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Evaluation of *Bilva Taila* prepared on the principle of *Snehapaka* through Quality Control Metrics

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ABSTRACT

The globalization of Ayurveda has led to an increased focus on the standardization and quality control of traditional formulations like *Sneha Kalpana*. It involves the process of infusing fats with herbal properties to enhance their therapeutic effects. Certain principles were given by *Acharya* Sharangdhara for the preparation of *Sneha Kalpana. Bilva Taila* was taken as an example of *Sneha Kalpana* and two samples were prepared according to the *Snehapaka* principles by two different references. This study aims to evaluate *Snehapaka* principle through the quality control metrics of *Bilva Taila.* The organoleptic characters, physico-chemical parameters, phyto-chemical screening, Marmelosin quantification through HPTLC analysis and heavy metal analysis of raw material i.e. *Apakva Shushka Bilva Phala* (Unripe Bael fruit powder), *Tila Taila* (Sesame oil) and finished products (Both the samples of *Bilva Taila*) were done. The results showed that the pH value for all three samples of *Taila* was similar, while all other physico-chemical parameters varied between samples. Phyto-chemical screening revealed the presence of various functional group in different samples. Marmelosin quantification also vary in different samples. Heavy metal analysis confirmed that all samples met the permissible limits for lead, arsenic and mercury, indicates the quality and safety for therapeutic use. The study concludes that there is difference found in preliminary analysis, Phyto-chemical screening and marmelosin quantification of both the samples of *Bilva Taila* prepared on the principle of *Snehapaka.*

Key words: *Snehapaka principles, Preliminary analysis, Phyto-chemical screening, Marmelosin quantification*

INTRODUCTION

The globalization of Ayurveda is currently expanding at a rapid rate. The entire world is ready to follow this traditional healthcare system as well. People all around the world are awakened regarding the necessity of drug standards and use every resource at their access to discover safe and effective medications. They also realized that it was essential to explore quality control of both raw material and finished product virtually.

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Sneha Kalpana is an important concept in Ayurveda, primarily related to the preparation of medicated oils and ghee. It involves the process of infusing fats with herbal properties to enhance their therapeutic effects. Certain principles were given by *Acharya Sharangdhara* for the preparation of *Sneha Kalpana.*[1] There are many *Taila* and *Ghrita* formulations in the classics which do not cite the proportion of liquid media and the use of four times of water to *Sneha* for proper extraction of active ingredients. Therefore, it is necessary to evaluate the difference between both the samples. In this study *Bilva Taila* was taken as an example of *Sneha Kalpana* and two samples were prepared according to the *Snehapaka* principles by two different references i.e., *Chakradatta*[2] and *Sharangdhara Samhita*.[3]

The implication of the most recent analytical techniques is the need for time to standardize various formulations of *Sneha Kalpana*. This process involves the evaluation of organoleptic parameters and the conduct of preliminary analyses, including pH, viscosity, specific gravity, acid value, saponification value, refractive index, iodine value, and qualitative

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phytochemical screening. Additionally, the quantification of marmelosin using high-performance thin layer chromatography (HPTLC) and heavy metal analysis are performed. By systematically applying and documenting these analytical parameters, the quality, safety, and efficacy of *Sneha Kalpana* formulations can be effectively maintained. The study aims to assess the *Snehapaka* principle through the quality control parameters of *Bilva Taila*.

Materials and Methods

Procurement of raw material

Apakva Bilva Phala (unripe Beal fruits) (*Aegle Marmelos* Corr.) were procured from the local traders of Umarpada, Gujarat in October 2022 and labelled as UBFP**.** *Tila Taila* (sesame oil) was procured from the Government Ayurved Pharmacy, Rajpipala, Gujarat and labelled as TT.

