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Original article

HPTLC and GC–MS finger-printing of two potential multifunctional siddha tailams: Mathan and maha megarajanga tailam

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A B S T R A C T

The Siddha system of medicine is an ancient medical lineage that is practiced primarily in the southern part of India. Siddha system of medicine has been in practice for thousands of years with documented evidence dating back to the 6th century BCE. According to siddha system of medicine's basic fundamental principle, the human body is made up of 96 thathuvam (primary components), which encompass physical, physiological, psychological, and intellectual aspects. Medicine (marunthu) is classified as a wide range of internal and external medicines. The major components of its medical formulations include plant parts, minerals and animal products. Various methods were carried out for the purification process to eliminate the toxins. Choornam, Guligai, Tailam, Parpam, Chendooram, Kattu, Pasai and Poochu are the most common medicines used in Siddha system of medicine for the treatment of various diseases. The pathophysiological classification of diseases is elaborated in detail in the classical Siddha literature. Siddha system of medicine plays an important role in protecting people from diseases such as COVID-19 by providing immune-protecting and immune-boosting medicines in today's world. Mathan tailam and maha megarajanga tailam are the two unique preparations used widely for various skin diseases including chronic wounds and burns. Scientific validation of both medicines will help in understanding their effectiveness against a typical wound condition. In the present study physio-chemical and phytochemical, HPTLC, and GC–MS analyses were carried out and discussed in detail on the multifunctional properties exhibited in the patient communities.

1. Introduction

The Siddha system of medicine (SSM) has its fundamental concepts, anatomy, physiology, and pathology, extensive pharmacopoeia, and different internal medicines and external therapies in use, with expertise in iatrochemistry far before the development of modern science. According to the literature, the practice of SSM for therapy and wellbeing of humans evolved before 4000 BCE (Karunamoorthi et al., 2012). As per the doctrine of SSM (Panchabootha Pancheekaranam), every object in the cosmos, including the human body is made up of five fundamental elements namely earth, water, fire, air and ether. The human body, food and medications, regardless of where they come from, are considered to be exact replicas of the cosmos by this five-fold combination theory. According to SSM, the human body is composed of 96 thathuvam (principle factors), which include physical, physiological, psychological and intellectual aspects. Three humours (uyir thathu) - vatham, pitham and kabham - as well as seven body tissues (udal thathu) -

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saram - plasma, senneer - blood, oon - muscle, kozhupu - fat, enbu - bone, moolai - bone marrow and sukkilam/suronidham - semen/ovum were considered more significant in the medical approach (Shukla and Saraf, 2011). The pharmacological aspect of the SSM includes plant, animal and mineral-based formulations, which comprise 32 types of internal medications and 32 types of external medications. They are primarily in the form of choornam (fine powder), maathirai (tablet), tailam (medicated oil) and higher levels of unique medicines like parpam and chendooram (a herbal-mineral blend) (Janani et al., 2017). Many classical Siddha formulations are prepared and practised for the treatment of various diseases such as viral fever, arthritis, skin disorders and diabetes in India especially in the south, by Government Siddha Physicians, registered private Siddha practitioners, and native traditional medicine practitioners (Thas, 2007).

The use of kabasura kudineer, a poly-herbal Siddha medicine, for the prophylaxis and management of mild/moderate disease conditions during the recent COVID-19 pandemic period demonstrates the intense efficacy of the classical formulation documented more than 200 years ago (Meenakumari et al., 2022). Siddha Central Research Institute (SCRI) completed a randomized control trial with higher level scientific support in COVID-19 patients and developed authentic data to support the literature evidence of SSM (Natarajan et al., 2021). Such meritorious medicines are required by the modern scientific fraternity to be scientifically analyzed for their genuine use by a large population. We intend to conduct an *in vitro* study on two such Siddha medications, mathan tailam (MT) and maha megarajanga tailam (MMRT), which have been widely used to treat skin diseases, especially wounds for more than 50 years.

1.1. MT and MMRT preparation

MT also termed pachai ennai, is a traditional herbomineral external medication used widely in Tamilnadu, India for all types of skin infections and burn wounds. It contains a combination of coconut oil, *Datura metel* leaves and purified blue vitriol (copper sulphate) (Thyagarajan, 2004). It is used in all the government Siddha hospitals of Tamilnadu for treating skin injuries like lacerations, abrasions and skin infections exclusively in diabetic wounds. It has been well-documented in the literature that MT possesses antiseptic and antispasmodic properties. SCRI (Siddha Central Research Institute, Chennai) has done standardization of MT preparations.

(Arunadevi et al., 2020). A case series published by SCRI on the usage of topical application of MT on chronic non-healing ulcers show a significant improvement in healing (Samraj et al., 2019) No *in vitro* studies were done on the original native form MT; either they have done more on the separate plant extract (Datura leaves extract). To understand the drug interaction with the body tissues and its mechanism of action *in vivo*, one needs to carry out an in-depth scientific analysis of the herbal preparation in its original form.

MMRT, a unique herbal preparation, used both internally and externally by registered Siddha medical practitioners and traditional vaidyars for more than 50 years to treat chronic ulcers, diabetic wounds, and extensive burn wounds. Furthermore, literature also shows its effectiveness against gangrene wounds, abnormal skin eczema, and leprosy (Subramaniya Pandithar et al., 1970). A case report explored its tissue healing properties in a diabetic ulcer patient (Subramanian, et al., 2022). No scientific evaluation has been done so far to explore the drug interaction with the tissues and mechanism of action.

2. Materials and methods

2.1. Materials and reagent

All chemicals and reagents used in the current work were of molecular and analytical grade and obtained from Sigma Chemical Company. The MT sample was procured from Indian Medical Practitioners Co-operative Pharmacy & Stores Ltd. (IMPCOPS) [MT 100 ml/Batch No S1-116/Mfg date Nov 2021]. MMRT was procured from Vaidyar Sachidananda Swamigal, Dharmapuri, Tamil Nadu. Toluene, Ethyl Acetate, Formic acid, vanillin-sulphuric acid, Dimethyl sulfoxide (DMSO), 1,1-diphenyl-2-picryhydrazyl free radical (DPPH) purchased from Sigma Chemical Company.

2.2. Preparation of MT

Fresh leaves of the *D. metel* plant were collected and cleaned with water and drained well to remove the excess water and macerate the leaves to get the fresh extract. To the extract, added 350 g of copper sulfate and stirred well for complete dissolution. To the above mixture added 1.4 L of fresh coconut oil and continue the stirring then transferred to a seasoned clay pot and heat under mild flame till it attains the required consistency. The mixture was then filtered when hot, using a muslin cloth, transferred to an amber-colored bottle, sealed properly, and stored at room temperature until use (The Siddha formulary of India, 1992).

