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QUALITY ASSESSMENT OF VRANROPAN TAILA: AN AYURVEDIC SIDDHA TAILA FOR WOUND HEALING AND ANTI-MARK PROPERTY

K. N. Yadav *, P. V. Kadam, C. L. Bhingare and M. J. Patil

Marathwada Mitra Mandal's College of Pharmacy, Kalewadi, Pune - 411033, Maharashtra, India.

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Correspondence to Author:
K. N. Yadav

Assistant Professor
Department of Pharmacognosy, Marathwada Mitra Mandal's College of Pharmacy, Kalewadi, Pune - 411033, Maharashtra, India.

E-mail: kavitan.yadav@gmail.com

ABSTRACT: Standardization can create trust and confidence in the products and increase market relevance. It is an essential requirement for the open exchange of information; without it, the network will simply not work. The practitioner, as well as the consumer, now seeks assurance from the manufacturer about the quality, safety, and efficacy of a readymade Herbal supplement or Ayurvedic formulations. An average person's diet, lifestyle and other social habits, all who play important roles in disease and treatment, are completely different today. Hence, the earlier recommendations for herbs for specific disease states may not hold today unless validated in today's times. Phenotypic changes in plant species. Hence, the original pharmacological claims of these formulations need to be revalidated. Pharmaceutical research is aimed at meeting the medical needs of the population for whom appropriate therapeutic remedies are not available or at those that are available are unsafe for prophylactic use for various disorders. While meeting medical needs, research also has to ensure that the market needs for such exist. Vranropantaila is a Siddha taila used in Ayurveda for wound healing as well as anti-mark property. It contains sixteen Herbal and mineral origin ingredients in it. It is prepared by special procedure mention in Ayurveda. But because of polyherbal formulation, it becomes difficult to standardize it. The present study is mainly aimed to standardize "Vranaropantaila" in terms of identity, purity, safety, and efficacy.

INTRODUCTION: Traditional systems of medicine (TSM) / complementary and alternative systems of medicines (CAM) have been used throughout the world for centuries. One such important system is Ayurveda - the holistic system of medicine from India still used extensively, in developed and developing countries [1]. Ayurvedic knowledge and practical database can provide new functional leads to reduce time, money and toxicity - three main obstacles in drug development. These records are particularly valuable since from ancient times people are using it for effective treatment.

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One of the characteristics of traditional preparations is that all the herbal medicines either presenting as a single herb or a group of herbs in composite formulae, which are extracted with boiling water during the decoction process [2]. This may be the main reason why quality control of traditional oriental drugs is more difficult than that of the western drug. Efforts are underway to establish pharmaco-epidemiological evidence base concerning safety and efficacy for the practice of Ayurvedic medicines [3].

Nowadays for rationalize the utility of positive and judicious use of traditional formulation; it becomes a prime need to standardize it by various quality control parameters. A concept of the golden triangle which comprises of Ayurveda, modern medicine and science will be helpful to search for new, safe and cheap with better efficiency of

formulations. This will be supportive for pharmaceutical companies, researchers and the global community to take the initiative in TSM [4]. "Vranaropantaila" is Siddha taila used in Ayurveda for wound healing with anti-mark potential. "Vrana" means wound and "Ropana" means wound healing. It is useful in the rapid healing of the wound. Vranaropantaila is a reputed Ayurvedic formulation which is used for wound healing treatment. The formula of vranaropantaila comprises of sixteen elements *viz*. Nagarmotha (*Cyperus rotundus)*, Khairsal (*Acacia catechu*), Manjishta (*Rubia cordifolia*), Loddhra (*Symplocos racemose*), Kath *(Catha edulis*), Lavang (*Syzygium aromaticum*), Padmakashtha (*Prunus cerasoides*), Vadsal (*Ficus bengalensis*), Yashtimadhu (*Glycyrrhiza glabra*), Kankol (*Piper cubeba*), Pimpalmul (*Ficus religiosa*), Dhyti (*Woodfordia fructicosa*), Kayphal (*Myrica esculenta)*, Nagkeshar (*Mesua ferrea*), suvarngairik, bhim-senikapur till taila [5, 6].

