IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Pharmaceutical And Analytical Profile Of *Pradarghna Rasa*

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Abstract

India having a rich heritage of traditional medicine constituting with its different components like *Ayurveda*, *Siddha* and *Unani*. The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of herbs as herbomineral formulations. *Pradarghna Rasa*, a herbomineral formulation, composed of five herbal drugs and one mineral drug. A combination of phytoconstituents from medicinal plants plays a crucial role to treat various ailments. The final product of the sample was analyzed by organoleptic characteristics, physicochemical parameters and advanced sophisticated instrumental technologies such as TLC, XRD, and Determination of heavy metals through ICP-OES were carried out.

Keywords: Ayurveda, Siddha, Unani, TLC, XRD, ICP-OES.

Introduction

Rasa Shastra is a well established branch of Ayurveda serving humanity with its unique heritage of drugs derived from mineral, metal and animal origin combined with certain herbs [(1)]. Herbal (Medicinal) plants contain various phytoconstituents which in single or in combination exert therapeutic efficacy for numerous disease [(2)]. The trend of quality control and standardization of drugs can be found in Ayurvedic scriptures. The earlier physician identifies and categorizes the drug based on its morphological characteristics and therapeutic class for various ailments [(3).] For maintaining the quality of formulation it is essential that the raw materials which are used should be of desired quality and process involved in its preparation should be followed correctly so that the final product will be a good quality product. The quality of the product is dependent on the quality of the raw material and the quality of the extraction, formulation and manufacturing processes [(4)]. The naming of

many Ayurvedic herbs represents their physical and certain chemical characteristic properties which are considered as standardization tools [5].

In the present study, a herbo-mineral formulation, *Pradarghna Rasa* is prepared containing five herbal drugs i.e. *Lodhra Twak Churna (Symplocos racemosa* roxb*), Rohitaka Twak Churna (Tecomella undulata* G.Don*), Dhataki Pushpa Churna (Woodfordia fruticosa* (L.) Kurz.*), Nimba Twak Churna (Azadirachta indica* A. Juss.*)* and one mineral drug *Kukkutanda Twak Bhasma (calcinied egg shell)* were mixed properly with one bhavana of *Daruharidra Kwath (Berberis aristata* Dc.*).* All these drugs had been selected because of their *Kapha Shamaka, Sangrahi, Shoshana, Krimighna* and *Sthambhana* properties as per Ayurvedic point of view to break the *Samprapti* of the disease. As per current context, these herbs have anti-microbial and anti-inflammatory properties which facilitates in breaking up the pathogenesis of the disease. By evaluating all the ancient as well as recent researches and publications related to *Shweta Pradara*, we have been prepared a formulation with the title *Pradarghna Rasa*.

Considering all these facts, to find out the analytical profile of Ayurvedic medicine is the need of hour to identify nature of final product. The present work was carried out to establish analytical profile of *Pradarghna Rasa* by evaluating organoleptic, physicochemical characterization, microbial evaluations and using sophisticated modern tools and techniques such as Thin Layer Chromatography (TLC), X-ray Diffraction (XRD) and Inductively Coupled Plasma Optical Emission Spectroscopic Analysis (ICP-OES).

Materials and Methods

Collection of raw materials

The *Lodhra Twak, Rohitaka Twak, Dhataki Pushpa, Nimba Twak, Daruharidra Kanda, Nausadar* (Ammonium chloride) and *Saindhava Lavana* (Rock salt) were purchased from local herbal drug market of *Gola Dinanath* & fresh *Changeri* (*Oxalis corniculata*) *panchanga* (whole plant) was collected from the garden of Ayurvedic Pharmacy, BHU. *Kukkutanda Twak* (egg shell) was collected from a local egg shopkeeper of Lanka, Varanasi and all the samples were authenticated by experts of concerned department of the Institute.

