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QUALITY CONTROL OF *VARUNA KWATHA* CHURNA, AN AURVEDIC FORMULATION AND ITS COMPARATIVE STUDY WITH MARKETED FORMULATIONS

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ABSTRACT

Pharmacognostic standardization of the *Varuna Kwatha* Churna an ayurvedic formulation formulated from various medicinal plants as bark of varuna, pashanbheda rhizome, fruit of gokshura and rhizome of sunthi which is used to treat urolithiasis. The varuna kwatha churna manufactured by the formula in the ratio as specified in the ayurvedic formulary of India given in specified quantities as cited in the Cakradatta, it has been coarsely powdered and passed through sieve, weighed, was carried out to determine its macro- and microscopical characters and also some of its quantitative standards. Various standardization parameters such as physicochemical standards, chemo profiles as preliminary analysis, TLC fingerprint profiles and safety evaluation as microbial contamination, heavy metal determination were also evaluated with the market formulations. These findings will be useful towards establishing pharmacognostic standards on identification, purity, quality and classification of the plant, which is gaining relevance in plant drug research.

INTRODUCTION: Plants and plant-derived products are part of health care system since ancient human civilizations. There are different types of ayurvedic preparations, such as asava, arishta, ghruta, taila, churna, kwatha. Thus, the churna is a fine powder of well dried drug or drugs described in ancient literature. The kwatha churna, type of churna is the combination of drugs made into coarse powder, kept for preparation of kasaya made with the ingredients in the formulation. The powder completely passed through sieve no. 85. It was found in yellow to brown in color with characteristic odor and taste, bitter as well as texture was like coarse powder.

Outer cork composed of thin walled, tangentially elongated cork cells, middle layer with parenchymatous cortex with a number of starch grains, stone cells vary consisting of sieve tubes, companion cells, parenchyma, alternating with medullary rays, few rhomboidal crystals of calcium oxalate also found in this region (Varun); cork found as outer layers of slightly compressed, brown colored cells, inner zone multilayered, thin-walled tangentially elongated and colorless cells, some cells contain rosette crystals of calcium oxalate and simple starch grains, vascular bundles, arranged in a ring and xylem consist of fibers, trachieds, vessels (Pashanbheda); the five wedge-shaped coccus lined by single layered epidermis, the cells protrude outwards to form long, unicellular trichome with zone of sclerenchymatous cells which in turn encloses a cavity for seed contains prismatic crystals (Gokshur); broad or reticulated vessel debris, long non-lignified fibers, starch grains large, upto 50 u, oval hilum were observed (Sunthi). Microscopic studies vary depending on the morphological groups to be incorporated in the

formulation examined as it has been combined powdered bark of varuna, pashanbheda rhizome, fruit of gokshura and rhizome of sunthi.

Thus, *Varuna kwatha* churna has been examined using these parameters and results were shown in experimental work.

It contains chemical constituents as glucosinolates, plant sterols including lupeol; saponins; alkaloids as cadabicine; tannins as (-) Epiafzelechin, (-) Epiafzelechin-5-β-D glucoside, Catechin; triterpenes as Diosgenin, β-Sitosterol, Lupeol, Varunol, Lupenone; flavonoids as Rutin, Quercetin, Isoquercetin, glucocappain and also contains bergenin, gallic acid, mucilage, wax, albumin and starch, catechin, gingerols.

The herbal formulation can be useful in urinary calculi as it removes the kidney stones. Demulcent, stomachic, laxative, diuretic, antipyretic, alternative, tonic, useful in calculus affections i.e. antiurolithiatic [1], disorders of urinary organs and used in snake bite, rubifacient. It shows a potent immunomodulatory [2] effect, astringent, cardiotonic, wound healer, antihelmentic, expectorant and anti-inflammatory. They are useful in renal and vesical calculi, helminthiasis, anaemia, scabies, ophthalmia and general weakness, digestive, carminative, aromatic and used widely for indigestion, malaria. It is said to be used for morning sickness, nausea, rheumatism, sore throat and vomiting.

