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Research Article

ANALYTICAL STUDY OF *NATAKUSTHADI YOGA* WITH SPECIAL REFERENCE TO THEIR *VISHAGHNA* EFFECTS

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ABSTRACT

Nata & *Kushta* medicinal plants are easily available in Indian diaspora and both are widely used in many *Agada* as *Vishaghna dravya* mentioned in classical texts. Both have reported acetylcholinesterase and Phospholipase A2 enzyme inhibiting property in many Research studies. The quality of *Nata, Kustha & Nata Kustha* Yoga was evaluated based on organoleptic, physico-chemical, and microbial quality parameters. Loss on drying at 105°C, Total ash, Acid Insoluble ash, Alcohol-soluble extractive, Water-Soluble extractive, HPTLC and pH value parameters were evaluated as per WHO guidelines, Ayurvedic pharmacopoeia and Indian pharmacopoeia. Analytic analysis of *Nata, Kustha & Nata Kusthadi Yoga* in this study found to be under standard limit. Standardization test done on *Natakusthadi Yoga* helped in authenticating the preparation and in ensuring quality of the same. The *Natakusthadi Yoga* prepared by following standard operative procedures should have Loss on drying at 105°C, Total ash, Acid Insoluble ash, Alcohol-soluble extractive, Water-Soluble extractive and pH value is 2.1%, 4.8%, 0.32%, 42.9%, 37.8% & 5.5 respectively.

INTRODUCTION

Since thousands of years plants have played a critical role in maintaining human health and civilizing the quality of human life. The use of various plants as medicines is as old as human civilization itself and out of about 258,650 species of higher plants reported from the world and more than 10% are used to cure ailing many communities. For primary healthcare people in many parts of world are still rely on traditional plant-based medicines. This is especially true for many rural communities in India where plants and knowledge of their traditional use are accessible and affordable.[1]

The anti-snake venom plants contain secondary metabolites that are responsible for venom neutralization.

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Most of the rural population in India and many parts of world are still rely on single plant or in combination as antidotes for snake envenomation. In most developing countries, up to 80% of individuals bitten by snakes first consult traditional practitioners before visiting a medical centre. For the treatment of snakebite in rural areas several traditional herbal medicines are easily available. Even today, indigenous and certain local communities practice herbal medicine to cure a variety of diseases, with plants particularly used as folk medicine to treat snakebites.[2]

Natakusthadi yoga is one of the herbal combination mentioned in *Charak Samhita*, *Chikitsasthan, Vishchikitsa* (23/194) & *Astang Hridya, Uttarsthan, Sarpvishpratished Adhyaya* 36/73, used for treatment of snake bite leading to severe risk of life. *Natakusthadi* yoga is prepared by the equal quantity (1 *Pala* each) of powder of *Tagar* (*Valeriana Wallichii* DC) and *Kustha* (*Saussurea Lappa* C.B. Clarke) added with Ghee and *Madhu* (2 *Pala* each) and consumed internally to destroy the *Takshak Sarp Visha*. In this herbal formulation, *Tagar* & *Kustha* are two major contents.

Tagara (*Valeriana wallichii*) in Ayurveda is a hairy perennial herb which is belongs to Valerianeaceae family, it grows in the temperate regions of the Himalayas and Khasia hills up to an altitude of 3,000m.[3] The plant leaves are hairy herb grown up to 45cm in height. Rootstalk is thick, long-petioled, cordate, and ovate, horizontal, and usually sinuate, 2.5-2.75cm in diameter, cauline leaves only a few, much smaller, entire or pinnate, often crowded stipules nil. Flowers of Valerian are deciduous, white to pink, in terminal corymbs and unisexual, male, and female in different plants.[4]

It has been used in Ayurveda as a medicine for various ailments and disorders from centuries. Their root has been used in the form of powder in a dose of 1-3g. Roots of *Tagara* contain Valerinic acid, Valepotriates which has been used as sedative and tranquilizers. Essential oils were usually obtained from the root and dried rhizomes. The essential oil contains sesquiterpene, valeric acid, camphene, terpineol and terpene alcohol. [5]

