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Physiochemical Analysis of Nilotpaladi Yoga

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ABSTRACT

In Ayurveda many Ayurvedic formulations are explained, which used to treat a variety of illness since ancient time and to protect patient faith in Ayurveda and Ayurvedic treatment, scientifically proven safety, standard and quality of herbs is the need of the hour. Nilotpaladi yoga (NY) is mentioned in Ayurvedic texts for the Treatment of Raktatisara (−haemorrhagic diarrhoea). Nilotpaladi yoga consists of four ingredients such as Nilakamala (Nymphaea nouchali Burm. f.), Mocharas (Salmalia malabarica Schott & Endl.), Lajavanti (Mimosa pudica Linn.) and Kamalakesara (Nelumbo nucifera Gaertn.). Nilotpaladi yoga ingredient like Mochras (Salmalia malabarica Schott & Endl.) is known to be effective as Shonita sthapaka gana (a group of drugs that act as haemostatics). Physiochemical analysis of Nilotpaladi yoga is not explored in detail. So, this article is an attempt to present a physiochemical analysis of ingredients of Nilotpaladi yoga by using Modern analytical Techniques. Physio-chemical analysis, HPTLC etc., were carried out as per standard methods.

INTRODUCTION

In Charaka Samhita, NilotpaladYoga is mentioned for the treatment of Raktatisara (Shukla and Tripathi, 2002b). Ayurveda described Raktatisara (−haemorrhagic diarrhoea), which has symptoms similar to ulcerative colitis, i.e. Shula (−pain in abdomen), Gudapadaka (−burning sensation in rectum) and Trishna (−excessive thirst) (Shukla and Tripathi, 2002a). Flowers of Nymphaea nouchali were found antibacterial functions against bacteria like Bacillus cereus and Staphylococcus aureus (Vasu and Singaracharya, 2008). Some Studies also indicated that Mochras (Salmalia malabarica Schott & Endl.) has haemostatic, antidiarrhoeal, anti-inflammatory, and antipyretic properties (Williamson, 2002; Singh, 2005). The Mocharas (Salmalia malabarica Schott & Endl.) is known to contain tannin and gallic acids, which acting as astringents and precipitate proteins which is helpful in the healing of the damaged mucosal lining of ulcerated mucosa (Jagtap et al., 2011; Hussain et al., 2015). The Nelumbo Nucifera is used to treat bleeding, gastritis and has anti-inflammatory effects (Bensky et al., 2015; Yang et al., 2007). Nelumbo leaf extract (NLE) was also shown antibacterial activity (Li and Xu, 2008).

MATERIALS AND METHODS

Nilotpaladi yoga (NY) ingredients were purchased from the Gola dinanath market of Varanasi.
identity was confirmed with the help of Sri Dharmasthala Manjunatheshwara centre for research in Ayurveda and Allied Sciences (AYUSH Centre for Excellence and Recognized SIROs by DSIR) Laxminarayana Nagar, P.O. Kuthpady–UDUPI Karnataka. Nilotpaladi yoga ingredients means gum of Salmalia malabarica Schott & Endl., Seeds of Lajavanti (Mimosa pudica Linn.), Stamen of Kamalakesara (Nelumbo nucifera Gaertn.) and petals of Nilakamala (Nymphaea nouchali Burm. f.) were converted into Churna (~powder form). Samples were sent to Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and allied Sciences (AYUSH Centre for Excellence and Recognized SIROs by DSIR). Kuthpady, UDUPI Karnataka, where Physicochemical parameters as depicted in (Table 1) were estimated as per Standard methods described in Ayurvedic Pharmacopeia of India and world health organization standard and HPTLC values were evaluated by using Linomat 5 TLC applicator.

**Methodology of Various Parameters and Results**

**Loss on drying at 105°C**

10 g of each ingredients sample of Nilotpaladi yoga were taken separately in a separate tared evaporating dish. It was then dried at temperature 105°C for 5 hours in the oven and then weighed. The drying continued till the difference between two continuous weights didn’t more than 0.01 after cooling by using a desiccator. Moisture percentage calculated in relation to the weight of the sample.

**Total Ash**

2 g of sample was incarnated in tared platinum crucible at a temperature not exceeding 450°C until carbon-free ash is obtained. The percentage of ash was calculated in relation to the 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 a 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 a 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.

