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Pharmacopoeial Standards for *Venpucani Ilakam* - A classical Siddha medicine

Research Article

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Abstract

Belief on the therapeutic values of herbal medicine is in increasing trend and the consumption of traditional medicine is also in upward trend. The quality control and assurance of these traditional medicines is a prime criterion for any herbal drug manufacturer. *Venpucani ilakam* (VPI) is a classical poly herbal Siddha medicine under the category Ilakam (Legiyam in Ayurveda). This poly herbal medicine is a combination of 18 ingredients of plants and animal origins. An attempt was made to prepare the drug with authenticated ingredients and subjected to physicochemical analysis, thin layer chromatographic photo documentation and high performance thin layer chromatographic finger printing. The TLC photo documentation of defatted chloroform extract showed nine spots under UV 254, ten spots under UV 366 nm and eleven spots after derivatization with vanillin sulphuric acid; defatted success ethanol extract showed five spots under short wavelength, six spots under long wavelength and seven spots after derivatization. HPTLC finger printing recorded eleven peaks under 254 nm, seventeen under 366 nm and fifteen peaks after derivatization in defatted chloroform extract; nine peaks under 254 nm, eleven peaks under 366 nm and eight peaks after derivatization in defatted ethanol extract. About twenty three percent loss in weight was observed on heating at 105^{o}C and drug was acidic nature. Not less than fourty percentage of the drug was soluble in alcohol and also in water. This drug has wide therapeutic use and the derived results may serve as reference standards.

Key Words: *Venpucani,* Aphrodisiac, Jaundice, Leucorrhoea*, Pandanus odoratissimus.*

Introduction

Venpucani ilakam is a Siddha polyherbal formulation consisting of 18 ingredients. It is prescribed for *Manjal noy* (Jaundice), *Utal* (edema), *Enpu Vettai* (Venereal diseases with bone manifestation), *Piramiyam* (Gonorrhea), *Vellai* (leucorrhoea), *Nirc curukku* (dysuria), *Veppu noy* (all types of fever) This drug is anabolic and aphrodisiac in nature. The therapeutic dosage is 5 to 10 g. Ethno pharmacological use of its ingredient *Benincasa hispida* Cogn is in leucorrhoea, fever, disease related to liver & edema (1), *Pandanus odoratissimus* Roxb in jaundice (2) edema (3) & dysuria (4) *Piper nigrum* in jaundice (5), edema (6), gonorrhea, leucorrhoea (7) & fever (8), *Citrus aurantifolia* in fever & jaundice (9), *Glycyrriza glabra* Linn. in edema (10), *Quercus infectoria* Oliv. in edema (11); anabolic nature of *Myristica fragrans* Houtt. (12) and aphrodisiac property of *P. nigrum* (7) & *P. odoratissimus* (4) validates the use of this drug in traditional medicine.

The pharmacopoeial standards for this drug are not available for assessing the quality. As the demand for herbal medicine is tremendously increasing, the necessity for arriving pharmacopoeial parameter is greatly sensed by the world community. Hence, the physicochemical parameters for Ilagam, thin layer chromatographic photo documentations under ultra violet light at short and long wave lengths, white light after derivatization with 5 % sulphuric acid and high performance thin layer chromatographic finger print profiles were carried out for defatted chloroform extract and successive ethanol extract.

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Materials and Methods

Procurement of Raw Drugs

The ingredients of *Venpucani ilakam* their common name, botanical name, the anatomical part used and quantity required are listed in Table 1. All the raw drugs from Sl.No.6 to 16 were procured from raw drug market in Chennai and other ingredients were collected from Chengalpat. All the items were authenticated by the Pharmacognosist of this Institute.

Preparation of VPI

Juices of items Sl.No.1 to 4 were taken. Powdered the ingredients 7 to 16 and sieved through

mesh sieve no.85. Mixed sugar with the juices of Sl.no.1 to 3 and boiled till the content reduces to half of the original. Heated the milk and lemon juice separately to 50 % of their volume. Mixed them to the condensed juice of item 1 to 3 & 6 and continued heating to reach syrup consistency. Added ghee and stirred well. Powdered drug ingredients were added to the syrup and lowered the flame till the consistency of semisolid. Removed from flame, added honey and stirred thoroughly. After cooling at room temperature, the drug VPI was stored in an air tight plastic container.