The formulation was stored in well airtight container in dry and cool place [3]. Pharmacognostic studies have not been reported for the formulations of *Varuna kwatha* churna. Therefore the main aim of the present investigation is to study the standardization parameters such as physicochemical standards, chemo profiles as preliminary analysis, TLC fingerprint profiles and safety evaluation as microbial contamination, heavy metal determination were also evaluated with the market formulations of *Varuna kwatha* churna which could be used to prepare a monograph for the proper identification of the plant.

MATERIALS AND METHODS:

Collection and Authentication: The market formulations were collected from the town of Lukhnow and Nagpur district, India. The plant species for the proposed study was identified and authenticated by senior scientist, MGIRI, Wardha-442001.

Formulation: The *Varuna Kwatha* Churna an ayurvedic formulation formulated from various medicinal plants as bark of varuna, pashanbheda rhizome, fruit of gokshura and rhizome of sunthi. Then, it has been coarsely powdered and passed through sieve no.85, weighed. The *Varuna kwatha* churna manufactured by the formula in the ratio as specified in the ayurvedic formulary of India [3] given in specified quantities as cited in the Cakradatta, ed. Jagadishvar Prasad, Varanasi: Chawkhamba Sanskrit Series Office, 1961 [4] given in the **table 1**.

TABLE 1: COMPOSITION OF FORMULATION OF DRUG

Name of Plant	Latin names	Part Used	Composition in parts
Varun	*Crataeva nurvala*	Brk.	1
Pashanbheda	*Bergenia ligulata*	Rz.	1
Sunthi	*Zingiber officinale*	Rz.	1
Gokshura	*Tribulus terrestris*	Fr.	1

Pharmacognostic Standardization: Morphological studies were done by using simple microscope. The shape, size, color, odor, taste were determined. Microscopic studies were done by warming a few mg with chloral hydrate, washed mounted in glycerin; few mg treated with iodine solution and mounted in glycerin; few mg heated in 2 per cent aqueous potassium hydroxide, washed in water and mounted in glycerin. Powders of the dried parts were used for the observation of powder microscopical characters [5-11].

Physico-Chemical Evaluations: Foreign matter, total ash, acid-insoluble ash, swelling and foaming index, assay to determine tannin content, successive extractive values were determined. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol soluble components. The moisture content was also been determined [5-11].

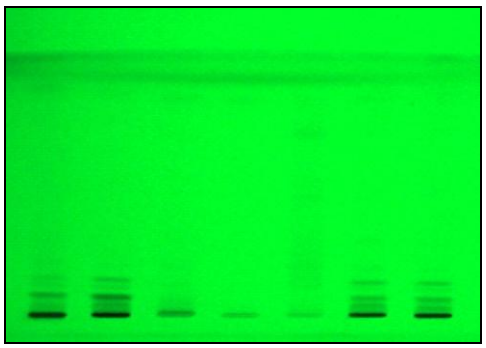
Chemo profiles: Preliminary phytochemical analysis gives the information about phytoconstituents present in the crude drug (**table 2**) [12-15].

TABLE 2: PHYTOCHEMICAL STUDIES OF VARIOUS EXTRACTS

Plant constituents	Identification test	n-Hexane extract	Chloroform extract	Alcoholic extract	Acetone extract	Aqueous extract
Alkaloids	Mayer's test	+	+	-	+	-
	Hager's test	+	+	-	+	-
	Dragendroff's test	+	+	-	+	-
Carbohydrates	Wagner's test	+	+	-	+	-
	Molisch test	-	-	+	-	+
	Fehling's Test	-	-	+	-	+
Glycosides	Borntrager's test	-	-	+	-	+
	Legal's test	-	-	+	-	+
Phenolic compound	$Fecl_3$ test	-	-	+	-	+
Tannins	Lead acetate test	-	-	+	-	+
	$Fecl_3$ test	-	-	+	-	+
	Alkaline reagent test	-	-	+	-	+
	Lead acetate test	-	-	+	-	+
Protein and amino acids	Millon's test	-	-	+	-	+
	Ninhydrin test	-	-	+	-	+
	Biuret test	-	-	+	-	+
Saponins	Foam test	-	-	+	+	+
Sterols	Liebermann Burchard test	+	+	-	+	-
Fixed oils, fats	Spot test	+	+	-	+	-
Flavonoids	Shinoda's test	-	-	+	-	+
	Alkaline reagent test	-	-	+	-	+

