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Research Article

ANALYTICAL STANDARDIZATION OF *TRIPHALADI TAILA*

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ABSTRACT

Analytical study plays an important role in the standardization of the drugs. *Ayurveda*, the ancient system of medicine is gaining recognition throughout the world and many herbal, metal and mineral drugs are now clinically tested and accepted. However, one of the impediments in the acceptance of the ancient systems of medical preparation is the lack of standard quality control profiles. The quality of the drugs, that is, the profile of the constituents in the final product has implication in efficacy and safety.

The *Sneha kalpa* are par excellent to other dosage forms due to their wider advantages like increased absorption and extraction of fat soluble active principles. *Sneha kalpas* are the only dosage from which can be administered conveniently both internally as well as externally. *Triphaladi Taila* is an important herbo mineral formulation mentioned in *Rasaratnakara* indicated for the management of the disease *Palitya* as *Kesharanjaka*. The ingredients present in the "*Triphaladi Taila*" are *Sodhita Lauha churna, Triphala churna*, *Bhringaraja swarasa* and *Tila taila. Shodhana, Swarasa nirmana, Kalka nirmana, Churna nirmana* and *Snehapaka* and are the main pharmaceutical procedures employed in the preparation of *Triphaladi Taila*. To assess the safety and to understand the physico chemical properties, organoleptic tests, moisture content, refractive index, fat, Iodine value, pH value, Saponification value, Acid value, ash value, weight/ml, viscosity, iron, volatile oil and microbial tests as well as chromatographically (TLC) for developing standards.

KEYWORDS: *Triphaladi Taila*, Saponification value, Analytical Standardization.

INTRODUCTION

Rasashastra and *Bhaishaja Kalpana* is the branch of Ayurvedic science that exclusively focuses on various aspects of preparation of medicines. It is known to establish change in the qualities and properties of the drug either by inducing a new property or improving the existing one and finally making the drug safe and more effective. *Taila Kalpana* (Medicated oil) is integral part of Ayurvedic treatment and *Taila* can be used for both *Bahya* (Externally) and *Abhyantara Chikitsa* (Internally). Standardization of Ayurvedic formulations is an important step for establishment of biological activity, consistent chemical profile, or quality assurance for production.

In the present study, *Triphaladi taila* was prepared by referring the method described in the text *Rasaratnakara*[1] and further studied organoleptically, physico-chemically and chromatographically for developing standards.

MATERIALS AND METHODS

The process was carried out in two steps:

1. Pharmaceutical Study
2. Analytical Study

Procurement of Raw materials

Lauha was collected from Chennai Market and foreign matter adhering to raw drugs was removed and cleaned. *Haritaki*, *Vibhitaki* and *Amalaki* were collected from Sri Srinivasa Ayurveda Pharmacy, TTD, Tirupati, fresh *Bhringaraja* was collected from the herbal garden, S.V. Ayurveda College, TTD, Tirupati. It was then identified macroscopically and studied for important botanical characteristics. The base, which was used for the preparation of this *Taila* i.e., *Tila taila* was also purchased from local market.

Procedure was carried out in Department of *Rasa Shastra* and *Bhaishajya Kalpana*, S.V. Ayurveda College, TTD, Tirupati.

Pharmaceutical Study

Table 1: The ingredients of the formulation

Name of the content	Quantity
Sodhita Lauha	3 Parts
Triphala churna	3 Parts
Bhringaraja	6 Parts
Tila Taila	6 Parts

- *Shodhita Loha churna* and *Triphala* were taken in equal quantity in a grinder.
- This mixture was made into paste by adding sufficient quantity of water through grinding.
- *Tila taila* was taken in wide mouthed vessel and placed over heating device on moderate fire.
- *Kalka* of *Triphala* and *Sodhita loha churna* was added to the *Tila taila*.
- *Bhringaraja swarasa* was added to *Taila* and the contents were stirred well.
- The heating was continued till the *Khara paka lakshnas* were obtained.
- Then the contents were filtered through a clean cloth to obtain *Triphaladi taila*.
- An earthen pot was taken, and innerside of the pot was smeared with *Ghrita*.
- Prepared *Triphaladi Taila* was poured into this earthen pot.
- Then pot was covered with lid and the joints were sealed with cloth and multanimitti. It was then dried.
- The pot was placed in a pit.
- Later, pit was covered completely with mud and kept undisturbed for 1 month.
- After one month earthen pot was taken out from the pit.
- Then *Triphaladi Taila* was transferred in to bottles.

Analytical study

The following studies were carried out in the college laboratory.