Saussurea lappa Clarke belongs to the family Compositae. It is a well identified medicinal plant and used in many medicines all over the world. It is a long erect herb found mostly in Northern mountainous regions of Pakistan and India.[6] Its flowers are dark purple or black in color, occupying terminal and axillary heads. Pappas is long, fluffy, feathery and fruit is cupped, curved, compressed and hairy. Leaves are radical with long lobately winged stalks. Different types of chemical compounds are isolated from the plant body and mainly the roots, these chemicals form many bioactive substances. Roots are stout, carrot like, 60cm long, possessing a characteristic penetrating sweet aromatic odour along with bitter taste.[7] Costunolide is one of the major bioactive constituents of *Saussurea lappa* root.[8-9]

Nata & Kushta medicinal plants are easily available in Indian diaspora and both are widely used in many *Agada* mentioned in classical texts. Both have reported acetylcholinesterase and PhospholipaseA2 enzyme inhibiting property in many Research studies. Present study is conducted to evaluate Organoleptic, Physicochemical & Microbiological analysis of *Natakusthadi Yoga*.

AIM

Study of the *Natakusthadi Yoga* on the basis of organoleptic, physico-chemical and Microbial quality parameters.

OBJECTIVE

1. To assess the organoleptic & physico-chemical observation.
2. To assess the Microbial Load.

MATERIAL & METHOD

Study Design Analytical study

Material

1. Collection of Drugs

The samples of Raw *Nata* and *Kustha* (rhizomes) were collected from Market.

2. Identification and authentication of study material

The raw samples of *Nata* and *Kustha* (Rhizomes) were identified and authenticated from Dravya Guna Department of G S Ayurveda Medical college and Hospital, Pilukhwa, Hapur, Uttar Pradesh.

METHOD

Drug Preparation

Drug prepared in Pharmacy of G S Ayurveda Medical College and Hospital, Pilukhwa, Hapur, Uttar Pradesh. *Nata* and *Kustha* were med into fine powder and sieved through 85 number mesh. Powdered drug is stored in air tight glass container, for further use.

Standardization of *Natakusthadi Yoga*

Test for Standardization of *Nata, kustha* & *Natakusthadi Yoga* was performed in Dattatraya Ayurveda Rasashala, Mahatma Gandhi Ayurved College, Hospital & Research Centre Salod (H), Wardha, DMIMS (Deemed University).

Organoleptic Parameter

It means the study of drug by using sense organs. The methods of analysis like colour, odour, taste, size, shape and special features, such as touch, texture, etc. the initial sight of the plant or extract is so specific that it tends to identify itself. Every plant or extract has a characteristic odour or taste. *Nata, kustha & Natakusthadi Yoga* were evaluated on (colour, odor & taste) parameters.

Physicochemical Parameter

1. Loss on drying at 105°C

Prepare the samples of *Nata* & *Kustha* 10-15gm each by cutting shredding into 3-4mm thickness. Then placed the samples in tared evaporating dish and dry at 105°C for 5-6 hours and weight. Continue the drying and weighing at one hour interval until difference two successive weighing corresponded to not more than 0.25%.

2. Total Ash

Incinerate continuously accurately weighed 3 gm of *Natakusthadi* Yoga in a silica dish at a temperature of 450°C until a constant weight was obtained.

3. Acid Insoluble Ash

Ashes of sampled drug (*Natakusthadi* Yoga) was taken with 25ml dilute Hydrochloric acid in a beaker boiled for 5 minutes then cooled. After that filtered the solution through filter paper (Whattman 41) and wash it with hot water repeatedly till filtrate become chloride free. Transferred the filter paper containing insoluble matter in the oven in a glass funnel for drying. Then dried paper along with

insoluble matter shifted to pre weighted muffle furnace and heated upto 600°C. The residue allow to cool in a suitable desiccator for 30 minutes and weigh without delay. Then calculate the water insoluble ash.

