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Physicochemical analysis and drug standardization of Pippalyadi yoga

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Abstract
Purpose: Pippalyadi Yoga is a combination of four drugs mentioned in Bhaishajya Ratnavali Strirogadhikara for strivandyatwa which is used in the form of curna. This work was carried out to standardize all the ingredients of Pippalyadi Yoga.
Methodology: Curna of Pippali, Maricha Shunti and Nagakesara are prepared, mixed together and stored in an airtight bottle. Analysis of the Pippalyadi yoga was done with following parameters physico-chemical analysis like pH, refractive index, specific gravity, total solids, viscosity and chromatographical analysis like High performance thin layer chromatography (HPTLC).
Result: Loss on drying value of trikatu curna (TC) is 12.87 % w/w and 10.74%w/w of nagakesara. Ash value of trikatu curna is 5.33% w/w and 11.79 % w/w of nagakesara. Acid insoluble ash value is 0.29 %w/w of TC and 7.93% w/w of nagakesara. Water soluble ash values are 2.89% w/w of TC and 1.01 % w/w of nagakesara. Water soluble extractive values are 14.94 of TC and 4.64% w/w of Nagekesara and alcohol soluble extractive values are 6.07 of TC and 10.90 % w/w of Nagakesara. HPTLC of Trikatu curna: 7 prominent spots with Rf values 0.01, 0.04, 0.10, 0.25, 0.32, 0.50, 0.60 at 254nm and 5 spots with Rf values 0.01, 0.10, 0.36, 0.61, 0.83 at 366 nm. Nagekesara curna: 10 prominent spots with Rf values 0.00, 0.03, 0.17, 0.36, 0.52, 0.59 0.65, 0.72, 0.86, 0.92 at 254nm. 9 spots with Rf values 0.01, 0.04, 0.09, 0.34, 0.46, 0.55, 0.61, 0.68, 0.81 at 366nm.
Conclusion: The analytical data generated here may be considered for the development of standard parameters for the formulation.

Keywords: Pippalyadi yoga, Stri Vandyatwa, standardization, physic-chemical analysis, HPTLC

1. Introduction
In modern era, however, there has been a metamorphosis of approach. The values have changed, needs have undergone unprecedented change and there has been need to prove consistency of medicine in its effectiveness and there is also need to ensure that there is no adulteration in the medicines used to ensure quality control as prescribed by Ayurvedic pharmacopoeia. Standardization of ayurvedic products is very much essential to face challenges and to accept in world market. Analytic parameters are developed to test defined characteristics of the drug substance or finished product against established acceptance criteria for that characteristic. In the light above statement a study was conducted on Pippalyadi Yoga [1] which is in the form curna with combination of Pippali, Shunti, Maricha and Nagakesara. The ingredients of this Yoga possess Katu, Tikta rasa and, Ushna veerya properties and acts as Deepaka, Amapachaka Garbhashaya shodhaka. Thus the yoga by acting on Agni helps in initiating the utpatti of beejarupi arthava from rasa dhatu, inducing Beejotsarga. As we are aware of infertility is a global public health issue in the present era. Prevalence of infertility is 60–80 million couples suffering from infertility every year worldwide, probably between 15 and 20 million (25%) are in India alone. According to a report by the World Health Organization (WHO), one in every four couples in developing countries is affected by infertility [3]. Pippalyadi yoga may be a hope to patients suffering from infertility. There is need to predetermine the fact that there is no adulteration and to ensure that the active constituents are present in drugs. Keeping the Ayurvedic ethos in centrality, a systematic study was conducted to standardize the finished product of Pippalyadi Yoga using Physico-Chemical parameters and HPTLC analysis. The authenticity, quality and purity of herbal drugs are established by references given in Pharmacopoeia.

Aim and Objective
The objective of the study was to prepare the Pippalyadi Yoga and to standardize it on the basis of Rasapanchaka, physico-chemical parameters and HPTLC.

Materials and Methods

Collection, Identification and authentication of raw drugs

The raw drugs for the study were procured from the Sri Dharmasthala Manjunatheshwara Ayurveda Pharmacy, Udupi. The ingredients were identified and authenticated in the department of Dravya Guna, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan.

Table 1: Ingredients of Pippalyadi yoga

Sl.No	Sanskrit Name	Botanical name	Part used
1	Pippali	Piper longum Linn.	Fruit
2	Shunti	Zingiber officinale Roxb.	Rhizome
3	Maricha	Piper nigrum Linn.	Fruit
4	Nagakesara	Mesua ferrea Lin.	Stamens

Preparation of Pippalyadi yoga

Dry powders of each ingredient were procured from Sri Dharmasthala Manjunatheshwara Ayurveda Pharmacy, Udupi. In the present study one batch of Pippalyadi Yoga was prepared and further studied for Rasapanchaka, physico-chemical and chromatographically evaluation.

Analytical study

The analytical study was carried out at S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi to determine the following standardization parameters like pH, refractive index, specific gravity, total solids, and viscosity were evaluated and tabulated below. HPTLC analysis was carried out using Ethyl acetate: acetic acid: water (3.0: 2.0: 0.8:0.2) as solvent system.

