# Standardization of *Madhumukthi Kudineer Chooranum* Using Pharmacognostic, Physicochemical and HPTLC Studies

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#### ABSTRACT

**Background:** Siddha system of medicine is considered to be foremost among other systems of medicine. Many medicines are available in traditional systems for all kind of diseases. Most people show their interest towards Siddha medicine for non-communicable diseases especially for diabetes, hypertension, etc., Poly herbal formulations like *Avarai kudineer* has been authorized for prescription for *Madhumegam. Madhumukthi Kudineer Chooranum* (MKC) is one such medicine which has been found efficacious in *Madhumegam* (Diabetes mellitus). The current study is aimed at standardizing MKC employing pharmacognostic and chemical analyses with the aim of developing standards for quality control of this important medicine of Siddha. **Materials and Methods:** MKC was analyzed for pharmacognostic, physiochemical and HPTLC standards following standard procedures mentioned in Pharmacopoeias. **Results:** Macroscopy, powder microscopy was matched with the characters of these ingredients as reported in pharmacopoeias. The physicochemical standards were correlated with standards. TLC of MKC showed 10 spots under UV 254 nm, 13 spots under UV 366 nm and after spray with 5% vanillin sulphuric acid solution. **Conclusion:** The reported results will be supportive for standardization and future studies of MKC.

**Keywords:** Antidiabetic, Antioxidant, *Kudineer chooranum, Madhumegam*, Polyherbal formulation.

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## **INTRODUCTION**

Diabetes is a major health issue affecting majority population worldwide, ranking among the ten leading causes of mortality. Diabetes has steadily increased in India and around the world over last 3 decades. By 2045, an International Diabetic Federation projections shows that 1 in 8 adults, approximately 783 million, will be living with diabetes. Over 90% of people with diabetes have type 2 diabetes. Many researchers have been found and still searching for safe and effective solutions from traditional medicines. WHO estimates that most of the populations from the underdeveloped society rely on traditional medicines. Furthermore, it has been identified that 21,000 varieties of



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therapeutic plants are present worldwide among which 2500 are present in India out of which 150 varieties are in commercial use. Several traditional medicinal practices are seen in India, among which Siddha is one of the ancient medicinal practice<sup>[1]</sup> that holds several high order medicines to treat non communicable diseases such as cancer, diabetes, hypertension, etc., In Siddha system of medicine diabetes has various synonyms like Miguneer, Inippu neer, Madhumegam. Siddha system has many medicines for Madhumegam, among which polyherbal formulations like Avarai Kudineer has been authorized for prescription. Madhumukthi Kudineer Chooranum (MKC) is one such medicine which has been found efficacious in treating diabetes. MKC is a combination of 5 herbal ingredients in *Kudineer Chooranum* (coarse powder) form prescribed to take internally at periodic intervals for patients with high blood glucose level. Each ingredient of this MKC formulation is used widely in traditional medicine and is widely evaluated for their phytochemical profiles and pharmacological actions. Tinospora cordifolia (stem), renowned in Indian

traditional medicine, has immunomodulatory,<sup>[2]</sup> hypoglyceamic<sup>[3]</sup> antiinflammatory<sup>[4]</sup> activities and used in diabetic nephrophathy.<sup>[5]</sup> *Aegle marmelos* (root), commonly known as Bael, is attributed with anti-nociceptive and antipyretic<sup>[6]</sup> and hypoglycemic<sup>[7,8]</sup> activities. *Salacia oblonga* (root), is a potential antidiabetic drug<sup>[9-11]</sup> as it contains 1.4% of mangiferin.<sup>[12]</sup> The stem bark of *Azadirachta indica* possess antidiabetic property.<sup>[13]</sup> *Cassia fistula* (bark), known as the Golden Shower tree, exhibits antidiabetic and analgesic properties.<sup>[14]</sup> A standardization study has been taken as a step further to assess the quality of this medicine. As per Siddha literature, 6 g of MKC is to be boiled with 240 mL of water until it reduces to 1/4<sup>th</sup> of the added quantity of water (60 mL) and filtered to prepare a dose for consumption. The kudineer has to be consumed within 3 hr from the time of preparation.