2.3. Preparation of MMRT

The parts of the plants mentioned in the Table 1 were collected separately and cleaned. The bark, root, and stem parts were cut into small pieces, and the flowers, fruits, and seeds were cleaned and added to 100 L of pure river water in the large vessel, where they should be boiled in a moderate fire until one-fourth (25 L) of their content is extracted and filtered. To that added 25 L of castor oil and transferred to a well-seasoned mud pot. Finally, added the parpam and heated under a moderate flame. Followed by the evaporation of water, added cow's milk (the required amount) and continue the heating until the watery content dissipates and transformed into a waxy phase. The waxy mass obtained was stored in an airtight container until use.

2.4. Phyto and physico-chemical analyses

Followed by procurement, both MT and MMRT samples were subjected to Phytochemical analysis as per the standard procedures described in AYUSH guidelines (Harborne,1973) and for physicochemical analysis, we followed the standard test procedures published by the Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory, Government of India.

2.5. Antioxidant activity by DPPH assay

DPPH radical scavenging activity of MT and MMRT samples was assessed as per the method of Colombo et al. (2018). Different concentrations of MT and MMRT (10–100 μL) were prepared. An ethanolic solution of DPPH (0.1 mM) was prepared, of which 0.35 ml was mixed with MT and MMRT respectively. Later, the mixture was incubated for 30 min and analyzed by a UV–visible spectrophotometer at 570 nm. Ethanol (0.5 ml) was used as a control (without drug) and results were compared with Ascorbic acid (10 mg/ml DMSO) as a reference. The percentage of DPPH scavenging activity was calculated using the following equation.

Table 1
The parts of the plants and their botanical name.

S.NO	Tamil Name	*Botanical Name*	Part used
1	Arugampul	*Cynodon dactylon*	Whole plant
2	Aada thodai	*Justicia beddomei*	Leaf
3	Aalam	*Ficus benghalensis*	Leaf, Bark, Fruit
4	Aavaram poo	*Cassia auriculata*	Flower
5	Annachi	*Ananas comosus*	Fruit
6	Arasu	*Ficus religiosa*	Leaf, Bark, Fruit
7	Atti	*Ficus racemosa*	Leaf, Bark, Fruit
8	Azhinjil	*Alangium Salvifolium*	Root
9	Boomi sakkaravali kizhangu	*Ipomoea Batatas*	Tuber
10	Chitha kathi poo	*Senna auriculata*	Flower
11	Chitiramoolam	*Plumbago zeylanica*	Root
12	Elikadilai	*Merremia emarginata*	Leaf
13	Elumbotti	*Ormocarpum sennoides*	Root
14	Elumichai	*Citrus Limon*	Fruits
15	Elumichan Thulasi	*Ocimum gratissimum*	Leaf
16	Gopuram Thangi	*Andrographis echioides*	Root
17	Kurukkaththi	*Hiptage benghalensis*	Leaf
18	Ilavanga poo	*Syzygium aromaticum*	Flower
19	Illupai ilai	*Madhuca longifolia*	Leaf
20	Iluppai pattai	*Madhuca longifolia*	Bark
21	Inji	*Zingiber officinale*	Tuber
22	Vengayam	*Allium cepa*	Whole plant
23	Kaata manakku	*Jatropha curcas*	Leaf, Stem, Root, Seed
24	Kadugu rohini	*Picrorhiza scrophulariiflora*	Seed
25	Kal thurinji	*Albizia amara*	Leaf
26	Kalli mulli	*Drosera indica*	Whole plant
27	kalli mulliyan	*Caralluma umbellata*	Whole plant
28	Kacheri poondu	*Alliaria petiolata*	Whole plant
29	Kandang kathiri	*Solanum surrattense*	Fruit, Leaf
30	Kanjaangkorai	*Ocimum basilicum*	Leaf
31	Kappu Thulasi	*Ocimum gratissimum*	Leaf
32	Karisalangkanni	*Eclipta prostrata*	Leaf, whole plant
33	Karu poola	*Phyllanthus reticulatus*	Flower
34	Karuda Kizhangu	*Corallocarpus epigaeus*	Tuber
35	karunj seeragam	*Nigella sativa*	Seed
36	Karunth Thulasi	*Ocimum tenuiflorum*	Leaf
37	Katralai	*Aloe barbadenesis*	Stem
38	Kizha nelli	*Phyllanthus amarus*	Leaf, whole plant
39	Kollu kothan	*Macrotyloma uniflorum*	Seed
40	Kotha malli	*Coriandrum sativum*	Leaf
41	Kottai karanthai	*Sphaeranthus Indicus*	Seed
42	Kovai	*Coccinia grandis*	Fruit
43	Kuchi kalli	*Opuntia ficus-indica*	Fruit
44	Kuppai meni	*Acalypha indica*	Leaf
45	Maa	*Mangifera indica*	Leaf
46	Malai Thaangi	*Sida acuta*	Root
47	Manathakalai	*Solanum nigrum*	Leaf
48	Maruthani	*Lawsonia inermi*	Leaf
49	Madana Kama poo	*Cycas Circinalis*	Flower
50	Mela Nelli	*Phyllanthus emblica*	Whole plant
51	Mukkirattai	*Boerhaavia diffusa*	Root
52	Moongil ilai	*Bambusa arundinacea*	Leaf
53	Mudakkatran	*Cardiospermum halicacabum*	Leaf
54	Mullu Kathiri	*Solanum trilobatum*	Leaf
55	Murungai keerai	*Moringa oleifera*	Leaf
56	Murungai pattai	*Moringa oleifera*	Bark
57	Murungai poo	*Moringa oleifera*	Flower
58	Murungai Vithu	*Moringa oleifera*	Seed
59	Musumuskkai	*Mukia maderaspatana*	Leaf
60	Muthukottai	*Ricinus communi*	Seed
61	Muthukottai ilai	*Ricinus communi*	Leaf
62	Naai Thulasi	*Ocimum americanum*	Leaf
63	Naayuruvi	*Achvranthes aspera*	Root
64	Naaval	*Syzygium cumini*	Fruits
65	Nannari	*Hemidesmus indicus*	Root
66	Naththai Churi	*Borreria hispida*	Root
67	Neermulli	*Hygrophila auriculata*	Root
68	Nerunjil	*Tribulus terrestris*	Root
69	Nilapanai Kizhangu	*Curculigo Orchioides*	Tuber
70	Nuna	*Morinda tinctoria*	Leaf
71	Panam poo	*Borassus flabellifer*	Flower
72	Pannai Keerai	*Celosia cristata*	Leaf
73	Parpatakam	*Hedyotis corymbosa*	Root
74	Peenari	*sterculia foetida*	Root

(*continued on next page*)

Table 1 (*continued*)

