Pharmacogn. Res., 2025; 17(1):78-84.
https://www.phcogres.com

Original Article

Standardization of Karisalai Madakku Tailam and Quantification of Wedelolactone by HPTLC

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ABSTRACT

Background: Herbal oils also known as Medicated oils, are oils infused with medicinal properties of herbs or medicinal plants. **Objectives:** To prepare the herbal oil, Karisalai tailam using gingelly oil and *Eclipta alba* Linn. (Karisalai) further to continue the process of madakku for two more times and to investigate the physico-chemical parameters for any changes. **Materials and Methods:** The herbal oil was prepared as per literature. Acid, saponification, iodine, peroxide values, rancidity, refractive index were estimated for the raw gingelly oil, gingelly oil heated in parallel without adding *Eclipta alba* Linn. juice, Karisalai Madakku Tailam (KMT1), second Madakku Tailam (KMT2) and third madakku tailam (KMT3). The content of major coumestane derivative present in *Eclipta alba* Linn., ie., wedelolactone which can serve as a bioactive marker was estimated in karisalai juice and Karisalai Madakku Tailams (KMT1, KMT2 and KMT3). **Results:** Physico-chemical results of Karisalai Madakku Tailam (KMT1, KMT2 and KMT3) were comparable with the values of base sesame oil. But the content of wedelolactone in first processed oil KMT1 was estimated as 0.039% which increased to 0.0715% in KMT2 and further increased to 0.1048% in KMT3. **Conclusion:** Physico-chemical parameters can be referred to examine the overall quality in the oil formulation at any of the madakku stage. HPTLC method would be a tool for the identification and quantification of wedelolactone in this medicated oil.

Keywords: *Eclipta alba*, Karisalai, Kaiyan tailam, Wedelolactone, Peroxide value.

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Received: 05-09-2024;
Revised: 14-10-2024;
Accepted: 03-12-2024.

INTRODUCTION

Demand on herbal medicines gains upward trend in the developing countries for preventive health care as herbal medicines are efficient, safe and less side effects. Recently, plant based medicines which are considered as eco-friendly and bio-friendly has been considerably utilized. Kaiyan thailam also called as Karisalai tailam, is a medicated herbal oil used for various ailments as per Siddha system of Indian medicine.[1] The juice of fresh leaves of *Eclipta abla* Linn. (Karisalai) is the ingredient of Kaiyan tailam. *E. alba* Linn. (Asteraceae) is a medicinal plant known as Bhṛṅngaja in Sanskrit; Bhangara or Bhangaraiya in Hindi; Karisalankanni, Karisalanganni, Karisalai in Tamil. Cañcivi Mattirai, Cūlaik Kuṭāram, Emataṇṭak Kulikai, Tālākak Kaṭṭu are some formulations of Siddha;[1] Bhṛṅgāmalakādi Taila, Bhṛṅgarāja Taila, Nīlī Bhṛṅgādi Taila, Bhṛṅgarājasava, Tekarāja marica are few preparations of Ayurveda.[2] *E. alba* (Linn.) Hassk, (syn. *E. prostrata)*, grows as a common weed throughout India. *Eclipta alba* has been used extensively in traditional medicine for a range of ailments for the treatment of coughing, blackening of grey hair, treatment of calculus, disorder of eyes and asthma, diabetes, gastric problems, inflammatory disorders, skin disorders and for long hair growth.[2-4] The plant is the active ingredient of many herbal formulations. It is often prescribed for regeneration of damaged liver cell, enlarged liver or spleen and in chronic skin diseases and found to be effective.[5,6] This plant has been established as a potent hepato protectant in the treatment of infective hepatitis in a clinical study.[7,8] The leaves of *E. alba* showed antihyperglycemic activity also.[9,10] Sesame oil (ben oil, benne oil, gingelly oil, sesame oil, thill oil) is derived from *Sesamum indicum* L. (family: Pedaliaceae) plant seeds. The present work was carried as standardization of drugs and estimation of wedelolactone of processed oil and drugs.

DOI: 10.5530/pres.20251927

MATERIALS AND METHODS

Collection of materials

The fresh leaves of *E. alba* Linn. were collected from Chennai, Tamil Nadu and was authenticated by Research Officer (Pharmacognosy), SCRI, Chennai. Cold pressed sesame oil was procured from the market, Chennai, Tamil Nadu.