4. **Alcohol -soluble Extractive**

5gm each, coarsely powdered sample of *Nata, Kustha* and *Natakusthadi Yoga* (air dried) was macerated with 100ml of alcohol in a flask and shaking frequently during initial 6 hours and allowed to stand for 18 hours. Thereafter filtered rapidly to minimize the loss of methanol. Evaporate 25mL of filtrate to dryness in a tared flat bottom shallow dish dried at 105°C and weighed. Then Calculate the percentage of alcohol-soluble extractive with reference to the air-dried sample of *Nata , Kustha* and *Natakushtadi Yoga*.

5. **Water-soluble Extractive**

Coarsly powdered and air dried sample of *Nata, Kustha* and *Natakusthadi* Yoga about 5gm transferred into 250ml reflux conical flask, followed by the addition of 50ml of boiled water. The flask was allowed to stand for 10 minutes after well shaken. Cooled and filtered then evaporate the filtrate on water bath and allowed to dry for 30 minutes in oven and weighed. Then calculate the percentage of water-soluble extractive with reference to the air-dried sample of *Nata, Kustha* and *Natakushtadi Yoga*.

6. **HPTLC (High Performance Thin Layer Chromatography)**

An extension of TLC is high-performance thin layer chromatography (HPTLC) which is robust, simplest, rapid, and efficient tool in quantitative analysis of compounds.

This is sophisticated and automated technique of thin layer chromatography. It has superior and advanced separation efficiency with detection limits and is often an exceptional alternative to high-performance liquid chromatography (HPLC) and gas chromatography (GC). HPTLC is also called as flat-bed chromatography or as planar chromatography.

The working principle of HPTLC is 'separation is adsorption' same as used in TLC. The mobile phase or solvent flows through the capillary action and the analytes move towards the stationary phase (adsorbent) according to their affinities. The component which has higher affinity moves slowly and which has lower affinity moves rapidly towards the stationary phase.

Principle and Procedure of HPTLC Chromatography

Experimental Procedure of HPTLC

- Before beginning an HPTLC experiment, we must recognize the various components essential to perform the process.
- A tool suitable for sampling as bands to monitor the size and position of the test, as well as the sample volume applied.
- An appropriate chromatographic chamber which provides developing distance and control of saturation.
- A device appropriate for controlling stationary phase behavior through relative humidity.
- A tool appropriate for reproducible drying of the developed plate.
- Appropriate equipment for reagent transfer and heating.
- A tool for electronic documentation of chromatograms.

Experimental Procedure for HPTLC

i. **Sample Preparation**

This requires a highly concentrated solution since much less sample quantity needs to be applied. The plate's solvents must be non-polar of the volatile type. Polar solvents are commonly used to dissolve samples for reversed- phase chromatography.

ii. **Selection of Chromatographic Layers**

The layer of HPTLC is available in the form of very fine particle size silica gel pre-coats which is widely used as adsorbent.

iii. **Pre Washing**

To water vapor or volatile impurities, the plates must be cleaned. It may be clean with a suitable solvent such as methanol.

iv. **Conditioning**

Plates are placed in an oven at 120°C for 15 to 20 minutes to perform conditioning.

v. **Sample Application**

The size of the sample spot is not greater than 1 mm in diameter. There are various methods for spotting samples in HPTLC. One is a self-loading capillary in which small quantities of samples can apply on the HPTLC plate.

vi. **Pre-Conditioning**

Saturation is necessary for highly polar mobile phases although there is no need for saturation for low polarity mobile phases.

vii. **Mobile Phase of HPTLC**

Through trial and error, the mobile phase of the suitable solvents is to be selective.

viii. Chromatographic Development

The linear development method in high-performance thin-layer chromatography is the most common technique here the plate is positioned vertically in an appropriate container with a solvent or mobile phase. The mobile phase is generally fed by capillary action and both sides may produce chromatograms.

ix. Detection of spot and Scanning

The HPTLC instrument has attached to computer and data recording devices. The development of spots is viewed as peaks at wavelengths of selected UV regions. The height and the area of the peaks are determined by the instrument and recorded as a percentage.

