

Trick in the manufacturing, the key to the therapeutic value of Ksheerabala thaila of Dr.JRK's

Amruthavalli G.V*¹, Aruna.V², Gayathri.R³

Dr.JRK's research and Pharmaceuticals Pvt Ltd, No.18 & 19, Perumal koil street, Kunrathur, Chennai-600069.

¹*Asst.Manager- Research and Pharmaceuticals Pvt Ltd, amruthavalli_gv@jrkresearch.com ,9940327847

²Manager-Quality Control & Production, aruna_v@jrkresearch.com, 9566165310

³Director, gayathri_rajagopal@jrkresearch.com, 9841027502

Abstract: Ksheerabala thaila is a sastric Ayurveda drug indicated for pain relief. It will be used as topical application. This product is prepared by two methods i.e., Astanga hridaya and sahasrayoga as per sastric methodology. Ksheerabala thaila contains actives of single herb *Sida cordifolia*.

In the present study, Ksheerabala thaila was prepared by various methods according to the sastric methodology and also by other methods as presented in paper. The efficacy and phyto-actives retention and its stability at various conditions of temperature and UV light are studied by hemodynamics, TLC etc., respectively.

The study results show that instability of the herbal constituents at high temperature when done by the standard methods. Solvent extraction and JRK's Ksheerabala thaila process showing the profile stability even at high temperatures and UV light exposure. Therefore, there is need of integration of modern technologies in the sastric drug preparations. Complete details are presented in the paper.

Keywords: Ksheerabala thaila, *Sida cordifolia*, pain relief, Ayurveda for pain relief.

1. INTRODUCTION

Ksheerabala thaila is a sastric Ayurveda preparation with proven therapeutic effect against cervical spondylosis, shoulder pain etc. of both inflammatory and non-inflammatory etiology. It is also effective for Ardita (~facial paralysis), Katishula (~low back ache), Katigraha (~gridhrasi, sciatica), Sandhigata Vata (~osteoarthritis), Greeva Hundana (~cervical spondylosis), cerebral palsy, Ardhangavata (~hemiplegia), Kampavata (~parkinson's disease), convulsions and other neurological disorders (including degenerative disorders).¹

The method of preparation of Ksheerabala thaila is quite cumbersome where the herbal decoction of *Sida cordifolia* in water is made first and then the decoction is boiled in gingelly oil with or without milk and then the residual water and milk is removed and the final oil is filtered and the filtered oil is thus called Ksheerabala thaila.

We have compared the pain relief benefit of Ksheerabala thaila manufactured by Dr.JRK's research and Pharmaceuticals vis-a- vis Ksheerabala thaila of other manufacturers in human volunteers by studying the hemodynamic profile in the glabrous skin. The study findings clearly show that the Ksheerabala thaila of Dr.JRK's was several folds' superior in its efficacy over the market products.

In order to understand the possible reason behind the superior therapeutic effect of JRK's Ksheerabala thaila in comparison with most of the market products we have further studied the thermal and photo stability of *Sida cordifolia* by thin layer chromatography. We have also studied how the herbal biogram in TLC gets altered when the extract(decoction) is incorporated into the boiling gingelly oil with or without milk, was also studied.

Our study has clearly shown that the method of process has a significant role in modifying the herbal biogram of *Sida cordifolia* and suggests that such modification may be responsible for the superior efficacy of JRK's Ksheerabala thaila. The company has integrated tradition and science in equal proportion in the manufacturing of Ksheerabala thaila and the details are presented in the paper.

2. MATERIALS AND METHODS

a. Preparation of Ksheerabala thaila by conventional method (As per Astanga hridaya²) at Dr.JRK's

In brief 960gm of *Sida cordifolia*(roots) was taken in 61.44 kg of water and boiled to reduce the final volume to 1/4th i.e., 15.36 kg. To this 960gms of sesame oil and 960gms of cow's milk were added and heated further to evaporate out both milk and water and the oil then was filtered, cooled and then used.

b. Preparation of Ksheerabala thaila made at lab with methanol

1.5gm of *Sida cordifolia* was extracted in 100 ml of methanol and then the filtrate was dried to evaporate the solvent and the extract thus obtained was incorporated with 100 ml gingelly oil.

c. Preparation of Ksheerabala thaila made at lab with water

1.5gm of *Sida cordifolia* was extracted in 100 ml of water and then filtered and the filtrate was incorporated in 100 ml gingelly oil and then boiled to remove the water completely.

d. Preparation of Ksheerabala thaila made in the lab as per Sahasrayoga^{3,4}

240gm of *Sida cordifolia* roots were taken and made into a paste with milk and the final quantity was adjusted to 960gm and was then mixed with 768 gms of gingelly oil, boiled to remove the milk and water, the oil was filtered and used.