Definition for tailam

Tailams are prepared by boiling to dehydration, the specified oil with milk, decoction, juices and some powders or pastes according to recipes.[1] Kaiyan Thailam (KMT1) is one kind of medicated oil preparation. It is prepared by boiling the juice of *E. alba* leaf with sesame oil for stipulated period and filtered.

Formulation Composition

Sl. No.	Ingredients	Botanical Name	Anatomical part	Quantity used
1.	*Karicālāic cāru*	*Eclipta alba* Linn.	Leaves	1 Part
2.	Nallenney (Sesame oil)	*Sesamum indicum* Linn.	Seed oil	1 Part

Method of Preparation

Squeezed the *E. alba* leaves to extract juice and filtered; equal volume of oil and leaf juice (100 mL). Boiled the oil in low flame and added the juice till it becomes dehydrated (Kaiyan Tailam or KMT1).[1] This process was repeated again for two times with fresh *E. alba* juice and the oils obtained from the prior process (KMT1 or KMT2).[11] At every process stage of tailam, the raw sesame oil was also boiled for the same time duration to evaluate the effect of repetitive heating of the edible oil.

Physico-chemical Analysis

The physico-chemical parameters of oil viz., acid, iodine, peroxide, saponification values, rancidity, refractive index and specific gravity were analyzed out by using procedures mention in standards[12-17] for Raw Sesame Oil (RSO), Heated Sesame Oil (HSO), KMT1, KMT2 and KMT3. The mean values and standard deviations were computed with the triplicate values.[1]

Oil sample extraction

The phytoconstituents present in Kaiyan thailam was extracted by adding 10 mL of Kaiyan tailam with 25 mL of methanol and constantly stirring for 1 hr. Then it was stored in freezer to solidify the oil. The unfrozen alcoholic part was filtered in Whatman no.41 filter paper and the filtrate was concentrated, made upto 10 mL volumetric flask and used for quantification of wedelolactone by using HPTLC method.

Preparation of standards

Procured wedelolactone (10 mg) from Natural Remedies. Dissolved 10 mg of wedelolactone in ethyl alcohol and made up to 10 mL in a standard flask (stock standard solution). From this, 1 mL was pipetted out and made up to 10 mL in a standard flask (working standard solution).

Calibration plot for wedelolactone

The sample and standard solutions were applied in the form of 6 mm band width with a Camag 100 µL sample syringe (Hamilton, Switzerland) on a silica gel precoated aluminium $60F_{254}$ plate (E. Merck, Germany; 20 cm×10 cm and 10 cm x 10 cm with 200 µm thickness) using an ATS4 applicator. Prewashed the plates with methanol and activated for 5 min at 110°C prior to application of sample and standards. Distance between the applied bands was 5 mm. The dimension of the slit was kept at 5 mm×0.45 mm and speed of the scanning was 20 mm/s. The mobile phase solvent mixture was *toluene: ethyl acetate: formic acid* (7: 2.5: 0.5, *v/v/v*) and plate development distance was 70 mm. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (27°C±2) at relative humidity of 40%±5. The photo documentation was 254, 366 nm by using UV visualizer chamber. Densitometric scanning was performed using a Camag's HPTLC scanner IV at 366 nm and operated by winCATS software. The source of radiation used was mercury lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression and amount of wedelolactone was computed from peak area.

Estimation of wedelolactone

Estimation of wedelolactone in samples of 1st, 2nd and 3rd processed oil. The test solutions of KMT 1st (10, 15 µL), 2nd (5, 6 µL), 3rd (4, 5 µL) samples and standard 2, 3 and 4 µL respectively, in triplicate, the sample was applied into the precoated silica gel $60F_{254}$ plates, with the Camag ATS-4 automatic TLC sample applicator. The plate was developed and scanned in similar manner. The peak areas and absorption spectra were recorded. The amount of wedelolactone in the 1st, 2nd and 3rd samples were calculated.

RESULTS AND DISCUSSION

Herbal medicine quality depends on the correctness of authentication of raw drugs, method of preparation. In this study, drug Kaiyan thailam was prepared by boiling sesame oil and fresh leaf juice of *Eclipta alba*. Standardization of drug, Raw sample Sesame Oil (RSO), throughout preparation drug Heated Sesame Oil only (HSO), first processed oil (KMT1), second processed oil (KMT2) and third processed oil its final drug (KMT3) using suitable physico-chemical parameters. The physico-chemical values are given in Table 1.

