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Comparative Quality Control Assessment of Different Marketed Thirikadugu Chooranam

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Abstract

Thirukadadugu choornam (TKC) is a traditional herbal medicine in Tamil Nadu, India. It has been widely used in Siddha medicine for the treatment of various diseases such as cancer, hepato protection, and fertility enhancement. This study aimed to compare and assess the quality control of various Thirikadugu Choornam products. The assessment included macro and microscopic analysis, physiochemical evaluation, and quantitative estimation of Piperine using the HPLC-UV method. The results obtained from this study suggest that there is a lack of consistency in the Physiochemical properties and quantity of marker compounds found in the same formulation produced by different manufacturers. The findings obtained can serve as a reference for establishing limits for the standards monograph value of Thirukadadugu Choornam, thereby ensuring quality control assessment and guaranteeing the safety and efficacy of patients.

Keywords: Thiru kadagu choornam, Quality control, Piperine, Siddha medicine, *Zingiber officinalis*, Piper.

INTRODUCTION

Siddha medicine is a traditional practice in certain regions of South India, particularly in Tamil Nadu. It is closely associated with Tamil culture and civilization. The word 'Siddha' is derived from 'Siddhi', which means attainment. Siddhars were individuals who attained great knowledge in the areas of medicine, yoga, and meditation. (Narayanaswamy, 1975) [1]. Siddha science is founded on the principles of 96 Thathuvams, which include Pancha boothas, Mukkunam, Five Kosam, Uyirthathukkal, 6 Adharangal, and 7 Udalthathukkal. The Panchapootham theory states that the universe is composed of five natural elements [2,3]. Thirikadugu chooranam is a popular Siddha formulation that is officially listed in the Siddha Formulary of India. It is a traditional poly herbal preparation that combines three essential spicy ingredients [4,5]. *Piper nigrum* L (Piperaceae), *Piper longum* L. (Piperaceae), and *Zingiber officinale* Roscoe. (Zingiberaceae). Piperine, an amide alkaloid found in *Piper longum* and *Piper nigruim* of Thirikadugu chooranam, possesses diverse properties such as antioxidant, anticancer, anti-inflammatory, antihypertensive, hepatoprotective, neuroprotective, and bioavailability and fertility enhancement activities [6-11]. Zingiber officinale as one of its active ingredients. This species has biologically active constituents, including non-volatile pungent principles like gingerols, shogaols, paradols, and zingerone, which create a hot sensation in the mouth. The major active components in the fresh rhizome are the gingerols, which are a series of chemical homologs differentiated by the length of their unbranched alkyl chains. Zingiberol is the primary aroma-contributing component of ginger rhizome [12-14]. The composition of phytochemical constituents in herbal formulations is influenced by the diverse climatic conditions, soil components, and geographical location of their cultivation. These factors pose a challenge in the standardization process. The escalating deforestation rates have led to an increase in the adulteration and substitution of herbal drugs, which ultimately jeopardizes their safety and effectiveness [15]. The wide variety of markets and regulatory environments in which herbs and extracts are supplied creates significant quality concerns. This necessitates the development of suitable analytical methods for their identification and standardization, as well as for the detection of adulterants and contaminants [16]. Therefore, this study aimed to compare and assess the quality control of various Thirukadadugu choornam (TKC) products. The assessment included macro and microscopic analysis, physiochemical evaluation, and quantitative estimation of Piperine using the HPLC-UV method.

MATERIALS AND METHODS

Materials

All the chemicals like Iodine, Acetonitrile, Hydrochloric acid, Saffroin red, Methanol were of analytical grade purchased from local suppliers

Collection of Samples

The Different Marketed Thirikadugu Chooranam drug was purchased from local retail pharmacy in Arumbakkam Chennai, they are CHA, CHB, CHC, CHD (Figure1).

Powder microscopy

Fine sample powder was mounted on clean glass slide and clarified with clearing solution. The powder sample was then treated with different chemical reagents. Stained samples were then mounted in glycerin water fluid and observed for identification of diagnostic characters. Different chemical reagents used for identification of diagnostic cellular characters [16-19].

