



## Research Article

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### COMPARATIVE ANALYTICAL STANDARDS OF TAMRADI TAILA AND ITS MODIFIED CREAM FORM

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#### ABSTRACT

Tamra is being used internally in the form of bhasma (residue after incineration) extensively. Tamra in the form of choorna (powder) is used in the preparation of Taila and is used externally. Tamradi Taila has been explained as one of the most effective medicine for the treatment of Vyanga (Melasma) in Ayurveda classics. Though the formulation is useful, its form poses certain inconveniences. Keeping these points in view the present study has been undertaken with the aim to modify Tamradi taila yoga into Cream form and to develop the physico-chemical profile of the product. Tamradi Taila Cream prepared by principle of Emulsification. The prepared Tamradi taila and Tamradi Cream was evaluated for organoleptic parameters, physico-chemical profiles like pH, loss on drying, Rancidity, Refractive index and Saponification value, also the product was subjected for and HPTLC profiles. It was inferred from the results that Organoleptic parameters and Physico-chemical profile of the product were in the acceptable range, HPTLC profile revealed the presence of 6 spots under 254nm and 366nm respectively for Tamradi taila and 8 spots each in 254nm, 366nm for Tamradi taila cream. Till date no analytical standards established for this modified formulation, hence this analytical profile may serve for future studies and to maintain standard quality of the formulation

**Keywords:** Tamra, Vyanga, Tamradi Taila, Tamradi Cream

#### INTRODUCTION

Rasasashtra is one of the very important offshoots of Ayurveda which mainly deals with above mentioned substances. Rasoushadhis (Mercurial preparations) have attributes like instant effectiveness, requirement in very small doses and extensive therapeutic utility which help in Deha siddhi (Enhance body health). Tamradi Taila has been explained as one of the most effective medicine for the treatment of Vyanga (Melasma) in Rajamarthanda<sup>1</sup> and Bharata Bhaishajya Ratnakara<sup>2</sup>. Physico-chemical and Analytical study provides the objective parameters to fix up the standards for quality of raw drugs as well as finished products. Classical texts of Ayurveda have mentioned analytical techniques to understand the quality of the end product. These are mostly qualitative and subjective. Modern analytical techniques can be applied to the product to ascertain its components which also gives an idea of its pharmacological action. Skin responds better to natural products than synthetic ones and this explains the latest trend of using traditional, natural products in dermatological formulation. In the present study Tamradi taila is prepared classically and is modified in to cream<sup>3</sup> form, for easy handling and use and a comparative analytical standards were analysed.

#### MATERIALS AND METHODS

**Major Drugs:** Tamra patra (Copper foil), Tila Taila (Gingelly oil), Godugdha, (Cow's milk), Kesara (*Crocus sativus*) are the major ingredients in the preparation of Taila. Stearic acid, Triethanolamine, Lanolin, Distilled water, Glycerin are the raw drugs in the preparation of Cream. These materials were collected from authentic sources according to the Grahya Agrahya Lakshnas, (Quantitative and Qualitative analysis)

**Associated Drugs:** The other raw materials used for the present study are Saindava lavana (Rock salt) Nimbu Swarasa (Lemon extract) and Nirgundi swarasa (*Vitex nigundo* extract) for Shodhana of Tamra. Tanka pisti (Borax paste) and Gomutra (Cow's urine) for preparation of Tamra choorna, (Copper powder).

Analytical tests were carried out at SDM Center for Research in Ayurveda & Allied Sciences, Udupi, Karnataka, India.

#### Organoleptic characters

Organoleptic characters of the test sample were documented by means of examination using sensory organs.

#### Loss on drying at 105 °C

10 g of sample was placed in tarred 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.<sup>4</sup>

#### Rancidity test

1ml of melted fat was mixed with 1ml of conc. HCl and 1ml of 1% solution of phloroglucinol in diethyl ether and then mixed thoroughly with the fat acid mixture. A pink color indicates that the fat is slightly oxidized while a red color indicates that the fat is definitely oxidized.<sup>5</sup>

### Refractive index

Placed a drop of water on the prism and adjusted the drive knob in such a way that the boundary line intersects the separatrix exactly at the centre. Reading was noted. Distilled water has a refractive index of 1.3325 at 25 °C. The difference between the reading and 1.3325 gives the error of the instrument. If the reading is less than 1.3325, the error is minus (-) then the correction is plus (+) if the reading is more, the error is plus (+) and the correction is minus (-). Refractive index of oil is determined using 1 drop of the sample. The correction if any should be applied to the measured reading to get the accurate refractive index. Refractive index<sup>6</sup> of the test samples were measured at 28 °C.

