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Preliminary standardization of *Vasavaleha* prepared by two different methods of extraction

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Abstract

Pharmaceutics is the science of dosage form design. *Ayurveda* carries high reputation for providing early thoughts relating to theories and techniques of different aspects of pharmaceuticals sciences. The present study deals with the physic-chemical analysis of *Vasavalehya* prepared by two different methods of extraction i.e., the direct squeezing method [*Swarasa*] and the *Putapaka* method. Hence in order to observe, compare and interpret the changes that might occur during the different methods of preparation, this study was planned incorporating the physicochemical analysis & TLC. Non reducing sugars were found more in *Vasavalehya* prepared by *Swarasa* extraction method than the *Putapaka* method. Successive extraction with ethyl alcohol has shown that *Putapaka* method yielded more organic constituents. TLC has revealed that there were two additional Rf values observed in *Putapaka* method using the Benxene: Ethyl acetate (6:1) as compared to the rest seen in *Swarasa* method. The results provide preliminary hints towards phytochemical mechanism involved in traditional method of preparation.

Key words – *Vasaka*, *Adathoda vasica, Putapaka, Vasavalehya,* Physico-chemical studies

Introduction

Vasaka [*Adhatoda vasica* (Nees)] possesses wide spectrum of medicinal activities [1]. It is an ingredient of many Ayurvedic polyherbal formulations used in the management of respiratory ailments including cough, bronchitis [2], and asthma [3]. It has effective mucolytic and expectorant properties [4]. Vasicine and vasicinone are the two major alkaloids of *A. vasica,* known to possess interesting biological activities including respiratory, stimulant, bronchodilator, and hypotensive activities [3]. The principles quite akin to current day pharmaceutical processes have been comprehensively described in treatise of Ayurveda known as Bhaishajya Kalpana [4]. *Vasavalehya* is a semisolid polyherbal medicinal formulation prepared mainly with the sap from the leaves of *Vasaka* with the addition of sugar candy. There are two methods of obtaining *Vasaka* sap, first method is to directly express the crushed leaves (known as *Swarasa* method) and second method is subjecting a bolus of crushed fresh leaf to heat followed by expressing the sap (known as *Putapaka* method). In Ayurveda, *Vasavaleha* prepared using *Vasaka* sap obtained as per first method is indicated in respiratory disorders, whereas that prepared using *Vasaka* sap obtained as per second method is indicated in bleeding disorders [5]. The differential indication is the moot research question of this study. The changes in physicochemical and thin layer chromatography parameters might give useful hints in deciphering the mechanisms involved in changes occurring in the *Vasaka* sap obtained with and without heat application used in the preparation of *Vasavaleha*.

Aim and Objective

Physicochemical evaluation of the two samples of *Vasavalehya* prepared by different methods prescribed in Ayurveda- *Swarasa* (fresh

expressed sap) and *Putapaka* method (sap extraction after heat application) and to compare the changes in the physicochemical parameters of above samples.

Ayurvedic description of *Vasaka* leaves

Pharmacodynamics and pharmaco-kinetics in Ayurveda is explained in terms of certain attributes of an ingredient used as medicine or food. The description in brief are as follows; *Rasa* [tastes viz., *Madhura* (sweet), *Amla* (sour) *Lavana* (saline), *Katu* (pungent) and *Kashaya* (astringent)]. *Guna* [properties (effect it has on the body after ingestion and assimilation including the nature of its interaction with digestive juices), viz., *Laghu* (light for digestion), guru (heavy for digestion). *Ruksha* (dryness) and so on]. *Veerya* [potencies (release or conservation of energy during digestion and metabolism) viz., *Ushna Veerya* (releases energy during digestion and metabolism) and *Sheeta Veerya* (conserves energy during digestion and metabolism)]. *Vipaka* [post digestive effect on metabolism viz., *Madhura* (sweet), *Amla* (sour), *Katu* (pungent)]. Based on these the probable action of a drug or food can be predicted and understood in terms of action on a *Dosha* (*Vata, Pitta* or *Kapha*) known as *Doshaghnata*. *Karma* is systemic action *Vyadhiharatva* or *Prabhava* is specific action on a particular disease. *Vasaka* has *Tikta* (bitter), *Kashaya* (astringent) *Rasa* with *Katu Vipaka*. It is *Laghu, Ruksha* and *Sheeta* in *Guna* with *Kaphapittashamaka* action. It is indicated in *Kasa, Shwasa*, *Rajayakshma* [6, 7]. The antitussive activity of *Vasaka* is similar to codeine against coughing induced by irritant aerosols [8] *Vasaka* possess a wide spectrum of medicinal properties including positive effects on inflammatory diseases [5]. *Pippali* also has *Katu* (Pungent) *Rasa,* is *Laghu, Snigdha, Tikshna* in *Guna* with *Anushna Sheeta Veerya* attaining *Madhura Vipaka*. It has *Kaphavatashamaka* action [5], thereby has a synergistic effect along with *Vasaka* in the treatment of *Kasa, Shwasa*. It is an expectorant, useful in asthma, bronchitis and other respiratory ailments.

