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Pharmaceutico-analytical Standardization of *Katupila Taila;* An Ayurvedic Dosage Form from Ethnobotany: *Securinega leucopyrus*

Research Article

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Abstract

Katupila - *Securinega leucopyrus* (willd) Muell. is ethnobotanic medicine, whose leaves are proven to exhibit wound healing property through in vitro, in vivo experimental studies and clinical study along with other properties like anti inflammatory, antioxidant etc. Present study was aimed to develop suitable Ayurvedic dosage form which can value add to ethnic practice and outcomes of recent studies on *Katupila* by utilizing probable potential of its alternate aria plant part; stem. Six batches of *Katupila taila* were prepared from decoction and bolus of stem of *Katupila* heated with Sesame oil in prescribed manner of *Snehapaka* as per Ayurvedic classics till *Madhyam Snehapaka.* Organoleptic, physicochemical evaluation, test parameters for evaluation of *Snehakalpa* as per Ayurvedic Pharmacopeia of India (API) were recorded in triplicate along with chromatographic pattern. Average 934.8 g (93.48% yield) of *Katupila Taila* was obtained from batch size of, 1 *l* of *Tila Taila* with 212 g of *Kalka dravya* and 4 *l* of *Kwatha* of *Katupila* stem prepared over 3 days with 13 hrs of total duration of actual heating. Mean specific gravity, refractive index, acid value, iodine value, saponification value and Bellier's turbidity temperature test (BTTT) value were 0.9179, 1.449 2.908 ± 0.0538, 108.075 ± 2.97, 169.66 ± 8.91 and 19.6 ± 0.3 Mean ± SD respectively. Adopted method of *Snehapaka* for preparation of *Katupila Taila* can be considered as standard. Observed organoleptic features, range of physicochemical parameters and chromatographic pattern as per API may be considered as analytical standard for *Katupila Taila*.

Key Words: *Ethnobotany, Formulation development, Katupila taila, Katupila stem, Securinega leucopyrus, Snehakalpana.*

Introduction

Ethnomedicine is the study of traditional or folklore medicine which is concerned with health, diseases and healing practices.(1) *Katupila* (*Securinega leucopyrus*) is not mentioned in any *Ayurvedic* classical text book, but in floras of some region it is recognized as very potent medicine.(2) Many researches are conducted on *Katupila* leaves in recent years which substantiate its Pharmacological as well as Clinical efficacy. Application of paste of *Katupila* leaves with *Tila taila* to patient's wound, exerts significant effect in wound healing.(3) But pharmaceutical, analytical & pharmacological studies of *Katupila* stem is still remained a wide area of exploration. An effort is made to provide stable pharmaceutical dosage form which is suitable for the industrial application. *Tila Taila* possesses wound healing activity internal as well as external.(4) Many Ayurveda dosage forms are unique in terms of their principles of formulation compounding, formulation design, mechanism of action etc. Most of the tests described in literature appear to be based on observations and seems to be subjective. It would be value addition to utilize contemporary methods of test parameters to monitor process control and assure their precision and assure repeatability and quality of derived dosage form. Standardization and development of reliable quality protocols are important especially in regard to formulations or practices of ethnobotanic origin or formulations developed from ethnobotanic practices to strengthen them, provide scientific base, maximize their therapeutic utility by increasing their chance to bring them to mainstream medical practice.(5) Uniqueness, potential of Ayurvedic dosage forms being easy to manufacture, economic processing, versatility to pharmaceutical operations in terms of therapeutic utility and safety (probably because of similar pharmaceutical operations in daily use and organic origin drugs, ingredients, base raw materials for preparation etc.) However, standardization of *Katupila Taila* is desirable for its greater recognition and acceptance. The standardization study of formulation never be achieved by one or two parameters and hence

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is preferable to achieve it in a multidisciplinary way. With this in mind, the study has been undertaken to develop pharmaceutical standardization with six batches of formulation *Katupila Taila.* Organoleptic characters, physicochemical parameters were done with six batches of *Katupila Taila* and *Tila Taila* (Sesame oil).

Aim and objectives

- To develop standard manufacturing procedure of *Katupila Taila* (KPT).
- To develop analytical profile of *Katupila Taila* (KPT).

