ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

QUALITATIVE AND QUANTITATIVE ANALYSIS OF *KARUNKOZHI THYLAM* PREPARED AS PER SIDDHA LITERATURE

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Article info

Article History:
Received: 16-08-2024
Accepted: 21-09-2024
Published: 20-10-2024

KEYWORDS:
Karunkozhi Thylam, Degenerative diseases, GC-MS, Oleic acid, Siddha medicine, *Vatha noigal*.

ABSTRACT

The Siddha system is a traditional system of south India especially in Tamil Nadu. *Thylam* (medicated oil) is one of the 32 internal medicines. *Karunkozhi thylam (KKT)* is used to treat *Vatha* disease (degenerative disease). Qualitative and quantitative analysis ensures the quality and safety of drugs and minimizing contamination and adulteration risks. Weight/ml, refractive index (RI), density, iodine value (IV), acid value (AV) were analyzed as standardization parameter and GC-MS were employed to identify the active compound and test for heavy metals (lead, cadmium, mercury, arsenic), microbial contamination, test for specific pathogen (E.coli, Salmonella spp., Staphylococcus aureus, Pseudomonas aeruginosa), pesticide residue (organochlorine, organophosphorus, pyrethroids), test for Aflatoxins (B1, B2, G1, G2) were used as a safety measure. The result shows RI was 1.457, density was 1.213g/cm^3, IV was 108.12g I/100g and AV was 0.86mg KOH/g. The safety parameters are within the acceptable limit. GC-MS shows the presence of oleic acid and n-hexodeconoeic acid that have anti-inflammatory activity. This study establishes a standardized protocol for KKT, ensuring quality, safety and efficacy. The findings have important implications for regulatory compliance and suggest areas for further research.

INTRODUCTION

Siddha system is a traditional system of South India, especially in Tamil Nadu. According to the Siddha system of medicine, imbalance of the three humours and seven elements *(Udal thathukkal)* is the main cause of all diseases. Therefore, the treatment is based on balance of three humours. Siddha medicine is broadly classified into internal and external medicines. *Karunkozhi thylam (KKT)* is the medicated oil form of internal medicine used to treat degenerative diseases. *Vadham* is one of the three humours, and imbalance of *vadham* is the basic factor causing degenerative diseases. According to the Siddha system, medicines with hot potency and viscous properties balance *vadham*[1]. *KKT* having the above properties, qualitative and quantitative analysis ensures the quality and safety of medicines, minimizing the risk of adulteration, and enables the establishment of standard parameters. Lack of quality control can affect the potency and well-being of drugs that may lead to health problems in consumers. In this modern world, the evidence of traditional knowledge is necessary for the globalization process and gives it added strength. Therefore, qualitative and quantitative analysis of *KKT* is one of the initial steps towards this goal.

Access this article online

Quick Response Code

https://doi.org/10.47070/ijapr.v12i9.3391

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MATERIALS AND METHODS

Standard Operative Procedure for the preparation of *KKT* [2]

Procurement of Active Ingredients

Karunkozhi (Kadaknath hen) was purchased from poultry farm at Krishnapuram, Tirunelveli. The other ingredients of *KKT* were purchased from reputed indigenous raw drug store at Thuckalay, Kanyakumari.

Ingredients

1. A egg laying *Karunkozhi* (Kadaknath hen) feather and gut removed – 908g
2. *Perungayam (Ferula asafoetida)* -10g
3. *Sukku (Zingiber officinale)* – 10g
4. *Parangipattai (Smilax china)* – 10g
5. *Poondu (Allium sativum)* – 10g
6. Neem oil *(Azadirachta indica)* – 2.5 liter

Identification and Authentication

The raw drugs for the preparation of *KKT* were identified and authenticated by the experts of Gunapadam department, Government Siddha Medical College and Hospital, Palayamkottai, Tirunelveli, Tamil Nadu.

Procedure

The drugs listed above were purified according to Siddha literature and the pound like a coarse paste, water was added and made a decoction. At the end, the neem oil was added in the decoction, boiled to the particular consistency that mentioned in the literature then filtered.

Fig 1: *Karunkozhi*

Fig 2: Ingredients of *KKT*

Fig 3: *KKT*

Dosage

15ml orally for 8 days at OD.