Organoleptic characters

Organoleptic characters such as form, colour, odor, taste and texture were observed.

Physico-chemical analysis

The physico-chemical parameters such as loss on drying, total ash, acid insoluble ash, water soluble extractives, alcohol soluble extractives, pH (10% solution), reducing sugar and total sugar were determined as per the standard methods (13)**.**

Sample Preparation for TLC/HPTLC

VPI (2 g) was first defatted with hexane, then successively extracted by chloroform and ethanol in a Soxhlet. The extracts were dried and weight were taken. Then the chloroform and ethanol extracts were dissolved in 10 ml of respective solvents. These solutions were taken in sample vials.

Solvent system for TLC/HPTLC

After many trials, the solvent system for defatted chloroform extract of VPI was finalized as *toluene: ethyl acetate: formic acid* (8:2:0.5, *v/v/v*). For successive ethanol extract the mobile phase chosen was *toluene: ethyl acetate: methanol: formic acid* (4:3:2:1, *v/v/v/v*).

Methodology for TLC/HPTLC of defatted chloroform extract

7, 9 and 11 µl of the extract were applied on silica ($60F_{254}$) coated aluminium plate using Camag's ATS4 applicator and developed in a twin trough chamber (CAMAG) (5× 10 cm), previously saturated with the selected mobile phase. The plate was developed up to 85 mm from the bottom. After development, the plate was photo documented using Camag's TLC Visualizer under UV λ254 nm and UV λ366 nm. Then the plate was scanned using Camag's Scanner 4 at λ254 nm (D_2 lamp, absorption mode) and λ366 nm (Hg lamp, fluorescence mode) respectively & finger print profiles of the extract was performed. Subsequently, the plate was dipped in 5% sulphuric acid solution in alcohol followed by heating at 105°C till development of the colored spots. The plate was then photo documented in white light using Camag's TLC Visualizer.

Methodology for TLC/HPTLC of successive ethanol extract

6, 8 and 10 µl of the extract were applied on silica ($60\ F_{254}$) coated aluminium plate using Camag's ATS4 applicator and developed in a twin trough chamber (CAMAG) (5× 10 cm), previously saturated with the chosen mobile phase The plate was developed up to 85 mm from the bottom. After development, the plate was photo documented using Camag's TLC Visualizer under UV λ 254 nm and UV λ 366 nm. Then the plate was scanned using Camag's Scanner 4 at λ254 nm (D_2 lamp, Absorption mode) and λ366 nm (Hg lamp, Fluorescence mode) respectively & finger print profiles of the extract was performed. Subsequently, the plate was dipped in 5% sulphuric acid solution in alcohol followed by heating at 105°C till development of the colored spots. The plate was then photo documented in white light using Camag's TLC Visualizer.

Results and Discussion

The prepared drug VPI was semisolid in nature, blackish green in colour, odor pleasant, initially sweet later pungent in taste and smooth and sticky.

The results of physicochemical parameters are presented in Table 2.

The TLC photo documentation under UV 254 nm, 366 nm and at white light after spray with vanillin-sulphuric acid followed by heating at 105°C till the colour development and the respective finger print profiles of defatted chloroform extract are presented in Fig. 1 and that of successive ethanol extract are presented in Fig. 2. The R_f values of the developed spots of defatted chloroform extract and successive ethanol extract under visualization and detection as mentioned above are presented in Table 3.

The loss on drying was determined as 23.09 %. The ingredients at Sl.No.1 to 5 & 17 are liquids which contributes to the high loss on drying content. The total ash was found to be 2.03 % which is very low indicating the presence of meager inorganic salts. And the acid insoluble ash was 0.41 % indicating the negligible amount of siliceous matter. The total sugar was calculated as 22.93 % which is due to the addition of sugar and honey. The drug is soluble in water and ethanol upto 49.64 % and 46.08 % respectively due the liquid ingredients and sugars. The pH value of 10 % solution was observed as 3.52 indicating the high acidic nature of the drug. Despite the high loss on drying the acidity of the drug would be helpful for preventing from the formation of moulds and fungus.