The Refractive Index (RI) is the ratio of the speed of light in vacuum to the speed of light through a given material.[18,19] The refractive index values (1.463-1.469) are nearly in line with the level recommended by FAO/WHO (1.465-1.456). The acid value are used to indicate the level of or Free Fatty Acid (FFA) and edibility of oils.[20] Free fatty acid means by-products of the metabolism of fat in adipose tissues. Iodine value is defined as the amount of unsaturated fatty acids present in the oil. Diet rich in unsaturated fatty acid is recommended for healthy food. Saponification value is a measure of average molecular weight (or chain length) of all the fatty acid present. The quality control parameters of all the tested samples (RSO, KMT1, KMT2 and KMT3) recommended are nearly line with the levels by FAO/WHO values (Table 1).

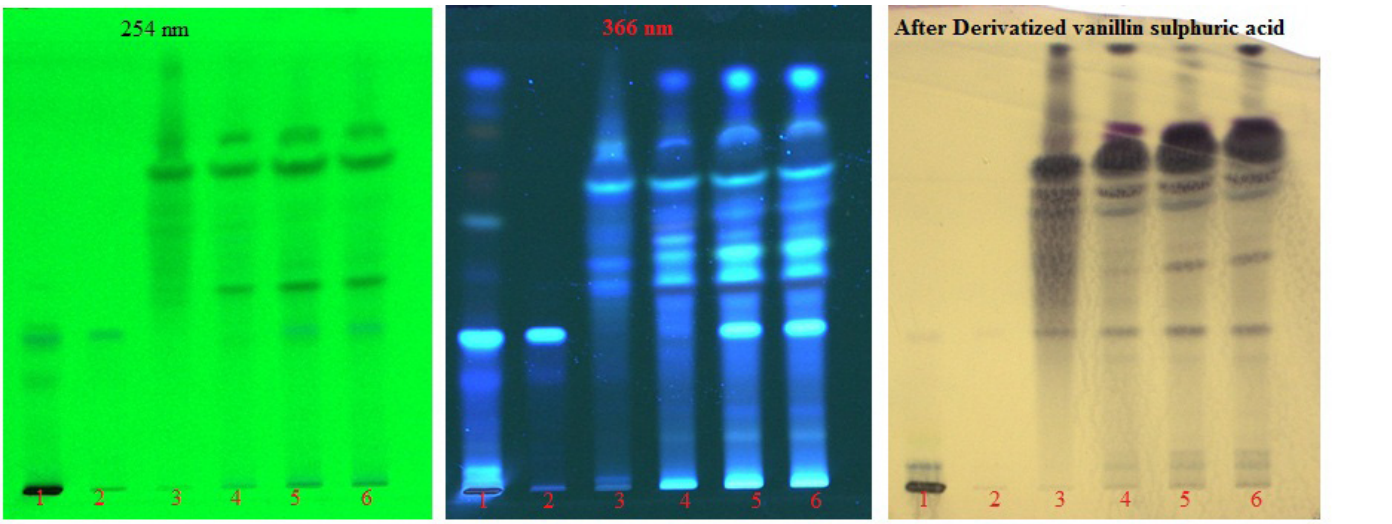
Peroxide value is used as a quantity of the amount to which rancidity reactions have occurred during storage and it is used as a good measure to determine of the quality and stability of oils.[21] High peroxide value could be resulted from high degree of unsaturation and found to increase with the light, temperature, storage time and contact with atmospheric oxygen.[18,22] However the peroxide value of heated sesame oil increased from 4.769 to 18.647 but there is a decrease with every processing which may be due to the antioxidants present in *E. alba* leaf juice controlling or preventing the formation of reactive oxygen species during the repeated heating processes. Edibility of the oil is increasing in every madakku process which is evident from the decrease in the peroxide value.

The comparative TLC and HPTLC photo documentation of *E. alba*, sesame oil, KMT1, KMT2 and KMT3 oils and R_f values are given in Figure 1. The marker compound of wedelolactone R_f 0.36 similar spot was present in *Eclipta alba*, KMT1, KMT2 and KMT3. Sesame oil spots 0.46, 0.50, 0.73 and 0.81 all spots were present in KMT1, KMT2 and KMT3 (Table 2, Figure 1). 3D Chromatogram of *Eclipta alba*, sesame oil, KMT1, KMT2 and KMT3 are given in Figure 2. UV spectral comparison of wedelolactone and corresponding spots in KMT oils is given in Figure 3.