TLC separation of methanolic extract of *Sida cordifolia*

Preparation of Extracts

10 gm of *Sida Cordifolia* was weighed and then incorporated into methanol. The mixture was then gently heated for 5 minutes and kept at room temperature for 24 hours with frequent stirring. After 24hrs the mixture was filtrated and the filtrate was evaporated to remove the solvent and then the residual extract was used for the study. Water extract was also prepared as described above.

TLC chromatogram:

TLC chamber was saturated with mobile phases such as Toulene: Ethyl acetate: Methanol (7:3:1) and Toulene: EA (9:1) for 30 minutes and then TLC plate loaded with sample and then TLC assay was done. Rf value was calculated using the following formulae.

$$R_f \text{ value} = \text{Distance travelled by the sample} / \text{distance travelled by solvent}$$

Thermal stability of aqueous and methanolic extracts of *Sida cordifolia*

Aqueous and methanolic extracts (after complete removal of methanol) were taken separately in duplicate and kept for 10 min and 30 min at 70C and then the samples were studied by biogram by thin layer chromatography.^{5,6}

Photo stability of aqueous and methanolic extracts of *Sida cordifolia*

Aqueous and methanolic extracts (after complete removal of methanol) were taken separately in duplicate and exposed to UV irradiation for 5, 10, 15 and 20 minutes and then studied for biogram by thin layer chromatography.

Hemodynamic profile of lab made Ksheerabala, manufactured by Dr.JRK's and Market sample

Human volunteers were employed for the study after identifying the test site in the volar forearm region and scoring the initial erythema value in the scale of 1-5 where 5 being highly erythematic and 1 being free from erythema, with the help of a highly illuminated lamp reflectance.

The test drugs were applied over the preselected volar forearm region and the oil was massaged gently. After 5 minutes of hold up time the erythema was scored as described above. Plain gingelly oil massage was done as control. The hemodynamic profile score was recorded in test drug applied region vis-à-vis control region.

3. RESULTS

Ksheerabala thaila manufactured by Dr.JRK's Research and pharmaceuticals, ksheerabala thaila prepared with methanolic extract (cold and hot process), and Ksheerabala thaila prepared with aqueous extract of *Sida cordifolia* showed comparable physiochemical characteristics with reference to odour, taste, specific gravity and acid value.

Ksheerabala thaila prepared by conventional methods show dissimilar physiochemical characteristics and bizarre acid values. Table-1

Table -1: Organoleptic characteristics of Ksheerabala thaila prepared by different methods

Test sample	Physical parameters				
	Colour	Odour	Taste	Specific gravity	Acid value
Ksheerabala thaila – Dr.JRK's	Dark Yellow	characteristic	Downy, viscous, slightly palatable, non-tempting taste	0.917	8
Ksheerabala thaila- lab made with methanol extract mixed in cold phase	Light green	characteristic	Downy, gingly oil predominant taste with intervening herbal feel	0.915	6
Ksheerabala thaila- lab made with methanol extract mixed in hot phase	Light green	characteristic	Downy, gingly oil predominant taste with intervening herbal feel	0.915	6.5
Ksheerabala thaila-lab made with water extract	Light green	characteristic	Gingly oil predominant taste with no herbal feel	0.917	8
Ksheerabala thaila – made as per sahasrayoga	Dark yellow	characteristic	Downy, viscous, slightly palatable, partly tempting taste	0.919	8
Ksheerabala thaila – market sample	Yellow	characteristic	Spiky, viscous, non-palatable, non-tempting taste	0.921	10

TLC separation of methanolic extract of *Sida cordifolia*

The thin layer chromatography of methanolic extract of *Sida cordifolia*, when separated with two different mobile phases such as Toulene: EA (9:1) and Toulene: EA: MEOH (7:3:1) showed 8 and 11 distinct bands respectively. The methanolic extract was incorporated in the gingly oil at hot phase and then the methanolic fraction of the oil was studied by TLC by using the above mobile phases. Interestingly the profile of *Sida cordifolia* concurs with the TLC profile of the extract. This suggests that methanolic extract does not seem to undergo any modification or disintegration due to heating.