Preparation of *Bilva Taila*

All the samples of *Bilva Taila* were prepared in the pharmaceutical laboratory of Rasashastra and Bhaishajya Kalpana Department of Government Ayurved College, Vadodara, Gujarat. Sample 1 of *Bilva Taila* was prepared according to the reference of *Chakradatta* (BTC) in the ratio of *Bilva Kalka*, *Tila Taila* and *Godugdha* were 1:4:16 parts respectively.[4] while Sample 2 of *Bilva Taila* was prepared according to the reference *Sharangdhar Samhita* on the principle of *Snehapaka* (BTS) in the ratio of *Bilva Kalka, Tila Taila, Godugdha* and *Jala* were 1:8:32:32 parts respectively.[5]

Place of Analytical study

The analysis was carried out at the Quality Testing Laboratory of Upgraded Department of Rasashastra and Bhaishajya Kalpana, Government Ayurved College, Vadodara, Gujarat and Vasu Research Centre, Division of Vasu Healthcare Pvt. Ltd. Vadodara, Gujarat.

Organoleptic characters

The organoleptic characters, i.e., colour, odour, taste, texture and appearance of samples were observed to verify the identity and authenticity of both raw materials as well as finished product.

Physico-chemical parameters

The physico-chemical parameters in this study were assessed according to the method mentioned in API. Preliminary analysis carried out were pH value,[6] loss on drying,[7] water soluble extractive,[8] alcohol soluble extractive,[9] total ash,[10] acid insoluble ash,[11] viscosity,[12] specific gravity,[13] acid value,[14] saponification value,[15] refractive index,[16] iodine value,[17] peroxide value[18] and rancidity test.

Phyto-chemical Screening

Alkaloids, flavonoids, terpenoids, glycosides, saponins, tannins and other phytochemicals are measured qualitatively through specifically described methods.[19]

High Performance Thin Layer Chromatography Study (HPTLC)[20]

A high-performance thin layer chromatography (HPTLC) study was conducted for the quantitative estimation of marmelosin in UBFP, BTC, and BTS samples. The HPTLC tracks were scanned under UV light at 313 nm to determine their Rf values. Methanol extracts of the samples were analyzed using a CAMAG system with a Linomat 5 applicator. Silica gel 60 F254 pre-coated plates were employed, utilizing a mobile phase consisting of toluene and ethyl acetate (9.3:0.7 v/v), with an application volume of 5 µl for the standard. The plates were developed to a distance of 10 mm in a CAMAG TLC twin trough chamber, and the TLC plate heater was preheated to 100 ± 5 °C for 3 minutes.

Marmelosin Quantification

Marmelosin was quantified through HPTLC method, by densitometric scanning at 313 nm, the wavelength of maximum absorbance. To prepare standard solution, 5 mg Marmelosin standard was taken in 5 ml volumetric flask and make volume up to mark with methanol. Use the Standard Solution thus obtained for HPTLC fingerprinting. For preparation of test solutions, accurately weighed 2.0 g of sample was taken individually in an iodine flask and add 20 ml Methanol into it. Reflux it on water bath for 20 minutes. Filter by Whatman no.1 filter paper and evaporate it till 8 to 10

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ml remains and make up volume up to mark with methanol in 25 ml volumetric flask. Use the Test Solution thus obtained for HPTLC fingerprinting.

Tests for Heavy metals by ICP-OES

Quantitative analysis of the heavy metals can be done with gravimetric analysis prescribed as limit test for heavy metals.[21] It was done by using ICP-OES (Inductive Coupled Plasma Optical Emission Spectrometer). Sample preparation was done by acid digestion of sample, take 0.5 g sample and add 5ml concentrated HNO_3: 1 ml concentrated HCL in a closed vessel, followed by heating on plate. Allow it to cool, filter the solution into 25 ml volumetric flask and make up by deionized water up to mark. Prepare blank in similar way. Calibrate using the blank and standard and then analyse blank and sample solution. Close the vessels tightly and keep on the turner. Set the method parameters in Microwave digester. Plot a calibration curve on atomic absorption spectrophotometer, detect the metals in samples using calibration curve.