Physiochemical analysis

Quantitative analysis for total ash, acid insoluble ash, water and alcohol soluble extractive values and loss on drying at 105 ºC, were carried out in triplicate for the Thirikadugu choornam according to the method recommended in Quality Control Methods for Medicinal Plant Materials by WHO, 1998 [16-21].

HPLC Quantification of Piperine

HPLC system and conditions-

The HPLC system (Shimadzu Co., Japan), consisting LC-20AT pump, UV detector (Shimadzu SPD-20 A), Rheodyne 7725 I (CA, USA) manual injector with 20 µl loop and phenomenex C-18(2) column (250 × 4.6 µm ID, 5 µm) with a compatible guard column was used. The mobile phase consisted water rand Acetonitrile (65:35 v/v).

Sample preparation for HPLC analysis-

The different marketed Thiru kadagu chooranam was weighed 50mg and extracted in 50 ml of HPLC grade methanol. The extract was filtered and 1 ml of this was transferred to a 50 ml volumetric flask and volume was made up to the mark. The resulting solution was used for quantification [20-25].

RESULTS

Macroscopic Studies

Upon conducting a macroscopic examination of various commercially available Thirikadugu choornam, it was observed that all of them exhibited a yellowish-brown color and were in the form of a fine powder. Additionally, these samples emitted an aromatic odor and were found to possess a pungent taste, as indicated in Table 1 and Figure 1.

Table 1: Comparative Macroscopic evaluation of different Marketed Thirikadugu Choornam CHA, CHB, CHC, CHD

Physical characters	TKC - CHA	TKC - CHB	TKC- CHC	TKC- CHD
Powder type	Fine powder	Fine powder	Fine powder	Fine powder
Colour	Light brown colour	Brown colour	Brown colour	Dull brown colour
Taste	Pungent	Pungent	Pungent	Pungent
Odour	Aromatic	Aromatic	Aromatic	Aromatic

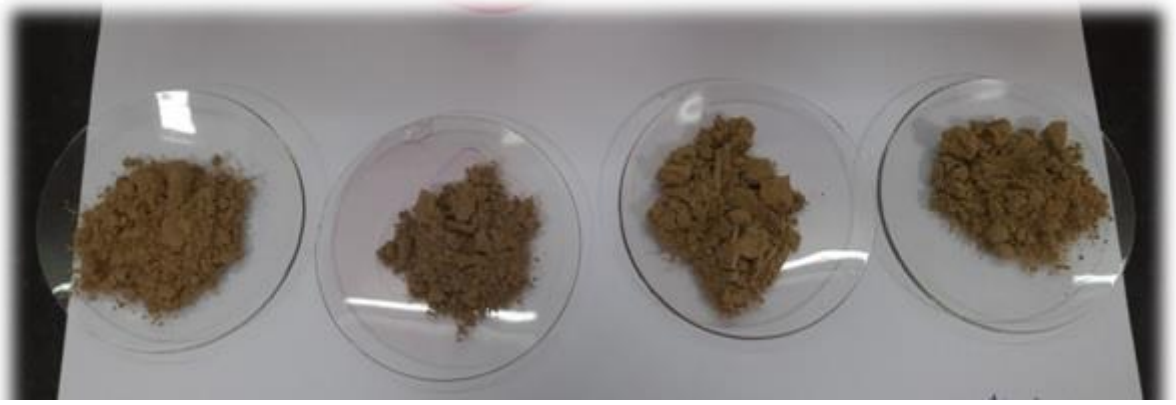

Figure 1: Comparative Macroscopic Evaluation of Different Marketed Thirikadugu Choornam CHA, CHB, CHC, CHD