S.NO	Tamil Name	*Botanical Name*	Part used
75	Peramutti	*Pavonia odorata*	Whole plant
76	Pirandai	*Cissus quadrangularis*	Stem
77	Pulicha keerai	*Hibiscus cannabinus*	Leaf
78	Ponnangkani	*Alternanthera sessilis*	Leaf
79	Ponnavarai	*senna occidentalis*	Leaf
80	Poolai poo	*Aerva lanata*	Flower
81	Poonaikkali vithu	*Mucuna pruriens*	Seed
82	Poovarasa	*Thespesia populnea*	Leaf, Bark
84	Por seenthil	*Tinospora sinensis*	Leaf
85	Pudina	*Mentha spicata*	Leaf
86	Sanga Kozhunthu	*Azima tetracantha*	Leaf
87	Sangu poo kodi	*Clerodendrum indicum*	Flower
88	Santhana	*Santalum album*	Leaf
89	Satti pannai keerai	*Amaranthus cristatus*	Leaf
90	Seendhil	*Tinospora cordifolia*	Stem
91	Seeragam	*Cuminum cyminum*	Seed
92	Semparuthi	*Hibiscus rosa-sinensis*	Flower
93	Senkazhu neer	*Nymphaea nouchali*	Root
94	Senthatti	*Tragia involucrata*	Root
95	Seppu nerunji	*Rotula aquatica*	Root
96	Seruppadai	*Coldenia procumbens*	Whole plant
97	Siru naga poo	*Mesua ferrea*	Flower
98	Citramutti	*Pavonia zeylanica*	Whole plant
99	Sivanaar vembu	*Indigofera aspalathoides,*	Whole plant
100	Sukku	*Zingiber officinale*	Root
101	Thanneer vittang Kizhangu	*Asparagus racemosus*	Tuber
102	Thennam poo	*Cocos nucifera*	Flower
103	Thippili	*Piper Longum*	Fruit
104	Thulasi	*Ocimum sanctum*	Leaf
105	Thumbai	*Leucas aspera*	Flower
106	Thuththi	*Abutilon indicum*	Leaf,Root
107	Udhayamaram	*Lannea coromandelica*	Leaf
108	Vaata manakku	*Ricinus communis*	Leaf
109	Vaazhai Thandu	*Musa paradisiaca*	Stem
110	Vazhai poo	*Musa paradisiaca*	Flower
111	Vellari	*Cucumis sativus*	Seed
112	Vellarugu	*Enicostemma axillare*	Whole plant
113	Veppilai	*Azadirachta indica*	Leaf
114	Vetrilai	*Piper betle*	Leaf
115	Villa	*Limonia acidissima*	Fruit
116	Aadu theenda palai	*Aristolochia bracteolata*	Leaf
117	Aagaya thamarai	*Pistia stratiotes*	Leaf
118	Aazha pazham	*Ficus benghalensis*	Fruit
119	Amman pacharisi	*Euphorbia thymifolia*	Leaf
120	Ammukira Kizhangu	*Withania somnifera*	Tuber
121	Arasa pazham	*Ficus religiosa*	Fruit
122	Arasu	*Ficus religiosa*	Leaf
123	Arukeerai	*Amarantus tristis*	Leaf
124	Echangkolunthu	*Phoenix sylvestris*	Leaf
125	Sathikkai	*Myristica fragrans*	Fruit
126	Kadukkai	*Terminalia chebula*	Fruit
127	Kalyanmurungai	*Erythrina variegata*	Leaf
128	Karumbu	*Saccharum officinarum*	Stem
129	Karuveppilai	*Murraya koenigii*	Leaf
130	Katralai	*Aloe vera*	Stem
131	Kizhanelli	*Phyllanthus amarus*	Leaf
132	Manathakkali	*Physalis minima*	Leaf
133	Manjal	*Curcuma longa*	Tuber
134	Masikkai	*Qurecus infectoria*	Fruit
135	Mookiratai	*Boerhavia diffusa*	Root, Whole plant
136	Mookuti poondu	*Pentanema indicum*	Whole plant
137	Naaga thali	*Opuntia dillenii.Haw.*	Leaf
138	Nochi	*Vitex negundo*	Root, Leaf
139	Omam	*Hyoscyamus niger*	Seed
140	Ooridhal thamarai	*Hybanthus enneaspermus*	Whole plant
141	Paalai keerai	*Leptadenia reticulata*	Leaf
142	Pasalai keerai	*Portulaca quadrifida*	Leaf
143	Perunelli	*Emblica officinalis*	Bark, Fruit
144	Piramanthandu	*Argemone mexicana*	Seed
145	Poduthalai	*phyla nodiflora*	Whole plant
146	Siru kurinjan	*Gymnema sylvestre*	Leaf
147	Siru nelli	*Phyllanthus acidus*	Bark, Fruit
148	Sirukeerai	*Amaranthus polygamus*	Leaf
149	Thakara	*Cassia tora*	Leaf
150	Thamarai ilai	*Nelumbo nucifera*	Leaf

Table 1 (*continued*)

S.NO	Tamil Name	*Botanical Name*	Part used
151	Thamarai thandu	*Nelumbo nucifera*	Stem
152	Thandrikai	*Terminalia bellirica*	Fruit
153	Thandu keerai	*Amaranthus tricolor*	Leaf
154	Theluvu	*Borassus flabellifer*	Fruit extract
155	Thengai	*Cocos nucifera*	Fruit extract
156	Vatha narayanan	*Delonix elata*	Bark, Leaf
157	Vendhaya keerai	*Trigonella foenum-graecum*	Leaf
158	Vetpalai	*Wrightia tinctoria*	Leaf
159	Vilvam	*Aegle marmelos*	Leaf, Fruit, Bark
160	Naarthai	*Citrus aurantum*	Fruit
161	Vengai	*Premna serratifolia*	Bark
162	Kaatu Vaaghai	*Albizia lebbeck*	Bark
163	Velvel	*Acacia ferruginea*	Bark
164	Karuvel	*Acacia nilotica*	Bark
165	Thetran	*Strychnos potatorum*	Bark
166	Vembu	*Azadirachta indica*	Bark
167	Itthi	*Dalbergia latifolia*	Bark
168	Kaatu athi	*Bauhinia tomentosa*	Bark
169	Murukku	*Aquilaria malaccensis*	Bark
170	Sarakondrai	*Cassia fistula*	Bark
171	Mul ilavu	*Bombax ceiba*	Bark
172	Pungu	*Derris indica*	Bark
173	Pei kadukkai	*Terminalia paniculata*	Bark
174	Maavilangu	*Crataeva magna*	Bark
175	Maruthu	*Terminalia arjuna*	Bark
176	Sangu parpam	*conch shell*	
177	Palagarai parpam	*cypraea moneta shells*	Ash powder
178	Silajithu parpam	*Asphaltum mineral pitch*	
179	Suthamana Vilakkuennai	*ricinus communis*	Castor oil
180	Pasu paal	Cow milk	Cow milk

$$\text{DPPH Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.6. Thin-layer chromatography (TLC)