Indication

Vaatha noi (degenerative diseases) and *soolai* (pricking pain)

Refractive index (RI)

The refractive index is measured by refractometer at 25º (±0.5) with reference to the wavelength of the D line of sodium (589.3 nm). The temperature should be carefully adjusted and maintained[3].

Density

The liquid sample filled into the container without air bubbles. Place the hydrometer into the liquid sample and record the density value.

Iodine value (IV)

To determine the iodine value, accurately weigh the substance and place it in a dry iodine flask. Dissolve the substance in 10ml of carbon tetrachloride, followed by the addition of 20ml of iodine monochloride solution. Insert the stopper, previously moistened with potassium iodide solution, and allow to stand in a dark place at 17°C for 30 minutes. Add 15 mL of potassium iodide solution and 100ml of water, then shake and titrate with 0.1 N sodium thiosulphate, using starch solution as indicator, and record the volume used (a). Concurrently, perform a blank test by repeating the process without the substance and record the volume of sodium thiosulphate required (b). Finally, calculate the iodine value using the formula: Iodine Value = ((b - a) × 0.01269 × 100) / W, where W is the weight of the substance in grams [3].

Acid value (AV)

Accurately weigh approximately 10 g of the substance (1-5) - specifically for resins - into a 250ml flask. Add 50ml of a neutralized alcohol-solvent ether mixture (1:1) containing 1ml of phenolphthalein solution. Gently heat the mixture on a water bath, if necessary, until the substance completely dissolves. Then, titrate with 0.1 N potassium hydroxide, shaking constantly, until a persistent pink colour lasts for 15 seconds. Record the volume of potassium hydroxide required. The acid value calculate by:

$$Acid\ value\ = \frac{a \times 0.00561 \times 1000}{W}$$

Where 'a' is the number of ml. of 0.1 N potassium hydroxide required and 'w' is the weight in g of the substance taken[3].

Gas chromatography and mass spectrometry (GC-MS)

The GC analysis was conducted on an Agilent 6890N gas chromatograph, equipped with a photon multiplier tube detector and utilizing a 1079 front injector. The chromatograph was fitted with HP 5. The column used for MS analysis was a 30m×0.25mm ×0.25µm capillary column. The injector temperature was set at 250°C, and the oven temperature was initially at 70°C hold for 4 mins then programmed to 200°C at the rate of 10°C/min and then held for 13 min at 200°C. Helium was used as a carrier gas with the flow rate of 1.5 ml/min. 0.2 microliter of the sample *KKT* (diluted with methanol 1:10) were injected in the splitless mode. The percentage of composition of the samples were calculated by the GC peak areas. GC–MS analysis of sample was performed by Agilent gas chromatography equipped with JEOL GC MATE-II HR Mass Spectrometer. The GC analysis utilized the same conditions and column as described earlier. Electron impact ionization at 70 eV was employed for mass spectrometry analysis. Ion source and transfer line

temperatures were kept constant at 250°C. The mass spectra were obtained by centroid scan of the mass range from 50 to 600 amu. Compound was identified by comparing retention indices (RI), retention times (RT), and mass spectra with WILEY and NIST library data, as well as literature references, using the GC-MS system. (Adams, 2009).

Heavy metal analysis by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

Measured drug is dissolved in a decomposition vessel with nitric acid into 10ml solution, then analysed in Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

Microbial Contamination

A test sample was inoculated into a sterile petri dish, followed by the addition of approximately 15ml of molten agar at 45°C. The agar and sample were thoroughly mixed by gently tilting and swirling the dish. The mixture was then allowed to solidify undisturbed for approximately 10 minutes, enabling the agar to completely gel. (about 10 minutes). Plates were then inverted and incubated at 37°C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for Colony forming units[3].

Test for specific pathogen

Test sample was inoculated into the specific pathogen medium (Eosin Methylene Blue, Deoxycholate Citrate, Mannitol, Cetrimide) by pour plate method. The plates were incubated under 37°C for 24–72 hour for observation. Presence of specific pathogen identified by their characteristic colour with respect to pattern of colony formation in each differential media.[3]

Pesticide Residue

The test samples underwent acetone extraction, followed by brief homogenization and filtration. Subsequent addition of acetone was performed. Solvent evaporation was achieved using a rotary evaporator at a controlled temperature (≤40°C), resulting in near-complete removal of the solvent. To the residue add a few milliliters of toluene and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter and analysis were done as per Pharmacopoeial Laboratory of Indian Medicines (PLIM) guidelines[3].