The amount of wedelolactone in KMT1, KMT2 and KMT3 were calculated for calibration plot is represented in Figure 4. Calibration curve shown by the standard of wedelolactone has

Table 1: Physicochemical parameters.

Sl. No.	Parameters	Sesame oil values (WHO/Codex)	Sesame oil (Fresh)	Sesame oil (Heated)	KMT1	KMT 2	KMT 3
1.	Specific gravity	0.915-0.924	0.843	0.849	0.8695	0.8406	0.831
2.	Refractive index	1.465-1.469	1.467	1.470	1.463	1.467	1.469
3.	Acid value	4.0	6.23	1.561	5.273	6.703	7.35
4.	Saponification Value	186-194	215.95	216.88	203.34	208.87	214.14
5.	Iodine value	104-120	110.03	109.64	109.287	104.3	101.12
6.	Peroxide value	3.0	4.7699	18.64	4.761	4.758	2.708



Track -1 –*Eclipta alba* ; Track-2-Wedelolactone; Track-3-Raw oil; Track -4- 1st Processed oil; Track-5- 2nd Processed oil; Track-6- 3rd Processed oil

Figure 1: TLC Photodocumentation of *Eclipta alba* juice, sesame oil, KMT1, KMT2 and KMT3.Track 1-*Eclipta alba* juice; Track 2-Wedelolactone, Track 3-Raw oil, Track 4-KMT1, Track 5-KMT2, Track 6-KMT3.

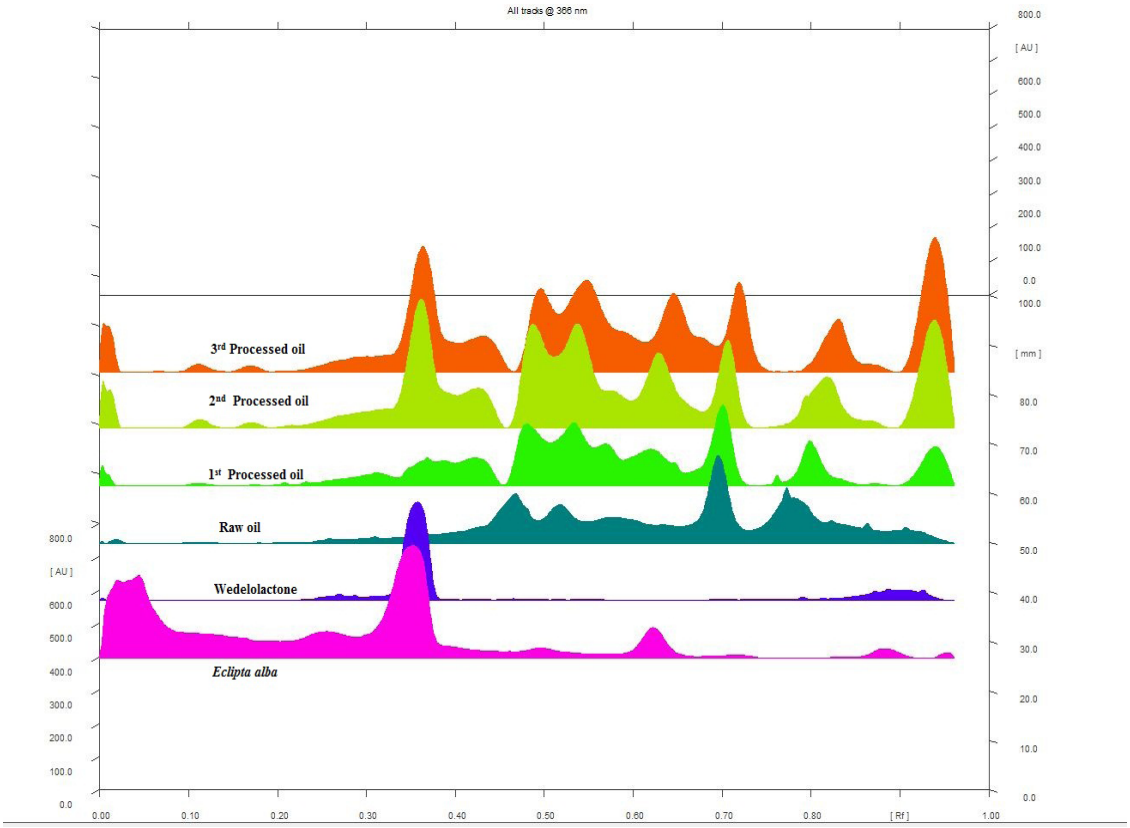


Figure 2: 3D Chromatogram of *Eclipta alba* juice, raw oil, KMT1, KMT2 and KMT3.