TLC separation of water extract of *Sida cordifolia*

The thin layer chromatography of the water extract of *Sida cordifolia*, when separated with two different mobile phases such as Toulene: EA (9:1) and Toulene: EA: MEOH (7:3:1) showed no clear bands

Table-2: TLC profile of various samples

Samples tested	Mobile phase, NM, RF range and No. of bands							
	Toulene : EA (9:1)				Toulene: EA: MEOH (7:3:1)			
	254 nm/ RF range 0.2-0.5	254 nm /RF range 0.51-0.96	366 nm/ RF range 0.2-0.5	366nm /RF range 0.51-0.96	254 nm/ RF range 0.2-0.5	254 nm /RF range 0.51-0.96	366 nm/ RF range 0.2-0.5	366nm /RF range 0.51-0.96
MeOH ext(Cold)	3	5	0	5	4	7	2	4
MeOH ext (Hot)	3	5	0	5	4	7	2	4
Aqueous ext	-	-	-	-	-	-	-	-
Milk extract	-	3	-	2	-	3	-	1
Hot oil ext (MeOH fraction)	-	3	-	1	3	6	-	3
Market sample(MEOH)	-	3	-	1	-	3	-	2

fraction)								
Dr.JRK's oil (MEOH fraction)	2	4	1	3	3	3	1	2
Oil-Sahasrayoga (MEOH fraction)	-	3	-	2	-	3	-	1
Oil-Astanga hridaya (MEOH fraction)	-	-	-	-	-	-	-	-

The water extract was incorporated in the gingly oil at hot phase and was then heated until the complete evaporation of water. The methanolic fraction of the above oil was studied by TLC by using the above mobile phases. Interestingly the profile of *Sida cordifolia* showed totally a different TLC profile with that of the aqueous extract. This suggests that the aqueous extract may have poor thermo stability.

Photo stability of aqueous and methanol extract of *Sida cordifolia*

Methanolic extract of *Sida cordifolia* (hot and cold), DR.JRK's Ksheerabala thaila did not show any photo instability by TLC analysis. However, all other samples showed high photo vulnerability. Table-3

Table-3: Photo-stability of various samples by TLC

Samples tested	Mobile phase ,No. of bands and RF range)							
	Toulene : EA (9:1)				Toulene: EA: MEOH (7:3:1)			
	254 nm/ RF range 0.2-0.5	254 nm /RF range 0.51-0.96	366 nm/ RF range 0.2-0.5	366nm /RF range 0.51-0.96	254 nm/ RF range 0.2-0.5	254 nm /RF range 0.51-0.96	366 nm/ RF range 0.2-0.5	366nm /RF range 0.51-0.96
MeOH ext (Cold)	3	5	0	5	4	7	2	4
MeOH extract (Hot)	3	5	0	5	4	7	2	4
Aqueous ext	-	-	-	-	-	-	-	-
Milk extract	-	2	1	1	-	3	-	1
Hot oil extract (MeOHfraction)	1	2	-	1	4	5	1	2
Market sample(MEOH fraction)	1	2	-	1	-	2	-	2
Dr.JRK's oil (MEOHfraction)	2	4	1	3	3	3	1	2
Oil-sahasrayoga (MEOHfraction)	1	2	-	2	1	2	-	1
Oil -Astanga hridaya (MEOH fraction)	-	-	-	-	-	-	-	-

Thermal and photostability of *Sida cordifolia* by boiling directly in oil:

Sida cordifolia when directly boiled in gingly oil and then the methanolic fraction of the oil was studied by TLC showed almost 80% similarity with that of the methanolic extract of *Sida cordifolia*. The photostability of the extract was almost 100% as it concurred fully with the methanolic extract of *Sida cordifolia*.

The thermal and photostability of *Sida cordifolia* extracted in water at room temperature over 3 days.

The cold aqueous extract of *Sida cordifolia* showed the TLC profile agreeing almost to 70% with that of methanolic extract of *Sida cordifolia*. The thermal and photostability of the cold fraction of *Sida cordifolia* extract showed no modification from its initial profile. Fig-2.