The finger print profile of defatted chloroform under UV 254 nm showed elevan peaks in which the peak at R_f 0.31 is the major peak with an area of 30.58 %. Other major peaks appear at R_f 0.48 (17.11 %), 0.60 (22.15 %), 0.71 (12.40 %). All other peaks are minor; under UV 366 nm, the major peaks are at R_f 0.31 (21.52 %), 0.41 (17.82 %), 0.51 (27.09 %) and 0.57 (9.51 %). After derivatization at 520 nm, the major peaks are at R_f 0.32 (9.14 %), 0.52 (16.88 %) and 0.73 (46.67 %).

In the finger print profile of successive ethanol extract under UV 254 nm, the major peaks are at R_f 0.21 (17.29 %), 0.45 (11.52 %), 0.65 (33.76 %) and 0.72 (16.48 %); under 366 nm, the major peaks appeared at R_f 0.15 (14.20 %), 0.58 (13.27 %), 0.64 (27.03 %), 0.73 (11.68 %); after derivatization with VSR at 520 nm, the major peaks appeared at 0.08 (37.28 %), 0.62 (14.14 %), 0.70 (26.14 %) and 0.86 (16.07%).

Table 1. List of ingredients, their common name, part and quantity used in the drug

Sl.No	Common Name	Botanical name	Part Used	Quantity
1	Venpucanikkayc caru	*Benincasa hispida* Cogn	Unripen fruit (juice)	5600 ml
2	Talai vilutic caru	*Pandanus odoratissimus* Roxb	Aerial stil root (juice)	1400 ml
3	Tennampuc caru	*Cocos nucifera* Linn.	Flower (juice)	1400 ml
4	Elumiccam Palac caru	*Citrus aurantifolia* (Christm.) Swingle	Fruit (juice)	1400 ml
5	Pacumpal	*Bos indicus* Linn.	Milk	2800 ml
6	Carkkarai	*Saccharum officinarum* Linn	-	350 g
7	Cirakam	*Cuminum cyminum* Linn.	Fruit	35 g
8	Kottumalli	*Coriandrum sativum* Linn.	Fruit	35 g
9	Kottam (Kostam)	*Saussurea lappa* C.B. Clarke	Tuber	35 g
10	Milaku	*Piper nigrum* Linn	Fruit	35 g
11	Macikkay	*Quercus infectoria* Oliv.	Gall	35 g
12	Elam	*Elattaria cardamomum* Maton	Fruit	35 g
13	Catikkay	*Myristica fragrans* Houtt.	Seed	35 g
14	Catippattri	*Myristica fragrans* Houtt.	Aril	35 g
15	Atimaturam	*Glycyrriza glabra* Linn.	Root	35 g
16	Talicapattiri	*Taxus baccata* Linn.	Leaf	35 g
17	Ney (Ghee)	*Bos indicus* Linn	-	700 ml
18	Ten (Honey)	*Apis mellifera* Linn.	-	700 ml

Table 2. Physico-chemical values

Parameters	Mean(n=2)
Loss on drying (%, w/w)	23.09
Total ash (%, w/w)	2.03
Water soluble ash (%, w/w)	0.82
Acid insoluble ash (%, w/w)	0.41
Total solid (%, w/w)	76.91
Fat content (%, w/w)	4.76
Water soluble extractives (%, w/w)	49.64
Alcohol soluble Extractives (%, w/w)	46.08
pH value (10 % solution)	3.52
Reducing sugar (%, w/w)	4.59
Total sugar (%, w/w)	22.93