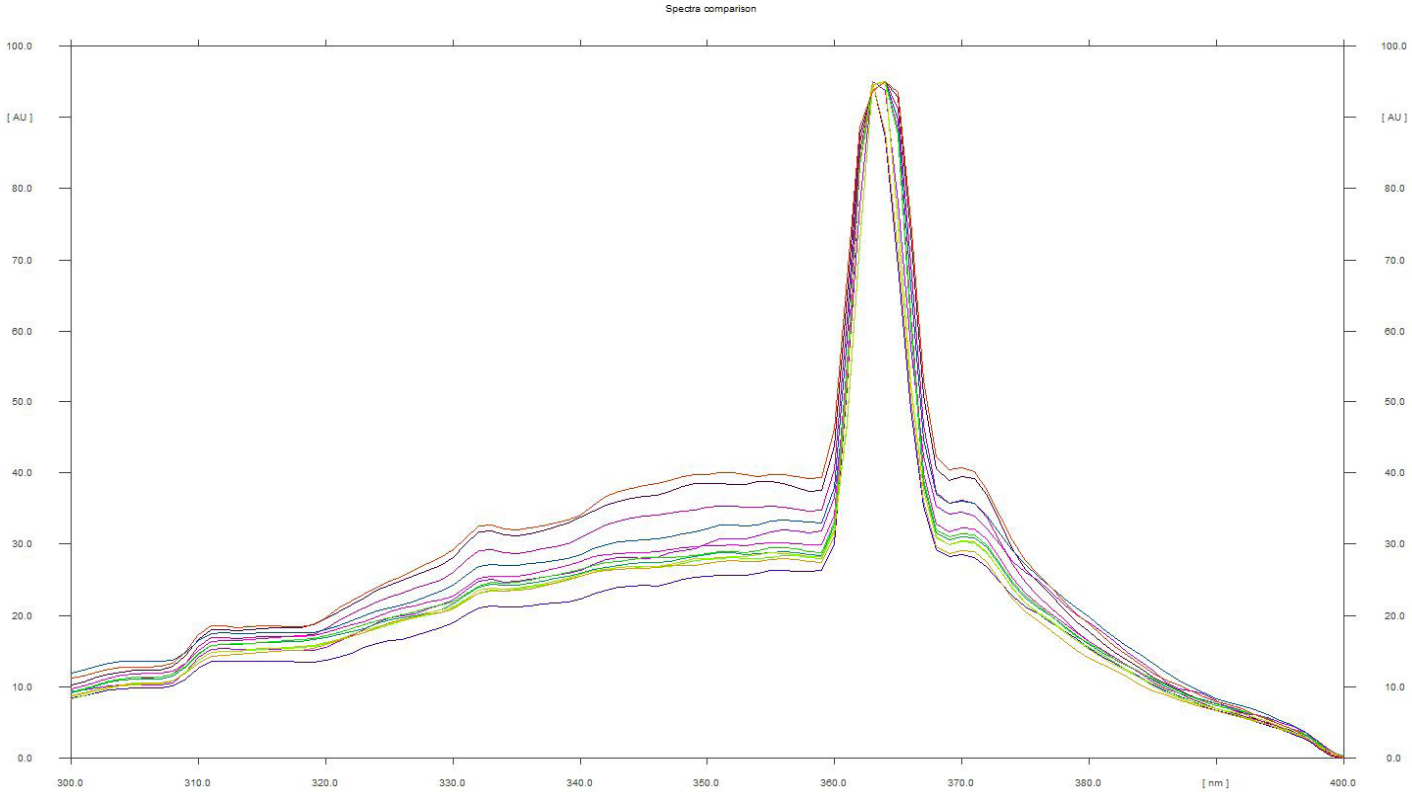


Figure 3: UV spectral comparison of wedelolactone and corresponding spots in KMT.