Mobile phase :-	**Rf Value :-**
Toluene : Ethyl Acetate [7:3]	***Tagar* :- 0.57, *Kushta* :-0.63, *Nata-kushtadi*:-0.63**

7. pH Value

pH means the quantitative indication of the acidity or alkalinity of a substance. It is a common logarithm of the reciprocal of the hydrogen ion concentration.

Microbiological Parameter

Some bacteria such as Salmonella typhi, styphylococcus aureus and E. coli are used to determine the antiseptic value (the degree of antiseptic activity e.g. phenol co-efficient of certain drugs). Microbiological assays by cylinder plate method and turbidimetric method were used in evaluation of Total viable count, Enterobacteriaceae, Total fungus count, E-coli, Salmonella, Staphylococcus aureus, Psedomonas aueruginosa.

OBSERVATION AND RESULT

Analysis of *Natakusthadi* Yoga, (Raw Sample 100 gm) and *Nata* & *Kustha* (50gm each).

Table 1: Analysis of *Natakusthadi* Yoga, *Nata* & *Kustha*

S.No.	Test Parameters	*Natakusthadi Yoga* Observation	Raw *Tagar* (*Nata*) 50gm	Raw *Kustha* 50gm
Organoleptic Parameters				
1	Colour	Greenish	Black	Greyish to dull brown
2	Odor	Strong, characteristically aromatic	Strong and reminiscent of isovaleric acid	Strong, characteristically aromatic
3	Taste	Slightly bitter	Bitter and somewhat camphoraceous	Slightly bitter
Physicochemical Parameter				
4	Loss on drying at 105°C	2.1%	5.5%	3.8%
5	Total ash	4.8%	7.4%	2.4%
6	Acid Insoluble ash	0.32%	2.3%	1.2%
7	Alcohol-soluble extractive	42.9%	35.7%	22%
8	Water soluble extractive	37.8%	23.9%	26%
9	pH Value	5.5	5.1	5.2
Microbiological Parameter				
10.	Total Viable Count	Absent	Absent	Absent
11.	Enterobacteriaceae	Absent	Absent	Absent
12.	Total fungus Count	Absent	Absent	Absent
13.	E-coli	Absent	Absent	Absent
14.	Salmonella	Absent	Absent	Absent
15.	Staphylococcus aureus	Absent	Absent	Absent
16.	Psedomonas aeruginosa	Absent	Absent	Absent
High Performance Thin Layer Chromatography				
Mobile Phase:- Toluene: Ethyl Acetate [7:3]			**Rf Value:-** *Tagar*:- 0.57, *Kushta*:- 0.63, *Natakusthadi*:- 0.63	

DISCUSSION

Tagar (*Valeriana Wallichii* DC) and *Kustha* (*Saussurea Lappa* C.B. Clarke) are two medicinal plants which are widely used in many *Agad's* and *Vishnashak yoga's* mentioned in different Ayurvedic text (Table 2).

Table 2: *Agad's* and *Vishnashak yoga's*

S.No.	***Tagar* (*Valeriana Wallichii* DC)**		***Kustha* (*Saussurea Lappa* C.B. Clarke)**	
1.	*Masyadi Yoga*	C. Chi.23/ 190	*Chandnadi yoga*	C.Chi. 23/ 192
2.	*Chandnadi yoga*	C.Chi. 23/ 192	*Takshrya Agada*	Su. K. 5/ 65
3.	*Vyoshadi Yoga*	Ch. Chi. 23/197-198	*Sarvkarmik Agada*	Su. K. 5/ 78-80
4.	*Kutajadi Yoga*	Ch. Chi. 23/206-207	*Rishabh Agada*	Su. K. 5/ 68-72
5.	*Ajit Mahagada*	Su. K. 5/ 64	*Natadi Agada*	A.H.U.36/ 73
6.	*Takshrya Agada*	Su. K. 5/ 65	*Katukadi Agada*	A.H.U.36/67
7.	*Sarvkarmik Agada*	Su. K. 5/ 7880		
8.	*Natadi Agada*	A.H.U.36/ 73		
9.	*Vajra Agada*	A.H.U. 36/ 82&83		
10.	*Bilwadi Agada*	A.H.U. 36/ 84&85		