Materials and Methods

Pharmaceutical Study

Collection and authentication of raw drugs

Katupila stem was collected at its fruiting stage from Nana Thavaria villege, near Vijarkhi dam, Jamnagar (Gujarat) in June month. (Plate 1 fig.a)

Katupila (*Securinega leucopyrus* [willd] Muell.) was authenticated in Department of Pharmacognosy, IPGT&RA, Gujarat Ayurved University; bearing Herbarium Voucher's Specimen no. 6317. (Plate 1 fig.b)

Tila Taila (FSSAI grade) was procured from the pharmacy, Gujarat Ayurved University. Formulation was prepared in Department of Rasa shashtra & Bhaishajya kalpana laboratory, I.P.G.T& R.A, Jamnagar.

Preparation of *Katupila Kwatha*

Physical impurities from raw material were removed manually. *Yavkuta* of *Katupila* stem was prepared in pharmacy, Gujarat Ayurved University. Then it was soaked in water overnight for 15 hours. Next day it was subjected to heat over *Mandagni* to *Madhyamagni* (80° C – 100° C) until half part is reduced. *Kwatha* was filtered in warm condition through cotton cloth and stored in stainless steel vessel for further process. (Table no. 1) (Plate 1 fig.d,e,f).

Table 1: Observations of Six batches of *Katupila Kwatha* (KPK)

Batches >	KPK 1	KPK 2	KPK 3	KPK 4	KPK 5	KPK 6	Mean ± SD
Initial Qty. of Katupila stem (gm)	1000	1000	1000	1000	1000	1000	1000
Size of the Pieces (c.m)	2.3 - 4.5	2.3 - 4.5	2.3 - 4.5	2.3 - 4.5	2.3 - 4.5	2.3 - 4.5	3.4 ± 1.1
Diameter of the Pieces (cm)	0.10 - 0.48	0.10 - 0.48	0.10 - 0.48	0.10 - 0.48	0.10 - 0.48	0.10 - 0.48	0.29 ± 0.19
Total Qty. of Water (ml)	8000	8000	8000	8000	8000	8000	8000
Total Time for Soaking (h)	15 Hrs	15 Hrs	15 Hrs	15 Hrs	15 Hrs	15 Hrs	15 Hrs
Temperature (after One hour)	101⁰ C	100⁰ C	101.5⁰ C	101⁰ C	100⁰ C	101⁰ C	100.75 ± 0.6
Duration (min)	150 min	156 min	165 min	148 min	148 min	152 min	153.16 ± 6.52
Obtained *Kwatha* (ml)	4180	4260	4290	4410	4340	4210	4281.66 ± 84.71
Fresh Residue (gm)	1870	1932	1867	1892	1951	1877	1898.16 ± 35.17

Preparation of *Katupila Kalka*

Katupila Yavakuta was taken in grinder machine to prepare fine powder. Fine powder was triturated with water to convert in to paste form. Boluses of *Kalka* were kept in stainless steel vessel for further process. (Table no. 2) (Plate 1 fig.c).

Table.2: Results obtained during Six batches of *Katupila Kalka*

Batch	I	II	III	IV	V	VI	Mean SD
Weight of ingredients (g)	167	167	167	167	167	167	167
Quantity of water *used* (g)	45	44	45	45	45	45	44.83 ± 0.40
Total weight of *Kalka* (g)	212	211	212	212	212	212	211.83 ± 0.40

Preparation of *Katupila Taila*

Tila Taila in the mentioned quantity was taken in a stainless-steel vessel and heated over mild flame (100⁰C for 8 min) till complete evaporation of moisture and then heating was stopped. After that boluses of *Kalka* were added to *Tila Taila* at 80⁰C. After mixing of *Kalka* into *Taila,* the specified quantity of *Katupila Kwatha* was added and the mixture was heated over a mild to medium heat. Heating was continued maintaining the temperature in between 87⁰C to 101⁰C with intermittent stirring. The mixture was left undisturbed after discontinuation of direct heating till next heating and heating was given for 3 days. Mixture was stirred continuously to avoid the settling of solid contents and overheating due to possibility of settling down. Heating was continued on 3rd day until *Sneha Siddhi Lakshana* were obtained. After obtaining desired *Sneha Siddhi Lakshana*, heating was discontinued and oil was filtered immediately through two folded cotton cloth in hot stage. After cooling, the prepared oil was kept in a vessel for a day for sedimentation allowed for settlement of suspended particles and filtered again and stored in labeled air tight bottle. (Table no: 3) (Plate 1 fig. g to l).