The test samples MT and MMRT were subjected to TLC analysis after dissolute in ethanol. In brief, 1 g of oil sample in 10 ml of ethanol was homogenized for 15 min and then filtered. The filtrate of 8 μL was placed in a TLC plate and developed a chromatogram using the mobile phase containing a mixture of Toluene: Ethyl Acetate: Formic acid (7.5:2.5:0.5, v/v). After the completion, the plate was photo documented using Camag's TLC Visualizer under UV 254 nm and UV 366 nm and then scanned using Camag's Scanner 4 at (D2 lamp/Absorption mode, Hg lamp/Fluorescent mode) fingerprint profiles of the extract were documented. Then the plate was dipped in 5% vanillin-sulphuric acid reagent followed by heating at 105 °C till the development of colored spots. The plate was then photo documented in white light and scanned at 520 nm for fingerprint profile**.**

2.7. GS-Ms

GC–MS analysis of MT and MMRT samples were assessed using Agilent 7000 Series Triple Quadrupole MS coupled with the GC Agilent 7890A, equipped with an Agilent capillary column (Jiang, et al., 2011; Ashmawy et al., 2019). The samples were dissolved in DMSO and then subjected to analysis, and 1 μL of the sample was injected. The oven temperature was programmed from 65 °C to 200 °C at 3 °C/minute; injector temperature, 250 °C; carrier gas, Helium (1 ml/minute); automatic sample injection, mode: Splitless. The MS operating parameters were: interface temperature: 300 °C, ion source temperature: 200 °C, EI mode: 70 eV, scan range: 40–400 amu. Compounds identification Mass spectra of the individual GC peaks were identified by a computer search of the commercial libraries (NIST).

3. Results

3.1. Phyto chemical screening

Fingerprinting of MT and MMRT initiated with an investigation on the phytochemical constituents of the products. Table 2 describes the phytochemical parameters of MT and MMRT in detail. Both the tailam showed the presence of phenols, proteins and acid content. Flavonoids, phytosterol and carbohydrates were absent in both MT and MMRT. Quinones and tannins were present only in MMRT and not in MT.

3.2. Physicochemical properties of MT and MMRT

The physiochemical profile of MT and MMRT were shown in Table 3. MMRT has not shown any peroxide value but has a high

Table 2
Result of the phytochemical analysis of MT and MMRT.

S. No.	Test	MT	MMRT
1.	Phenol	Positive	Positive
2.	Tannin	Negative	Positive
3.	Saponin	Negative	Negative
4.	Acid	Positive	Positive
5.	Protein	Positive	Positive
6.	Carbohydrate	Negative	Negative
7	Glycosides	Negative	Negative
8.	Phytosterol	Negative	Negative
9.	Alkaloids	Positive	Negative
10.	Flavonoids	Negative	Negative
11.	Quinones	Negative	Positive
12.	Reducing sugars	Negative	Negative

Table 3
MT and MMRT physicochemical values.

S. no	Name of the Experiment	Mean Value (MT)	Mean Value (MMRT)
	ph Value	5.520	6.51
	Saponification Value	199.43	174.96
	Iodine Value	38.96	71.81
	Peroxide Value	5.46	Nil
	Refractive Index	1.457	1.474
	Rancidity	Absent	Absent
	Specific gravity	0.92	0.95

Iodine value compared to MT. Both the tailams showed nil rancidity. Based on the physiochemical analysis, the pH of MT and MMRT was determined as 5.59 and 6.57 respectively indicating the suitability of these agents for the healing of wounds. According to Tang *et al.*, [13], the pH value of an external application agent for wound healing should be between 6.0 and 7.0. With respect to specific gravity both the samples were shown 0.92 and 0.95 may be due to the increased consistency in the presence of oil.

3.3. Antioxidant assay of MT and MMRT invitro DPPH analysis

Free radical scavenging tests were performed to evaluate the antioxidant capacity of MT and MMRT. Results showed more than 50% of DPPH radical quenching exhibited by MMRT (Table 4). However, these results indicate that compared to MT MMRT has presented a high antioxidant capacity.

3.4. MT thin layer chromatography and HPLC

Chromatogram analyses, Fig. 1 (A-C) illustrates the HPLTC of MT followed by the relative chromatogram shown in Fig. 2 (A-F) observed under wavelengths 254, 366 and 520 nm. At 254 nm, we observed the spots at Rf 0.23 and 0.78; at 366 nm, the spots were at Rf 0.24 and 0.76 and at 520 nm the spots at Rf 0.21 and 0.76 were observed. Table 5 detailed the percentage peak area with respect to MT and MMRT samples.

3.5. MMRT thin layer chromatography and HPLC

Fig. 3 (A-C) depicts the HPTLC analysis of MMRT and the relative chromatogram shown in Fig. 4 (A-F) observed under 254, 366 and 520 nm. Spots with Rf 0.73, 0.82 and 0.93 were observed under 254 nm; Rf 0.50, 0.73 0.81 and 0.92 were observed under 366 nm; and Rf 0.45 and 0.47 were observed under 520 nm. Table 6 detailed the percentage peak area with respect to MT and MMRT samples.

3.6. GC–MS chromatogram of MT and MMRT

GC–MS chromatogram of MT and MMRT shown in Table 7 indicates the respective list of molecules present in the samples. It has

Table 4
Antioxidant activity of MT and MMRT. Data are expressed as mean ± mean ± SEM of observations (n = 4).

S.No	Sample concentration (µl)	Standard % of inhibition	MT % inhibition	Standard % of inhibition	MMRT % inhibition
1	10	9.48	5.255	42	13.515
2	40	10.23	5.59	48.81	20.845
3	80	11.725	8.82	59.9	40.52
4	100	14.8	13.81	70.95	59.705