Preparation of *Kukkutanda Twak Bhasma*

Shodhana of *Kukkutanda Twak* [6]

Shodhana of *Kukkutanda Twak* was done by following classical guidelines. Initially raw *Kukkutanda Twak* was washed with running tap water to remove stickiness of egg shells after that cleaned Raw KT was taken in a stainless steel vessel and filled with tap water and add *Nausadar* and *Saindhava Lavana*, heated till the water boils up to 100^0C and set aside for 3 days. At last it becomes soft, removes the inner layer then washed with water and dried in sunlight.

Marana of *Kukkutanda Twak* [7]

In the first *marana*, *shodhita Kukkutanda Twak* was kept in earthen *Sharava* and covered with another *Sharava*. Joint of both *Sharava* was sealed by clay smeared cotton cloth up to seven times and every coating was allowed to dry in sunlight. This *Sharava samputa* was subjected to heat in EMF at 850^0C and the temperature was maintained up to 3 hours. Next day, after self-cooling, *Sharava samputa* withdrawn from EMF and after removing clay smeared cotton, *Kukkutanda Twak* powder was collected. For second Marana process, collected *Kukkutanda Twak* powder was triturated with *Changeri Swarasa* for two hours, followed by preparation of small, round, flat pellets and subjected for sun drying. The dried pellets were taken in *Sharava* and covered with another *Sharava*, sealed with clay smeared cloth and after drying subjected to heat in EMF at 850^0C for three hours. The same procedure was repeated up to three times and finally desired quality of *Kukkutanda Twak Bhasma* obtained.

Preparation of churna

The foreign materials from the raw material were separated manually. After washing and cleaning, the drug was dried in sunlight, and then powdering was done with the help of pulverizer. All the powdered drugs were passed individually through 120 mesh size sieve and collected in air tight container.

Preparation of *Daruharidra* kwatha

The dried *Daruharidra* Kanda was converted into coarse powder from with the help of hammer mill. The required quantity of *Daruharidra* kwatha was prepared as per the classical reference [8].

Preparation of *Pradarghna Rasa*

Initially powder form of each ingredients of *Pradarghna Rasa* (*Kukkutanda Twak Bhasma, Lodhra Twak Churna, Rohitaka Twak Churna, Dhataki Pushpa Churna* and *Nimba Twak Churna*) was mixed properly and *Daruharidra Kwath* was poured into the mixture till the powder got fully impregnated and continues triturated in edge runner machine. The levigated material was kept in a stainless steel tray and kept in sunlight until it becomes dry. The dried material was powdered and filter through a clean cotton cloth to obtain the *Pradarghna Rasa* which was kept in air tight container with proper labeling.

The details are depicted in Table 1.

Table 1. Composition of *Pradarghna Rasa* (6 Kg)

Ingredients	Quantity
Kukkutanda Twak Bhasma	0.460 kg
Lodhra Twak Churna,	1.845 kg
Rohitaka Twak Churna	1.845 kg
Dhataki Pushpa Churna	0.925 Kg
Nimba Twak Churna	0.925 Kg

Three batches of *Pradarghna Rasa* had been prepared to maintain the SOP. The details are depicted in Table 2.

Table 2. Summary of three batches of *Pradarghna Rasa*

Batch	Quantity of Bhavana Dravya *(DHK)	*Pradarghna Rasa*		Total gain in Wt. %	Color of final product
		Wt. before Bhavana	Wt. after Bhavana		
1st	3000ml	6000g	6250g	4.17%	Dark Brown
2nd	3000ml	6000g	6265g	4.42%	Dark Brown
3rd	3000ml	6000g	6255g	4.25%	Dark Brown

*DHK- *Daruharidra Kwath*

Organoleptic Analysis

Organoleptic Analysis of the prepared sample of *Pradarghna Rasa* was carried out by classical parameters such as *Shabda* (sound), *Sparsha* (touch), *Roop* (color), *Rasa* (taste), *Gandha* (odor). Physicochemical Analysis was also carried out by following the standard methods for estimation of loss on drying [(9)], total ash [(10)], acid-insoluble ash [(10)], alcohol soluble extractive value [(9)], water soluble extractive value [(9)] and determination of pH [(11)]. In addition, TLC, XRD, SEM, EDAX and Determination of heavy metals through inductively coupled plasma optical emission spectroscopy (ICP-OES) were carried out.