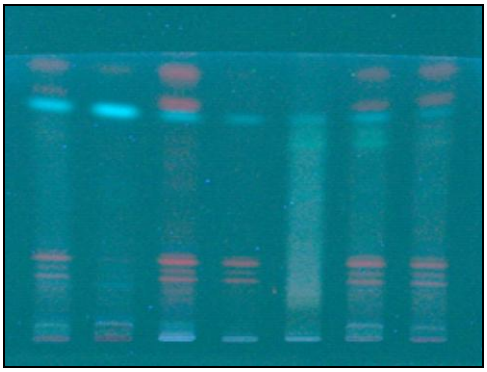
+ : Test positive; – : Test negative

Fluorescence study of powdered drug shows the color change due to varied chemical constituents at different wavelength. Along with this powdered study, the comparison with the marketed samples can be made on the basis of the TLC profile (**figure 1-4**) which shows different bands on various wavelengths and TLC fingerprint profiles carried out by preparing the extract with 50ml methanol and 2.5g powdered drug, filtered with whatman filter paper. TLC of this alcoholic extract on Silica gel "G" plate using Toluene: Methanol: Ethyl acetate (6:1:1) shows spots; on spraying with methanolic sulphuric acid reagent and heating the plate for ten minutes at 105^{o}C were determined.



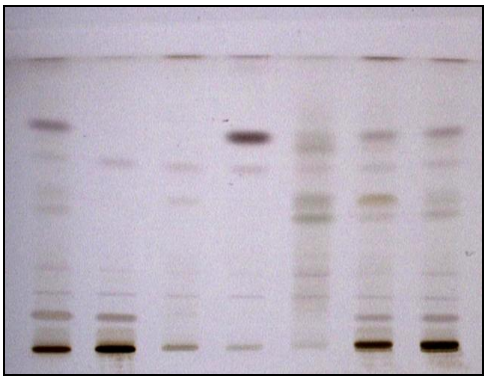
S: 1 2 3 4 5 6 7

FIG. 1: 254 UNDERIVE



S: 1 2 3 4 5 6 7

FIG. 2: 366 UNDERIVE



S: 1 2 3 4 5 6 7

FIG. 3: WHITE R 366 UNDERIVE

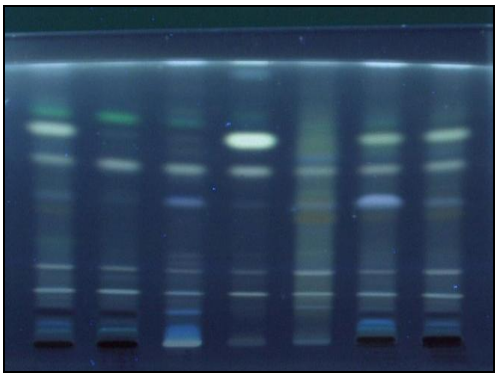


FIG. 4: 366 UNDERIVE

S: Sample; 1: *Varuna kwatha* churna; prepared drug, 2: Bergenia, 3: Gokshur, 4: Varun, 5: Zingiber, 6: Market drug (A), 7: Market drug (B)

Safety evaluation: The drug was evaluated for the various safety [16] and toxicological parameter like microbial content and metal determination. The various tests for microbial contamination [17-19] had been performed such as, *E. coli, salmonella, staphylococcus aureus* etc. may contaminate the herbal drugs and cause serious health hazard. In literature information is available regarding the presence of metals in herbal drugs. If these are present beyond the certain limit may causes toxic effects. Quantitative determinations of heavy metals [20] such as lead, cadmium were carried out.

RESULT AND DISCUSSION: The powder of *Varuna kwatha* churna were observed to be light yellow to brown in color having specific odor and taste. In microscopic studies of powder shows the presence of epidermis, starch grains, stone cells, cork cells trachieds, trichomes, cortex, phloem, endodermis. The study revealed the presence of fibers, vessels, xylem fibers, trichomes and parenchyma along with crystals.