Materials & Methods

Extraction of *Vasaka* sap:

Vasaka leaves were procured from the Survey of Medicinal Plants Unit (SMPU), National Ayurveda Dietetics Research Institute, Bangalore (NADRI-B). After cleansing them of physical impurities the sap from the leaves of *Vasaka* was extracted in two different methods as follows [9]:

1) *Swarasa* method (SM): the leaves were crushed and fresh expressed sap was collected separately.
2) *Putapaka* method (PM): separate set of crushed leaves were subjected to heat and then the sap was collected.

Equipments used were mortar and pestle, sieve, tray for drying, mixer, and cooker, stove.

Method of Preparation of *Vasavalehya*

The *Vasavaleha* was prepared in the Drug Standardization Research Unit of NADRI-B. The details of the ingredients used are given tables 1 & 2 and Figures.1-5. *Vasaka* sap is prepared as per two different methods as mentioned above. *Sharkara* (Ing. 3) and *Pippali* (Ing.5) were powdered separately.

***Vasavalehya*-SM (SMV)**

Step 1: Vasavalehya-SM (SMV) Step 1: Vasaka sap is mixed with Sharkara (sugar) and *Vasaka* syrup is formed. The preparation at this state is known as *Paka* and is examined by the following markers; when a small drop of the preparation is put between pressed opposing fingers, a thread like consistency connects the separating fingers; When a drop of the said preparation is put into water (of room temperature) in a glass beaker, the drop sinks to the bottom. These are as per ayurvedic principles of preparation

Step 2: After separating the preparation from fire, fine powder of *Pippali* (Ing. 5) was added and stirred vigorously to form a homogenous mixture.

Step 3: The hot mixture was mixed completely with clarified butter (ghee; Ing. 4).

Step 4: After the mixture cooled to room temperature it was again mixed completely with honey [10-13]

The final homogenous semisolid mixture was then kept in an airtight container and labeled.

***Vasavalehya*-PM (PMV)**

The *Vasavaleha* was prepared in the same method as above by using *Vasaka* sap of *Putapaka* method in place of *Swarasa* method [10-13], (Fig.6).

Methodology of Physicochemical Analysis

Both samples of SMV and PMV were used for the analysis. Physico-chemical & preliminary phytochemical analysis of two samples were carried out employing standard procedures and using GPR grade reagents [(WHO, 1996) British Pharmacopoea, Indian Pharmacopoea].

Sl. No.	Name of the drug	Botanical /English name	Parts used	Quantity in gms
1.	*Vasaka* sap (*Swarasa*/ *Putapaka*)	*Adhatoda vasica* Nees.	Fresh Leaf	384
3.	*Sita/Sharkara*	Sugar candy	As it is	192
4.	*Sarpi/Ghrita*	Clarified Butter	As it is	48
5.	*Pippali*	*Piper longum*	Fruit	48
6.	*Madhu*	Honey	As it is	192

Table -1 Ingredients of *Vasavalehya* (*Swarasa* & *Putapaka* method)

Soxhlet Extraction: Solvents like Petroleum ether, benzene, chloroform and alcohol were used for extraction. The resin powder of sample 1 was filled in the thimble of soxhlet apparatus. The material was exhaustively extracted with petroleum ether (40°C) for about 48 hours. The solvent was distilled off at low temperature and under vacuum and concentrated on water bath to get semisolid liquid. After extracting with petroleum ether, the material was refluxed with other solvents like benzene, chloroform and alcohol [2]. The same procedure was repeated for sample 2.

