Original Research Article (Experimental)

Preparation and standardization of Mathan Tailam: A classical Siddha formulation for diabetic ulcerative wound healing

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ABSTRACT

Background: Increasing popularity of Mathan Tailam (mattan tailam, pachai ennai) for the treatment of diabetic foot ulcer necessitated standardization and quality control of this medicated oil both in large scale production and quality check in marketed drug.

Objective: Present study aims to develop standard operating procedure for the preparation of Mathan Tailam as per Siddha Formulary of India and its standardization using suitable analytical techniques.

Materials and methods: Mathan Tailam was prepared as per Siddha Formulary of India. Physicochemical parameters and preliminary phytochemical screening were carried out using standard methods. The in-house prepared sample underwent physico-chemical analysis, qualitative phytochemical analysis, Gas chromatography-mass spectrum (GC-MS) analysis, High performance thin layer chromatographic (HPTLC) fingerprinting profile and inductively coupled plasma-optical emission spectrosopic (ICP-OES) analysis.

Results: Physico-chemical parameters of the prepared formulation were comparable to that of coconut oil. Aqueous methonolic extract of this drug was found to be positive for alkaloid, saponin, coumarin, steroid, tripterpinoid, quinine and furan. The GC-MS values were comparable to that of the base used i.e., the coconut oil. HPTLC fingerprinting profile revealed the presence of phytochemicals in the medicated oil derived from both coconut oil and Datura metel. ICP-OES addressed the mineral portion of the formulation and its safety in heavy metal aspect.

Conclusion: All these parameters can be utilized for the overall quality check over its preparation and formulation.

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1. Introduction

Mathan Tailam, a herbomineral classical Siddha formulation [1] is used as a remedy for healing supplicative wound and is very useful in healing diabetic ulcers [2]. Medicated oil is prepared by protracted-boiling of base oil with juice of prescribed herbal drug and mineral drug till it is dehydrated or near dehydration. This process results in the transfer of some therapeutically active principles of the ingredients into the base oil [3]. Thus, traditional method of tailam preparation assures the enrichment of oil with active principles. Mathan Tailam is a herbomineral Siddha formulation prescribed widely for several conditions such as eczema, weeping eczema, itches, wounds, chronic ulcers, bed sores, anal fistula, ear infections, carbuncle ulcer of diabetes, per anal abscess, non-healing of external ulcers, folliculitis, alopecia and burn wound etc. [4]. Real standard of a drug should be of its therapeutic value, a biological parameter, which varies from individual to individual and species to species. The biological standards can be supplemented and complemented by enhancing the physico-chemical parameters already employed by making use of latest techniques. Information on quality control standards for Mathan Tailam described in CCRAS publication covers only physico-chemical parameters already employed by making use of latest techniques. There is a need that as and when more aspects on scientific knowledge are unfolded, new parameters can be included which will make the tests and standards more meaningful. Increasing
popularity of this medicated oil for the treatment of diabetic foot ulcer necessitated the improved methods of standardization. Hence the present work was carried out to develop Standardized Operating Procedure (SOP) for the preparation of Mathan Tailam and standardization using suitable analytical techniques namely physico-chemical parameters, qualitative phytochemical parameters, Gas chromatography-mass spectrum (GC–MS) analysis, High performance thin layer chromatographic (HPTLC) and inductively coupled plasma-optical emission spectroscopic (ICP-OES) analysis.

2. Material and methods

2.1. Standardized operating procedure for preparation

*Datura fastuosa* L. (*Datura metel* L.) belongs to the family Solanaceae, Tamil name- Karuoomathai was collected from Chennai, Tamil Nadu. The plant was authenticated by Prof. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy and Research Centre, Chennai, based on the organoleptic, and macroscopic examination of fresh sample [6]. The specimen (voucher No: PARC/2015/3055) was deposited in his Institute for future reference. Edible quality oil of *Cocos nucifera* Linn. was procured from local market and its quality was checked by using physico-chemical parameters like iodine value, saponification value, acid value, peroxide value, refractive index, specific gravity and rancidity [7]. Required quantity of copper sulfate was procured from local market and its quality was checked by using physico-chemical parameters, qualitative phytochemical parameters, Gas chromatography-mass spectrum (GC–MS) analysis, High performance thin layer chromatographic (HPTLC) and inductively coupled plasma-optical emission spectroscopic (ICP-OES) analysis.