Table 3. R_f value and colour of the TLC

Extract	Visualization under UV 254 nm		Visualization under UV 366 nm		Detection after spray with VSR in white light	
	Color	R_f value(s)	Color	R_f value(s)	Color	R_f value(s)
	Green	0.09	Blue	0.09	Violet	0.03
Defatted chloroform	Green	0.13	Red	0.16	Violet	0.10
	Green	0.17	Red	0.18	Blue	0.13
	Green	0.21	Red	0.20	Violet	0.19
	Green	0.25	Blue	0.26	Brown	0.23
	Green	0.28	Yellow	0.33	Violet	0.28
	Green	0.33	Fluorescent Blue	0.37	Violet	0.35
	Green	0.50	Fluorescent Blue	0.44	Violet	0.40
	Green	0.62	Fluorescent Blue	0.53	Violet	0.46
	-	-	Red	0.60	Black	0.54
	-	-	-	-	Blue	0.57
Successive Ethanol	Green	0.21	Blue	0.24	Yellow	0.22
	Green	0.45	Blue	0.37	Grey	0.34
	Green	0.50	Blue	0.45	Pale Pink	0.50
	Green	0.65	Fluorescent Blue	0.64	Violet	0.58
	Green	0.74	Blue	0.72	Violet	0.62
	-	-	Red	0.80	Violet	0.71
	-	-	-	-	Violet	0.85

Figure 1. TLC photo documentation and HPTLC finger pring profiling of defatted chloroform extract of VPI

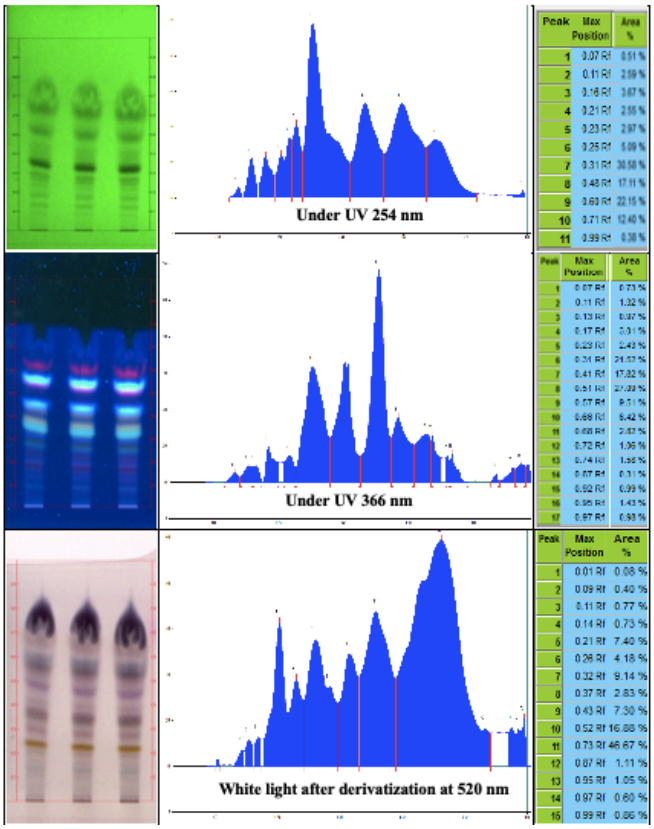
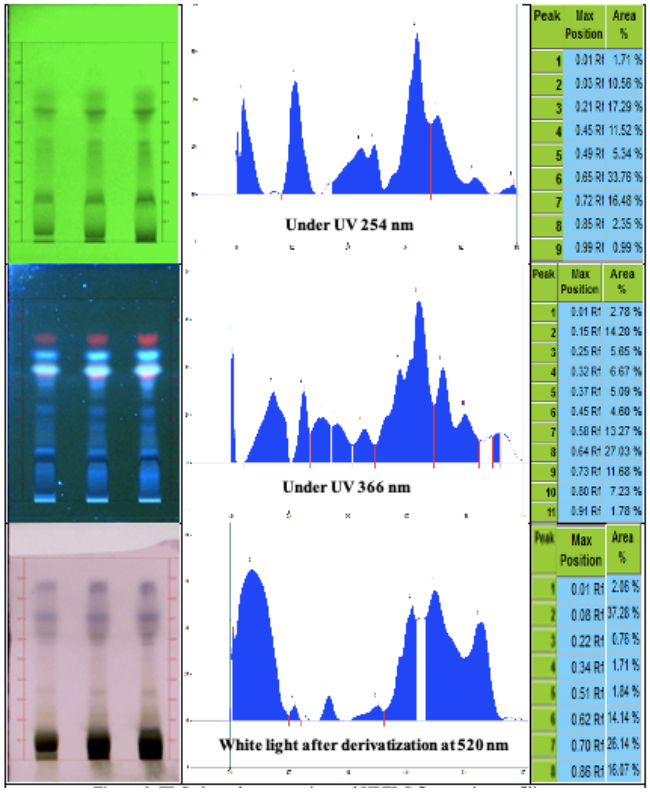