Organoleptic Characters

Organoleptic tests helps in providing basic information about drugs. This generally includes tests that can be done by one's sensory organs

Table 2: Showing result of organoleptic test of *Triphaladi Taila*

S. No.	Parameter	Observation
1.	Colour	Brown color
2.	Taste	Metallic
3.	Odour	Like *Bhringaraja*
4.	Touch	Unctuous
5.	Appearance	Viscous liquid

Determination of pH value

The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in gm/litre. The pH value of a liquid can be determined potentiometrically by means of the pH meter.[2]

Determination of Refractive Index

The refractive index of a substance is the ratio of the sine of the angle of incidence to the site of the angle of refraction. In other words, it is the ratio of the velocity of the light in vacuum to the velocity in the substance or a chosen media. Refractive index of oils increases with the increase in unsaturation and also chain length of fatty acids. [3]

Loss on drying [4]

An excess of water in medicinal plant materials will encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis. Limits for water content should therefore be set for every given plant material. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water. It is the loss in weight of sample after heating at 105°C until it attains constant weight.

Determination of Iodine Value [5]

The Iodine value of a substance is the weight of iodine absorbed by 100 part by weight of the substance.

$$\text{Iodine value} = \frac{(b-a)\ X\ 0.01269\ X\ 100}{W}$$

Where 'W' is the weight in gm of the substance taken.

The iodine value is a measure of the amount of unsaturation (number of double bonds) in a fat.

Determination of Saponifiction Value [6]

The saponification value of an oil or fat is defined as the number of milligrams of Potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1grm of the sample.

$$\text{Saponification value} = \frac{(b-a)X\ 0.02805\ X\ 1.000}{W}$$

Where 'W' is the weight in gm of the substance taken.

Determination of Acid Value [7]

The acid value of an oil or fat is defined as the number of milligrams of Potassium Hydroxide required to neutralize the free acid in one gram of the sample.

$$\text{Acid value} = \frac{a\ X\ 0.00561\ X\ 1000}{W}$$

Where 'a' is the number of ml of 0.1 N potassium hydroxide required and 'W' is the weight in gm of the substance taken.

Determination of Ash Value [8]

It is the total quantity of ash obtained by igniting the sample at 450°C until it gets free from carbon.

Water soluble Ash Value

It is the percentage of water soluble content of the sample which is soluble in non-ionic water.

This procedure determines quantity of water soluble content contributing to the weight of ash.

$$\frac{weight\ of\ ash\ dissolved\ in\ water}{weight\ of\ sample\ taken} \times 100 = \%\ value\ of\ water\ soluble\ ash$$

Weight / ml at 40°C

The weight per millilitre of a liquid is the weight in gm of 1 ml of a liquid when weighed in air at 40^0, unless otherwise specified. [9]

Determination of Viscosity [10]

Viscosity is the quantity that describes a fluid's resistance to flow. Fluids resist the relative motion of immersed objects through them as well as to the motion of layers with differing velocities within them.

Viscosity is a property of a liquid, which is closely related to the resistance to flow.

$$\text{Kinematic Viscosity} = \frac{\text{Dynamic viscosity}}{Density}$$

Determination of Volatile oil

Volatile oils are characterized by their odour, oil like appearance and ability to volatize at room temperature. Chemically, they are usually composed of mixtures of, for example, monoterpenes, sesquiterpenes and their oxygenated derivatives. Aromatic compounds predominate in certain volatile oils. Because they are considered to be the "essence" of the plant material, and are often biologically active, they are also known as "essential oils". The term "volatile oil" is preferred because it is more specific and describes the physical properties.[11]

Microbiological Tests

Microbial contamination is determined by the total viable aerobic count, which is the sum of the bacterial count and the fungal count. The tests allow quantitative enumeration of mesophilic bacteria and fungi that may grow under aerobic conditions. Membrane filtration, plate count methods and most-probable-number method are used for determination of total viable aerobic count. According to IP the acceptance limit for this is not more than 10^3 bacteria and not more than 10^2 fungi per gm or ml of the preparation.

Chromatographic Study of TLC

Thin- layer chromatography is particularly valuable for the qualitative determination of small amounts of impurities. As it is effective and easy to perform, and the equipment required is inexpensive, the technique is frequently used for evaluating medicinal plant materials and their preparations.[12]

RESULT

Table 3: Showing results of analytical tests of *Triphaladi Taila*

pH value	5
Refractive index at 40 deg C	1.46500
Fat, w/w	99.74%
Moisture content	0.26 %
Iodine value	105.78
Saponification value	175.30
Acid value	1.70
Ash value, w/w	0.17%
Weight / ml at 40 deg C	0.9319 g
Viscosity	46.64 (Oswald viscometer 0%)
Iron as Fe	0.01
Volatile oil	0.64 %
Total bacterial count	Less than 10 CFU/ml
Total fungal count	Less than 10 CFU/ ml

DISCUSSION

- Organoleptic tests of a substance are done with the help of sense organs. It plays a major role when the medicine is ingested orally.
- Brown colour of the final product is due to *Triphala.*
- Taste of *Triphaladi Taila* is metallic.
- Odour of *Bhringaraja swarasa* dominated when compared with other ingredients.
- Viscous nature of the drug indicates the proper formation of *Triphaladi Taila.*

Physico- Chemical tests

Investigating Physico-Chemical systems makes possible to determine the nature of interactions between the components of sample through a study of relationship between sample physical properties and composition.