The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign matter such as metallic salts or silica. The moisture content of the drug is not too high, thus it could discourage bacterial, fungi or yeast growth, as the general requirement for moisture content in crude drug is not more than 11.5% w/w. The ash values, extractive values and moisture content of leaves were determined. As varuna bark, gokshur, sunthi shows negligible foam and tannins, so foaming index and tannin content could not be determined. As bergenia, gokshur, sunthi shows negligible foam, so swelling index could not be determined.

Phytochemical analysis gives the information about phytoconstituents present in the crude drug (table 2). Along with this powdered study, the comparison with the marketed samples (**table 5, 6**) can be made on the basis of the TLC profiles (figure 1-4) which shows different bands on various wavelengths. The safety evaluations carried out the various tests were microbial load and heavy metals detection. The study shows that lead and cadmium presents within the permissible limits as per WHO. The herbal drug formulations passed all tests and it revealed that the herbal drug formulations were safe for human consumption as like marketed drug. The results are depicted in **Table 3**.

Since the *Varuna kwatha* churna is useful in traditional medicine for the treatment of urolithiasis, it is important to standardize it for use as a drug.

TABLE 3: MONOGRAPH OF VARUNA KWATHA CHURNA; PREPARED DRUG

Name of Product	***Varun kwatha* churna**	
Category	Antiurolithiatic powder	
Ingredients	Varun Pashanbheda Gokshur Sunthi	
PHYSICO-CHEMICAL SPECIFICATIONS		
PARAMETER	**LIMIT**	**PROTOCOL**
Appearance	Coarse powder	Visual inspection
Color	Yellow to brown	Organoleptic evaluation
Taste	Characteristic	
Foreign matter	-	In House Specification

Powder microscopy	Parenchymatous cortex cork cells in surface, stone cells, trachied, starch grains, non-glandular trichomes, vascular bundles, vessels.	
Loss on drying	Not more than 11.5 per cent	
Total ash	Not more than 7.11 per cent	
Acid insoluble ash	Not more than 0.93 per cent	
Water soluble extractive	Not less than 13.5 per cent	
Alcohol soluble extractive	Not less than 10.12 per cent	
Swelling index	Not less than 0.13 per cent	
Foaming index	Not less than 0.12 per cent	
Assay	Tannin content 15.56 per cent	
TLC	TLC of alcoholic extract on Silica gel "G" plate using Toluene: Methanol: Ethyl acetate (6:1:1) shows spots; on spraying with methanolic sulphuric acid reagent and heating the plate for ten minutes at 105^0C.	
Extractive values	Not less than 6.97 per cent	
n-Hexane	Not less than 6.4 per cent	
Chloroform	Not less than 3.52 per cent	
Acetone	Not less than5.64 per cent	
Methanol	Not less than 5.76 per cent	
MICROBIOLOGICAL SPECIFICATIONS		
Total Viable Count	$<10^5$cfu/g	The Ayurvedic Pharmacopoeia of India
E-coli	Absent	
Salmonella/gm	Absent	
Staphylococus aureus/gm	Absent	
Pseudomonas aeruginosa/gm	Absent	
Yeast &Mould Count	$<10^3$cfu/g	
HEAVY METALS	Lead: NMT 0.7736 ppm Cadmium: NMT 0.0171ppm	AOAC Methods
PRODUCT PACKAGING		
Storage instruction	At dry place, away from direct sun light.	