### Determination of pH

Preparation of buffer solutions: Standard buffer solution: Dissolved one tablet of pH 4, 7 and 9.2 in 100 ml of distilled water.

Determination of pH<sup>7</sup>: 1 ml of sample was taken and makes up to 10 ml with distilled water, stirred well and filtered. The filtrate was used for the experiment. Instrument was switched on. 30 minutes time was given for warming pH meter. The pH 4 solution was first introduced and the pH adjusted by using the knob to 4.02 for room temperature 30 °C. The pH 7 solution was introduced and the pH meter adjusted to 7 by using the knob. Introduced the pH 9.2 solution and checked the pH reading without adjusting the knob. Then the sample solution was introduced and reading was noted. Repeated the test four times and the average reading were taken as result.

### Determination of Saponification value

About 2g of the substance was weighed in tared 250 ml round bottom flask. 25ml of the alcoholic solution of KOH was added and a reflux condenser was attached. Kept it for boiling on water bath for 1hour, the contents of the flask was rotated frequently. The flask was cooled and 1ml phenolphthalein solution was added and excess of alkali titrated with 0.5N HCl. The number of ml (a) required was noted. The experiment was repeated with the same quantities of reagents in the same manner omitting the substance. The number of ml required (b) was noted. The experiment was repeated twice to get concordant values.

Saponification value<sup>8</sup> =  $56.1 \times (b-a) \times \text{Strength of Hydrochloric acid} / \text{Weight of the sample taken}$

### Determination of Unsaponifiable matter

Weighed 5g of the substance into the flask. Added 50ml alcoholic KOH into the sample. Boiled gently but steadily under reflux condenser for one hour. The condenser was washed with 10ml of ethyl alcohol and the mixture was collected and transferred to a separating funnel. The transfer was completed by washing the sample with ethyl alcohol and cold water. Altogether, 50ml of water was added to the separating funnel followed by an addition of 50ml petroleum ether. The stopper was inserted and shaken vigorously for 1 minute and allowed it to settle until both the layers were clear. The lower layer containing the soap solution was transferred to another separating funnel and repeated the ether extraction six times more using 50ml of petroleum ether for each extraction. All the

extracts were collected in a separating funnel. The combined extracts were washed in the funnel 3 times with 25ml of aqueous alcohol and shaken vigorously. And drawing off the alcohol-water layer after each washing. The ether layer was again washed repeatedly with 25ml of water until the water no longer turns pink on addition of a few drops of Phenolphthalein indicator solution. The ether layer was transferred to a tarred flask containing few pieces of pumice stone and evaporated to dryness on a water bath. Placed the flask in an air oven at 85°C for about 1 hour to remove the last traces of ether. A few ml of Acetone was added and evaporated to dryness on a water bath. Cooled in a desiccator to remove last traces of moisture and then weighed<sup>9</sup>.

### HPTLC

Unsaponifiable matter of the given sample is dissolved in 10 ml of chloroform. 9µl of the each of the above sample was applied on a precoated silica gel F254 on aluminum plates to a band width of 8 mm using Linomat 5 HPTLC<sup>10</sup> applicator, the Linomat 5 company name is Camag made in Switzerland. The plate was developed in Toluene – Ethyl acetate (8: 1) and the developed plates were visualized under 254 and 366 nm and after derivatisation in vanillin-sulphuric acid spray reagent and scanned under UV 254nm, 366 nm and 620nm. R<sub>f</sub> colour of the spots and densitometric scan were recorded.

### RESULTS AND DISCUSSION

The results obtained from the Analytical study were depicted in the tables. The Organoleptic parameters were presented in Table 1. The results of Standardisation parameters were shown in Table 2. R<sub>f</sub> values were presented in table 3. The Densitometric scan at 254 nm, 366nm, 620 nm were presented in Figure 2,3 and 4 respectively. Densitometric scan at 366nm following derivitisation presented at Figure 5 and Chromatogram represented at Figure 6.