2.2. Quality evaluation of MT

2.2.1. Physico-chemical parameters

The following physico-chemical parameters namely iodine value, saponification value, acid value, peroxide value, refractive index, specific gravity and rancidity were carried by using standard procedure [7]. Three replicates were prepared and the mean values were computed.

2.2.2. Gas chromatography mass spectrum analysis

Sample was injected into the GC–MS unit (Instrument; Agilent 5975 series, column; DB5 MS 30 m × 0.25 mm × 0.25 mm, single quadrupole detection system with carrier gas of Helium). Oven temperature and injection temperatures were maintained at 280 °C. Injection volume was 10 μL. It was separated into various constituents with different retention times which are detected by mass spectrophotometer. The chromatogram was plot for intensity against retention time and recorded by the software attached to it. From the graph, the compounds were identified comparing the data with the existing software NIST-11.

| S. No | Test | Observation | Inference |
|-------|------|-------------|-----------|
|       | Coconut oil | in-house prepared sample | D. metel leaf |
| 1     | Alkaloids (Dragendorff's reagent) | Brick red precipitate | -Absent | +Present | +Present |
| 2     | Flavonoids (Magnesium + Concentrated HCl) | Magenta color | +Present | +Absent | +Absent |
| 3     | Sugar (Anthrone + Conc. H₂SO₄) | Green color | +Absent | +Present | +Present |
| 4     | Saponins (Frothing test) | Froth formation | +Absent | +Present | +Present |
| 5     | Coumarin (10% NaOH) | Formation of yellow color, color disappears with the addition of Conc. H₂SO₄ | +Absent | +Present | +Present |
| 6     | Tannins (a) Ferric chloride test | Green precipitate | +Absent | +Absent | +Present |
| 7     | Steroids (Conc. H₂SO₄ test) | Reddish brown precipitate at interface | +Present | +Present | +Present |
| 9     | Acid compounds test | Color change in moist blue litmus | +Absent | +Absent | +Absent |
| 10    | Quinone (reaction with Conc. H₂SO₄) | Red color | +Present | +Present | +Present |
| 11    | Phenol (Ferric chloride) | Green color | -Absent | -Absent | +Present |
| 12    | Furan Extract dissolved in alcohol P-dimethyl amino benzaldehyde and Conc. HCl was added | Pink color | +Present | +Present | +Present |
2.2.3. Extractive value
Extraction of phytochemical constituents from Mathan Tailam was done by mixing 5 ml of Mathan Tailam with 15 ml of 90% aqueous methanol and subjected to constant stirring by using magnetic stirrer on hot top at 60 °C for 1 h. It was then stored in freezer for solidification. The alcoholic portion was separated and filtered through Whatman filter paper no. 41 and filtrate was used for qualitative phytochemical testing and HPTLC profiling. The extraction was repeated for three times for effective extraction of phytochemicals from the medicated oil. Same procedure was followed for coconut oil [3].

2.2.4. Qualitative phytochemical parameters
Analyses for the following qualitative phytochemical parameters were carried out: steroids, triterpinoids, flavonoids, alkaloids, sugar, coumarine, quinine, saponine, tannic acid, furan, phenol [11].

2.2.5. High performance thin layer chromatographic (HPTLC) profile
HPTLC studies were carried out by following standard methods [12–14]. The extract prepared for extractive value was utilized for HPTLC fingerprinting of coconut oil and Mathan Tailam. D. metel leaf extract was prepared by 4 g shade dried and coarse powdered leaves. The prepared powder was extracted with 40 ml of 90%
aqueous methanol. It was then filtered and concentrated to 10 ml.

15 ml of this extract was used for HPTLC fingerprinting. Aliquots of samples were applied on a pre-coated silica gel 60 F254 (E. Merck) of 2 mm thickness aluminum plates to a bandwidth of 6 mm using CAMAG HPTLC system equipped with ATS 4 applicator. The plate was developed using solvent system of Chloroform: Methanol in the proportion of 9:1 up to 80 mm, removed from the chamber and allowed to dry. The developed plate was visualized under UV 254, 366 nm using deuterium, mercury lamp source, with slit dimensions 5.00 mm × 0.45 mm and scanned using TLC Scanner 4 and analyzed with winCATS software version 1.4.4. The chromatograms were recorded. After scanning, the plate was dipped in vanillin-sulfuric acid reagent and dried at 105 °C in hot air oven till the color of the spots appears. Then the plate was scanned at 520 nm using tungsten lamp. The Rf values and fingerprint data were recorded by winCATS software.