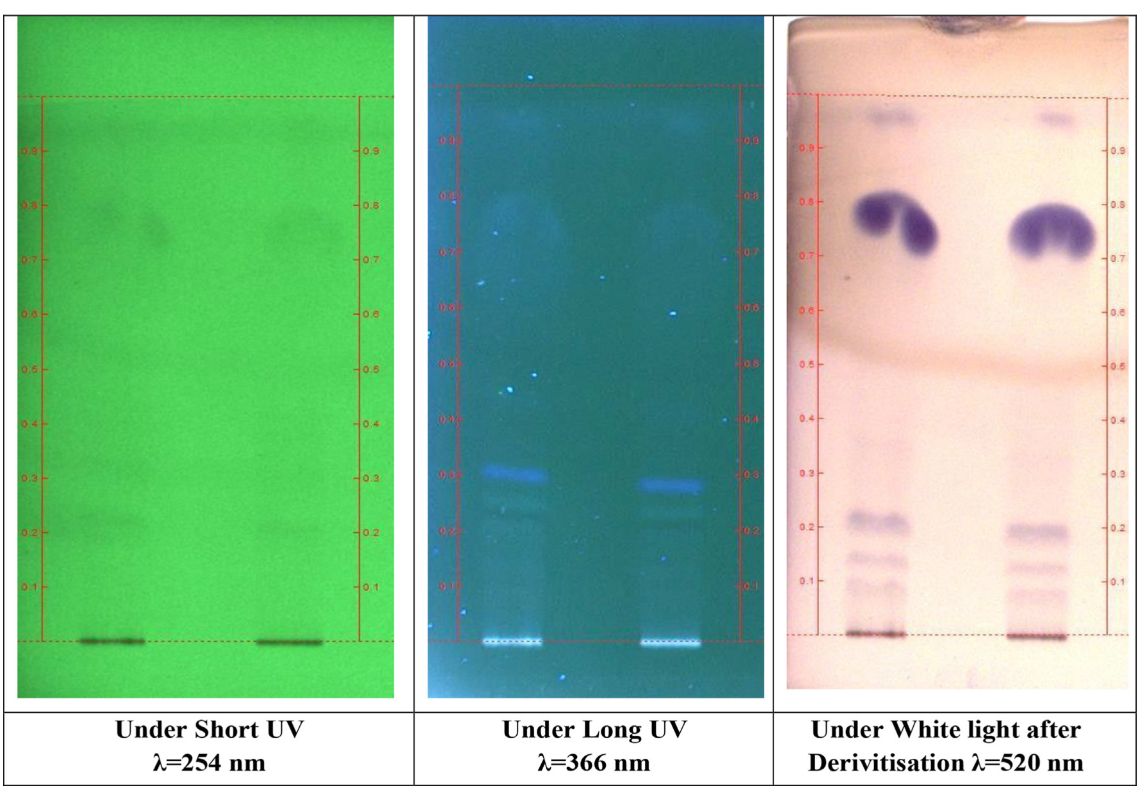


Fig. 1. MT Thin Layer Chromatography observations. (A)Under short UV λ = 254 nm, (B) Under long UV λ = 366 nm, (C) Under white light after derivatisation λ = 520 nm.

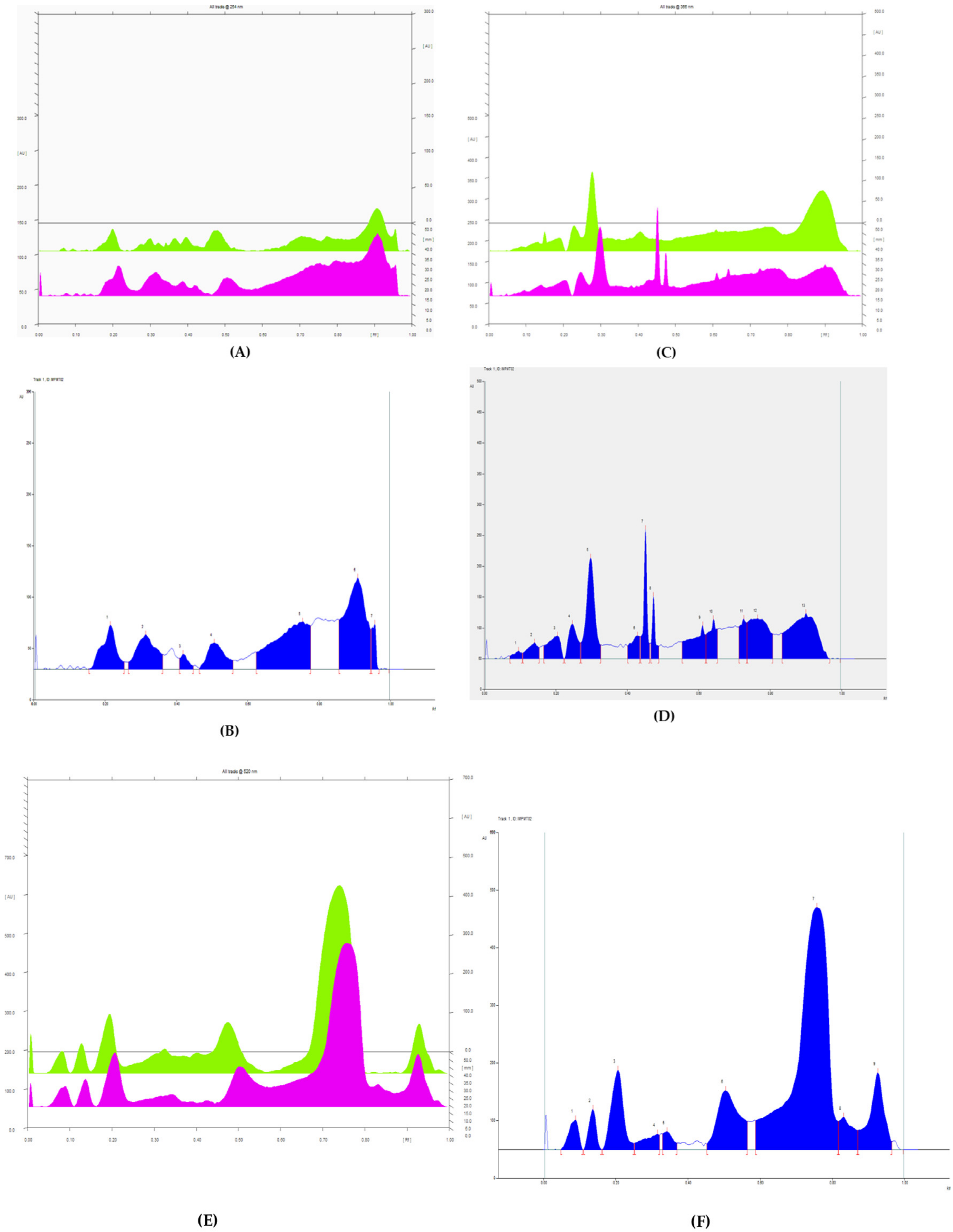


Fig. 2. (A-B) MT HPTLC Chromatogram and fingerprint profile @ 254 nm, (C-D) HPTLC Chromatogram and fingerprint profile @ 366 nm, (E-F) HPTLC Chromatogram and Peak table @ 520 nm.

been observed that MT samples showed the presence of a higher percentage of fatty acids and fatty esters. MMRT samples also displayed several fatty acids and fatty esters.