This study is aimed at standardizing MKC by employing pharmacognostic and chemical analyses.

## **MATERIALS AND METHODS**

## **Procurement of plant material**

The required bark and roots are collected from natural habitat at Jolarpettai and foot hills of Elagiri. The raw drugs are authenticated by Department of Pharmacognosy, Siddha Central Research Institute, Chennai.<sup>[15]</sup>

## **Preparation of MKC**

All the ingredients collected were washed and air dried. Air dried materials, as mentioned in Table 1 and Figure 1 were taken, made into coarse powder and mixed as per proportion specified

in the formula composition to obtain *Madhumukthi Kudineer Chooranum*.

## **Powder microscopy**

A small amount of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol after treating with chloral hydrate for clearing and potassium iodide solution for testing starch grains. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented.<sup>[18]</sup>

## **Physico-chemical Parameters**

All the physicochemical parameters were carried out as per the standard test procedures.<sup>[19]</sup>

## **Extract Preparation**

MKC (5 g) was soaked in 50 mL of ethanol for 18 hr. Then sonicated for 30 min, filtered and concentrated using rotary evaporator under reduced pressure (100 mbar) and reduced temperature (55°C). It was dissolved in 10 mL of ethanol and used for HPTLC.

## **HPTLC Methodology**

Applied 15µl ethanol extract on TLC plate using Camag Linomat 5 applicator and developed by the mobile phase (toluene: ethyl acetate: formic acid (6:2.5:0.5, v/v/v)) to 8 cm. After development, the plate was photo documented using Camag TLC Visualizer under UV 254 nm and UV 366 nm. The plate was then scanned



Figure 1: Ingredients of Madhumukthi Kudineer Chooranum.

using Camag Scanner 4 at UV 254 nm ( $D_2$  lamp, Absorption mode), UV 366 nm (Hg lamp, fluorescence mode) fingerprint profiles of the extracts were documented. Then the plate was dipped in 5% vanillin sulphuric acid reagent followed by heating to 105°C till development of coloured spots. The plate was then photo documented white light and Scanner 4 at UV 520 nm (W lamp, absorption mode) finger print profiles of the extracts were documented.

## RESULTS

The powder is creamish brown in colour with characteristic odour and slightly bitter taste, shows fragments of cork, parenchyma with starch grains, bordered pitted vessels, fibre, parenchyma, groups of prismatic crystals and starch grains from *Tinospora cordifolia* (stem), fragment of cork, sclereids, crystal fibre, fibre bundle, bordered pitted vessel and starch grains from *Aegle marmelos* (root); fragments of cork, parenchyma with brownish content, fibrosclerids, bordered pitted vessel, reddish brown content and starch grains from *Salacia oblonga* Wall L. (root); cork, cells filled with tannin, fibre, crystal fibre, stone cells and prismatic crystals from *Azadirachta indica* (stem bark) and fragment of cork in surface view, cortical parenchyma, medullary ray, crystal fibre and prismatic crystal from *Cassia fistula* (stem bark) (Figure 2).

#### **Physico-chemical parameters**

The results of physico-chemical parameters are presented in Table 2.

### Thin Layer Chromatography

The TLC photos of MKC documented under short UV, long UV and post derivatization with vanillin-sulphuric acid are presented in Figure 3. The colour and  $R_f$  of spots visualized under UV condition and after derivation with vanillin-sulphuric acid are presented in Table 3.

The TLC photo-documentation of MKC viewed under short UV showed 10 spots with  $R_f$  from 0.1 to 0.96. The TLC photo-documentation viewed under long UV and in white light after derivatization with 5% alcoholic vanillin sulphuric acid showed 13 spots each. The  $R_f$  and color of the spots are presented in Table 3 and Figure 4.

## DISCUSSION

Diabetes mellitus is a heterogeneous metabolic disorder characterized by altered carbohydrate, protein and lipid metabolism resulting from insufficient insulin secretion, action or both which causes hyperglycemia. Oxidative stress is produced during normal metabolic process in body as well as induced by a variety of environmental factors and chemicals. Oxidative stress has been shown to have a significant effect in the causation of diabetes as well as diabetes related complications. Oxidative stress may have significant effect in Glucose Transport Protein (GLUT) or at insulin receptors.