2.2.6. Inductively coupled plasma-optical emission spectroscopic (ICP-OES) analysis

Copper and lead estimation was carried out using ICP-OES. 2 g of oil was weighed and treated with 20 ml of 0.1N HNO3 for 1 h using magnetic stirrer at 60 °C. The aqueous portion was separated by using separating funnel. This aqueous portion was filtered using Whatman filter paper number 40. The volume was made up to 50 ml using de-ionized water. This clear aqueous solution was used for analysis of copper and lead concentration by ICP-OES using the wavelength of copper — 327.393 and lead — 220.353.

3. Results

Results of physico-chemical analyses are presented in Table 1. The results showed that the in-house prepared drug represented the values similar to that of coconut oil [15]. The result of GC-MS analysis of in-house prepared drug is given in Fig. 1 & Table 3. The results of the GC—MS analysis represented the coconut oil composition but did not reveal the composition of herbal and mineral portion of the drug.

Extractive value of in-house prepared sample was found to be 11.5 mg/ml. This value directly represented the phytochemicals of medicinal herb. Results of the qualitative phytochemical parameters are given in Table 2.

The results of HPTLC analysis are given in Fig. 2 and Supplementary file. At 254 nm, number of spots observed in coconut oil, Mathan Tailam and D. metel plant extract were 5, 15 & 12 respectively. Among these spots, the spots with Rf 0.32 and 0.87 were common for both Mathan Tailam and D. metel plant extract. At 366 nm, number of spots observed in coconut oil, Mathan Tailam and D. metel plant extract were 0, 12 and 11 respectively. Among the spots, the spot with Rf value 0.28 was common for Mathan Tailam and D. metel plant extract. At 520 nm, number of spots observed in coconut oil, Mathan Tailam and D. metel plant extract were 9, 10 and 11 respectively. Among the spots, the spot with Rf value 0.49 was common for coconut oil and Mathan Tailam. Another spot with Rf value 0.59 was common for coconut oil, Mathan Tailam and D. metel

Fig. 1. GC—MS chromatogram of in-house prepared Mathan Tailam.

Fig. 2. TLC photo documentation of 90% aqueous methanolic extract of coconut oil (Track 1), in-house prepared Mathan Tailam (Track 2) and D. metel leaf extract (Track 3) at 254, 366 nm and 520 nm after derivatization with vanillin sulfuric acid. Note: 1 denotes track 1; 2 denotes track 2 and 3 denotes track 3.
plant extract. Two other spots with Rf values 0.78 and 0.92 were common for Mathan Tailam and D. metel plant extract.

Result of ICP-OES for all the samples indicated that the level of lead was below detectable limit. Copper content of in-house prepared drug and coconut oil was 33.2 and 0.55 ppm respectively.