Table.3: Formulation composition of *Katupila Taila*

No.	Ingredients	Latin/ English name	Part used	Quantity
Kalka dravya				
1	*Katupila churna*	*Securinega leucopyrus* Muell.	Stem	167 gm
Sneha dravya				
2	*Til Taila*	*Seasamum indicum* Linn.	Seed oil	1000 gm
Drava dravya				
3	*Katupila Kwatha*	*Securinega leucopyrus* Muell.	Stem	4000 gm

Plate No. 1

Pharmaceutical steps for preparation of *Katupila Taila*

Pharmaceutical steps of *Katupila Taila* preparation
Fig. 1 : Plant of *Katupila* (Source)
Fig. 2 : Herbarium of *Katupila* (*Securinega leucopyrus*)
Fig. 3: Preparation of *Kalka*
Fig. 4: Soaking of *Katupila* stem *yavakuta* in water
Fig. 5: Preparation of *Katupila Kwatha*
Fig. 6: Filtration of *Katupila Kwatha*
Fig. 7: Heating of *Tila Taila*
Fig. 8: Addition of *Katupila Kalka* boluses
Fig. 9: Addition of *Katupila Kwatha* in mixture of *kalka* and *Tila taila*
Fig. 10: Phenodgam stage
Fig. 11: *Mardanevartikotpatti*
Fig. 12: Filtration of *Katupila Taila*

Analytical Study

Analytical evaluation of *Katupila Kwatha* (KPK) & *Katupila Taila* (KPT) was carried out to develop standards for the reproducibility of product. The samples were analyzed on the basis of organoleptic characters. It includes sensory characters of drug i.e. *Sparsha* (Consistency and texture), *Rupa* (Colour), *Gandha* (odor). Physicochemical parameters such as Specific gravity @ 25° C, Refractive Index, pH, Total Solid contents, Acid Value, Iodine Value, Saponification value, Bellier's turbidity temperature test() & HPTLC were done for analysis of *Katupila Taila* at pharmaceutical Chemistry laboratory of I.P.G.T& R.A, Jamnagar.

High Performance Thin Layer Chromatography (HPTLC)

0.1 ml of oil was taken and 1 ml of hexane was added. The prepared solution was used for chromatography. Thereafter pre chromatographic derivatization was carried out. Alcoholic KOH (base) and thereby heated for 10-15 minutes in CAMAG TLC plate heater. Sample application was done using CAMAG linomat 5.

HPTLC of *Katupila taila* was carried out using solvent system Petroleum ether: Diethyl ether: Acetic acid (70:30:2 v/v). HPTLC study was performed for the normal phase separation of components of product.

Chromatographic study (HPTLC) was carried out under 254 nm and 366 nm to establish fingerprinting profile.

Observations and Results

Yield of dried *Katupila* stem from 17.170 kg quantity of fresh stems was 22.100 kg i.e. 77.69 %. While preparing *Yavakuta* of *Katupila* stem, 15.500 kg quantity of *Yavakuta* was obtained with 70.13%. Total 2.090 kg (92.88%) of fine powder was derived from 2.250 kg of *Yavkuta* tailing 7.12%. Mean diameter & length of stem were 0.49 ± 0.19 cm & 3.4 ± 1.1 cm respectively. Average weight of *Kalka* was 212 g after mixing with water. Prepared *Kalka* was light brown in color, having characteristic smell. During heating of *Tila Taila* (Sesame oil), foaming was observed which was probably due to moisture content of *Taila*. When temperature of oil reached at 75°C boluses of *Kalka* were added & fried for around 10-12 minutes. The *Kalka draya* were added while discontinuation of heating to avoid overheating of *Kalka dravya*. After mixing of *Kalka* into *Taila* and four times of *Katupila kwatha* (4000 ml) was added and again heating process was done with maintaining temperature in between 87°C to 101° C with occasional stirring. After 15 minutes of heating, bubbling was visible with peculiar odor of started with specific odor of *Katupila*. After half hour of heating, contents became dark brownish in color and upper layer of mixture was light brown in color with more viscuousness of mixture and frothing. After 1 hour of heating, light brown color of mixture was changed to dark brown having excessive frothing was observed during the process. The *Taila* was heated intermittently maintaining temperature in between 87 °C – 101 °C for 3 days. On the third day, after 11 hrs of heating process *Phenodgama* was observed. *Kalka* was examined at regular intervals. *Mridu paka & Madhyama Paka* stages were observed at specific temperature which presented in Table no: 4.