TABLE 4: MONOGRAPH OF THE INGREDIENTS OF *VARUNA KWATHA* CHURNA

Name of Product	***VARUNA KWATHA* CHURNA INGREDIENTS**			
Category	Antiurolithiatic powder			
Ingredients	Varun Pashanbheda Gokshur Sunthi			
PHYSICO-CHEMICAL SPECIFICATIONS (%)				
PARAMETER	**Varun**	**Bergenia**	**Gokhru**	**Zingiber**
Part used	Bark	Rhizome	Fruit	Rhizome
Colour	Cream yellow	Dark brown	Pale yellow	Golden brown
Taste	Bitter	Characteristic	Characteristic	Astringent
Foreign matter	NMT 0.2	NMT 1.3	NMT 2.0	NMT 0.15
Powder microscopy	Cork cells, stone cells, vessels.	Cork cells, starch grains, stone cells, trachied,	Non-glandular trichomes, crystal.	Ridges, starch sclereids, crystals
Loss on drying	NMT 8.5	NMT 12.1	NMT 5.5	NMT 10.5
Total ash	NMT 10.32	NMT 7.66	NMT 9.15	NMT 6.2
Acid insoluble ash	NLT 0.75	NLT 0.5	NLT 0.51	NLT 1.0
Water soluble extractive	NLT 15.5	NLT 22.75	NLT 15.0	NLT 17.37
Alcohol soluble extractive	NLT 3.25	NLT 20.1	NLT 9.1	NLT 3.5
Swelling index	NLT 0.44	-	-	-

Foaming index	-		NLT 0.45	-	-
Assay	-		NLT 28.4	-	-
TLC	Shown in figure 1-4				
EXTRACTIVE VALUES					
n-Hexane	NLT 7.0		NLT4.66	NLT 9.96	NLT 7.48
Chloroform	NLT 2.15		NLT 4.4	NLT 3.74	NLT 3.75
Methanol	NLT 0.76		NLT 8.7	NLT 1.54	NLT 0.5
Acetone	NLT 2.3		NLT 13.93	NLT 6.36	NLT 2.37
Water	NLT 6.95		NLT 9.23	NLT 8.18	NLT 11.92
MICROBIOLOGICAL SPECIFICATIONS					
Total Viable Count	$<10^5$cfu/g		$<10^5$cfu/g	$<10^5$cfu/g	$<10^5$cfu/g
E-coli	Absent		Absent	Absent	Absent
Salmonella/gm	Absent		Absent	Absent	Absent
Staphylococus aureus/gm	Absent		Absent	Absent	Absent
Pseudomonas aeruginosa/gm	Absent		Absent	Absent	Absent
Yeast & Mould Count	$<10^3$cfu /g		$<10^3$cfu /g NMT 0.05	$<10^3$cfu /g NMT 0.045	$<10^3$cfu /g NMT 0.5
HEAVY METAL(ppm)	Lead	NMT 0.03			
	Cadmium	NMT 0.01	NMT 0.03	NMT 0.01	NMT 0.09
PACKAGING					
Storage instruction	At dry place, away from direct sun light				

TABLE 5: MONOGRAPH OF VARUNA KWATHA CHURNA MARKET FORMULATION (A)

Name of Product	***VARUNA KWATHA* CHURNA MARKET FORMULATION (A)**	
Category	Antiurolithiatic powder	
Ingredients	Varun Pashanbheda Gokshur Sunthi	
PHYSICO-CHEMICAL SPECIFICATIONS		
PARAMETER	**LIMIT**	**PROTOCOL**
Appearance	Coarse powder	Visual inspection
Colour	Yellow to brown	
Taste	Characteristic	Organoleptic evaluation
Foreign matter	-	In House Specification
Powder microscopy	Parenchymatous cortex cork cells in surface, stone cells, trachied, starch grains, non-glandular trichomes, vascular bundles, vessels.	
Loss on drying	Not more than 9.7 per cent	
Total ash	Not more than 7.66 per cent	
Acid insoluble ash	Not more than 1.23 per cent	
Water soluble extractive	Not less than 15.0 per cent	
Alcohol soluble extractive	Not less than 9.75 per cent	
Swelling index	Not less than 0.13 per cent	
Foaming index	Not less than 0.11 per cent	
Assay	Tannin content 15.8 per cent	
TLC	TLC of alcoholic extract on Silica gel "G" plate using Toluene: Methanol: Ethyl acetate (6:1:1) shows spots; on spraying with methanolic sulphuric acid reagent and heating the plate for ten minutes at 105^0C.	
EXTRACTIVE VALUES		
n-Hexane	Not less than7.2 per cent	
Chloroform	Not less than 6.37 per cent	