Epidemiological studies and clinical trials strongly support that hyperglycemia is the principal cause of complications. Chronic hyperglycemia significantly increases the risk of diabetic complications, which involves microvascular, macrovascular and neuropathic issues. Effective blood glucose control is the key for

SI. No. Name of the Plants Part used Parts 1 Tinospora cordifolia (Willd.) Miers ex Hook.f. and Thomson 2 Stem 2 3 Aegle marmelos (L.) Correa Root 3 Salacia oblonga Wall. Root 4 3 4 Azadirachta indica A. Juss. Stem bark 5 Cassia fistula L. Stem bark 1

 Table 1: Formula composition of Madhumukthi Kudineer Chooranum.<sup>[16,17]</sup>

## Table 2: Physico-chemical parameters of Madhumukthi Kudineer Chooranum.

Parameters	Results (Mean SD)		
Loss on drying at 105°C.	7.67±0.172%		
Total ash.	6.42±0.204%		
Water soluble ash.	$1.15 \pm 0.080\%$		
Acid insoluble ash.	0.93±0.006%		
Water soluble extractive.	9.57±0.140%		
Alcohol soluble extractive.	8.23±0.088%		
pH value (10% solution).	5.75±0.005%		





Figure 2: Powder microscopy of Madhumukthi Kudineer Chooranum.

preventing or reversing diabetic complications and improving the quality of life. Scavengers of oxidative stress may have an effect in reducing the increased serum glucose level in diabetes and reduces its secondary complications.

Ingredients of MKC namely, *Seenthil (Tinospora cordifolia)* stem helps in quenching thirst which is common in diabetes. Recent researches in *T. cordifolia* was found to exhibit insulinotropic effect thereby significantly lowering blood glucose level and increasing plasma insulin levels in alloxan-induced moderately diabetic rats and stem extract shows potent antioxidant activity by inhibiting lipid peroxidation and super oxide and hydroxyl radicals *in vitro*.<sup>[20,21]</sup>

*Vilvaver* (*Agele marmelos*) root being good source of several antioxidant components such as betacarotene, glutathione, alpha tocopherol, ascorbic acid, polyphenols and flavonoids, it cures *Mukutram* (imbalance in *tridoshas*) and helps to overcome emaciation, by giving nourishment to body tissues.<sup>[22,23]</sup>

*Ponkorandi* (*Salacia oblonga*) root is the most promising antioxidant, cytoprotective drug of choice for treating diabetes and added as main ingredient in many formulations due to the presence of mangiferin, phenolics, flavonoids and other phytochemicals and it decreases post prandial glucose sugar by inhibiting alpha glucosidase and alpha pancreatic amylase and also inhibits aldose reductase which otherwise results in microvascular complications.<sup>[24]</sup>

*Vembu* (*Azadirachta indica*) its stembark and gum has astringent, tonic properties respectively and has cytotoxic, antibacterial, antioxidant activities so that it plays significant role in management of type 2 diabetes by improving the insulin signaling molecules and glucose utilization in the skeletal muscle.<sup>[25]</sup>

Sarakondrai (Cassia fistula) stem barks cures megam (diabetes) and strengthen body. Stem bark showed high alpha amylase



Figure 3: TLC photo documentation of ethanolic extract of Madhumukthi Kudineer Chooranum.

Under short UV		Under long UV		Under white light (Derivitized)	
R <sub>f</sub>	Color	R <sub>f</sub>	Color	R <sub>f</sub>	Color
-	-	0.03	Blue	-	-
0.10	Green	-	-	-	-
-	-	0.14	Blue	-	-
-	-	-	-	0.17	Purple
-	-	0.20	Blue	-	-
-	-	-	-	0.23	Purple
0.26	Green	-	-	0.25	Blue
-	-	-	-	0.28	Green
0.30	Green	0.29	Cyan	0.31	Purple
0.33	Green	0.33	Cyan	-	-
-	-	-	-	0.37	Purple
0.41	Green	0.40	Cyan	-	-
0.44	Green	0.44	Cyan	-	-
-	-	0.49	Blue	-	-
0.53	Green	0.53	Blue	0.53	Purple
-	-	-	-	0.58	Purple
-	-	0.64	Blue	0.63	Purple
-	-	-	-	0.68	Purple
-	-	0.70	Blue	-	-
0.75	Green	-	-	0.75	Blue
-	-	0.81	Blue	0.80	Purple
0.84	Green	-	-	-	-
-	-	0.92	Red	-	-
0.96	Green	-	-	-	-
-	-	-	-	0.98	Dark Purple