Table 2: Rasapanchaka of ingredients of Pippalyadi yoga

Sl. No	Dravya	Rasa	Guna	Virya	Vipaka	Karmukatha
1	Pippali	Katu	Teekshna, laghu, Snigdha	Anushna	Madhura	Vatakaphahara
2	Shunti	Katu	Teekshna, Guru, Ruksha	Ushna	Madhura	Vatakaphahara
3	Maricha	Katu	Laghu, Teekshna	Ushna	Katu	Vatakaphahara
4	Nagakesara	Katu, tikta, kashaya	Ruksha, laghu	Ushna	Katu	Kaphahara.

Physico-chemical analysis

The following tests were done

Loss on drying at 105oC

10 g of sample was placed in tared evaporating dish. It was dried at 105˚C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.

Total Ash

2 g of sample was incinerated in a tared platinum crucible at temperature not exceeding 450˚C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.

Acid insoluble Ash

To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ashless filter paper (Whatmann 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight.

Allow the residue to cool in suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air dried drug.

Water soluble ash

Boil the ash for 5 min with 25 ml of water; collect insoluble matter on an ashless filter paper, wash with hot water, and ignite for 15 min at a temperature not exceeding 450˚C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash with reference to the air-dried sample.

Alcohol soluble extractive

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95%). Shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value.

Water soluble extractive

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent.

Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice. Take the average value.

HPTLC of Trikatu curna

1g each of Trikatu curna was suspended in 10 ml of alcohol, kept for cold percolation for 24hrs followed by fliteration. 3, 6 and 9µl of the above extract were applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator.

The plate was developed in Toluene: Ethyl acetate (7.0: 1.0). The developed plates were visualized under short UV, long UV and then derivatised with vanillin sulphuric acid, observed under white light and scanned under UV 254nm, 366nm. Rf, colour of the spots and densitometric scan were recorded.

HPTLC of Nagakesara curna

1g of Nagakesara curna was suspended in 10 ml of alcohol, kept for cold percolation for 24hrs followed by fliteration. 3, 6 and 9µl of the above extract were applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate: Formic acid (5.0: 4.5: 0.5).

The developed plates were visualized under short UV, long UV and then derivatised with vanillin sulphuric acid, observed under white light and scanned under UV 254nm, 366nm and 620nm. Rf, colour of the spots and densitometric scan were recorded.

Results

Physicochemical results: Trikatu and Nagekesara curna.

Table 3: Results of standardization parameters of Trikatu curna

Parameter	Results n = 3 %w/w
Trikatu curna	
Loss on drying	12.87 ± 0.01
Total Ash	5.33 ± 0.04
Acid Insoluble Ash	0.29 ± 0.01
Water soluble Ash	2.89 ± 0.01
Alcohol soluble extractive value	6.07 ± 0.00
Water soluble extractive value	14.94 ± 0.01

Table 4: Results of standardization parameters of Nagakesara curna

Parameter	Results n = 3 %w/w
Nagakesara curna	
Loss on drying	10.74±0.01
Total Ash	11.79±0.31
Acid Insoluble Ash	7.93±0.01
Water soluble Ash	1.01±0.01
Alcohol soluble extractive value	10.90±0.01
Water soluble extractive value	4.64±0.02

HPTLC results

Trikatu curna: 7 prominent spots with Rf values 0.01, 0.04, 0.10, 0.25, 0.32, 0.50, 0.60 at 254nm and 5 spots with Rf values 0.01, 0.10, 0.36, 0.61, 0.83 at 366 nm.

Nagekesara curna: 10 prominent spots with Rf values 0.00, 0.03, 0.17, 0.36, 0.52, 0.59 0.65, 0.72, 0.86, 0.92 at 254nm. 9 spots with Rf values 0.01, 0.04, 0.09, 0.34, 0.46, 0.55, 0.61, 0.68, 0.81

Discussion

Loss on drying method is applied to determine the amount of water, all or a part of water for crystallization, or volatile matter in the sample. Loss on drying value of trikatu curna (TC) is 12.87 % w/w and 10.74%w/w of Nagakesara. Total ashes are designed to measure the total amount of material remaining after ignition. It includes both physiological (which is derived from the plant tissue itself) and non-physiological ash (residue of the extraneous matter etc. adhering to the plant substance) Ash value of powder is 5.33% w/w Trikatu curna and 11.79 % w/w of Nagakesara. Acid insoluble ash indicates the genuinity of the product; value is 0.29 %w/w of TC and 7.93% w/w of Nagakesara. Water soluble ash indicates percentage of solubility of contents of the sample in water, values are 2.89% w/w of TC and 1.01 % w/w. Water soluble extractive values are 14.94 of TC and 4.64% w/w of Nagekesara and alcohol soluble extractive values are 6.07 of TC and 10.90 % w/w of Nagakesara which indicates alcohol soluble constituents such as tannins, alkaloids. HPTLC of Trikatu curna contains piperine and gingerol as chemical constituents at 0.36 Rf of 366nm[3] and 0.50 Rf of 254nm [4] respectively. HPTLC of Nagakesara contains eugenol as chemical constituent at 0.86 Rf of 254nm [5] when compared with standard HPTLC reports of piperine, gingerol and eugenol.

Conclusion

Physicochemical analysis and HPTLC of Pippalyadi yoga provides substantial information for the proper identification, authenticity, quality and purity of the final product/drug. On the basis of observations made and results of study, this study may be beneficial for future researchers and can be used as a reference standard in the further quality control researches.

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