Thin Layer Chromatography

Benzene: Ethyl acetate (6:1) ; Benzene: Ethyl acetate (4:1) Benzene:Ethyl acetate (1:1) Comparative TLC was done using three different concentrations of Benzene: Ethyl acetate solvent system at 6:1, 4:1 & 1:1 (Igon & Stahl, 1969). The iodine vapor and long wave length (365 nm) ultra violet images were evaluated.

RESULTS AND DISCUSSION

Vasavaleha is a preparation produced by application of heat per se. the difference between the two samples of *Vasavaleha* used in the study is, the use of cold extract of *Vasa* in SMV and heat extract of *Vasa* in PMV. The following discussion will highlight the differences between the two samples rendering their distinct usage.

Physicochemical analysis

The results of the preliminary analysis are given in the table 2. The preliminary physicochemical parameters were compared between SMV and PMV. Both the samples were very dark brown in colour with the smell of ghee and were aromatic. Both had bitter taste and were oily and sticky. Results of loss on drying at 105° C, pH of 10% w/v aqueous solutions, ash values, extractive values are given in (table 2) Non reducing sugars were found more in *Vasavalehya* with the *Swarasa* extraction (VS=13.89 %) than the *Putapaka* method (VP=4.59%). Solvent extraction of *Vasavalehya* by *Putapaka* method, with the petroleum ether (0.28%), chloroform (1.31%) and ethyl alcohol (78.21%) revealed extractives as indicated in brackets under each fraction. Solvent extractions of *Vasavalehya* by *Swarasa* method, with the petroleum ether (1.69%), chloroform (3.53%) and ethyl alcohol (38.30%) revealed extractives. Successive extraction with ethyl alcohol has shown that *Putapaka* method (VP) yielded 78.21% which indicates that the organic constituents were more in *Putapaka* method than the *Vasavalehya* with *Swarasa* method (VS) which yielded 38.3%. Increase in petroleum ether extracts is indicative restructuring of steroids. Increased ethyl alcohol extracts is suggestive of increased glycosides, flavonoids and tannins. Increased chloroform extracts points towards increase in steroids, triterpenes and alkaloids.

Thin Layer Chromatography Studies

Samples of SMV and PMV were subjected to Thin Layer Chromatography (TLC) (Table-3). Petroleum-ether extracts of both the samples were subjected to TLC in Benzene: Ethyl acetate (6:1) solvent system which is specific among others, for poly-phenols. Compounds corresponding to 0.41, 0.66, and 0.81 were common to both the samples. Two Rf values (0.04, 0.24) were unique to the PMV and one Rf value 0.31 was unique to SMV, suggesting that presence of unique additional polyphenols because of the use of cold and heat extracts. Similarly Chloroform extracts of both the samples were subjected to TLC in Benzene: Ethyl acetate (4:1). Compounds corresponding to 0.06, 0.23, 0.45 & 0.66, were common to both the samples. Two Rf values (0.78, 0.90) were unique to the SMV. This suggests presence of unique additional polyphenols in SMV. Ethanol extracts of both samples were subjected to TLC in Ethyl acetate mobile phase which is specific principally to glycosides and oils. Interestingly only one Rf value 0.92 was common to both samples and the PMV sample had four unique Rf values 0.21, 0.57, 0.64, 0.74, 0.84. This indicates that presence of certain specific glycosides might be

contributing to discrete therapeutic property. A similar study conducted for the validation of different methods of preparations of *Adhatoda vasica* leaf juice reveals that steaming of fresh leaves under 15 lb pressure yielded same quantity of juice as the traditional method and its total alkaloid content and *vasicine* content (4.05+/-0.12 and 3.46+/-0.06 mg/ml, respectively) were very high, though the traditional method was found to give the best quality juice with highest amount of total alkaloids (5.93+/-0.55 mg/ml) and *vasicine* (5.64+/-0.10 mg/ml) content[11].