Being used in many *Vish Nashak* formulations, the standardization of *Natakusthadi Yoga* was conducted by physico-chemical and phyto-chemical analysis.

Both *Nata* and *Kustha* were Identified & authenticated after procurement from market. After disintegrate into smaller parts, *Nata* and *Kustha* were med into fine powder with the help of pulverizer and stored in glass container for further analysis.

Organoleptic evaluation, is a scientific method also referred as sensory analysis. It provides the objective information on consumer experience on product. Sensory evaluation is an invaluable tool for Quality Control as well as Research and Development.

The age old most common and fundamental method for preservation of medicinal plants post-harvest is drying. It allows for the quick conservation of the medicinal qualities of the plant material in simple manner. The loss on drying Test is designed to measure the amount of water and volatile matters in a sample when the sample is dried under specified conditions. e.g., 105°C. If water/moisture content is more likely to get infected by mould and fungi growth in drug. The sample of *Natakusthadi Yoga* showed loss during drying within standard limit which indicates both are authentic drugs.

The purity and quality of a crude drug is determined by Ash values, especially for powdered drug. It remove the traces of organic matter in crude drug which may be interferes in an analytical determination. On incineration, the crude drugs normally produce ash which is usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. Total ash values of *Natakusthadi Yoga* is observed within limit prescribed in API.

The analyzed values of acid insoluble ashes in *Natakusthadi Yoga* were found within considerable range which indicates the standard samples of drug.

Water-soluble ash means the total ash content, which is soluble in water. It indicates the incorrect preparation of drug and previous extraction of water-soluble salts in the drug. The water-soluble ash values of the *Natakusthadi Yoga* were found in the range. It's indicates the normal quality of the drugs.

The adulteration in exhausted and incorrectly processed drugs, quality and purity can be determined by extractive values by different solvents.

The HPTLC procedure was optimized with a view to develop a stability indicating assay method. The solvent system of the mobile phase having toluene: ethyl acetate (7:3, v/v) gave dense, compact and well separated spots of the single herbal ingredient.

pH value refers to acidic and alkaline nature of the drug, which indicate the suitability of drug for human use. The pH values of *Natakusthadi* Yoga was found in standard limit and acceptable for their therapeutic uses.

No microbial growth was found in the samples of *Natakusthadi Yoga*, which indicates strict and sterilized measures has been taken during preparation of drug.

CONCLUSION

Nata and *Kustha* are used in many *Vishnashak Agad*, mentioned in *Agad Tantra* text, so genuineness of the raw drug and their standard preparations are most important. Hence, the analytic analysis of *Natakusthadi* Yoga in this study found to be under standard limit as described in API and this study can be considered for future research. The *Natakusthadi Yoga* prepared by following standard operative

procedures should have Loss on drying at 105°C, Total ash, Acid Insoluble ash, Alcohol-soluble extractive, Water-Soluble extractive and pH value is 2.1%, 4.8%, 0.32%, 42.9%, 37.8% & 5.5 respectively. The microbial loads on the sample of *Natakusthadi Yoga* is found absent which specify the quality of the drug. The analytical specification of *Natakusthadi Yoga* established by this study bridging the gap in between ancient and modern knowledge of *Natakusthadi Yoga*.