Table 2: R_f **and color of spots of *E. alba*, wedelolactone, raw oil, KMT1, KMT2 and KMT3.**

Track No	UV 254 nm	UV 366 nm	After dipping in vanillin sulphuric acid
1. *Eclipta alga* juice	0.25 (green) 0.35 (light blue) 0.47(green)	0.26 (blue) 0.35 (fluorescent blue) 0.50 (blue) 0.62 (blue) 0.81 (red) 0.88 (blue)	0.05 0.34 (both grey)
2. Wedelolactone	0.35 (light blue)	0.35 (fluorescent blue)	0.34
3. Raw oil	0.43 0.46 0.50 0.53 0.60 0.64 0.73 0.81 (all green)	0.29 0.47 0.52 0.57 0.70 0.77 0.82 0.86 (all blue)	0.35 0.63 0.67 0.73 0.84 (all grey)
4. KMT1	0.36 (light blue) 0.46(green) 0.50(green) 0.54(green) 0.57(green) 0.60(green) 0.65(green) 0.73(green) 0.81(green)	0.31 (blue) 0.36(fluorescent blue) 0.42(blue) 0.48(blue) 0.53(fluorescent blue) 0.57(fluorescent blue) 0.62(blue) 0.70(fluorescent blue) 0.76(blue) 0.80(blue)	0.04 (grey) 0.34 (grey) 0.63 (grey) 0.67 (grey) 0.73 (grey) 0.84 (violet)
5. KMT2	0.16(green) 0.36 (light blue) 0.46(green) 0.51(green) 0.55(green) 0.62(green) 0.74(green) 0.81(green)	0.11(blue) 0.17(blue) 0.36(fluorescent blue) 0.43, 0.49(blue) 0.54(fluorescent blue) 0.63(blue) 0.71(fluorescent blue) 0.82(blue)	0.04 0.08 0.34 0.63 0.67 0.73 (all grey)
6.KMT3	0.16(green) 0.36 (light blue) 0.47(green) 0.53(green) 0.65(green) 0.74(green) 0.82(green)	0.11 (fluorescent blue) 0.17 (fluorescent blue) 0.30 (fluorescent blue) 0.36 (fluorescent blue) 0.43 (blue) 0.50(fluorescent blue) 0.55 (fluorescent blue) 0.65 (blue) 0.72 (fluorescent blue) 0.83 (blue)	0.04 0.08 0.34 0.63 0.67 0.73 (all grey)