Microbial limit test [(12)]

Microbial limit test for *Pradarghna Rasa* was carried out according to FSSAI, Ministry of Health and Family Welfare, GOI, New Delhi. The *Pradarghana Rasa* was tested for the presence of contaminating *Escherichia coli, Salmonella sp*. and total aerobic microbial count.

Results

Pradarghna Rasa is a smooth, bitter in taste, dark brown colored powder having a specific smell of *Daruharidra*. It is a mixture of *Kukkutanda Twak Bhasma, Lodhra Twak Churna, Rohitaka Twak Churna, Dhataki Pushpa Churna, Nimba Twak Churna* and triturated with *Daruharidra Kwath* .

Physicochemical analysis of *Pradarghna Rasa* was carried out and detailed results are depicted in Table 3.

Table 3. Physicochemical analysis of *Pradarghna Rasa*

S.No.	Parameters	Results
01.	Loss on Drying at 105^0C	9.71%
02.	Total Ash	17.56%
03.	Acid Insoluble Ash	4.03%
04.	Alcohol soluble extractive value	2.34%
05.	Water soluble extractive value	10.54%
06.	pH	8.45

Thin-layer Chromatography

The preliminary TLC was carried out the methanolic extracts of *Pradarghna Rasa* with its ingredients by using mobile phase (chloroform: methanol, 9:1). The developed TLC spots were visualized in normal daylight and under ultraviolet (UV)- visible light at 254 nm and 365 nm. The TLC profile showed good separation of alkaloids, turpentine, phenols and sugar contents. (Figure 1: a-d)

X-ray diffraction analysis of *Pradarghna Rasa*

The raw XRD pattern of *Pradarghna Rasa* shows amorphous structure. The detailed results of XRD analysis are depicted in Table 4.

Table 4. XRD analysis of *Pradarghna Rasa*

2-theta (degree)	d- spacing (^{0}A)	FWHM (degree)	Height	Size	Rel. Int.
14.32(2)	6.181(9)	0.24(3)	217(15)	350(43)	14.03
14.928(11)	5.930(4)	0.253(12)	665(26)	331(16)	45.53
15.265(11)	5.799(4)	0.12(2)	337(18)	673(116)	11.68
22.62(8)	3.927(14)	1.61(8)	293(17)	53(3)	100.00
24.344(8)	3.6533(11)	0.216(6)	2037(45)	392(11)	93.62
26.608(14)	3.3474(18)	0.237(19)	538(23)	359(29)	33.70
28.69(8)	3.109(8)	0.48(11)	102(10)	177(42)	17.94
30.043(11)	2.9720(11)	0.281(17)	702(27)	306(19)	64.21
30.70(2)	2.910(2)	0.40(5)	253(16)	213(26)	32.22
31.401(18)	2.8466(16)	0.16(3)	282(17)	551(109)	13.80
32.154(10)	2.7815(8)	0.24(3)	350(19)	358(49)	25.28

Inductively Coupled plasma optical emission spectroscopic analysis (ICP-OES)

The ICP-OES of *Pradarghna Rasa* shows the presence of Arsenic in the sample was 1.67 ppm i.e. within the permissible limit. The other heavy metals like Cadmium, Lead and Mercury were not detected in the sample. The result of ICP-OES analysis is tabulated in Table 5.

Table 5. ICP-OES analysis of *Pradarghna Rasa*

Elements	Results mg/ltr
Arsenic (As)	1.67 ppm
Cadmium (Cd)	Not detected
Mercury (Hg)	Not detected
Lead (Pb)	Not detected

Microbial limit test

Microbial limit test of *Pradarghna Rasa* for specific pathogens like *Escherichia coli, Salmonella sp.* was found negative. In the total Fungal count, no colony was seen but in bacterial count $4.3x10^3$ CFU/gm colony was seen in Table 6.