Consistency of *Kalka* became sticky to touch with no moisture and was able to make *varti (Mardanevartikotpatti)* and smooth in texture. At this stage of *Madhyam Paka*, *Taila* was filtered through cotton cloth without squeezing and in hot stage to get maximum yield of final product. On an average, total duration for *Taila Paka* and yield for each batch of *Katupila Taila* was observed to be approximately 13 hours and 934.8 ml respectively after 3 days. (Table no: 5).

Table 4: Observation of six batches of *Katupila Taila* at different temperature

Sr. No	Observation	Temperature	Time
1	Starting Temp.	27^0 C	0 min
2	On addition of *Kalka*	88^0 C	21 min
3	On addition of *Kwatha*	84^0 C	30 min
4	During *Phenodgama*	87^0 C	11 hrs
5	During *Mrudu paka*	89^0 C	12 hrs
6	During *Madhyam Paka*	91^0 C	13 hrs
7	Duration between initiation of *Mrudu paka* & end of *Madhyam paka*	89^0 C	72 min
8	Duration (start from *Mrudupaka*) for appearance of *Kalka Varti Pariksha*	-	75 min
9	Duration (start from *Mrudupaka*) for disappearance of sound upon burning of wick.	-	75 min
10	Total duration of heating (Heating-cooling)	-	13 Hrs
11	Total Time for the Process- (h)	-	52 Hrs

Table 5: Results obtained during preparation of *Katupila Taila* (KPT)

Parameters	KPT 1	KPT 2	KPT 3	KPT 4	KPT 5	KPT 6
Quantity taken of *Tila Taila* (g)	1000	1000	1000	1000	1000	1000
Quantity obtained of *Katupila Taila* (g)	936	941	940	936	923	930
Total loss (g)	64	59	60	61	77	70
Loss in (%)	6.4	5.9	6.0	6.1	7.7	7.0
Colour of *Kalka*	Dark Brown	Dark Brown	Dark Brown	Dark Brown	Dark Brown	Dark Brown
Form of *Kalka*	Paste	Paste	Paste	Paste	Paste	Paste

Specific observations

Physico chemical parameters: physico chemical parameters of *Katupila Kwatha* are presented in Table no: 6

Table 6: showing physicochemical characteristics of *Katupila Kwatha* (KPK)

Parameters	*KPK* 1	*KPK* 2	*KPK* 3	*KPK* 4	*KPK* 5	*KPK* 6	Mean SD
Specific gravity	0.9848	0.9920	0.9806	0.9880	0.9840	0.9838	0.9855 ± 0.0039
Refractive index	1.346	1.333	1.324	1.329	1.331	1.336	1.333 ± 0.0074
pH	5.64	5.64	5.62	5.63	5.65	5.64	5.63 ± 0.0103
Total Solid Contents (%w/v)	0.2918	0.2818	0.2990	0.2927	0.2920	0.2924	0.2916 ± 0.0055

Organoleptic characters: The organoleptic characters of six samples of six batches of *Katupila Taila* are presented in Table no: 7.

Table 7: showing organolepic characters of *Katupila Taila* (KPT)

Organoleptic Characters	*KPT* 1	*KPT* 2	*KPT* 3	*KPT* 4	*KPT* 5	*KPT* 6
Colour	Brown	Brown	Brown	Brown	Brown	Brown
Smell	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Touch	Oily	Oily	Oily	Oily	Oily	Oily

Comparative physico chemical parameters of *Tila Taila* (Sesame oil) and *Katupila Taila* are presented in Table no: 8.