Test for aflatoxins (B1, B2, G1, G2)

Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5µL, 5µL, 7.5µL and 10µL. The test sample was applied to the chromatographic plate and allowed to dry. The chromatogram was then developed in an unsaturated chamber using a solvent system comprising chloroform, acetone, and isopropyl alcohol (85:10:5 v/v) until the solvent front had migrated at least 15cm from the origin. After development, the plate was removed from the chamber, the solvent front was marked, and the plate was air-dried. Locate the spots on the plate by examination under UV light at 365nm[4].

RESULTS

Physiochemical Analysis

Table 1: Physiochemical analysis of *KKT*

Colour	Dark yellow
Density	1.213 g/ cm^3
Acid value	0.861 mg KOH/g
Iodine value	108.12 g I/100g
Refractive index	1.457

GCMS analysis

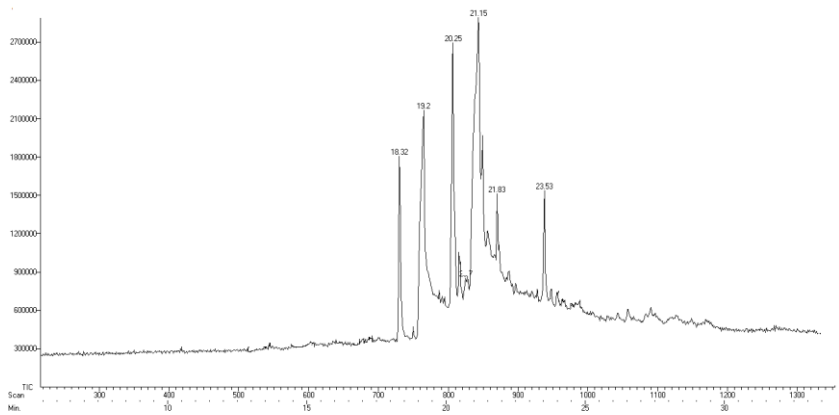


Fig 4: Shows the graph of *KKT*

Table 2: Shows GC-MS results indicating retention time (RT), compound name, peak area % of *KKT*

S.No	RT	Compound name	Peak area %
1	18.32	Hexa decanoic acid, methyl ester	15.60
2	19.20	n-hexadecanoic acid	20.20
3	20.25	6-octadecenoic acid	23.01
4	21.15	Oleic acid	24.30
5	21.83	12-methyl-e,e-2,13-octadecadian-1-ol	7.64
6	23.53	Octadec-9-enoic acid	8.90

Heavy metal analysis by ICP OES

1. Lead (Pb)-BDL
2. Cadmium (Cd) -BDL
3. Mercury (Hg)-BDL
4. Arsenic (As)-BDL

*BDL-Below Detection Limit.