**HPTLC**

1g of each of Nilakamala, Mocharasa, Lajavanti, Kamalakesara and Churna formulation (1part of each of the ingredient) was extracted with 10 ml of alcohol. 5µl of each of the above extract was 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 (9:0. 1.0). The developed plates were visualized in under short UV, long UV and then derivatised with vanillin sulphuric acid and scanned under UV 254nm, 366nm and 620nm (the following derivatisation). Rf, colour of the spots and densitometric scan were recorded (Tables 2, 3 and 4) and Figures 1 and 2 a-d, Figure 3 a-d & Figure 4 a-d).

**RESULTS AND DISCUSSION**

Physiochemical analysis is an important tool to find out the quality, standard and efficacy of Ayurvedic drugs and by correct identification, it can be helpful in preventing adulteration (Srikanth et al., 2019). Ash values used to be an important tool to assess the purity of herbs. The Ash and extractive values is the fastest means of determining the quality and efficacy of Herbs. Acid insoluble ash is a part of total ash and tells us the amount of siliceous earth (Srikanth et al., 2019). Physiological Parameters evaluation of Nilotpaladi yoga is depicted in (Table 1). Loss of drying was maximum seen in Mocharasa (Salmalia malabarica Schott & Endl.) (10.97%) and minimum in Lajavanti (Mimosa pudica Linn.) (6.77%). Total ash values maximum Nilakamala (Nymphaea nouchali Burm. f) (10.83) and minimum Lajavanti (Mimosa pudica Linn.) (5.44%). Each Nilotpaladi yoga ingredients Standardization Parameters Results depicted in (Table 1).

With HPTLC, authentication of many types of plant possible, as well as the analysis of stability and con-
Table 1: Results of *Niltpaladi yoga* ingredients standardization parameters

| Parameter                               | Nilakamala | Mocharasa | Lajavanti | Kamalakesara |
|-----------------------------------------|------------|-----------|-----------|--------------|
| %w/w (Avg)                              |            |           |           |              |
| Loss on Drying                          | 9.46       | 10.97     | 6.77      | 9.22         |
| Total Ash                               | 10.83      | 8.22      | 5.44      | 7.18         |
| Acid Insoluble Ash                      | 0.89       | 3.47      | 0.29      | 2.58         |
| Water soluble Ash                       | 4.08       | 3.18      | 0.29      | 2.67         |
| Alcohol soluble extractive value        | 4.88       | 12.40     | 16.59     | 5.06         |
| Water soluble extractive value          | 14.19      | 20.47     | 47.80     | 16.03        |

Table 2: *R*$_f$ values At short UV

| Nilakamala  | Mocharasa | Lajavanti | Kamalakesara |
|-------------|-----------|-----------|--------------|
| -           | -         | -         |              |

*D– dark; L – light; F – fluorescent*
Figure 2: Densitometric scan at 254nm

Table 3: Rf values At long UV

|             | Nilakamala | Mocharasa | Lajavanti | Kamalakesara |
|-------------|------------|-----------|-----------|--------------|
| -           | -          | -         | -         | -            |
| -           | -          | -         | -         | -            |
| 0.38 (F red)| 0.48 (F aqua blue) | -         | 0.48 (F green) |
| 0.48 (F blue) | 0.55 (FD blue) | 0.55 (FD blue) | 0.55 (F blue) |
| 0.69 (F blue) | 0.69 (FL blue) | 0.69 (FL blue) | 0.69 (F blue) |

*D– dark; L – light; F – fluorescent
Figure 3: Densitometric scan at 366nm

Table 4: \( R_f \) values After derivatisation

|               | Nilakamala | Mocharasa | Lajavanti | Kamalakesara |
|---------------|------------|-----------|-----------|--------------|
| Nilakamala    | -          | -         | -         | -            |
| Mocharasa     | -          | -         | -         | -            |
| Lajavanti     | -          | -         | -         | -            |
| Kamalakesara  | -          | -         | -         | -            |