The vranaropantaila and raw material for the preparation of the same are procured from SG Phytopharma Pvt. Ltd., which has been prepared according to the traditional method mention in Ayurveda. The present study has to emphasis the standardization of Siddha taila using traditional and modern techniques of chromatography for determining quality control standards.

MATERIALS AND METHODS: The existing work relates to standardization of Ayurvedic polyherbal formulation, as per traditional and WHO guidelines.

Procurement of Sample: For the current study, a polyherbal formulation, vranaropantaila and raw material for the preparation of the same were procured from S. G. Phytopharma Pvt. Ltd., Kolhapur.

Evaluation of Physicochemical Parameters: Physicochemical parameters such as colour, smell, appearance, rancidity, specific gravity, saponification value, unsaponification value [7], peroxide value, acid value and iodine value were determined in vranropan tail according to standard official procedure [8, 9, 10].

Determination of Rancidity: Mix 1 ml of Vranaropantaila and 1 ml of conc. HCl in a test tube. Add 1 ml of a 1% solution of phloroglucinol in diethyl ether and mix thoroughly with the fat acid mixture [11, 12].

Determination of Viscosity: Viscosity of the formulation was determined by Brookfield Viscometer at 100 rpm, using spindle no 61.8.[13]

Qualitative Phytochemical Parameters: Qualitative analyses of various phytoconstituents like steroids, triterpenoids, flavonoids, alkaloids, sugar, coumarin, quinine, saponins, tannic acid, furan, phenol were carried out using routine chemical tests[14].

Determination of Extractive Value: Extraction of phytochemical constituents from vranaropantaila was done by mixing 5 ml of vranaropantaila with 15 ml of 90% methanol and subjected to constant stirring by using magnetic stirrer on hot top at 60ºC for 1 h. It was then stored in the freezer for solidification. The alcoholic portion was separated and filtered through Whatman filter paper no. 42 and filtrate were used for qualitative phytochemical testing. The extraction was repeated for three times for effective extraction of phytochemicals from the medicated taila. The same procedure was followed for till taila as a placebo [15].

Determination of Heavy Metals, Pesticide, Aflatoxin, and Microbial Load: Determination of heavy metals, pesticide, aflatoxin and the microbial load was done as per WHO guidelines [16, 17].

High-Performance Thin Layer Chromatographic (HPTLC) Fingerprinting: HPTLC study was carried out by following the routine method. The unsaponifiable content of vranaropantaila & till taila was utilized for HPTLC fingerprinting. The samples were prepared in methanol and were used for HPTLC fingerprinting. Aliquots of samples were applied on the stationary phase used for the same is 12 × 10 cm TLC silica gel 60 F_{254} (Merck) using CAMAG Linomat 5 system equipped with ATS 4 applicator. The plates were developed using a solvent system of *n*-hexane: ethyl acetate: chloroform (1.7:0.3:3.0) to 80 mm from the lower edge of the plate, removed from the chamber and allowed to dry.

The plates were analyzed in daylight and under short and log UV light finally it was immersed into

anisaldehyde reagent for 1 sec then heated at 100ºC for 5 min in hot air oven till the color of the spots appears as a part of derivatization. Then the plate was scanned at 520 nm using a tungsten lamp. The R_f values were calculated [18, 19].

Skin Irritation Test: Skin irritation test (OECD guidelines no. 404) of vranropantaila, till taila was carried out on five healthy rats (Wistar strain) in each group and permission was taken from Institutional Animal Ethical Committee (IAEC No: MMCOP/IAEC/002/2016, dated: 20/02/2016). Approximately 24 h before the test, 1 to 2-inch fur was removed by closely clipping the ventral area of the neck of each rat. Vranropantaila was applied on the ventral side of the intact epidermis of the rats considered as a treated group, while the other group received till taila as a placebo, the third group is without treated act as a control group. Observations for irritation in terms of erythema and oedema (according to Draize Test) were recorded at 24, 48, and 72 h after topical application of vranropantaila, till taila in order to check any adverse effects [20].

