Both general and specific purifications were carried out. Subsequently Bhanupaka (sun cooking), Sthalipaka (pot cooking) and incineration in muffle furnace were carried out. Seasame oil, buttermilk, cow's urine, sour gruel and decoction of horse gram were used to quench the iron heated up to 750 to 850^0 C. The heating and dipping in each liquid was done seven times. The observations are as follows:[1]

Sl.No.	Liquid	Change in iron	Change in liquid
1	Sesame oil	Colour became blackish. Brittleness not altered	On quenching caught fire. Color turned blackish. Became more thick.
2	Buttermilk	Started breaking into pieces	Consistency disturbed. Color slight blackish
3	Cow's urine	Further reduction in size	Ammoniac smell emerged
4	Sour gruel	Further reduction in size	Typical smell. Turned purple in color.
5	Decoction of horse gram	Further reduction in size	Slight blackish discoloration.

Iron obtained after general purification was subjected to special purification by heating and quenching in the decoction of the three myrobalans for seven times[2]. The purified iron was subjected to Bhanupaka (sun cooking) with the decoction of the three myrobalans. Sun cooked iron was subjected to Sthalipaka (Pot cooking) with the decoction of the three myrobalans.

For Putapaaka (incineration) of iron, the product of pot cooking was wet ground for three hours in an end runner and the obtained thick paste was made into pellets and dried in sun. These pellets were incinerated in a muffle furnace. This incineration process was repeated for 20 times. The bhasma (ash) was examined after incineration. The final ash floated on water (varritara), filled in the finger grooves (rekhaa-poorna), was stable (not heat labile – apunarbhava) and was purplish red in color (pakva-jambo-nibha).

Section II

For the preparation of Trushanaadi Loha, all the herbs were taken from Davasaz (herbal shops) located at Begumbazar, Hyderabad. The purified and incinerated sample of iron was from the procedures detailed above.

Sl. No.	Name of the Drug	Latin Name	Parts Used
1	Shunti	*Zingiber officinalis* - Roxb	Rizome
2	Pippali	*Piper langum* linn	Roots,Dried spices
3	Pippalimoola	*Piper langum* root	Root
4	Maricham	*Piper nigrum* linn	Fruits
5	Hareethaki	*Terminalia chebula* ret Z	Fruit coat
6	Amlaki	*Emblica officinalis* gaertn	Fruit coat
7	Vibhithaki	*Terminalia bela rica* roeb	Fruit coat
8	Chitramoola	*Plumbago zeylanica* linn	Root bark
9	Backuchi	*Psoralia carlifolia* linn	Seeds(Purified)
10	Saindava lavana	Rock salt	-
11	Souvarcha lavana	-	-
12	Bida lavana	-	-
13	Kacha lavana	-	-
14	Loha bhasma	Purified iron catalyst(ash)	-

Preparation of trushanaadiloha

The drugs 1,3,4,5,6,7,8,9,10,12,13 mentioned above are powdered and kept separately, No.2 i.e Pippali are fried in cow's ghee then powdered, No.9 i.e. Bakuchi seeds purification was done in cow's urine for above 7 days, by doing bhanana. After 7 days bhanana, it was dried and powdered. No.14 i.e. Loha (Iron) was taken in the

form of pure bhasma (ash/catalyst) and all 14 ingredients in equal quantity are mixed together thoroughly and kept in a glass container[4]. After preparation the blended powder was filled in bottles sealed and labeled. This packed sample was used for further studies.

Analytical study of trushanadiloha

Organoleptic Tests

Texture: Fine powder
Odour: Aromatic
Colour: Reddish Brown
Taste: Astringent & salty

Physicochemical Analysis[3,4,5]

pH: 2.95
Moisture content: 7.34%
Total ash: 41.5%
Water Soluble matter: 53%
Alcohol soluble matter: 25%
Acid in soluble ash: 13.5%
Inorganic/Organic contents: Total Chloride estimation 2.8% (In terms of NaCl)

Phytochemical Screening

The sample was negative for carbohydrates, starch and positive for the tests of alkaloids, steroids, proteins, glycosides, tannins-phenolics and for flavonoid. [5,6,7]

The TLC of sample was done by using solvent system – Toluene : Ethyl acetate and spraying reagent vanillin and sulphuric acid and detected 3 spots with RF values of 0.22 (purple), 0.15 (yellow), 0.18 (Brown). Monograph is not available to Trushanadi Loha in volumes of Ayurvedic Pharmacopeia of India and in Ayurvedic Formulary of India Vol.I and II for reference of analysis till date.

Inductively coupled plasma mass - Spectrometer (ICP-MS)

The sample was examined by ICP-MS to estimate the heavy metals. After examination the observations were shown in table No:3 by correlating with permissible limit of heavy metals in the dietary contents as per W.H.O.