Table 3: R, a	and color of the s	oots from ethanolic ex	tract of Madhumuktl	hi Kudineer Chooranum.
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inhibitory activity and shows significant radical scavenging by inhibiting lipid peroxidation.<sup>[26,27]</sup>

The efficacy of MKC can be attributed to the rich phytochemical profiles of each botanical component. *Tinospora cordifolia* contains alkaloids, flavonoids and terpenoids, contributing to all pharmacological effects.<sup>[28]</sup> *Aegle marmelos* is a source of coumarins and alkaloids providing anti-inflammatory and digestive support.<sup>[29]</sup> *Salacia oblonga* contains kotalanol, kotalagenin 16acetate and salacinol which have metabolic benefits.<sup>[30]</sup> *Cassia fistula* contains oxyanthraquinone, dihydroxyanthraquinone and rhamnetin-3-O-gentiobioside.<sup>[31]</sup> *Azadirachta indica* is rich in nimbin, nimbinin and nimbidin compounds.<sup>[32]</sup>

In spite of the aforesaid evidences of efficacy of MKC ingredients individually, the formulation consisting of all these ingredients together has been analyzed for pharmacopoeial standards following standard procedures. The macroscopy and powder microscopy correlated with standards and matched with their characters.<sup>[33]</sup> Powder microscopy of MKC has been documented as per standard procedures. Microscopic features of *Tinospora cordifolia* ((Willd.) Miers ex Hook.f. and Thomson) Stem, *Aegle marmelos* (L.) Corrêa root, *Salacia oblong* Wall L. root, *Azadirachta indica* A. Juss. stem bark and *Cassia fistula* L. stem bark were observed under microscope showing authenticity of the formulation.<sup>[34-37]</sup>

The loss on drying of MKC was determined as 7.67 % which is comparatively less and the drug may have better shelf life.<sup>[38]</sup> The total inorganic content of the drug, total ash was calculated as 6.42 % which indicates the presence of micro-macro nutrients in the drug.<sup>[39-41]</sup> The siliceous matter, calculated as acid insoluble ash was less than one percent which is negligible and the drug may be considered free from foreign inorganic matter. Both the



Figure 4: HPTLC Finger print profiling of ethanolic extract of *Madhumukthi Kudineer Chooranum* at different wavelengths.

water soluble extractives and alcohol soluble extractives were determined and found to be less than 10 % which indicates that the drug is rich in fibre and primary metabolites. In addition to the above physicochemical parameters, the principle phytochemical markers of each ingredient have to be estimated for assessing the quality of the drug by HPTLC<sup>[42]</sup> or HPLC<sup>[43]</sup> methods. The content of phytochemical markers may also be mentioned in the label claims of the drug once it is brought in the commercial market which will help in the quality control of the drug.

## CONCLUSION

As per Siddha system of Medicine, there exists a huge collection of plants with antidiabetic, antioxidant potential. Only few of them have been scientifically proven and a lot more have yet to be explored and proved. In this standardization study, all the ingredients of MKC are analyzed for its identity, quality and purity using pharmacognostic, physicochemical, HPTLC studies. Results revealed many standards for authenticity of this formulation. Standardization studies help researchers to assess the quality and purity of formulations. This paper will be helpful for new researchers to collect information and do further research in this Siddha Medicine.

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## **CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

### ABBREVIATIONS

MKC: Madhumukthi Kudineer Chooranum.

## **SUMMARY**

*Madhumukthi Kudineer Chooranum* (MKC) is one such medicine which has been found efficacious in *Madhumegam* (Diabetes mellitus). The current study is aimed at standardizing MKC employing pharmacognostic and chemical analyses with the aim of developing standards for quality control of this important medicine of Siddha. Macroscopy, powder microscopy was matched with the characters of these ingredients as reported in pharmacopoeias.

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