It has been therefore evidenced that the discrete therapeutic action of *Vasavalehya* prepared out of *Swaraa* and *Putapaka* is due to available compounds corresponding to Rf values as elucidated above. The authors of Ayurveda have observed change of pharmacological action by the application of heat known as *Agni Sannikarsha* and the process by which change in the action of the drug can be brought about is known as *Samskara*[12]. Many such processing techniques find mentioned in age old texts of Ayurveda which can stand the modern test of reasoning. The attempt is to document the phytochemical modulation that is accompanied with these processes to give a lucid corroboration and validation to distinctive therapeutic properties of pharmaceutical preparations.

Conclusion

Vasaka is an important herbal ingredient of wide range of medicinal formulations used in Ayurveda. Its properties and action according to ayurvedic principles have been described in detail but reports of phyto-chemistry and pharmacological rationale in terms of Modern scientific methods are inadequate. Hence, this study was undertaken to document properties as per modern scientific methods and attempt to give an interpretation of Ayurvedic descriptions. From the above observations, it can be concluded that these parameters can be utilized as marker parameters for monitoring the quality of the formulation. The physicochemical parameters, quantitative analysis may be used for qualitative evaluation and the standardization of *Vasavalehya*. This was a preliminary study and provides significant leads to undertake future endeavors. Further studies with HPLC will strengthen the above views. Although, differences observed in the two preparations are minute, some of these differences might be the basis for change in the action of the two preparation viz., SMV being haemostatic and PMV antitussive and useful in respiratory disorders. It is a matter of admiration that ancient Ayurvedic scientists could decipher this subtle difference in property without advanced techniques differences. Based upon these findings further studies with advanced phytochemical techniques and randomized double blind clinical studies could be initiated to evaluate these differential properties clinically.

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Fig.1 to 6: Ingredients of *Vasavalehya*

Fig.1. *Vasaka* (Leaf) -Adhatoda vasica Nees

Fig.2. P*ippali* (Fruit)-Piper longum Linn

Fig.3.*Sita* (*Sarkara*)

Fig.4.*Ghritha* (Ghee)

Fig.5.*Madhu* (Honey)

Fig.6. *Vasavalehya*

***Table-2.Physico-chemical Analysis**

Parameters	Results of *Putapaka* method	Results of *Swarasa* method
1) Description • Colour • Odour • Taste • Touch	• Very dark brown • Smell of ghee & aromatic • Sweet and bitter • Oily &sticky	• Very dark brown • Smell of ghee & aromatic • Sweet and bitter • Oily & sticky
2) Loss on drying at 105℃	12.48 %	12.53 %
3) Total ash	1.8 %	1.27 %
4) Acid insoluble ash	0.17 %	0.26 %
5) pH	6.3	6.6
6)Specific gravity at 25℃	1.21	1.27
7) Total solids	87.52 %	87.47 %
8) Fat content	1.09 %	1.69 %
9) Total sugars • Reducing sugars • Non reducing sugars	41.58 % 36.99 % 4.59 %	53.92 % 40.03 % 13.89 %
10) Successive extraction • Petroleum ether 60-80°C • Chloroform • Ethyl alcohol	 0.28% 1.31% 78.21%	 1.69% 3.53% 38.30 %

Table 3: TLC findings of *Vasavalehya* (*Swarasa/ Putapaka* Method)

Sl. No	Extractives	Adsorbent	Solvent system	Viewing medium	R_f Values *Putapaka* method	R_f Values *Swarasa* method
1	Petroleum-ether 60-80°C	Silica gel 60 F_{254} pre coated sheets	Benzene: Ethyl acetate (6:1)	Iodine vapour	0.04, 0.24, 0.41, 0.66, 0.81.	0.31,0.41, 0.66,0.81
2	Chloroform	Silica gel 60 F_{254} pre coated sheets	Benzene: Ethyl acetate (4:1)	Iodine vapour	0.06,0.23, 0.45,0.66.	0.06,0.23, 0.45,0.66, 0.78,0.90
3	Ethanol	Silica gel 60 F_{254} pre coated sheets	Benzene: Ethyl acetate (1:1)	Iodine vapour	0.21,0.57, 0.64,0.74, 0.84,0.92.	0.92.