Powder Microscopy

The examination of different Thirikadugu choornam samples involves using a light microscope and applying phloroglucinol solution and iodine to assess specific cellular attributes. These investigations help observe small details and identify cell characteristics of active ingredients like *Piper longum*, *Piper nigrum*, and *Piper longum*. In microscopic view, *Zingiber officinalis* has a few thick-walled rectangular cork cells and reticulate xylem vessels with spiral thickening. There are also numerous oleoresin cells with a yellowish-brown color. Iodine solution reveals round and oval-shaped starch grains, as well as aleuronic grains and oil globules within the perisperm cells found in all formulations. *Piper longum* and *Piper nigrum* contain lignified stone cells of different sizes and shapes, as well as fragments of multicellular trichomes, aleuronic grains, and oil globules that are embedded in the perisperm. Furthermore, oval-shaped starch grains are found in all of these formulations. (Table 2, Figure 2).

Physiochemical Studies

Various formulations underwent loss on drying analysis, revealing that CHA had the highest loss on drying, followed by CHB, CHC, and CHD in descending order. The total ash content analysis was also conducted on the different formulations, with CHD having the highest total ash content, followed by CHA, CHB, and CHC. Similarly, the acid insoluble ash analysis showed that CHD had the highest acid insoluble ash content, followed by CHB, CHC, and CHA. The water-soluble extractive analysis was carried out, and CHD had the highest water-soluble extractive, followed by CHC, CHA, and CHB. Lastly, the alcoholic extractive

analysis was performed, and CHD had the highest alcoholic extractive, followed by CHC, CHA, and CHB (Table 3 and Figure 3).