RESULTS: Results of organoleptic characters are presented in **Table 1**. The results showed that the vranaropantaila represented the increased values to that of till taila. Results of the qualitative phytochemical parameters are given in **Table 2**. Extractive value of vranaropantaila was found to be 9.06 mg/ml. The elevated extractive value represented the presence of phytochemicals from raw material. The results of the qualitative phytochemical analysis are given in **Table 3**.

TABLE 1: ORGANOLEPTIC CHARACTERIZATION

S. no.	Parameters	Vranaropantaila	Til taila
1	Colour	Brown	Yellowish
2	Smell	Characteristic	Characteristic
3	Appearance	Viscous	Less viscous
4	Touch	Sticky	Sticky
5	Clarity	Clear	Clear

TABLE 2: PHYSICO-CHEMICAL PARAMETERS

S. no.	Physico-chemical parameters	Values	
		Till taila	Vranaropantaila
1	Viscosity (poise)	1248	3497
2	Specific gravity	0.90	1.03
3	Refractive index	1.468	1.332
4	Saponification value (mg/g)	131.835	260.865
5	Iodine value (gI_2/100g)	3.1725	2.09385
6	Acid value (mg KOH/gm)	1.568	2.147
7	Ester value	130.267	258.718
8	Rancidity	Slightly oxidized	Slightly oxidized

TABLE 3: QUALITATIVE PHYTOCHEMICAL ANALYSIS

S. no.	Test	Observation	Inference		
			Till taila	Vranaropan-taila	Vranaropantaila extract
1	Alkaloids (Dragendorff's reagent)	Brick red precipitate	Absent	Present	Present
2	Flavonoids (Magnesium and concentrated HCl)	Magenta color	Absent	-	Present
3	Saponins (Frothing test)	Froth formation	Absent	-	Present
4	Tannins Ferric chloride test	Green precipitate	Absent	Present	Present
5	Steroids (Conc. H_2SO_4 test)	Reddish brown precipitate at the interface	Absent	Present	Present
6	Triterpenoids (Libermann Burchard)	Red precipitate	Absent	Present	Present
7	Phenol (Ferric chloride)	Green color	Absent	Present	Present

TABLE 4: SKIN IRRITANCY TEST

Rat no.	24 h Total			48 h Total			72 h Total			Average
	Erythema + Oedema			Erythema + Oedema			Erythema+ Oedema			
	Test	Control	Placebo	Test	Control	Placebo	Test	Control	Placebo	
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
Mean	0	0	0	0	0	0	0	0	0	P. I. I. = 0

Placebo = Application of Till taila, Test = Application of Vranaropantaila, P.I.I= Primary Irritation Index, Control = Without treated

TABLE 5: PESTICIDE RESIDUE

Testing Parameter	Method (WHO)	Testing Parameter	Method (WHO)	Testing Parameter	Method (WHO)
Dichlorvos	Complies	*α*-Endosulfan	Complies	Trans-Permethrin	Complies
Parathion-methyl	Complies	Dieldrin	Complies	Cypermethrin	Complies
Chlorpyrifus-methyl	Complies	p,p'-DDE	Complies	Fenvalerate	Complies
Pirimiphos-methyl	Complies	Endrin	Complies	Delta Methrin	Complies
Malathion	Complies	*β*-Endosulfan	Complies	o,p'-DDT,HCH	Complies
Parathion	Complies	o,p'-DDT	Complies	o,p'-DDT,HCL	Complies
Chlophyrifos	Complies	Carbophenothion	Complies	PCB (Polychlorinated bophenyl)	Complies
Ethion	Complies	p,p'-DDT	Complies	o,p'-DDE	Complies
Carbophenothion	Complies	Cis-Permethrin	Complies	ε- Hexachlorocyclohexane	Complies
Lindane	Complies	α-Hexachlorocyclohexane	Complies	Heptachlor	Complies
Gamma-hexachlorocyclohexane	Complies	Hexachlorocyclohexane	Complies	*β*- Hexachlorocyclohexane	Complies
Aldrin	Complies	Cis-Heptachlorepoxide	Complies		