RESULT

Organoleptic characters

Organoleptic characters of the drugs, including colour, odour, taste, texture, and appearance, were evaluated. The sample of UBFP was cream coloured, with a characteristic odour, and exhibited a taste that was astringent, sweet, and bitter, along with a smooth textured powder appearance. The TT sample was yellow coloured, had a sweet and astringent taste, a characteristic odour, and an unctuous textured liquid appearance. Both BTC and BTS samples displayed similar organoleptic characters, being mustard yellow in colour with characteristic odour and unctuous textured liquid appearance.

Physico-chemical parameters

pH value, Loss on Drying (LOD) value, Total Ash value, Acid-insoluble Ash,Water-Soluble Extractive and Alcohol Soluble Extractive value of UBFP sample was 6.85, 8.01, 2.49, 0.294, 61.12 and 11.34 (%w/w) respectively.

The result of physico-chemical parameters of TT, BTC and BTS were mentioned in table no. 1

Table 1: Physico-chemical parameters of TT, BTC and BTS

Parameters	TT	BTC	BTS
pH Value	5	5	5
Specific gravity	0.917	0.9180	0.9184
Viscosity by Ostwald	22.02	23.36	17.24
Refractive Index	1.466	1.467	1.467
Acid value	2.77	1.76	2.05
Peroxide value	5.36	5.71	10.29
Saponification value	204.96	199.06	206.97
Iodine value	103.10	79.76	94.22
Rancidity	Not rancid	Not rancid	Not rancid

Phytochemical screening

Screening of various phyto-chemicals was performed by using different reagents. The result showed that the maximum phytochemical was found in the UBFP sample, which was identified as alkaloids, glycoside, flavonoids, tannin, saponin and carbohydrates. In TT only flavonoids and terpenoids were present. In BTC and BTS samples alkaloids, glycoside, tannin and terpenoids were present.

High Performance Thin Layer Chromatography (HPTLC)

In the HPTLC analysis of the UBFP sample, four prominent spots were observed under 254 nm, with Rf values of 0.18, 0.15, 0.31, 0.40, and 0.50. (Figure 1 and Figure 2). In the BTC sample, five spots were detected under the same wavelength, with Rf values of 0.13, 0.22, 0.37, 0.45 and 0.59. In the BTS sample, four spots were noted under 254 nm, with Rf values of 0.37, 0.47, 0.59, and 0.62. (Figure 3 and Figure 4)

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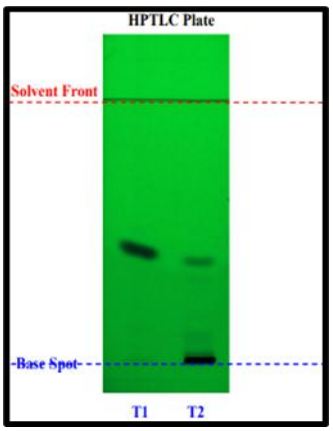

Figure 1: HPTLC photo-document of UBFP Track

T1: Standard Marmelosin

Track T2: Unripe Bael fruit powder

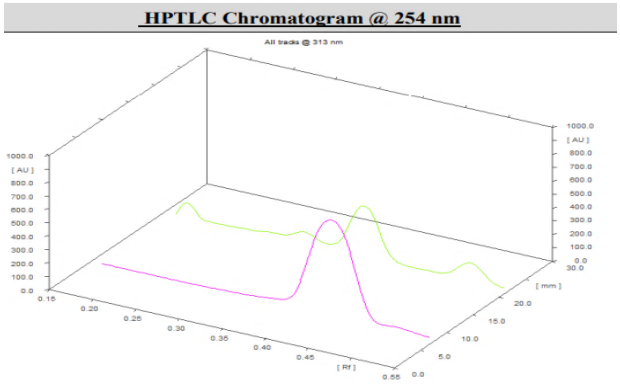

Figure 2: HPTLC chromatogram of UBFP

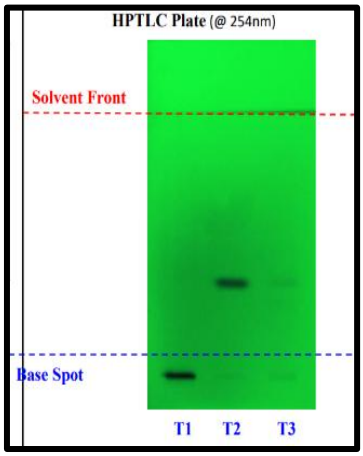

Figure 3: HPTLC photo-document of BTC and BTS

Track T1: *Bilva Taila* sample – 2 (BTS)