4. Discussion

As described in the introduction, finger-printing of two potential siddha tailam was the major objective of the study because of the non-availability of information on the constituents present and the prolonged claim on the multifunctional properties. With reference to the documents on the Siddha medical system (Uthamarayan, 1968) and Siddha Vaidhiya Thirattu management of chronic wounds and skin problems are through oil-based formulations, where in sesame oil, castor oil, and coconut oil were in use. With regard to MT, SCRI has standardized and validated the preparation of MT. It has been widely used in government Siddha health

Table 5
MT HPTLC Peak observations. (A) HPTLC Peak table @ 254 nm, (B) HPTLC Peak table @ 366 nm, (C) HPTLC Peak table @ 520 nm.

(A)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.16 Rf	0.4 AU	0.22 Rf	42.8 AU	14.46 %	0.25 Rf	7.4 AU	1569.1 AU	11.23 %
2	0.26 Rf	7.0 AU	0.31 Rf	33.8 AU	11.42 %	0.36 Rf	14.4 AU	1688.9 AU	12.09 %
3	0.41 Rf	11.0 AU	0.42 Rf	14.6 AU	4.93 %	0.45 Rf	3.2 AU	305.0 AU	2.18 %
4	0.46 Rf	1.1 AU	0.51 Rf	25.6 AU	8.64 %	0.56 Rf	8.7 AU	1175.5 AU	8.41 %
5	0.62 Rf	16.7 AU	0.75 Rf	46.4 AU	15.66 %	0.78 Rf	42.4 AU	4094.5 AU	29.30 %
6	0.86 Rf	48.4 AU	0.91 Rf	89.0 AU	30.05 %	0.94 Rf	38.5 AU	4639.2 AU	33.20 %
7	0.95 Rf	39.0 AU	0.96 Rf	44.0 AU	14.85 %	0.97 Rf	0.1 AU	500.5 AU	3.58 %

(B)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.07 Rf	4.4 AU	0.10 Rf	12.6 AU	1.30 %	0.11 Rf	9.3 AU	250.2 AU	1.07 %
2	0.11 Rf	9.2 AU	0.14 Rf	26.0 AU	2.69 %	0.15 Rf	18.0 AU	704.9 AU	3.01 %
3	0.17 Rf	21.2 AU	0.20 Rf	36.9 AU	3.82 %	0.22 Rf	0.0 AU	1213.8 AU	5.19 %
4	0.22 Rf	0.9 AU	0.25 Rf	56.2 AU	5.81 %	0.27 Rf	25.8 AU	1286.8 AU	5.50 %
5	0.27 Rf	26.1 AU	0.30 Rf	164.0 AU	16.97 %	0.33 Rf	22.1 AU	3644.4 AU	15.58 %
6	0.40 Rf	20.9 AU	0.43 Rf	37.4 AU	3.87 %	0.44 Rf	36.2 AU	845.3 AU	3.61 %
7	0.44 Rf	35.9 AU	0.45 Rf	210.1 AU	21.74 %	0.46 Rf	25.3 AU	1788.5 AU	7.65 %
8	0.47 Rf	24.7 AU	0.47 Rf	101.8 AU	10.53 %	0.49 Rf	20.6 AU	842.6 AU	3.60 %
9	0.55 Rf	27.6 AU	0.61 Rf	53.8 AU	5.57 %	0.62 Rf	39.0 AU	1883.0 AU	8.05 %
10	0.62 Rf	39.5 AU	0.64 Rf	63.8 AU	6.60 %	0.65 Rf	48.6 AU	1256.5 AU	5.37 %
11	0.71 Rf	52.1 AU	0.73 Rf	64.6 AU	6.69 %	0.74 Rf	58.6 AU	1102.4 AU	4.71 %
12	0.74 Rf	58.7 AU	0.76 Rf	65.4 AU	6.77 %	0.81 Rf	40.3 AU	3404.7 AU	14.56 %
13	0.83 Rf	41.1 AU	0.90 Rf	73.7 AU	7.63 %	0.97 Rf	0.3 AU	5165.1 AU	22.08 %

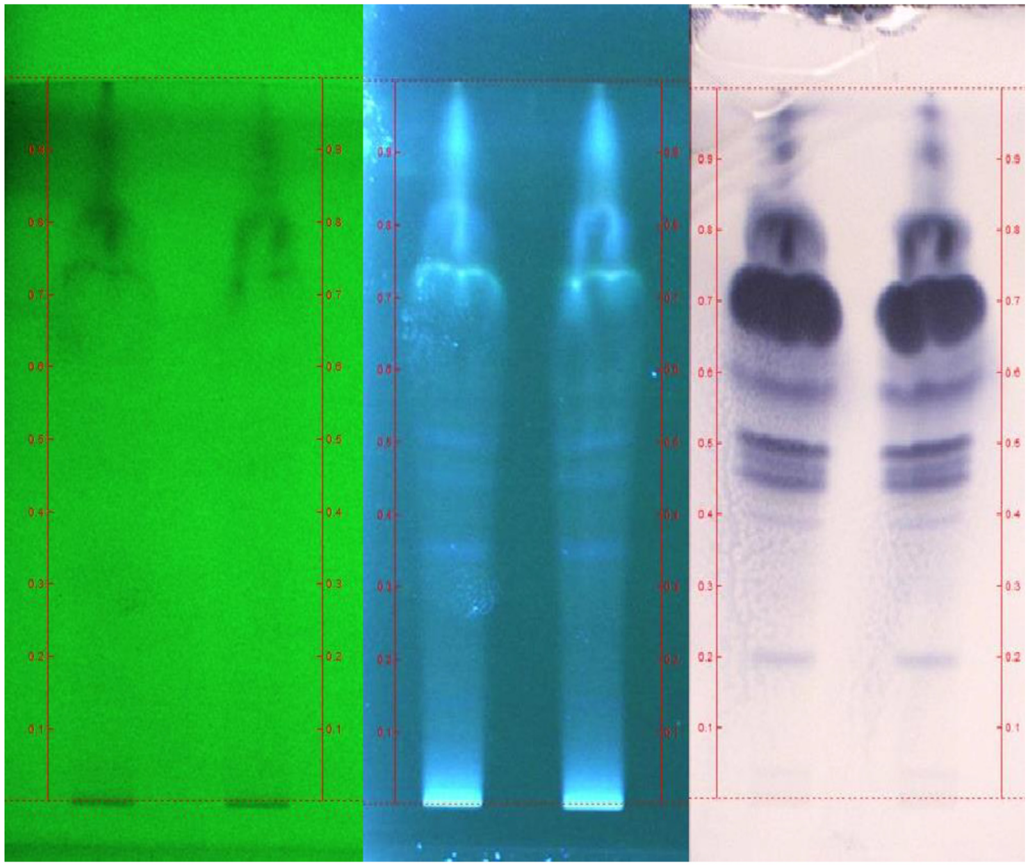
(C)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	0.2 AU	0.09 Rf	51.5 AU	5.00 %	0.11 Rf	0.3 AU	1314.4 AU	2.44 %
2	0.11 Rf	0.4 AU	0.14 Rf	69.5 AU	6.75 %	0.16 Rf	0.1 AU	1311.4 AU	2.44 %
3	0.16 Rf	0.5 AU	0.21 Rf	137.8 AU	13.38 %	0.25 Rf	11.1 AU	4286.5 AU	7.97 %
4	0.25 Rf	11.3 AU	0.32 Rf	27.0 AU	2.62 %	0.32 Rf	25.6 AU	1092.6 AU	2.03 %
5	0.33 Rf	27.7 AU	0.34 Rf	31.0 AU	3.01 %	0.37 Rf	11.9 AU	777.6 AU	1.45 %
6	0.45 Rf	10.2 AU	0.50 Rf	102.3 AU	9.94 %	0.56 Rf	49.5 AU	5802.8 AU	10.79 %
7	0.59 Rf	50.8 AU	0.76 Rf	420.5 AU	40.85 %	0.82 Rf	49.6 AU	32344.1 AU	60.12 %
8	0.82 Rf	49.6 AU	0.83 Rf	56.4 AU	5.48 %	0.87 Rf	33.5 AU	1940.8 AU	3.61 %
9	0.87 Rf	33.6 AU	0.93 Rf	133.5 AU	12.97 %	0.96 Rf	14.1 AU	4933.3 AU	9.17 %