Aflatoxins Analysis: Total aflatoxins (B1, B2, G1, G2)≤5 µg/kg.

TABLE 6: MICROBIAL ANALYSIS

S. no.	Test	Result
	Microbial Limit tests	
1	Test for *E. coli*	Absent
2	Test for *Salmonella*	Absent
3	Test for *P. aeruginosa*	Absent
4	Test for *S. aureus*	Absent
	Total Aerobic microbial count	
1	Total bacterial count	70 cfu/gm
2	Total fungal count	< 10 cfu/gm
3	Total yeast & mould count	< 10 cfu/gm

TABLE 7: HEAVY METAL ANALYSIS

S. no.	Heavy metals	Result	Maximum limit
1	Lead	0.694 ppm	10 ppm
2	Cadmium	Nil	0.3 ppm
3	Mercury	Nil	1.0 ppm
4	Arsenic	Nil	10 ppm

DISCUSSION: According to the World Health Organization (WHO) guidelines for herbal drugs, standardized herbal products of consistent quality and containing well-defined constituents are to be used to provide consistent beneficial therapeutic effects. Quality of herbal medicine depends on the uniqueness of raw drugs, method of preparation, correct dose and dosage regimen. As far as the preparation used in the Ayurvedic system of medicine, a drug formulation may not be a problem, because many formulations are well documented in classical texts. But, there is confusion concerning standards to be followed while preparing a formulation as well as basic parameters to assess the quality of the finished product. Efforts are made here to standardize vranaropantaila in terms of various physico-chemical and phytochemical, analytical and biological parameters to establish reliable standards and correlate with further work on instrumental analytical methods like HPTLC. In present study involves qualitative and quantitative evaluation of vranaropantailaa reputed marketed formulation containing sixteen ingredients, used for wound healing treatment.

Generally, the taila are given different characteristic color and odor relative to the herbs and other materials which were used to prepare the taila. In this Siddha taila red color is due to *R. Coraopolis* and katha. The characteristic odor is due to bhimseni camphor, one of the important ingredients of vranaropantaila added at the end of the procedure. The vranaropan taila is reddish brown viscous liquid with characteristic odor [21].

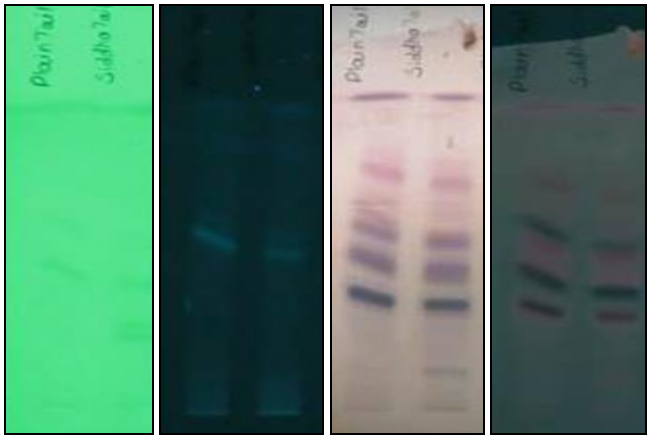
Refractive index indicates the density of sample compared to air and liquid media; the value of vranropantaila was within the limit. Specific gravity indicates the presence of solute content in the solvent; the value for the same was appropriate for vranaropan oil. The amount of alkali needed to saponify a given quantity of taila will depend upon a number of -COOH group present; the saponification value also indicates the average molecular weight/chain length of all fatty acids present. Longer the chains, fatty acid have low saponification value, and shorter chain fatty acid have high saponification value. Shorter chain fatty acid (high saponification value) have a faster rate of absorption than longer chain fatty acid; a saponification value of vranaropantaila was found to be 260.865 mg/g.