Track T2: Standard Marmelosin

Track T3: *Bilva Taila* sample – 1 (BTC)

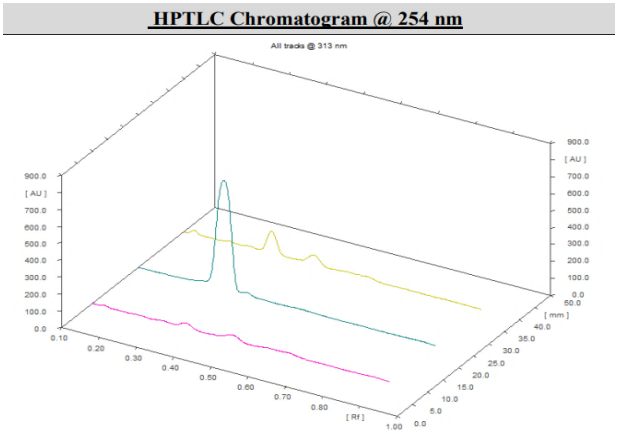

Figure 4: HPTLC Chromatogram of BTC and BTS

Marmelosin quantification

The retention factor (Rf) of marmelosin was detected at 0.40 in the UBFP sample, while for the BTC and BTS samples, the Rf value was 0.37. The percentage of marmelosin in the UBFP, BTC and BTS samples was 0.17 %, 0.097 %, and 0.023 %, respectively.

Test for Heavy metal

In UBFP, heavy metals like Lead, Arsenic and Mercury were present in the amount of 0.270 ppm, 0.627 ppm and 0.902 ppm respectively. In BTC sample, it was present in amount of 0.01 ppm, 0.01 ppm, 0.35 ppm respectively while in BTS 0.04 ppm, 0.01 ppm and 0.48 ppm respectively. Cadmium was not detected in any sample.

DISCUSSION

pH value of UBFP sample was consistent with findings from a previous research work in which it was ranged from 4.70 to 10 in different varieties of Bael fruit.[22] The Loss On Drying(LOD) value of UBFP was similar with a previous research article (i.e. 8.1 %).[23] The data reveals that value of the loss on drying in the UBFP samples indicates that it has an appropriate moisture content, ensuring stability and preventing microbial growth.[24] Total Ash value in UBFP complied with the API value (Not more than 4 %)[25] This value signifies the inorganic residue remaining after complete combustion of the herbal sample, reflecting its mineral content and offering insights into the purity and quality of the material.[26] Acid-insoluble Ash value for the

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UBFP sample was complies with the API standard (not more than 1 %).[27] This parameter primarily indicates the presence of non-soluble minerals, such as silicates or other impurities, that may come from soil or contamination.[28] The Water-Soluble Extractive value for the UBFP sample was meets the API standard (not less than 50 %).[29] This value represents the amount of soluble constituents that can be extracted from the herbal material using water, reflecting the presence of water-soluble compounds such as sugars, amino acids, vitamins, and specific active phytochemicals. The Alcohol Soluble Extractive value of UBFP, complies with API (not less than 6 %).[30] Alcohol-soluble extractive value reveals the existence of polar components.[31]

A consistent pH value of 5 was observed across all three samples i.e. TT, BTC, and BTS, which aligns closely with previous research works, which reported a value of 4.33.[32] pH value around 5 is generally favourable for oil absorption through the skin, supporting optimal skin health and product efficacy.[33]