Microbial contamination

Total bacterial count-absent

Total fungal count-absent

Test for specific pathogen

E.coli-absent

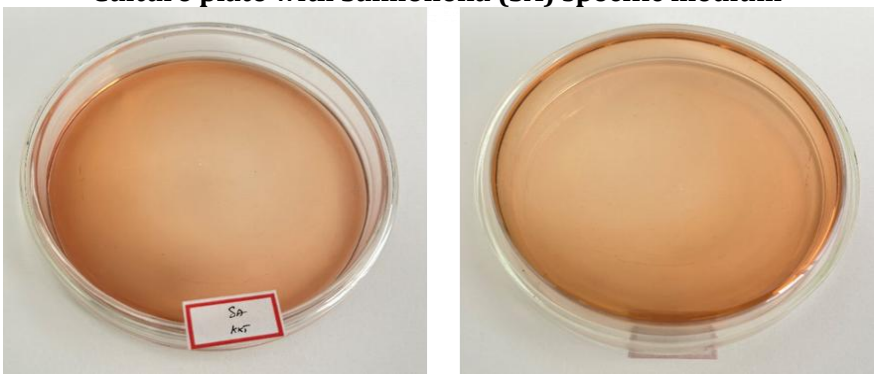
Salmonella-absent

Staphylococcus aures-absent

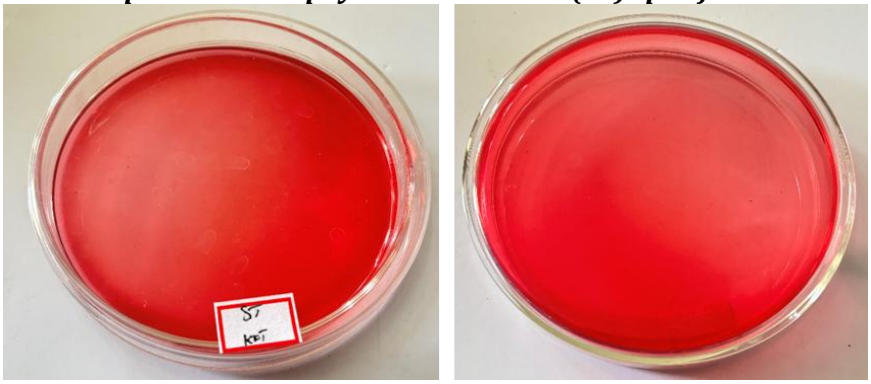
Pseudomonas aeruginosa-absent

Culture plate with E-coli (EC) specific medium

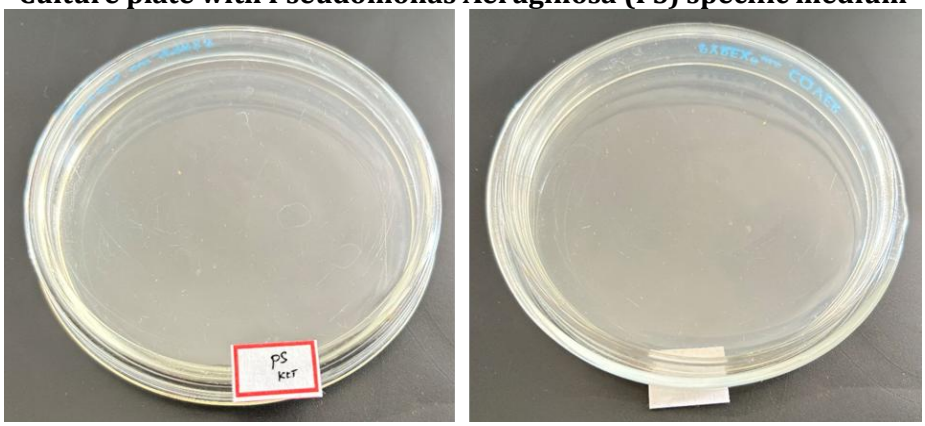
Culture plate with Salmonella (SA) specific medium



Culture plate with Staphylococcus Aureus (ST) specific medium



Culture plate with Pseudomonas Aeruginosa (PS) specific medium



Pesticide Residue

Table 3: Pesticide residue of *KKT* with AYUSH limit

I. Organo Chlorine Pesticides	**Sample KKT**	**AYUSH Limit (mg/kg)**
Alpha BHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
II. Organo Phosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
IV. Pyrethroid		
Cypermethrin	BQL	1mg/kg

*BQL- Below Quantification Limit

Aflatoxin

Table 4: Aflatoxin level with Ayush limit

Aflatoxin	**Sample KKT**	**Ayush specification limit**
B1	Not Detected - Absent	0.5 ppm (0.5mg/kg)
B2	Not Detected - Absent	0.1 ppm (0.1mg/kg)
G1	Not Detected - Absent	0.5 ppm (0.5mg/kg)
G2	Not Detected - Absent	0.1 ppm (0.1mg/kg)

DISCUSSION

Density

A density of 1.2g/ml indicates that the *KKT* is Relatively denser than water (density: 0.998 g/ml), but not extremely heavy.

Iodine value

The Iodine Value of *KKT* is 108.12 g I/100g signifies a higher proportion of unsaturated fatty acids, Increased IV indicates increased fatty acid digestibility [5]. This renders *KKT* an optimal selection for intake.

Acid value

The Acid Value (AV) is a measure of the amount of potassium hydroxide (mg KOH) necessary to neutralize one gram of sample. The AV of *KKT* is 0.86 mg KOH/g indicates low to moderate acidity.