Quality of a medicine is very important in health care system. Standardization is an essential measurement for ensuring the quality control of the herbal and mineral drugs. Analytical study brings standards for the quality drugs and helps in explaining pharmacokinetics and pharmacodynamics of a drug. Uniform consistency is also a mandatory criterion for topical applications, which can be elicited by rubbing the sample on the back of the hand; no solid components should be noticed. From the data of organoleptic analysis of the both formulations, it is clearly evident all the parameters found to be satisfactory.

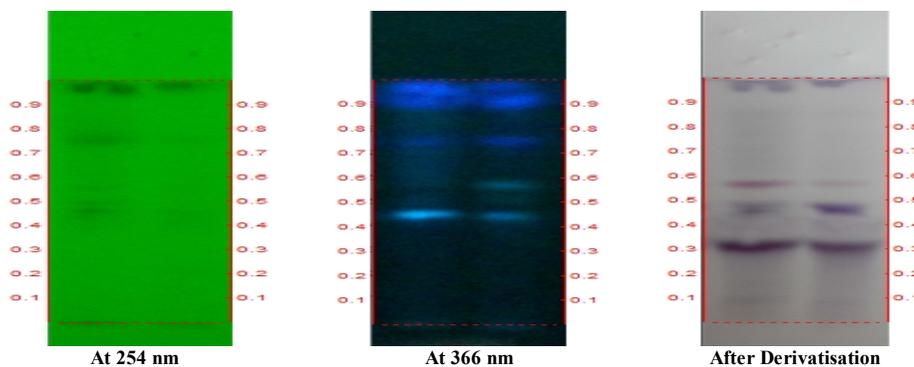
pH of Tamradi taila is 4.7 being slightly acidic, as that of Tamradi cream is 5.5, which lies within the normal pH of human skin ie between 5- 6.5. But both the formulations did not produce any skin irritation. Previous studies shows that the drug Tamra (Copper) does not produce any toxic effect in experimental models.<sup>11</sup> Loss on drying is a measure of amount of water and volatile matters in the sample, when the sample is dried under specified condition. Loss on drying of Tamradi cream was found to be 16.15, reason may be the presence of water soluble components in Tamradi cream. Rancidity usually characterizing fat that is undergoing oxidation or bacterial decomposition. Auto oxidation occurring in natural edible fats is called Rancidification. Both formulations found to be non rancid, since the fat is not oxidized. The Refractive index of Tamradi taila is 1.47.

**Table 1: Organoleptic characters of the Tamradi Taila and Tamradi Taila cream**

Parameter	Tamradi taila	Tamradi cream
Colour	Dark greenish	Pale greenish
Appearance	Semi solid	Semi solid
Odour	Greasy odour	Greasy odour

**Table 2: Standardisation parameters for Tamradi taila and Tamradi Taila cream**

Parameters	Tamradi taila	Tamradi Taila cream
LOD	-	16.15
Rancidity	Fat is not oxidised	Fat is not oxidized
Refractive index	1.4724	-
pH	4.7	5.5
Saponification value	148.25	134.93
Unsaponifiable matter	10.35	9.34

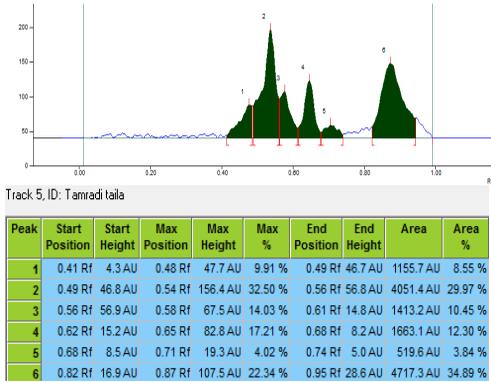


**Figure 1. HPTLC photo documentation of chloroform extract of Tamradi taila and Tamradi Taila Cream**  
Track 1-Tamraditaila – 9µl, Track 2– Tamradi cream – 9µl, Solvent system: Toluene: Ethyl acetate (8:1)

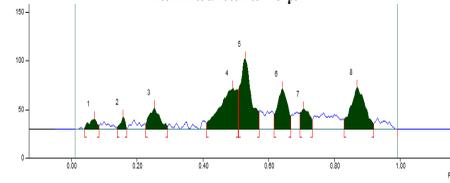
**Table 3: R<sub>f</sub> values of Tamradi Taila and Tamradi Taila Cream**