Table 6. Total Microbial Count of *Pradarghna Rasa*

Sample	*E. coli*	*Salmonella sp.*	Total Fungal Count	Total Bacterial Count
Pradarghana Rasa	-ve	-ve	-ve	$4.3x10^3$ CFU/gm

Discussion

Pradarghna Rasa is a herbo-mineral formulation containing *Kukkutanda Twak Bhasma, Lodhra Twak Churna, Rohitaka Twak Churna, Dhataki Pushpa Churna* and *Nimba Twak Churna* were mixed properly and triturated with one *Bhavana* of *Daruharidra Kwath.*

Recent classical texts of 20th century i.e. *Ayurveda Sara Sangraha, Rasa Tantra Sara evam Siddha Prayog Sangraha* and *Siddha Yoga Sangraha* had added *Kukkutanda Twak Bhasma* to be used in *Shweta Pradara* as it possesses *Kashaya Rasa, Stambhana* and *Ruksha Guna*[13, 14, 15].

Lodhra (Symplocos racemosa) is important Indian traditional drug used in many *Ayurvedic* and herbal formulations for treatment of liver as well as uterine disorders and leucorrhea. The stem bark of *Symplocos racemosa* is incorporated in various formulations used for the diseases of the uterus, menorrhagia and leucorrhea [16].

Acharya Charak prescribed powder bark, its decoction and extracts of *Rohitaka (Tecomella undulate)* with different mediating vehicles (*Anupana*) in treating jaundice, enlarge spleen, anaemia, intestinal worms, urinary disorders and gynaecological disorders [17]. Phytochemical screening of the crude powder of bark of *Tecomella*

undulate shows that the plant is rich in phytosterols, glycosides, tannins and phenolic compounds which had been reported to have antibacterial, antifungal and anti inflammatory properties[18,19,20] .

Nimba bark contains condensed tannins such as gallic acid, gallocatechin, epicatechin, catechin and epigallocatechin, of which gallic acid, epicatechin and catechin are primarily responsible for inhibiting the generation of chemiluminescence by activated human polymorphonuclear neutrophil (PMN)[21], indicating that these compounds inhibit oxidative burst of PMN during inflammation. Oil from the leaves, seeds and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms [22] , including *M. tuberculosis* and streptomycin resistant strains [23].

The presence of different phytosterols and sterol-like molecules, *e.g.*, β-sitosterol, lupeol, betulinic acid, betulin and ursolic acid [24] justifies the widespread ethnomedical success of *Woodfordia fruticosa* based herbal medicines in managing general uterine disorders [25, 26].

Daruharidra (*Berberis aristata*) an Indian medicinal plant, is useful as anti-pyretic, anti-bacterial, anti-microbial, anti-hepatotoxic, anti-hyperglycaemic, anti-cancer, anti-oxidant and anti-lipidemic agent. It is also useful in treatment of gynaecological disorders [27].

By screening all the ancient as well as recent researches and publications related to Shweta Pradara, we have been prepared a formulation with the title *Pradarghna Rasa*. All these drugs had been selected because of their Kapha Shamaka, Sangrahi, Shoshana, Krimighna and Sthambhana properties as per Ayurvedic point of view to break the Samprapti of the disease. As per current context, all these herbs have anti-microbial and anti-inflammatory properties which facilitates in breaking up the pathogenesis of the disease.

Pradarghna Rasa had been prepared by inculcating five herbal drugs and one mineral drug i.e. *Lodhra Twak Churna, Rohitaka Twak Churna, Dhataki Pushpa Churna, Nimba Twak Churna and Kukkutanda Twak Bhasma,* were mixed properly in the ratio of 4:4:2:2:1 and triturated with one *Bhavana* of *Daruharidra Kwath,* finally formulation prepared.