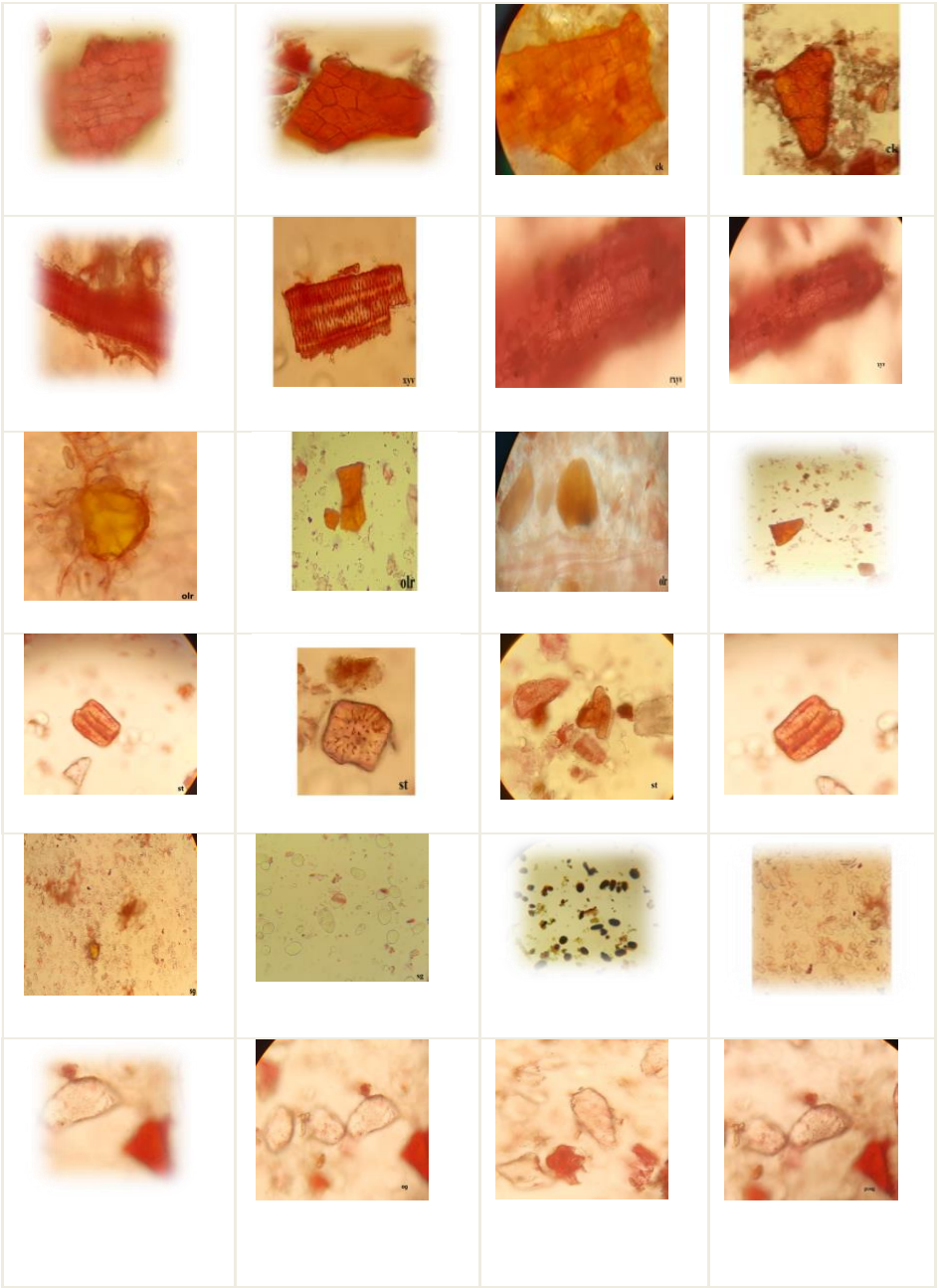

Figure 2: Characteristic cell features of Zinger

Table 2: Comparative Powder microscopic evaluation of different Marketed Thirikadugu Choornam CHA, CHB, CHC, CHD

Characteristics cell features	**TKC -CHA**	**TKC -CHB**	**TKC-CHC**	**TKC-CHD**
Cork cells	+	+	+	+
Pitted xylem vessels	-	-	+	-
Reticulate xylem vessels thickening	+	+	+	+
Oleoresin cells(yellow)	+	+	+	+
Oil globules and aleuronic grains	+	+	+	+
Septate fibre	+	+	+	+
Trichomes	+	-	-	-
Starch grains	+	+	+	+
Stone cells	+	+	+	+

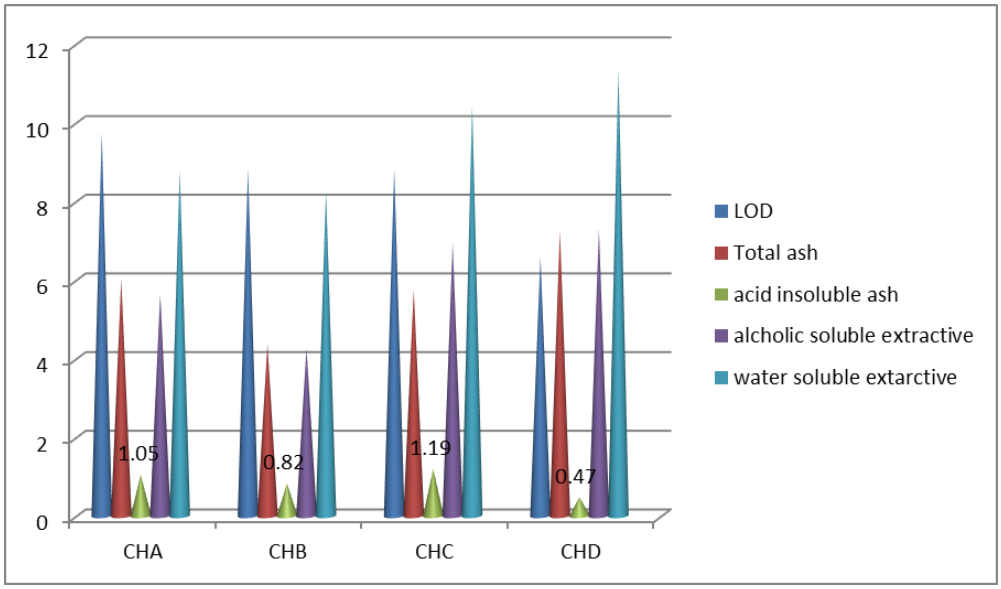

Figure 3: Comparative Physiochemical analysis of different marketed Thirikadugu choornam CHA, CHB, CHC, CHD