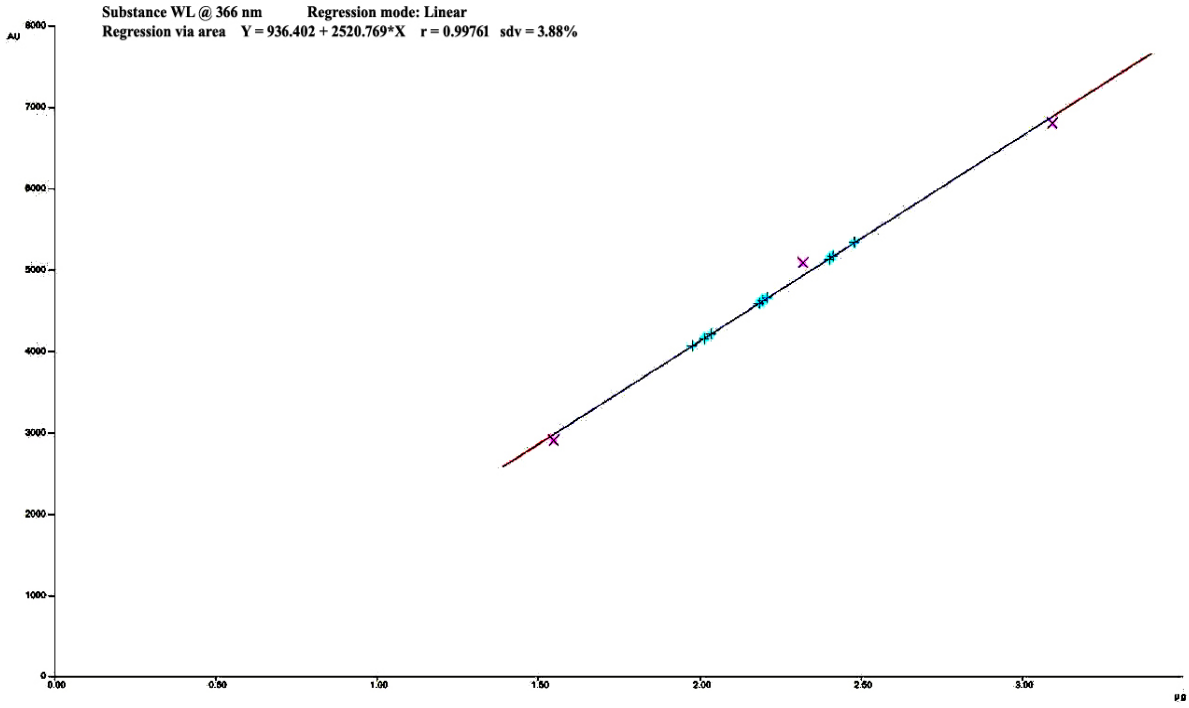


Figure 4: Linearity of calibration graph.

Table 3: Linearity data of wedelolactone.

Sl. No.	Parameters	Values
1	Linearity Range	1.5 to 3 µg
2	Limit of detection	0.00495 µg
3	Limit of quantification	0.015 µg
4	Standard deviation	3.88 %
5	Correlation coefficient	0.99761
6	% of wedelolactone 1st processed oil (KMT1).	0.03054 %
7	% of wedelolactone 2nd processed oil (KMT2).	0.09754 %
8	% of wedelolactone 3rd processed oil (KMT3).	0.01307 %

linear coefficient correlation in the range of concentration 1.5 to 3 µg (r=0.9976). The linearity of calibration graph was validated by correlation coefficient and the Standard Deviation (SD) for intercept value was 3.88%. The linearity study data is given in Table 3 and linearity curve is given in Figure 4.

This drug will be used in cough (cold cough) and taking oil bath also. Dosage of the drug is ½ to 1 teaspoon.[11] The present study may be useful for the quality control of drug and identification of phytochemical compounds.

CONCLUSION

The physico-chemical results of Kaiyan Tailam (KMT1) and KMT2 and KMT2 are comparable with the values of starting material (sesame oil). Whereas the peroxide value of heated sesame oil increased from 4.769 to 18.647 but there is a decrease with every madakku processing. *E. alba* leaf juice has controlled the oxidation of lipids, fats and acids of the sesame oil during heating. Similarly the wedelolactone content in KMT1 was estimated as 0.039% which is increased in KMT2 (0.0715%) and KMT3 (0.1048%). The madakku process fortified the wedelolactone content in the oil thereby increases its therapeutic efficacy in multiple folds.

AUTHOR CONTRIBUTION

The work was first designed by Subhasri R, Priyanka T, Hemavathy R and prepared the drugs. Murugammal S quantified the wedelolactone and drafted the manuscript. Shakila R restructured the design, carried out the analytical studies and revised the manuscript. All authors gave their consent to the manuscript.

ACKNOWLEDGEMENT

Authors are grateful to The Director General, CCRS and the Incharge, SCRI for the facilities The Principal, Government Siddha Medical College, Chennai, for encouragement.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

KMT: Karisalai madakku tailam; **KMT1:** Karisalai Madakku Tailam First time processed; **KMT2:** Karisalai Madakku Tailam Second time processed; **KMT3:** Karisalai Madakku Tailam Third time processed; **RSO:** Raw sesame oil; **HSO:** Heated sesame oil; **HPTLC:** High performance Thin Layer chromatography; **R_f:** Retention factor; **r:** Correlation coefficient.

SUMMARY

Kaiyan Tailam (KMT) is an oil preparation made out of *E. alba* juice and gingelly oil. It is internally taken to cure cough and externally applied for hair growth. It is further rejuvenated by repeating the process of adding *E. alb*a juice for two more times (KMT2; KMT3). The rejuvanated oil can be useful for asthma. In this study; KMT1; KMT2; KMT3; raw sesame oil and heated sesame oil were analysed for the physicochemical parameters related to oil. The values of KMT1; KMT2 and KMT3 were found to be decreasing in specific gravity; peroxide values; iodine value and increasing refractive index; acid value and saponification value. When compared to fresh sesame oil; the peroxide value and iodine values are very much decreasing which indicates the value addition of the oil. The percentage content of wedelolactone were estimated and found to be increasing which may increase the potency of the drug.

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Cite this article: Murugammal S, Subhasri R, Priyanka T, Hemavathy R, Shakila R. Standardization of Karisalai Madakku Tailam and Quantification of Wedelolactone by HPTLC. Pharmacog Res. 2025;17(1):78-84.