Methanol	Not less than 4.14 per cent	
Acetone	Not less than 5.62 per cent	
Water	Not less than 10.67 per cent	
MICROBIOLOGICAL SPECIFICATIONS		
Total Viable Count	$<10^5$cfu/g	The Ayurvedic Pharmacopoeia of India
E-coli	Absent	
Salmonella/ gm	Absent	
Staphylococus aureus / gm	Absent	
Pseudomonas aeruginosa / gm	Absent	
Yeast & Mould Count	$<10^3$cfu /g	
HEAVY METALS	Lead: NMT 0.515 ppm Cadmium: NMT 0.0189 ppm	AOAC Methods
PRODUCT PACKAGING		
Storage instruction	At dry place, away from direct sun light	

TABLE 6: MONOGRAPH OF VARUNA KWATHA CHURNA MARKET FORMULATION (B)

Name of Product	**VARUNA KWATHA CHURNA MARKET FORMULATION (B)**	
Category	Antiurolithiatic powder	
Ingredients	Varun Pashanbheda Gokshur Sunthi	
PHYSICO-CHEMICAL SPECIFICATIONS		
PARAMETER	**LIMIT**	**PROTOCOL**
Appearance	Coarse powder	Visual inspection
Color	Yellow to brown	Organoleptic evaluation
Taste	Characteristic	
Foreign matter	-	In House Specification
Powder microscopy	Parenchymatous cortex cork cells in surface, stone cells, trachied, starch grains, non-glandular trichomes, vascular bundles, vessels.	
Loss on drying	Not more than11.7 per cent	
Total ash	Not more than7.52 per cent	
Acid insoluble ash	Not more than0.95 per cent	
Water soluble extractive	Not less than 13.71 per cent	
Alcohol soluble extractive	Not less than 13.27 per cent	
Swelling index	Not less than 0.12 per cent	
Foaming index	Not less than 0.12 per cent	
Assay	Tannin content12.46 per cent	
TLC	TLC of alcoholic extract on Silica gel "G" plate using Toluene: Methanol: Ethyl acetate (6:1:1) shows spots; on spraying with methanolic sulphuric acid reagent and heating the plate for ten minutes at 105^0C.	
EXTRACTIVE VALUES		
n-Hexane	Not less than 6.89 per cent	
Chloroform	Not less than 6.59 per cent	
Methanol	Not less than 3.49 per cent	
Acetone	Not less than 5.65 per cent	
Water	Not less than 3.14 per cent	
MICROBIOLOGICAL SPECIFICATIONS		
Total Viable Count	$<10^5$cfu/g	The Ayurvedic

E-coli	Absent	Pharmacopoeia of India
Salmonella/ gm	Absent	
Staphylococus aureus / gm	Absent	
Pseudomonas aeruginosa / gm	Absent	
Yeast & Mould Count	$<10^3$cfu /g	
HEAVY METALS (ppm)	Lead: NMT 0.671 Cadmium: NMT 0.016	AOAC Methods
PRODUCT PACKAGING		
Storage instruction	At dry place, away from direct sun light	

CONCLUSION: From the present investigation various standardization parameters such as physicochemical standards, chemo profiles and safety evaluation were carried out, it can be concluded that the formulation of *Varuna kwatha* churna contains all good characters of an ideal churna and it was found to be harmless, more effective, and economic.

The monograph of the *Varuna kwatha* churna having the parameters as per pharmacopoeia can be used as a standard by the pharmaceutical companies as the ingredients and the preparation is authentic and standardized. The comparison with the marketed samples can be made on the basis of the TLC profile which shows satisfactory results, but the efficacy of the products can only be judged by doing the pharmacology of which is suggested as future scope of R and D.

The study shows that the contents of formulation presents within the permissible limits as per WHO, all these investigations are not specified in the standard literature such as in pharmacopoeia, which could helpful in authentication of *Varuna kwatha* churna. The result of present study will also serve as reference monograph in the preparation of drug formulation.

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