Table 3: Comparative Physiochemical evaluation of different Marketed Thirikadugu Choornam CHA, CHB, CHC, CHD

Physiochemical parameters	**TKC-CHA,**	**TKC - CHB**	**TKC- CHC**	**TKC- CHD**	**AVERAGE VALUE**
	Mean Value (n=3) ± SD				
Loss on drying %	9.74 ± 0.76	8.83 ± 0.42	8.83 ± 0.42	6.59± 1.27	8.50%
Total ash %	6.02 ± 0.18	4.37 ± 0.18	5.75 ± 0.05	7.25 ± 3.4	5.85%
Acid insoluble ash %	1.05 ± 0.21	0.82 ± 0.30	1.19 ± 0.13	0.47 ± 0.16	0.86%
Alcoholic soluble extractive %	5.65 ± 3.64	4.26 ± 3.00	6.93 ± 0.95	7.29 ± 5.5	7.63%
Water soluble extractive %	8.78 ± 1.62	8.27 ± 3.45	10.43 ± 2.28	11.34 ± 0.50	9.70%

HPLC Quantification

The HPLC method was employed to quantify the piperine content in various commercially available choornam products. The HPLC chromatogram revealed distinct peaks for the different marketed TKC samples, namely CHA, CHB, CHC, and CHD. Notably, the highest concentration of piperine was detected in the CHB TKC, followed by CHA. HPTLC quantifications and detailed chromatograms can be showed in (Figures 4-9).