At 254 nm		At 366 nm		After Derivatisation	
Tamradi taila	Tamradi cream	Tamradi taila	Tamradi cream	Tamradi taila	Tamradi cream
-	-	-	-	0.09 (L. purple)	0.09 (L. purple)
-	-	-	-	0.16 (L. purple)	-
-	0.22 (L. green)	-	-	-	-
-	-	-	-	0.32 (D. purple)	0.32 (D. purple)
-	-	-	-	0.40 (L. pink)	-
-	-	0.44 (F. blue)	0.44 (F. blue)	-	-
0.46 (L. green)	0.46 (L. green)	-	-	0.46 (D. purple)	0.46 (D. purple)
0.50 (L. green)	-	-	-	0.50 (D. purple)	-
-	-	-	0.53 (FL. blue)	-	-
0.56 (L. green)	-	-	-	-	-
-	-	0.57 (FL. blue)	0.57 (F. blue)	0.57 (D. pink)	0.57 (L. purple)
-	-	0.70 (FL. blue)	-	-	-
-	-	-	-	0.72 (L. purple)	-
0.75 (D. green)	0.75 (L. green)	0.75 (FD. blue)	0.75 (FD. blue)	-	-
-	-	-	-	0.78 (L. purple)	-
-	-	-	-	0.86 (L. purple)	-
-	-	0.92 (FD. blue)	0.92 (FD. blue)	-	-

Tamradi Taila showed 4 spots at 254 nm, 5 spots at 366 nm and 10 spots at post derivatisation. Tamradi Taila Cream showed 3 spots at 254 nm, 5 spots at 366 nm and 4 spots at post derivatisation.