EXPERIMENTAL AND INSTRUMENTAL DETAILS

One gram of each original sample was taken in an aluminium cup and pressed into a pellet using a hydraulic press (HERZOG,type:TP40\2D) At 15 tons to obtain pellet of moderate thickness. Samples were characterized by using WD-XRF spectrometer (Bruker S4 Pioneer), equipped with a 4 KW, Rh anode x-ray tube with six analyzer crystals [Lif (220), PET,OVO-55, OVO-N, OVO-C and OVO-B]. It has sealed proportional counter for lighter elements and a scintillation counter for heavy element detection. X-ray exposure time and power conditions were adjusted for each element by a pre-calibrated program[15].The method as measured by Fast-Vac.34**.**

RESULTS AND DISCUSSION

Table-1 shows the concentration of various elements determined in Trushanadi Loha by WD-XRF. Out of 19 elements listed, which are considered to be essential to the life systems[8,9], 9 are macro-nutrients (Na, Mg, Al, Si, P, S, K, Ca, & Ti) and 5 are defined as micro-nutrients (Mn, Fe, Cu, Zn & Ni). The concentrations of four heavy elements As, Cd, Pb, & Hg in the drug are shown in above mentioned table-2. Ca and Na which play a critical role in basic biological processes and are required in larger quantities, and, therefore, are suitably recognised as macro-nutrients. The Ca which is present in the sample is in the form of portlandite [$Ca(OH)_2$. Manganese functions as an enzyme activator and is a constituent of several metalloenzymes[10].It is up to o.595% in the drug. Fe is an essential trace element and necessary for proper functioning of the human body and present in the form of hematite (Fe2O3).

Arsenic, one of the four heavy metals is present in the sample is currently used as a drug in the form of arsenic trioxide to treat acute leukemia[11]. The concentration of it in sample is 0.005% by WD-XRF and 14.05 PPM determined by ICP-MS. Mercury is a toxic element, it's concentration in sample is 3.07 PPM, as determined by ICP-MS.

The compound was prepared and analysed after General and Special Shodana (means purification of minerals, metals and poisonous herbal drugs in order to remove inherent impurities and poisonous effects) and Marana (incineration) according to the textual reference. So, the heavy metals like Hg, As (which are just above the permissible limits as per W.H.O, shown in Table No:3) present in the compound mayn't produce any adverse events or toxic symptoms.

The trace elements-Cu and Zn which occur in body tissues and fluids have some essential activities[12]. The concentration of zinc is 0.0133% and Cu is 0.0424%. Chromium works with insulin in the metabolism of sugar to stabilize blood sugar levels and also cleans the arteries by reducing the cholesterol and triglyceride levels[10]. The concentration of Cr determined in the sample is 0.0304%.

Lead is one of the heavy and toxic metals that have known to biological functions[13]. The lead absorption increases in case of protein and iron deficiency[14]. The concentration of lead in the sample is 3.78 PPM determined by ICP-MS.

The naturally occurring Sr (strontium) in the form of Sr detected in the sample rebuilds bone lost due to osteoporosis and osteopenia. A recent *in-vitro* study conducted by the NY Collage of Dental Sciences using Sr on osteoblasts showed marked improvement on bone building osteoblasts. The result is stronger bones. (Ref: The effect of Sr citrate on osteoblast prolifelation and differentiation" Retrieved 2009-07-07).

CONCLUSION

Herbomineral or mineral preparations revolutionized the entire medical system since medieval period. The adoption of modern knowledge in terms of any analytical studies facilitates acceptability of the system to a greater extent. Basing on the observations recorded during the course of study of trushanadiloha in pharmaceutical and analytical aspects the following conclusions are drawn:

For general and special purification of iron the procedure mentioned in Sarangadhara Samhitha Madhyama Kanda 11/2-2^1/2 and Rasa Ratna Samuchaya 5/13 and 5/103 may be considered as standard procedures. Heating at 750-850^0 C during the process was effective.

The process of incineration is to be repeated 20 times. The Lohabhasma was examined by ICP-MS and proved the absence of free iron in the ash and the WD-XRF spectrometry proved the bhasma (ash) is in the form of hematite (Fe_2O_3).

The presence of bioactive compounds (after phytochemical tests) in the drug has been linked to their activities against disease causing microorganisms and also offering the plants to protect themselves against infections by pathogenic microorganisms. The 19 elements present in the sample which are confirmed by WD-XRF spectrometry are considered to be essential to the life systems. The heavy metals detected in the sample are within in the permissible limits as per the WHO which is evaluated by using ICP-MS.

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Table-1: Characterization of Trushnadiloha using WD-XRF Spectrometry

Table1: percentage concentration of the elements in the drug sample																		
Na	Mg	Al	Si	P	s	K	Ca	Ti	Cr	Mn	Fe	Ni	Cu	Zn	As	Cl	Br	Sr
10.46	0.595	0.346	1.55	0.436	1.830	4.673	1.853	0.0436	0.0304	0.0615	14.04	0.0129	0.0424	0.0133	0.005	15.42	0.0131	0.0099

T A B L E 2: Result of determination of heavy elements in Trushanadiloha by ICPS-MS

Constituent	Protocol	Value
ARSENIC	AOAC 18th EDN: 2006 by ICPMS	14.05 ppm
CADMIUM	AOAC 18th EDN: 2006 by ICPMS	0.03 ppm
LEAD	AOAC 18th EDN: 2006 by ICPMS	3.78 ppm
MERCURY	AOAC 18th EDN: 2006 by ICPMS	3.07ppm

Name of the heavy metal	Protocol	Result	WHO permissible limits
Lead	AOAC 18th EDTN : 2006 by ICPMS	3.78 PPM	10 PPM
Arsenic	AOAC 18th EDTN : 2006 by ICPMS	14.05 PPM	10 PPM
Mercury	AOAC 18th EDTN : 2006 by ICPMS	3.07 PPM	1 PPM
Cadmium	AOAC 18th EDTN : 2006 by ICPMS	0.03 PPM	0.3 PPM

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