centers for the treatment of skin lacerations, burns, and other skin pathologies (Samraj Karunanithi and Parameswaran, 2019). It is also used in the veterinary college for surgical wounds (Selvaraju et al., 2022).

In phytochemical analysis, results showed that both the tailam has the presence of phenols, proteins, and acid content. Flavonoids, phytosterol and carbohydrates were absent in both MT and MMRT. Quinones and tannins were present only in MMRT and not in MT. MMRT has not shown any peroxide value but has a high Iodine value compared to MT. Both the tailams showed nil rancidity. Based on the physiochemical analysis, the pH of MT and MMRT was determined as 5.59 and 6.57 respectively indicating the suitability of these agents for the healing of wounds. According to Tang et al.(2021), the pH value of an external application agent for wound healing should be between 6.0 and 7.0. With respect to specific gravity both the samples were shown 0.92 and 0.95 may be due to the increased consistency in the presence of oil. The use of oil in siddha formulation imparts shelf life and therapeutic value and according to Thirunarayanan and Sudha (2010), the shelf life of traditional Siddha oil formulations ranges from 1 to 3 years.

Priya et al. (2002) done the antimicrobial profile of MT and the authors concluded that the main constituents such as *Datura metel* leaves extract, coconut oil, and copper sulphate could be responsible for the antimicrobial as well anti-MRSA activities of MT. However, the present study on GC–MS analysis of MT displayed a list of constituents as described in the following paragraph could also play an important role in the antimicrobial property of MT. Further, the antioxidant profile of MT and MMRT also exemplifies the role played by phytoconstituents present in the product. Results showed more than 50% of DPPH radical quenching exhibited by MMRT reasoned to the presence of tannins and quinones. However, MT samples showed only the minimum percentage of quenching which may be due to the presence of phenols. Das et al. (2018) observed the strong relationship between the phenolic constituents and the antioxidant potential, which corroborates well with the present study. According to Fraga-Corral et al. (2021), the phenolic rings and the hydroxyl groups facilitate the protein binding properties and the antioxidant properties of phenolic constituents in plant extracts. With reference to the literature available on the wound healing properties exhibited by MT and MMRT suggested that the presence of alkaloids and their derivatives in MT and quinones derivatives in MMRT facilitate the cellular proliferation. Gadgoli reported that quinones assist proliferative regeneration of fibroblasts and alkaloids to stimulate the required precursors (Gadgoli, 2016). Moreover, MMRT preparations involve plants such as *Acacia arabica, Phyllanthus amarus, Punica granatum, Terminalia chebula, Emblica officinalis, Terminalia bellarica*, and *Quercus*



(A) (B) (C)

Fig. 3. MMRT (A) Under short UV λ = 254 nm, (B) Under long UV λ = 366 nm, (C) Under white light after derivatisation λ = 520 nm.

*pubescens, Smilax chin*a, etc., and were responsible for the presence of quinones and their derivatives in the final product.

GC–MS chromatogram of MT samples showed the presence of a higher percentage of fatty acids and fatty esters in addition to Silane and Vanillin lactoside. As reported in the literature, the presence of fatty esters and fatty acids may be responsible for the acceleration of wound healing. Similarly, MMRT samples also displayed several fatty acids and fatty esters and in addition, to p-cresol, Caryophyllene, Cis-Vaccenic acid, Ricinoleic acid, Squalene, Mequinol and Eugenol indicated that the active compounds of the oils used during the preparation of MMRT. In addition, the appreciable antioxidant activity shown by MMRT compared to MT may also be due to the presence of a higher percentage of fatty esters and other important plant active molecules such as Mequinol.

In general, the selection of fatty acids and fatty esters and the preparation of mixtures by employing synthetic methods are highly challenging and it is difficult to select the particular fatty acids and fatty esters and their concentrations for specific functions. However, researching or fingerprinting of well-established and currently in use siddha formulations showed the various percentages of the list of fatty esters and fatty acids and their combinations in addition to the presence of the most active molecules may be the solid reason for the healing efficacy shown by MMRT and MT. The current practice of MMRT and MT for burn wounds and other skin wounds as an antimicrobial, antioxidant and wound healing agent has been well evidenced from the fingerprinting analyses done in the present study. The presence of dimethylsulfoxide, a known free radical scavenger distributes topical medicaments through biological membranes (Moskot et al., 2019). According to Swaminathan et al. Octanoic acid (Caprylic acid) decreases gastrointestinal inflammation and tissue interleukin-8 secretions to treat Crohn's disease. Antioxidant skin care products contain Caprylic acid, which is one of the significant ingredients in moisturizers, lipsticks, anti-ageing creams, sunscreen lotions, foundations, lip and eyeliners. Caryophyllene oxide is one of the important molecules identified, and studies show that caryophyllene oxide eliminated onychomycosis faster than ciclopiroxalamine and sulconazole in a 15-day clinical trial (Yang et al., 1999). This drug reduces platelet aggregation *in vitro*(Lin et al., 2003). Further, Eugenol, another important molecule screened in MMRT. Clove essential oil contains volatile phenolic eugenol. Eugenol is an antibacterial, anti-inflammatory, analgesic, and antioxidant. Eugenol improves medication absorption through the skin. Recent research shows that phenolic compounds like eugenol are antioxidants and free radical scavengers (Mohammadi Nejad et al., 2017). Squalene is a component that is identified and the study proves that the water-soluble cationic steroid squalamine destroys Gram-negative and Gram-positive bacteria, fungi, angiogenesis, and tumors. Squalamine may treat lung and ovarian cancer (Kazakova et al., 2022). Undecylenic acid has antifungal properties and Oleic acid may locally influence cytokine production (i.e., inside the viable epidermis). Ricinoleic acid is the most abundant compound found in MMRT because castor oil is the base, and ricinoleic acid and its esters are primarily used in modern science as skin conditioners and emulsion stabilizers (Final report on the safety assessment of Ricinus Communis, 2007). Though MT contains only the *Datura metel* leaves extract and co-