4. Discussion

Quality of herbal medicine depends on the originality of raw drugs, method of preparation, proper storage, proper indication, correct dose and dosage regimen. In this study, the drug Mathan Tailam, was prepared from D. metel leaf juice, coconut oil and copper sulfate. D. metel Linn. (D. fastulata L.; D. alba Nees) is a poisonous herb used in Siddha system for various ailments. Leaf powder taken internally and rolled leaf for smoking were indicated for respiratory ailments. The leaves wilted in coconut oil used for fomentation for the conditions like joint swelling, abscess pain and inflammatory pain [16]. Major chemical constituents of Datura are scopolamine, daturadiol, hyoscyamine, fastudine, allantoin, niacin, vitamin C, tropine, noratropine, meteolodine hyosine, fastusic acid etc. Tropane alkaloids present in all parts of Datura are toxic. The plant is common in tropical part of India to temperate parts of Himalayas. Parts used are seed, flower, root, bark and leaves. Datura, on external application improves skin quality, helps in quick wound healing, helps to relieve pain and inflammation. It also detoxifies skin and relieves itching when applied as a paste. In this preparation, leaves were used for preparing the drug. There was no mention about the purification process for leaves used in this particular formulation. Purple variety of the plant is called Krishna mentioned in Rajangandu. Folklore claim states that the purple variety is medicinally more valued than the white variety [17]. Copper sulfate can be purified by dissolving in water and recrystallizing. For internal use, it is purified by being rubbed with honey or ghee and exposed to heat in a crucible. It is then soaked for three days in whey or water and dried in sun [18,19]. For external application purification of copper sulfate is not much indicated. However, the drug available in local market may have iron, lead and other contaminants. Hence, in order to remove such contaminants, recrystallization is always preferred. Reference states that addition of copper sulfate in medicated oil for external application increases its shelf life [20]. In the present study, finished drug did not show the presence of copper in qualitative assays. But the ICP-OES analysis revealed the presence of copper in ppm level and the absence of lead content also ensures the safety of the product from heavy metal toxicity. Further, copper sulfate reacts with coconut oil forming a copper soap at low concentration [21] which possesses anti-fungal property. A patented combination of soluble copper fertilizer and a naturally occurring fatty acid produces copper soap which has been reported to act as a fixed copper fungicide at a concentration range of 90 ppm and is also said to be biodegradable [22]. Coconut oil is preferred for drug used in wound healing among the medicated oils used in traditional system. In allergic conditions, it is used for external application. It can be justified by the presence of wound healing constituents like capric acid and lauric acid [23]. The extractive value holds its importance in deciding the dose of effective fractions so that the drug is devoid of toxicities. Still, there is a need to test its safety upon topical application over skin and mucous membrane. In spite of the lag behind advanced treatment and awareness, a 1984 clinical study [2] revealed much about the wound healing property of Mathan Tailam in diabetic cases where the chronic ulcerative wound upon its application in diabetic ulcers had shown its quick healing potential ranging from one to three months in accordance to the depth and affected area of ulcer. Another report indicates that in the presence of metabolic activation in Salmonella/microsome mutagenicity assay, the dichloromethane leaf extract shows anti-mutagenic property [24]. This further supports the evidence of protective and preventive action of Mathan Tailam against chronic wounds that are persisting and progressing towards malignancy. In addition, another report states that the leaf extract effectively inhibits mycelia growth of ringworm fungi Epidermophyton floccosum, Trichophyton mentagrophytes and Microsporum gypseum [25]. Literature search revealed that the plant possesses anti-bacterial activity. A new anti-bacterial agent 5', 7'-dimethyl 6'-hydroxy 3', phenyl 3 α - amine β - yne sitosterol 1 has been isolated from D. metel leaves [26]. A study states that the methanolic and chloroform extracts showed promising results against resistant strains of pathogenic bacteria [27]. Literature survey and traditional claim states that Mathan Tailam possesses anti-spasmodic, anodyne and antiseptic properties [28]. Literature available on standardization parameters of Mathan Tailam states only about its physico-chemical properties and not much about the phytochemical constituents derived from the plant. Hence, we carried out qualitative phytochemical screening for Mathan Tailam, coconut oil and plant extract. Marker compound gives an idea about the identity of plant in many cases. Most of the time, identified marker compound may not be a biologically active marker. In drugs using herbs in crude form, certain chemical will act in targeting the specific condition; other chemicals either potentiate the dynamics and facilitate the kinetic of this compounds or nullify the toxicity of the toxic principles. Therefore, in our study, the leaves extract was used as marker instead of specific single marker. Phytochemical constituents of Mathan Tailam revealed that the ingredients were derived from both coconut oil and plant. HPTLC fingerprinting was carried out for coconut oil, Mathan Tailam and plant extract using a solvent system that detects other than alkaloids. For detecting the presence of alkaloids, qualitative phytochemical analysis with dragendorff reagent was carried out and found to be positive. GC–MS method is more suitable for quantitative analysis of natural volatile components and lipophilic compounds with low melting points and good thermal stability in medicinal herbs. Advantage of GC–MS analysis is spectrum identification along with quantification for a sample by one injection. But highly polar heat liable non-volatile macromolecular organic compounds could not be detected. In the present study the drug in its native form was used for GC-MS analysis. Majority of the constituents detected were the fatty acid constituents of the base oil. The presence of copper from copper sulfate, lauric and capric acids from coconut oil and other phyto chemicals from leaves of D. metel ensures wound healing and counteract the bacterial and fungal infection thereby decides the quality of the drug.

5. Conclusion

All the parameters and result of this study provides quality standards for Mathan Tailam representing its source from both herbal and mineral origin. This can be utilized for the overall quality check over its preparation and formulation.

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Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jaim.2017.08.011

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