Table 8 : Showing physicochemical characteristics of *Katupila Taila(KPT)*

Parameters	*Tila Taila*	*KPTM* 1	*KPTM* 2	*KPTM* 3	*KPTM* 4	*KPTM* 5	*KPTM* 6	Mean SD
Specific gravity	0.9103	0.9059	0.9109	0.9198	0.9063	0.9118	0.9123	0.9179 ± 0.0050
Refractive index	1.386	1.459	1.434	1.456	1.449	1.453	1.443	1.449 ± 0.009
Acid value	3.263	2.877	2.885	2.995	2.955	2.863	2.873	2.908 ± 0.0538
Iodine value	109.860	108.012	106.032	103.726	112.186	108.345	110.153	108.075 ± 2.9793
Saponification value	161.32	170.39	186.93	166.18	163.45	162.92	168.13	169.66 ± 8.9132
BTTT value	21^0 C	19.2^0 C	19.5^0 C	20.1^0 C	19.5^0 C	19.8^0 C	19.6^0 C	19.6^0 C ± 0.3^0 C

HPTLC fingerprinting of *Katupila Taila* showed spots at 254 nm, spots at 366 nm before spray. (Table no: 9) (Plate 2)

Table 9 : HPTLC fingerprinting of *Katupila Taila*

Solvent system	Wavelengths	No of Spots	Rf value	AUC (Area Under Curve)
Petroleum ether (70) : Diethyl ether (30) : Acetic acid (2)	254 nm	3	0.03, 0.24, 0.86	14.09, 11.98, 73.93
	366 nm	2	0.03, 0.89	16.29, 83.71

Plate no 2

HPTLC of *Katupila Taila at* 254 nm

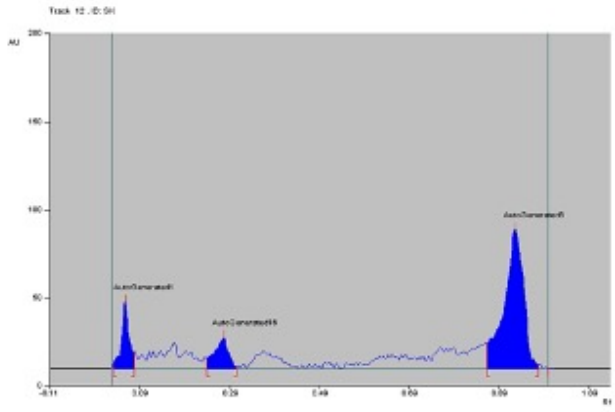
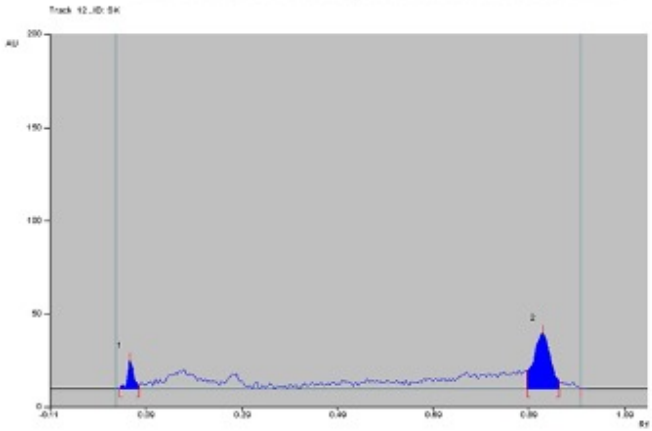


Plate no 3

HPTLC of *Katupila Taila* at 366 nm



Discussion

In some previous researches application of *Katupila* leaf paste and sesame oil showed effective wound healing in *Dushta Vrana* (chronic wound).(7) Studies on wound healing effect of *Tila Taila* extract exhibits significant reduction in wound length in rats and it has potential to be developed into therapeutic agent for wound healing (8). Although *Katupila* leaves are widely used and several researches are done on leaf; stem may provide more biomass for medicament preparation as compared to leaves. *Sneha kalpana* is selected & plant is processed with *Tila Taila* so fatty acids, lipogenic compounds & plant basis complexes can be obtained.