*D – dark; L – light; F – fluorescent

sistency of their preparations from various man-
ufactures (Dhalwal et al., 2008). HPTLC Finger-
printing, by examining \( R_f \) values and color of
the spots, is one of the easiest pharmacopeial pa-
rameters to evaluate the qualitative compositional
characteristics of any crude drug extracts. HPTLC of
ethanolic extract of Nilpotpaladi Yoga TLC photo
documentation confirmed the detection of various pho-
toconstituents with separate \( R_f \) values (Figure 1,
Tables 2, 3 and 4). HPTLC photo documentation of
Alcoholic fraction of Nilakamala, Mocharasa, Laja-
vanti, Kamalakesara Churna (Figure 1), under Short
UV and After derivatization showed no any spots
(Figure 1). Therefore, \( R_f \) Value at short UV and
after derivatization can not be observed (Figure 1,
Tables 2 and 4). HPTLC photo documentation at
Long UV of Nilkamala showed 3 spots (in differ-
cent colors) at \( R_f \) Values ranging from 0.38 to 0.69.
Mocharasa (Salmalia malabarica Schott & Endl.)
showed 2 spots (both F.blue) at \( R_f \) values ranging
Lajavanti (Mimosa pudica Linn.) showed 2 spots (F. Dark & F. Light) at Rf values ranging from 0.55 to 0.69. Kamalakesara (Nelumbo nucifera Gaertn.) showed 5 spots (in different colors) at Rf values ranging from 0.08 to 0.69 (Figure 1, Table 3).

**High-performance thin-layer chromatography**

Nilakamala (Nymphaea nouchali Burm. f.). On the densitometric scan of the plates, 3, 9 and 10 bands were detected under 254nm, 366nm and 620nm, respectively (Figure 2a, Figure 3a & Figure 4a). Out of 3 peaks seen on densitometric scan at 254nm, compounds with Rf 0.03 (78.31%) was the major peaks (Figure 2a); at 366, out of 9 peaks, peak with Rf 0.67 (41.26%) was the major peak detected (Figure 3a); at 620nm, out of 10 peaks, peak with Rf 0.08 (34.09%) was the major peak seen (Figure 4a).

Mocharasa (Salmalia malabarica Schott & Endl.). On a densitometric scan of the plates, 3, 8 and 5 bands were detected under 254nm, 366nm and 620nm, respectively (Figure 2b, Figure 3b & Figure 4b). Out of 3 peaks seen on densitometric scan at 254nm, compounds with Rf 0.03 (92.92%) was the major peaks (Figure 2b); at 366, out of 8 peaks, peak with Rf 0.66 (37.26%) was the major peak detected (Figure 3b); at 620nm, out of 5 peaks, peak with Rf 0.05 (89.80%) was the major peak seen (Figure 4b).

Lajavanti (Mimosa pudica Linn.) on densitometric scan of the plates, 9, 3 and 5 bands were detected under 254nm, 366nm and 620nm, respectively (Figure 2c, Figure 3c & Figure 4c). Out of 3 peaks seen on densitometric scan at 254nm, compounds with Rf 0.03 (78.31%) was the major peaks (Figure 2c); at 366, out of 9 peaks, peak with Rf 0.67 (41.26%) was the major peak detected (Figure 3c); at 620nm, out of 10 peaks, peak with Rf 0.08 (34.09%) was the major peak seen (Figure 4c).
pounds with $R_f$ 0.04 (52.85%) was the major peaks (Figure 2c); at 366, out of 3 peaks, peak with $R_f$ 0.66 (57.64%) was the major peak detected (Figure 3c); at 620nm, out of 5 peaks, peak with $R_f$ 0.07 (34.05%) was the major peak seen (Figure 4c).

*Kamalakesara* (*Nelumbo nucifera* Gaertn.) on a densitometric scan of the plates, 4, 5 and 7 bands were detected under 254nm, 366nm and 620nm, respectively (Figure 2d, Figure 3d & Figure 4d). Out of 4 peaks seen on densitometric scan at 254nm, compounds with $R_f$ 0.04 (64.48%) was the major peaks (Figure 2d); at 366, out of 5 peaks, peak with $R_f$ 0.65 (60.34%) was the major peak detected (Figure 3d); at 620nm, out of 7 peaks, peak with $R_f$ 0.04 (39.10%) was the major peak seen Figure 4d). These fingerprints will be helpful to serve the purpose of evaluation of the identity of chemical composition qualitatively.

**CONCLUSION**

The results and the standards used in Physiochemical analysis and HPTLC findings of *Nilotpaladi Yoga* ingredients surely be useful as an important part for validation and quality checking of crude drugs used in NY. This study may be useful for Researches etc. As this article is only dealt with Physio-chemical analysis, the implementation and outcome of this Nilotpaladi Yoga will be published separately.

**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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