As per *Ayurvedic* Pharmacopeia of India, all the herbal drugs has been discussed and their powder form drug dosing mentioned as 3-6 gm and the dose of *Kukkutanda Twak Bhasma* given in authoritative books of *Ayurveda* is 125 mg to 1 gm. We have combined all the drugs in their minimal dose such as *Lodhra Twak Churna* (1 gm), *Rohitaka Twak Churna* (1 gm), *Nimba Twak Churna* (500 mg), *Dhataki Pushpa Churna* (500 mg), *Kukkutanda Twak Bhasma* (250 mg) in a single dose of *Pradarghna Rasa*.

The physicochemical analysis exposed that the pH of *Pradarghna Rasa* was 8.75 which is alkaline in nature and thus it may interfere pharmacokinetic of the drug. Basic drugs are not absorbed until they reach the alkaline environment of the small intestine. The alkaline environment, in which the major component of the drug exists in a unionized form, facilitates their absorption.

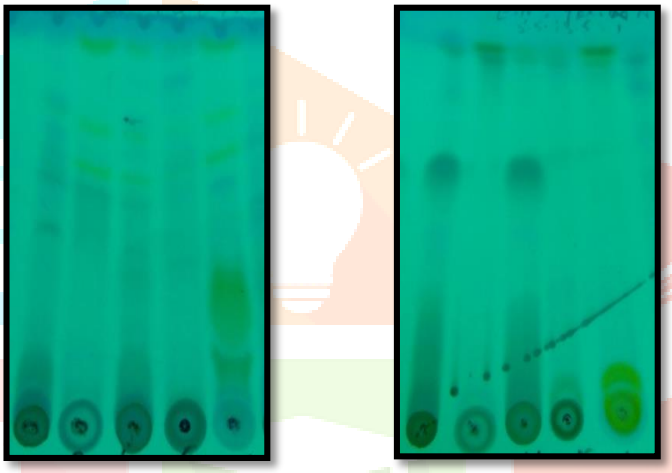
The quantitative analysis by the TLC method and UV spectroscopy proved the presence of alkaloids, turpentine, phenols and sugar contents in *Pradarghna Rasa*. These phyto-constituents aid different pharmacotherapeutic

effects of *Pradarghna Rasa*. The heavy metals analysis in Pradarghna Rasa detects the presence of Arsenic i.e. within the permissible limit concerning the Food and Drug Administration, WHO and Ayurvedic Pharmacopoeia guidelines. The microbial load of specific pathogens like *Escherichia coli, Salmonella sp.* was found negative and the preparation under permissible limits.

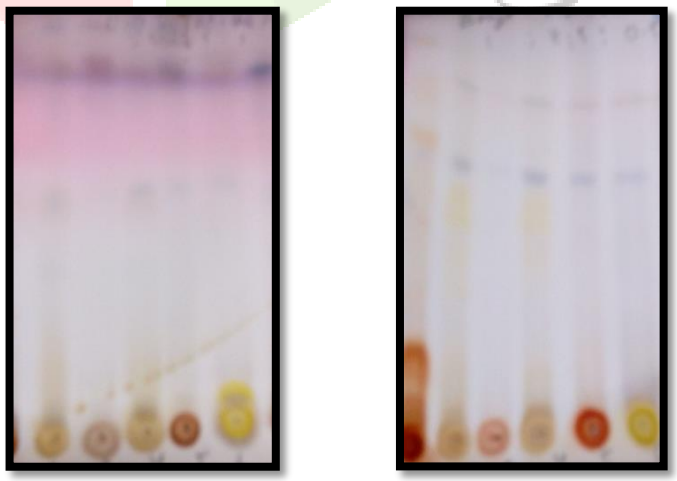
Conclusion

In the present study, preliminary morphological, physicochemical and chromatographic parameters were developed. The chromatographic data of *Pradaraghna Rasa* showed the presence of alkaloids, turpentine, phenols and sugar contents. Further studies should be undertaken to elucidate the mechanism of action of *Pradaraghna Rasa* on *Shweta Pradara*.

Figure 1: (a & b) TLC Spots under UV Visible Light at 254nm



(c & d) TLC Spots under Normal Day Light at 365nm



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