Hemodynamic profile of lab made Ksheerabala, manufactured by Dr.JRK's and Market sample

An increased erythema value was noticed in all volunteers treated with methanolic extract of *Sida cordifolia* in cold and hot, aqueous extract treated by UHT, Dr.JRK's Ksheerabala thailam, *Sida cordifolia* extracted in hot oil. All other samples did not alter the erythema value. Table -4

Table-4: Hemodynamic profile of various oils

Samples tested	No. of Human volunteers	Score 1-5	
		Initial erythema	Erythema after 15 min
Methanolic extract (Cold)	6	2 ± 0.75	4.1 ± 1.71
Methanolic extract (Hot)	6	1.3 ± 0.69	4.16 ± 1.61
Aqueous extract	6	2 ± 0.75	2 ± 0.75
Milk extract	6	1.66 ± 0.78	1.66 ± 0.78
Hot oil extract (Methanol fraction)	6	2 ± 0.75	4.83 ± 1.86
Dr.JRK's oil (MEOH fraction)	6	2 ± 0.75	4.52 ± 1.77
Market sample(MEOH fraction)	6	2 ± 0.75	2 ± 0.75
Oil as per sahasrayoga (MEOH fraction)	6	1.66 ± 0.78	1.66 ± 0.78
Oil as per Astanga hridaya (MEOH fraction)	6	1.66 ± 0.78	1.66 ± 0.78

4. DISCUSSION

Our present study has thrown a new insight about the photo and the thermal stability of *Sida cordifolia* as well as the method of process employed in making Ksheerabala thaila plays an important role in the efficacy.

The conventional method of preparation of Ksheerabala thaila whether it is by sahasrayoga or Astanga hridaya, the *Sida cordifolia* juice is prepared initially in water and then it is boiled in gingelly oil and milk until the water and milk is completely evaporated out. The oil thus prepared is called ksheerabala thaila.

Our study has clearly shown that the aqueous preparation of *Sida cordifolia* was unstable due to both temperature and UV exposure. This was confirmed by preparing *Sida cordifolia* extract in aqueous medium devoid of heating showed TLC profile exactly identical to that of the methanolic extract of *Sida cordifolia*. However, on heating the profile got totally distorted. This clearly suggest that *Sida cordifolia* bio-actives are highly unstable and thermolabile in aqueous medium.

Ksheerabala preparation by conventional methods demands prolonged heating of the aqueous juice of *Sida cordifolia* in oil until the total evaporation of water. Such prolonged heating is bound to affect the therapeutic value of *Sida cordifolia* that we have proved both by TLC study and which was again confirmed in human volunteers by studying the hemodynamic profile. Further the use of milk was found to engulf the bioactives of *Sida cordifolia* preventing them to be miscible in oil medium thus making the final oil mostly without any constituents of *Sida cordifolia*. However, such oil is treated as Ksheerabala by conventional norms without ascertaining the therapeutic value. The interesting twist of the tale was that the methanolic extract of *Sida cordifolia* prepared under hot or cold process as well as the *Sida cordifolia* directly boiled in gingelly oil did not show any photo or thermal instability.

We could not clearly ascertain whether just temperature and or the presence of moisture is causing distortion of *Sida cordifolia* as reflected in the TLC profile. The Ksheerabala thaila prepared with various methods of extraction as well as the methanolic fraction of the oils was studied by TLC and again the profile showed perfect concordance with the result obtained thereof.

Dr.JRK's Ksheerabala thaila showed superior therapeutic efficacy and intact chromatogram of *Sida cordifolia*. Whereas the market sample failed completely both in therapeutic evaluation as well as TLC profiling. Further the TLC profile of the market sample did not show any identifiable characteristics of *Sida cordifolia*.

Ksheerabala thaila is used for alleviating pain. Massage with Ksheerabala thaila is widely recommended for all types of chronic pain in the joints, spinal cord joints, muscular region as well as for the arthritic problems.

The Ksheerabala thaila is a single herb based preparation and therefore the entire therapeutic has to be sourced from the single herb called *Sida cordifolia*. The photo and temperature vulnerability of *Sida cordifolia* is, if not clearly understood and addressed in the beginning the drug thus made with *Sida cordifolia* may not be effective.