Figure 2. TLC photo documentation and HPTLC finger pring profiling of successive ethanol extract of VPI



Conclusion

Venpucani ilakam is a poly herbal Siddha combination of ingredients. Since the preparation process involves heating after addition of herbal powder, powder microscopy is not done and pharmacopoeial standard includes only physicochemical, TLC photo documentation and HPTLC fingerprint profile of defatted chloroform and successive ethanol extracts. It can be useful in assessing the quality of the drug from different batches.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Lee KH, Choi HR, Kim CH. Anti-angiogenic effect of the seed extract of *Benincasa hispida* Cogniaux. J Ethnopharmacol. 2005; 97(3); 509-13. doi: 10.1016/j.jep.2004.12.008.
2. Ilanchezhian R, Joseph R. Hepatoprotective and hepatocurativeactivity of the traditional medicine ketaki (*Pandanus odoratissimus* Roxb.) Asian J. Trad. Med. 2006; 5(6); 212–218.
3. Londonkar R, Kamble A, Reddy VC. Anti-inflammatory activity of *Pandanus odoratissimus* extract. Int. J. Pharmacol. 2010; 6; 311–314.
4. Adkar PP, Bhaskar VH. *Pandanus odoratissimus* (Kewda): A Review on ethnopharmacology, phytochemistry, and nutritional aspects. Adv. Pharmacol. Sci. 2014; 2014; 120895. doi: 10.1155/2014/120895.
5. Sharma J, Gairola S, Gaur RD, Painuli RM. The treatment of jaundice with medicinal plants in indigenous communities of the Sub-Himalayan region of Uttarakhand, India. J Ethnopharmacol. 2012; 143(1); 262–291. doi: 10.1016/j.jep.2012.06.034.)
6. Tasleem F, Azhar I, Ali SN, Perveen S, Mahmood ZA. Analgesic and anti-inflammatory activities of *Piper nigrum* L., Asian Pac. J. Trop. Med. 2014; 7(1); S461-S468. https://doi.org/10.1016/S1995-7645(14)60275-3.
7. Behera SK, Misra MK. Indigenous phytotherapy for genito- urinary diseases used by the Kandha tribe of Orissa, India. J Ethnopharmacol. 2005; 102(3); 319–25. doi: 10.1016/j.jep.2005.06.024.
8. Sabina EP, Nasreen A, Vedi M, Rasool M. Analgesic, antipyretic and ulcerogenic effects of piperine: An active ingredient of pepper. J. Pharm. Sci. Res. 2013; 5; 203–206.
9. Arias BA, Ramon-Laca L. Pharmacological properties of citrus and their ancient and medieval uses in the Mediterranean region. J Ethnopharmacol. 2005; 97(1); 89-95. doi: 10.1016/j.jep.2004.10.019.

10. Halder RM, Richards GM. Topical agents used in the management of hyperpigmentation. Skin Therapy Lett. 2004; 9(6); 1-3.
11. Kaur G, Hamid H, Ali A, Alam MS, Athar M. Antiinflammatory evaluation of alcoholic extract of galls of *Quercus infectoria*. J Ethnopharmacol. 2004; 90(2-3); 285-92. doi: 10.1016/ j.jep.2003.10.009.
12. Abourashed EA, El-Alfy AT. Chemical diversity and pharmacological significance of the secondary metabolites of nutmeg (*Myristica fragrans* Houtt.). Phytochem Rev. 2016; 15(6); 1035-1056. doi: 10.1007/s11101-016-9469-x.
13. Lohar DR . Protocol for testing of Ayurveda, Siddha and Unani Medicine. New Delhi, Pharmacopoeial Laboratory for Indian Medicine, Department of AYUSH, Ministry of Health and Family Welfare, Government of India. 2008.

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