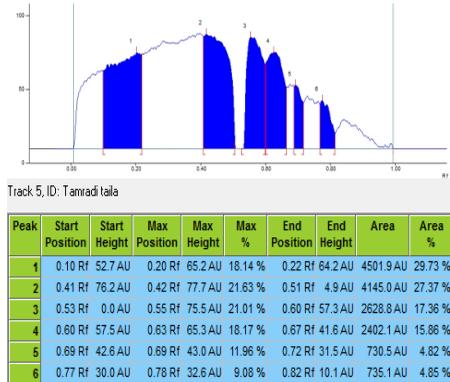


Tamraditaila - 9µl

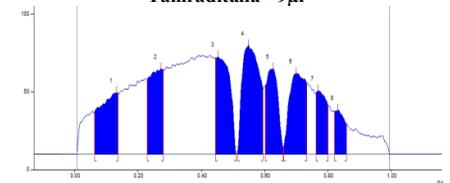


Tamradi cream - 9µl

Figure 2: Densitometric scan of the sample at 254nm

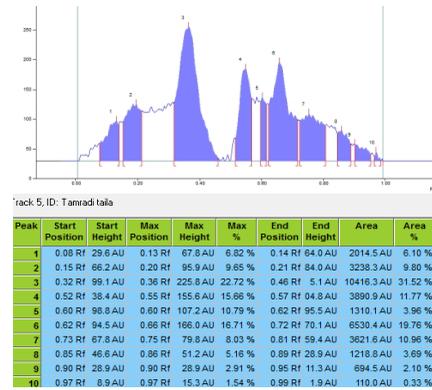


Tamraditaila - 9µl

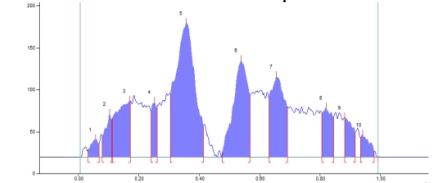


Tamradi cream - 9µl

Figure 3: Densitometric scan of the sample at 366nm

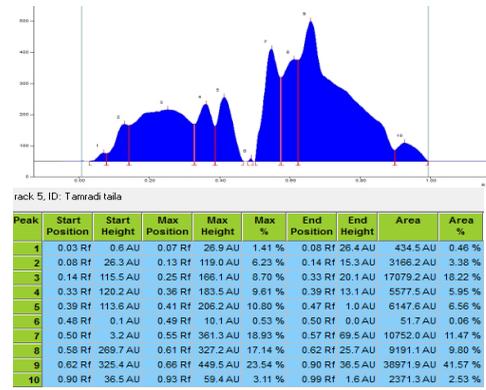


Tamraditaila - 9µl

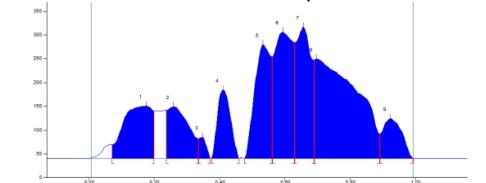


Tamradi cream - 9µl

Figure 4: Densitometric scan of the sample at 620nm following derivatisation



Tamraditaila - 9µl



Tamradi cream - 9µl

Figure 5: Densitometric scan of the sample at 366nm following derivatisation

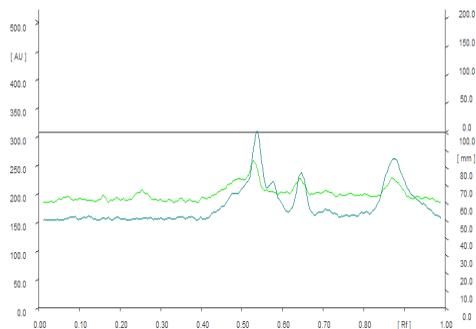


Figure: 6.1 At 254nm

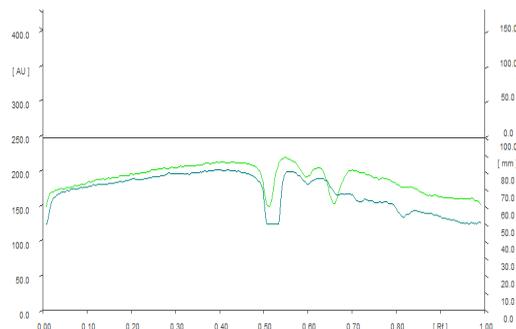


Figure: 6.2 At 366nm

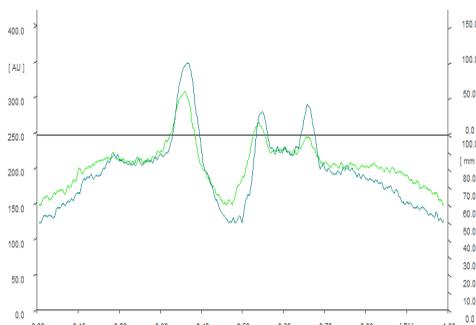


Figure: 6.3 At 620nm

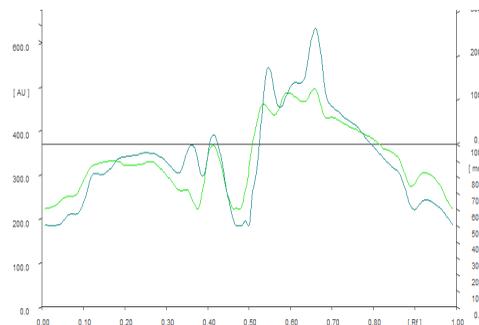


Figure: 6.4 At 366nm after derivitisation

Figure 6: Chromatogram

Medicated oil with high saponification value has better absorption. High saponification value also indicates the presence of fatty acids of low molecular weight (molecules are in simple form). Low saponification value indicates the molecules are in complex form. Tamradi taila got a saponification value of 148.25 and that of Tamradi cream was 134.93. It indicates the better absorption of Tamradi taila than that of Tamradi cream. Whereas unsaponification value is 10.34 in Tamradi taila and 9.34 in Tamradi cream. On analyzing the HPTLC results, Densitometric scan at 254nm of Tamradi taila showed 6 peaks and under 366nm, it showed 6peaks. Under 254nm, Tamradi cream showed 8 peaks, under 366nm, showed 8 peaks in densitometric scan. From these it can be assessed that more active principles are present in Tamradi cream when compared with Tamradi taila. Spots at 366nm were almost matching with each other, indicating presence of similar compounds but not same in case of visualization at 254nm. The differences indicate the presence of variable functional groups in both the formulations

#### CONCLUSION

HPTLC reports revealing more active constituents are present in Tamradi taila compared to Tamradi cream. Saponification value reveals that Tamradi Taila is having more absorption rate compared to Tamradi Cream .PH of Tamradi Taila is being slightly acidic while PH of Tamradi Taila cream with in the normal ph of skin.Both formulations found to be non rancid, since the fat is not oxidized. The Refractive index of Tamradi taila is 1.47.

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