As *Snehapaka* is carried out with *Kwatha*, quantity of *Kalka* was take as 1/6th as that of Oil. During *Snehapaka*, temperature reached at 75°C after 12 minutes of demoisturization, boluses of *Kalka Draya* were added to protect the overheating of *Kalka dravya.* After 30 minutes & 60 minutes of moderate heating, contents became light brownish & dark brownish in color respectively which may be due to some physical/ chemical changes during heating process. On third day, after 11 hours of heating process (actual heating from begining) *Phenodgama* was observed, it may be due to presence of unsaturated fatty acids in *Taila*. When heated unsaturated fatty acids it was formed oxides which released foam. *Taila* was filtered in hot condition to get maximum yield. An average 6.51 % loss was found during preparation of *Katupila Taila* (KPT) which may be due to more absorption of *Taila* in to *Kalka* and few losses may be due to *sneha paka pariksha* which may be due to highly polar nature of *Kwatha* and its constituents (phytochemicals) which is immiscible in fatty (polar) liquid media, thus there is need of time for reactions with to take place for formation of complexes of constituents of Kwatha with altered and probably continuously altering matrix of composition of sesame oil (trans fats, free fatty acids (FFA), volatile compounds, alteration in fatty acids composition like generation of saturated fats and continuous changes like oxidation, hydrolysis, saponification, etherification etc.) as a result of *Snehapaka*.(9)

As per tabled data (Table no:8), apart from RI and Saponification value, rest of the standards (rest of all analytical physico-chemical parameters) of *Tila Taila* chosen for present study are within limits for Sesame oil prescribed by regulatory authorities, which matches with API(10), BIS(11), CODEX(12), standards. This suggests need of development of inprocess standards and phytochemical marker studies of this formulation. Refractive index of samples i.e., *Tila taila* (1.386), finished product (1.449) were very different. Refraction of light in base oil compared to formulation may be due to addition of aqueous media, presence of suspended particulate matter, and change in concentration of extracted chemical moieties, addition /extraction of different chemical constituents in to Sesame oil or neo formation of different chemical constituents in to Sesame oil.(13) Specific gravity may also get altered (increased) due to some logically relevant reasons in final product (0.9179) as that of *Tila taila* (0.9103) which may be-formation of micelles due to heat treatment of oil with excess of aqueous media, suspension of solid particulate matter in to prepared *Sneha*, new generation of complexes with extracted constituents of formulation ingredients or their derivatives having comparatively more density than that of oil.(14) Inspite of known fact of increase in acid value after heat treatment of oils, comparatively lower Acid value of finished product (2.908) as compared with *Tila taila* (3.263) suggests formation of complexes of generated free fatty acids (FFA) and trans fatty acids (TFA) with phytoconstituents of Drug *Katupila*. More Saponification value in finished product (169.66) as that of *Tila taila* (161.32) may suggests formation of shorter chain fatty acids, with lower molecular weight and thus supporting fast and better absorption of generated phytoconstitution of oil.(15) Iodine value is a measure of the amount of unsaturation (number of double bonds) in a fat.(16) Increased Iodine value observed in *Katupila Taila* (108.075) as that of *Tila taila* (109.86) due to increased rate of oxidation during *Sneha paka*. Present study has incorporated maximum numbers of parameters of standardization of *Sneha Kalpana* advocated by pharmacopeia and evaluated by maximum number of research studies where to propose product standards of *Katupila Taila*. Present formulation may be

modified to conventional dosage form like gel, cream, emulsion, etc. to provide Hydration of skin, increase in duration of contact of medicament at affected area and ease in cleansing of residual medicament during daily dressing.

Conclusion

Adopted method of *Snehapaka* for preparation of *Katupila Taila* can be considered as standard. Average 934.8 g (93.48% yield) of *Katupila Taila* was obtained from batch size of, 1 *l* of *Tila Taila* with 212 g of *Kalka dravya* and 4 *l* of *kwatha* of *Katupila* stem prepared over 3 days with 13 hrs of total duration of actual heating. The changes in physicochemical parameters as that of *Tila taila* (Sesame oil) can be merely correlated to effect of heating, suggesting collective effect of formulation ingredients and pharmaceutical processing of *Snehapaka*.

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