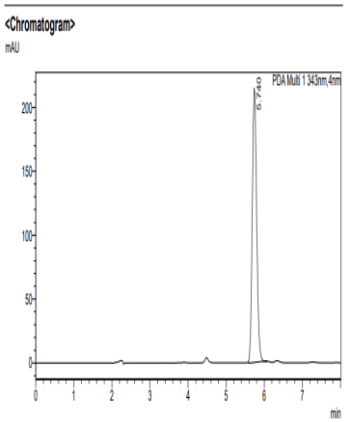

Figure 4: HPLC chromatogram of Standard Piperine

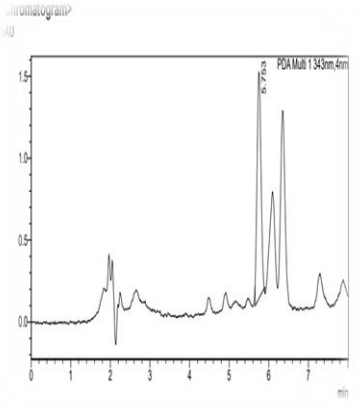

Figure 5: HPLC chromatogram of TKC-CHA

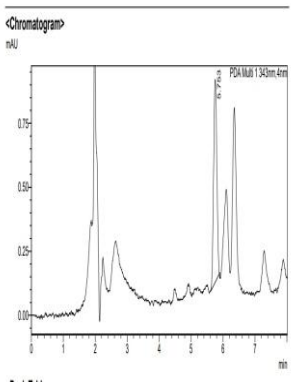

Figure 6: HPLC chromatogram of TKC-CHB

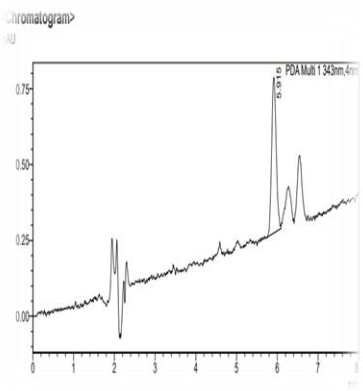

Figure 7: HPLC chromatogram of TKC-CHC

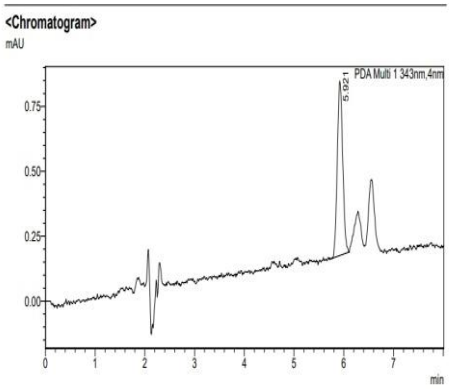

Figure 8: HPLC chromatogram of TKC-CHD

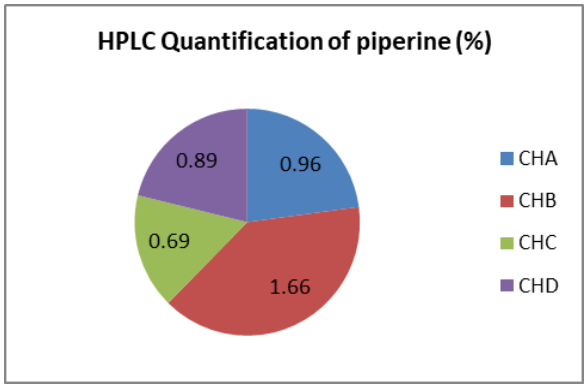

Figure 9: Comparative HPLC Quantification of piperine from different marketed Thirikadugu choornam

DISCUSSION

The lack of drug standardization in traditional system medicine has been identified as one of its main drawbacks, with many drugs not yet developed for inclusion in pharmacopeia monographs. Quality control of Siddha drugs is a complex task due to various factors affecting their bio efficacy and reproducible therapeutic effects. The current research aimed to conduct a comparative quality control assessment of different commercially available Thirukadagu Choornam products and establish monograph limits. Powder microscopy studies serve as a key quality control tool for identifying and authenticating drugs, as well as detecting any adulteration in formulated products. The results of the powder microscopy studies indicated no adulteration in the marketed Choornam products, confirming the presence and authenticity of *Piper longum*, *Piper nigrum*, and *Zingiber officinale* in the formulated products, thus ensuring the safety and efficacy of the drugs. Various physicochemical parameters were evaluated to provide different insights into the results. The moisture content of the drug was determined through Loss on Drying at 105 °C. Maintaining low moisture content is crucial to prevent the decomposition of crude drugs, whether through chemical changes or microbial contamination. An ideal moisture content range of 10-20% is recommended to minimize bacterial and fungal growth. Total ash content is a significant factor in assessing the purity and quality of drugs. Two methods were used to determine the ash value, measuring total ash and acid-insoluble ash. The total ash method quantifies the material left after ignition, which may include carbonates, phosphates, silicates, and silica. A high ash value may indicate contamination, substitution, adulteration, or negligence in preparing the crude drug for the market. Comparing the total ash value with the same sample can help differentiate between different types of ash content [27]. The quantification of phytochemical in a formulated product serves as an additional quality control measure to ensure its efficacy and safety. The HPLC-UV method was used to estimate the piperine alkaloids in thirukadagu choornam, with at least one percent of piperine being detected. Piperine primarily functions to enhance the absorption of other active ingredients and food components.

CONCLUSION

The findings obtained suggest that there is a lack of consistency in the physiochemical properties and quantity of marker compounds found in the same formulation produced by different manufacturers. This inconsistency in physiochemical characteristics and assay of piperine can be attributed to variations in geographical factors and improper adherence to Good Agricultural Practices. The only reliable method to ensure quality is through powder microscopy authentication. However, it is worth noting that there is currently no Pharmacopeia monograph available for Thirikadugu choornam. Comparative quality control studies have not revealed which marketed TKC product is of superior quality and which is of inferior quality. Therefore, it is imperative to establish a set of standards for every traditional formulation prior to its introduction into the market. The results obtained from this study can serve as a reference for establishing limits for the standards monograph value of Thirikadugu choornam, thereby ensuring quality control assessment and guaranteeing the safety and efficacy of patients.

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Conflict of Interest

There is no conflict of interest.

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None declared.

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