Refractive index

Refractive index defined in terms of the angle of an incident light upon a new medium and the angle of light refraction inside the new medium. Additionally, the refractive index can be defined as the ratio of light

speeds in two distinct media. The refractive index (RI) of *KKT* has been determined to be 1.457, which is slightly higher than that of water (1.332). This value serves as a standard parameter for identifying and characterizing KKT in pharmaceutical applications.

GC-MS analysis

GC-MS (fig 1) showed six compound. The biosynthesis of eicosanoids in mammalian cells typically begins with the activation of phospholipase A2, leading to the liberation of arachidonic acid from membrane phospholipids. Prostaglandin thromboxane and leukotrienes are collectively known as eicosanoids. Eicosanoid production is considerably increased during inflammation[6]. *n*-hexadecanoic acid shown significant inhibitory activity of PLA2 by enzyme kinetics study. Isothermal titration calorimetric study of *n*-hexadecanoic acid with PLA2 shows high active site binding affinity. Therefore it function as an anti-inflammatory agent[7]. Invitro study of lipopolysaccharide (LPS) stimulated inflammatory response in murine RAW264.7 macrophages with oleic acid analysed significantly reduced the inducible nitric oxide synthase (iNos), cyclooxygenase (Cox2), and interleukin-6 mRNA[8].

Heavy Metal Analysis

ICP-OES analysis of *KKT* has confirmed the absence of toxic heavy metals, ensuring the product's safety and purity for oral administration.

Microbial contamination and specific pathogen

Specific pathogen test shows absence of *E.coli, salmonella spp*, *Staphylococcus Aureus, Pseudomonas Aeruginosa* and absence of microbial growth shows purity and safety of the *KKT*.

Pesticide residue

While pesticides have increased crop productivity and helped prevent disease transmission, they also have detrimental consequences for human well-being and ecological balance. The range of these adverse health effects includes acute and persistent injury to the nervous system, lung damage, injury to the reproductive organ dysfunction of the immune and endocrine system, birth defects, and cancer[9]. However, our analysis reveals that (table 3) the levels of harmful pesticides, including organo chlorine pesticides, organo carbamates, and pyrethroids, are below the quantification limit (BQL), ensuring the safety and purity of *KKT*.

Aflatoxins

Aflatoxins are a group of toxic and carcinogenic compound produced by fungi such as *Aspergillus flavus, Aspergillus parasiticus.* They are divided into four group as Aflatoxin B_1 (AFB_1), Aflatoxin B_2 (AFB_2), Aflatoxin G_1 (AFG_1), and Aflatoxin G_2 (AFG_2). Aflatoxin M_1 (AFM_1) is a less toxic metabolite of AFB_1 produced in farm animals that consume aflatoxins contaminated feed. AFB_1 is the most occurring one and has been identified as the group-1 hepatocarcinogen in animals and humans[11]. Shows absence of aflatoxins B1, B2, G1, G2.(table 4) Ensuring the quality and safety of *KKT*.

CONCLUSION

The physiochemical parameters (color, RI, IV, AV, and Density) of *KKT* demonstrate reliability and establish standardization benchmarks. Moreover, safety assessments reveal compliance with quality standards, evidenced by absence of microbial contamination and specific pathogens (E.coli, Salmonella, Staphylococcus aureus, Pseudomonas aeruginosa). Aflatoxin levels (B1, B2, G1, G2) and undetectable pesticide residues (organo-chlorine, organophosphorus, pyrethroids). Furthermore, GC-MS analysis identifies n-hexadecanoic acid and oleic acid, compounds with documented anti-inflammatory properties. These findings scientifically validate the traditional indication of *KKT* for treating inflammatory conditions. This study underscores the potential therapeutic value of *KKT* and warrants further research too.

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Cite this article as:
Jothikaviyarasan G, R. Antony Duraichi. Qualitative and Quantitative Analysis of Karunkozhi Thylam Prepared as Per Siddha Literature. International Journal of Ayurveda and Pharma Research. 2024;12(9):12-18.
https://doi.org/10.47070/ijapr.v12i9.3391
Source of support: Nil, Conflict of interest: None Declared

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