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Conflict of Interest: None Declared

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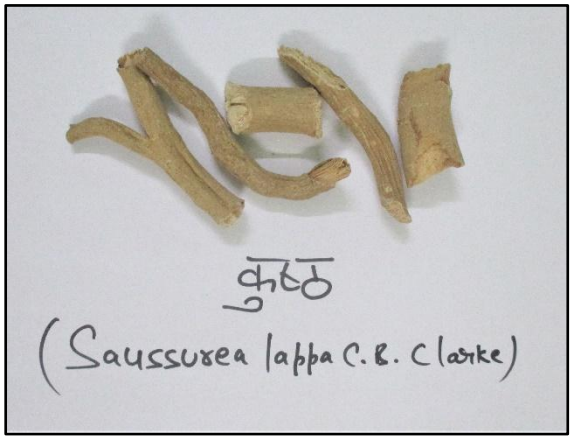
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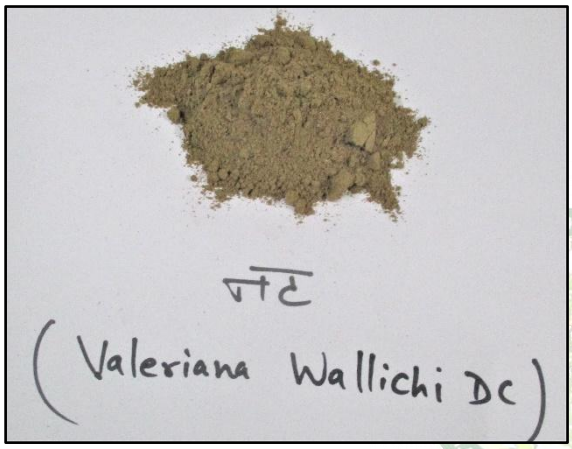
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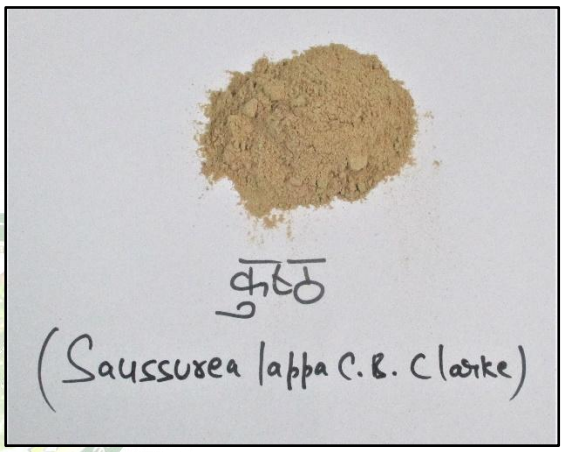
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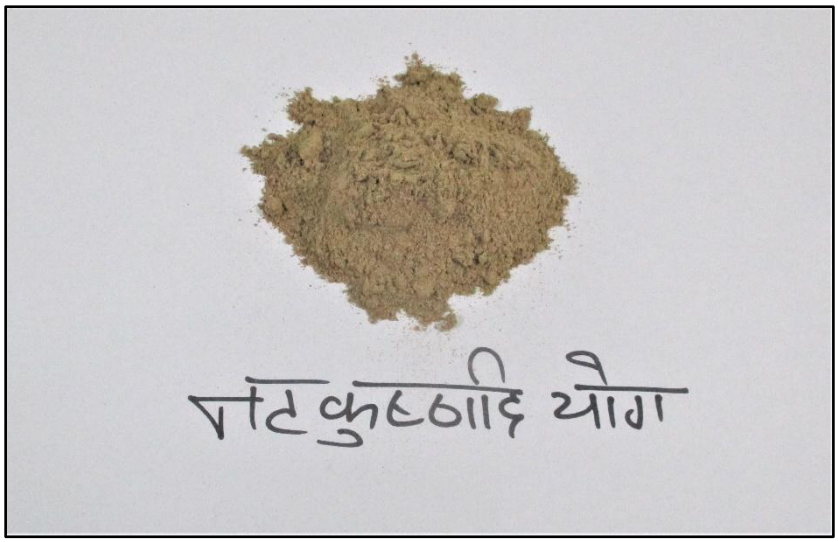
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