Dr.JRK's research and Pharmaceuticals has studied the process and other intricacies of *Sida cordifolia* and after integrating all necessary precautions the Ksheerabala thaila is made.

We have also found that the addition of milk in the process engulf several constituents of *Sida cordifolia* making them not available in the final oil. When *Sida cordifolia* is boiled in milk and then incorporated in gingelly oil and heated it further

to remove residual milk the final filtered oil also did not show the presence of *Sida cordifolia* where as such situation we did not encounter with water.

Whether the milk completely engulf *Sida cordifolia* constituents or impairs the miscibility in oil is not clear. Whereas in the case of water medium, the heat labile nature of *Sida cordifolia* gets pronounced only during heating. If the temperature of heating is regulated the thermolabile constituents can be preserved.

Although the solvent extraction may help to overcome all such odds observed above, such method is not approved in the sastric scriptures of Ayurveda. We are quite curious and unable to explain why when *Sida cordifolia* is directly boiled in oil showed well preserved chromatogram and therapeutic effect. Cold extraction in oil we presume may be the best option but it may consume more time.

We have employed hemodynamics as the therapeutic point to measure the possible clinical efficacy. Hemodynamics is widely accepted method for evaluating the analgesic preparations. During massaging with anti-inflammatory agents' vasodilatation takes place and as a result of increase blood flow is likely to occur. Therefore, the reading of erythema is the direct indicator of the analgesic being effective on the skin.

The present study clearly shows that a proper research on both the process of manufacture and the therapeutic benefit of all Sastric drugs is required. Ksheerabala thaila being an important drug for the treatment of pain in Ayurveda, a rigorous study on the method of manufacture and therapeutic value of *Sida cordifolia* is essential.

During our informal discussion with several AYUSH vidyas, we learned that the Ksheerabala thaila often show cryptic therapeutic benefits then consistent performance. We presume that the heat and light sensitivity of *Sida cordifolia* and the villaneous effect of milk may be affecting the clinical efficacy of Ksheerabala thaila. We have also found that the *Sida cordifolia* show great variability in its TLC profile among samples collected from different sources.

Among the various constituents present in *Sida cordifolia*, certain constituents are thermo and photo-stable and are also therapeutically effective. However, the proportion of such constituents appears to be quite low in *Sida cordifolia*, therefore a specialized chemical and pharmacognostic approach is needed to achieve their optimal concentration in ksheerabala thaila to be therapeutically effective.

Dr.JRK's Research and Pharmaceuticals is seriously engaged in identifying several phyto chemical groups in *Sida cordifolia*, the therapeutic value, the relative proportion and the stability of all such phytochemicals in order to make Ksheerabala thaila a reliable, effective and consistent as xyloquine or Diclofenac. The integration of science into the traditional wisdom has given an extra edge to the therapeutic value of Ksheerabala thaila made by Dr.JRK's Research and Pharmaceuticals Pvt Ltd.

REFERENCES

- [1] Jatinder Verma, Gopesh Mangal. New Perspectives of Ksheerabala Taila (oil): A Critical Review. IJATM, VOL 1 , ISSUE 1, DEC 2019; 25-31.
- [2] Ashtanga Hridaya – by Vagbhatta, Published by Chaukhambha Sanskrit Sansthan, Varanasi, 10th edition, 1992 Chikitsa sthan – 22/45-46.
- [3] Charaka Samhita – by Agnivesh, Published by chaukhambha Bharati academy, Varanasi 14th edition, 1987, Chikitsa Sthan – 29/119-120.
- [4] Sushruta samhita- by sushruta, Published by chaukhambha Sanskrit sansthan, Varanasi, 8th edition, 1993, Chikitsa sthan – 15/40-43.
- [5] Singhal S., Singhal N., Agarwal S., Pharmaceutical Analysis II, Thin layer chromatography, Pragati prakashan, First edition, 2009, 98-111.
- [6] Kasture A.V., Mahadik K.R, Wadodkar S.G, More H.N., A textbook of pharmaceutical analysis, Instrumental methods, Nirali Prakashan, 9th edition, 2005, vol II, 18-30.
- [7] Aruna V, Amruthavalli GV, Gayathri R. Peripheral Blood Flow: A New Indicator for Measuring the Analgesic Effect of Dolowhite Emulsion. Research and Reviews: A Journal of Unani, Siddha and Homeopathy. 2017; 4(1): 1–3p.