An average specific gravity of TT, BTC and BTS, were almost similar in all the samples. It is increased when there is an increase in solid content. Following the *Snehapaka* process, the solid content in the oil increases, resulting in a corresponding rise in specific gravity. Viscosity of the TT sample is comparable to that reported in previous research (24.47).[34] Viscosity, which is influenced by the composition of the oil, measures the ease of flow; it is also affected by the temperature of the material. There was difference found in the viscosity of all the three samples. It is significantly decrease in BTS in compare to TT and BTC. Heating process led to decrease in consistency of oil which results in decreased viscosity. Lower viscosity allows for freely flow and improved absorption into the body.[35]

The refractive index of TT aligns with the API standard (1.4650-1.4665).[36] A higher refractive index can indicate an increased potential for rancidity in oils, as well as a greater risk of spoilage.[37] However, in this study, minimal variation was observed in the refractive index across the samples. The acid value quantifies the amount of free fatty acids in the oil, serving as an indicator of quality variations during storage.[38] According to API standards, the acid value for TT, should not more than 2.77.[39] However, a previous study reported an acid value of 5.46.[40] In this study, BTS exhibited a higher acid value compared to BTC, while both BTC and BTS had lower acid values than TT. This suggests a reduction in free fatty acids following the *Snehapaka.*

The peroxide value indicates its oxidative stability. Peroxide value for TT is not mentioned in API, previous research work indicates a range of 3.9 to 15.4.[41] It was much higher in BTS. The higher the peroxide value there is more chance of oxidative degradation.[42] It also increases with an increase in unsaturated fatty acid. Which is absorbed more efficiently in the human body.[43] API specifies a saponification value range of 188 to 195 for TT.[44] however, a previous study reported a value of 212.45.[45] A higher saponification value suggests the presence of shorter-chain fatty acids, which have been proposed to reduce inflammation when administered exogenously.[46]

Iodine value is a measure of the degree of unsaturation in fat. Which has neuroprotective, antioxidant and anti-inflammatory activity.[47] The API specifies a normal range for the iodine value of TT as 103 to 116.[48] In this study, iodine value of BTC and BTS was less in compare to TT which can be due to heating process.

In BTC and BTS samples, alkaloids, glycoside and tannin were extracted from UBFP while Terpenoids was extracted from TT. Flavonoids was presented in TT and UBFP sample but was not found in BTC and BTS may be due to its thermolabile nature. Carbohydrates and saponins are present in UBFP but not found in BTC and BTS probably because that was polar compounds, which enhances their solubility in water. Consequently, they are not extracted in oil from Bael fruit due to their hydrophilic nature.[49] Based on these findings, it can be said that *Sneha Kalpana* is the dosage form in which water soluble and lipid soluble compounds can be extracted from medicinal plants.

Probable reason for less marmelosin in BTS may be due to less quantity of Bael fruit paste added during

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Snehapaka. To further assess the findings, an additional sample of *Bilva Taila* was prepared for the quantification of marmelosin, utilizing a composition of *Bilva Kalka, Tila Taila, Aja Kshira* and *Jala* in the ratio of 1:4:16:16, respectively. This sample also resulted in marmelosin quantification of 0.087 %, which is almost similar to the results obtained from the sample of BTC. Additionally, one research article mentioned that high temperatures may lead to the degradation or transformation of these compounds and potentially reduce their concentration.[50] The lower percentage of marmelosin observed in the BTS sample may be attributed to the extended duration of pharmaceutical preparation compared to the BTC sample.

In this study, the analysis of heavy metals, specifically lead, cadmium, arsenic, and mercury, was conducted in accordance with API guidelines. The results for UBFP, BTC and BTS samples were found to be within the permissible limits as specified. Therefore, the drug is considered safe for therapeutic use.

Conclusion

The study concludes that there is a difference found in quality control parameters of both the samples of *Bilva Taila.* The results of physico-chemical parameters (increased value of acid value, peroxide value, saponification value and iodine value) suggest that prolong heating generates lipid peroxidation and rise in unsaturated fatty acids in BTS sample. Additionally, the decrease in viscosity of BTS sample suggests a reduction in its consistency. HPTLC analysis indicates that there was difference found in quantification of marmelosin in with more percentage in BTC in compare to BTS.

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