a. pH

pH value of *Triphaladi Taila* is **5** being slightly acidic, which lies within the normal pH of human skin i.e., between 5 – 6.5.

b. Refractive index

Refractive index is a fundamental physical property of a substance often used to identify a particular substance, confirm its purity, or measure its concentration. Value of Refractive Index at 40^{o} C of *Triphaladi Taila* is 1.46500.

c. Acid value

The acid value indicates presence of free fatty acid in the oil which is responsible of rancidity of compounds; higher the free fatty acid more is the rancidity, this helps to decide the shelf life of the oil; acid value for *Triphaladi taila* was found to be 1.

d. Ash value

To estimate the Total Ash value, the drug was kept in muffle furnace at temperature of 450°C. Ash value indicates the presence of inorganic contents. Total Ash value of *Triphaladi Taila* was 0.17 w/w.

e. Saponification value

Saponification value is a measure of the average molecular weight of all the fatty acid present. The long chain fatty acids found in fats have low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat as compared to short chain fatty acids. In general, saponification value of plant origin oils ranges from 188- 196 mg/g. Saponification value of *Triphaladi Taila* is 175.30.

f. Iodine value

Iodine value indicates the degree of unsaturation of oil; greater the degree of unsaturation higher will be the possibility of absorption and atmospheric oxidation leading to rancidity. The more iodine number, the more unsaturated fatty acid bonds are present; unsaturated fatty acid better absorbed than saturated fatty acids, the iodine value of *Triphaladi taila* was found to be 105.78.

g. Loss on drying

Loss on drying signifies the amount of residual water in the finished product. Temperature was set to 105°C to facilitate complete evaporation of water. This value determines the quantity of moisture a given sample contains. Stability, shelf life and microbiological safety depend on this value. Loss on drying at 105^{0}C, of *Triphaladi Taila* is 0.26%.

h. Determination of Iron as Fe

Determination of Iron as Fe in *Triphaladi Taila* is 0.01.

i. Fat value

Fat / water soluble active principles of drugs are extracted into *Taila.* Total fat value of *Triphaladi Talia* is 99.74% w/w.

j. Weight / ml at 40^{o}C

The weight per millilitre of a liquid is the weight in grams of 1 ml of a liquid when weighed in air at 40^{o}C. Weight/ml at 40^{o}C of *Triphaladi Taila* is 0.9319 g/ml.

k. Determination of volatile oil

Volatile oils are characterized by their odour, oil – like appearance and ability to volatize at room temperature. Aromatic compounds predominate in certain volatile oils. Because they are considered to be the "essence" of the plant material, and are often biologically active, they are also known as "essential oils". Volatile oil content of *Triphaladi taila* is 0.64%.It indicates aromatic compounds present in *Triphaladi taila*.

l. Viscosity

Viscosity is index of resistance offered by the surface to flow a liquid; higher the viscosity of a liquid, greater is the resistance to flow, if viscosity of the oil preparation is increased, the rate of absorption decreases. If oil is less viscous this means rate of absorption is very much high; viscosity of *Triphaladi taila* is found to be 46.64 cps.

Microbiological Tests

The *Triphaladi Taila* was subjected for microbial contamination test to rule out the presence of pathogens in the preparation which may affect the efficacy and stability of the *Taila.* The test revealed that the *Triphaladi Taila* was free from bacterial and fungal contamination indicating it can be used safely in therapeutics.

Total bacterial count and fungal count of *Triphaladi Taila* is less than 10 CFU/ml.

Chromatographic Study of TLC

TLC is a simple, quick, and inexpensive procedure that gives a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the Rf of a compound is compared with the Rf of a known compound (preferably both run on the same TLC plate). The Rf value is the retention factor, or how far up a plate the compound travels

In Thin-layer chromatography gallic acid and alkaloids is observed. The fruit of *Haritaki*, *Vibhitaki* and *Amalaki* herbs contains tannic acid and gallic acid.

Gallic acid is an organic acid. Gallic acid is a trihydroxybenzoic acid, a type of phenolic acid. Hair dyes utilising gallic acid is a safe alternative. It is found both free and as part of hydrolysable tannins. it is a powerful antioxidant.

Formula of Gallic acid: $C_7H_6O_5$

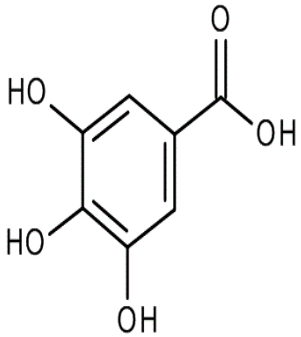

Image: TLC Chromatogram under short UV Gallic Acid $C_7H_6O_5$

CONCLUSION

Triphaladi taila is one of the *Kesharanjana taila* detailed method of preparation is available in *Rasaratnakara*. With that classical reference in backdrop, *Triphaladi taila* was prepared in lines with standard operating procedures (SOP) and subjected for different analysis thereon. The result of analytical study with TLC has been proposed as a monograph to identify and check quality of *Triphaladi taila*.

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