Which signify the quality of prepared vranropantaila is good enough and stable for longer duration [22]. The acid value indicates the presence of free fatty acid in the taila which is responsible for rancidity of compounds; higher the free fatty acid more is the rancidity, this helps to decide the shelf life of the taila; acid value for vranropantaila was found to be good thus indicating the longer shelf life of taila. Iodine value indicates the degree of unsaturation of taila; greater the degree of unsaturation higher will be the possibility of absorption and atmospheric oxidation leading to rancidity. The more iodine number, the more unsaturated fatty acid bonds are present; unsaturated fatty acid better absorbed than saturated fatty acids, the iodine value of vranropantaila was found to be fair enough which indicates the less rancidity of vranropantaila.

Viscosity is an index of resistance offered by the surface to flow a liquid; higher the viscosity of a liquid, greater is the resistance to flow, if the viscosity of the taila preparation increases, the rate of absorption decreases. If taila is less viscous this means the rate of absorption is very much high; viscosity of vranropantaila is found to be fair enough which enhances its absorption indices. The rancidity of vranropantaila was slightly oxidized because of low iodine value, unsaponification matter was used for phytochemical analysis, and ester value was within limit [23].

The pesticide residue, aflatoxin, and microbial load are responsible for the early deterioration of crude drug, but results of the same for vranropan tail was within the limit. The result of the study shows the presence of heavy metals in the vranropantaila was less. These constants can be used as standard values to derive quality parameter for vranropantaila. These values are within the Pharmacopoeial limits mentioned in the Ayurvedic Pharmacopoeia of India.

HPTLC is a well-appreciated separation technique and accepted all over the world, which has many applications in complicated traditional formulation systems. HPTLC remains one step ahead when compared with other tools of chromatography and one of the most flexible, reliable and cost-efficient separation technique ideally suited for the analysis of botanicals and herbal drugs. The HPTLC data of the vranropantaila signify the separation of various Phytoconstituents presents thereby in the formulation, which is a great tool of identification using analytical marker system. The proposed HPTLC methods for simultaneous estimation of active pharmaceutical ingredients seem to be accurate, precise, reproducible and repeatable. It is for the first time when different samples of this formulation and its raw materials are estimated and compared for the respective active constituents [24].



At 250 nm **At 366 nm** **At 540 nm** **At 366 nm**
After Derivatization

FIG. 1: IMAGES OF CHROMATOGRAM

TABLE 8: HPTLC FINGERPRINTING

Wavelength	R_f value	Sample	Track
254 nm	0.40 (Green)	Siddha tail	2
254 nm	0.44 (Sky blue)	Plain tail	1
540 nm	0.33 (Dark blue)	Plain tail	1
540 nm	0.37	Siddha tail	2
366 nm	0.51 (Pink)	Plain tail	1
366 nm	0.51	Siddha tail	2

Skin irritation test of vranropantaila on rats showed no adverse effects on its topical application. Vranropantaila did not cause any dermal irritation to the skin of rats. Thus safety of the formulation was established, and the vranropantaila may be treated safely for human skin. These methods will help in establishing the quality and efficacy of the traditional formulation vranropantaila. Vranropantaila is a rare type of Ayurvedic formulations mentioned in classical texts of Ayurveda. The normal types of taila (taila) are prepared from fresh drugs while vranropantaila is a unique type of preparation prepared by special process mention in Ayurveda. There are reports of standardization of normal type of taila (taila), while this study is the first time report of standardization of vranropantaila which can be important medication for wound healing.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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