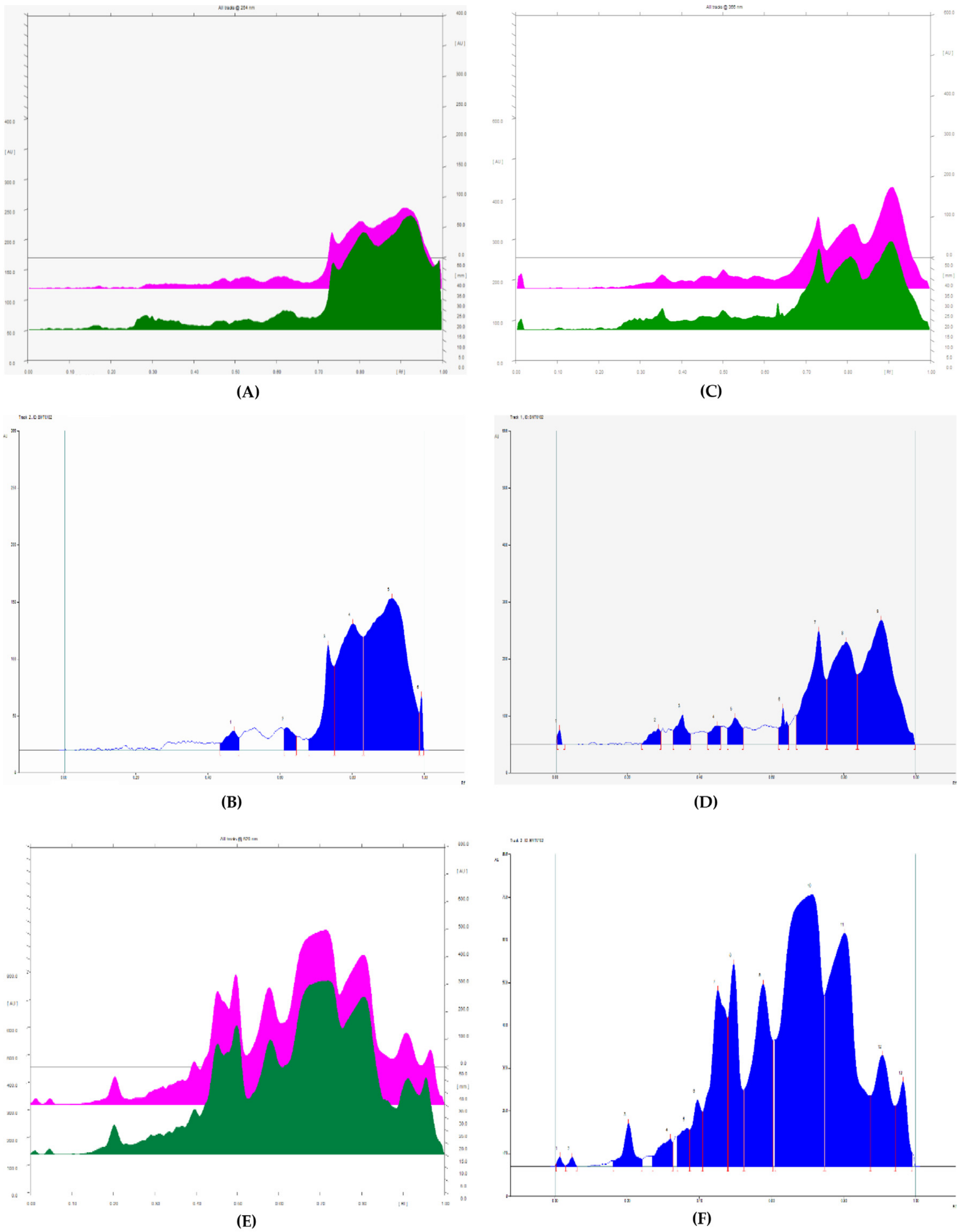


Fig. 4. MMRT (A-B) HPTLC Chromatogram and fingerprint profile @ 254 nm, (C-D) HPTLC Chromatogram and fingerprint profile @ 366 nm, (E-F) HPTLC Chromatogram and Peak table @ 520 nm.

conut oil, the presence of fatty acids and fatty esters as observed in GC–MS analysis of MT may be due to the preparation processes followed and there is nil report showing the presence of these compounds in MT (Hanif et al., 2022).

Thus, it has been understood that GC–MS analysis provided the quantitative presence of natural volatile components and lipophilic compounds with low melting points and good thermal stability in oil-based formulations with medicinal herbs and minerals. The presence of these compounds was found responsible for the enhanced wound healing property as accepted by the patients and proved as both MT and MMRT are effective siddha tailams for the management of skin wounds.

MT and MMRT have a long history of usage in the healing of wounds without side effects, however, the reports on the constituents responsible for the healing are missing. Similarly, MT and MMRT tailam has been prescribed by vaidhiyars for many

Table 6
MMRT HPTLC Peak observations. (A) HPTLC Peaks @ 254 nm, (B) HPTLC Peaks @ 366 nm, (C) HPTLC Peaks @ 520 nm.

(A)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.43 Rf	6.2 AU	0.47 Rf	16.9 AU	4.03 %	0.49 Rf	11.2 AU	545.0 AU	2.36 %
2	0.61 Rf	18.1 AU	0.62 Rf	19.9 AU	4.73 %	0.65 Rf	12.6 AU	492.9 AU	2.13 %
3	0.68 Rf	9.5 AU	0.73 Rf	92.2 AU	21.93 %	0.75 Rf	73.7 AU	2438.7 AU	10.54 %
4	0.75 Rf	74.1 AU	0.80 Rf	110.9 AU	26.37 %	0.83 Rf	99.6 AU	6382.5 AU	27.59 %
5	0.83 Rf	99.7 AU	0.91 Rf	133.3 AU	31.68 %	0.99 Rf	32.4 AU	12957.5 AU	56.02 %
6	0.99 Rf	32.6 AU	0.99 Rf	47.4 AU	11.26 %	1.00 Rf	0.0 AU	312.8 AU	1.35 %

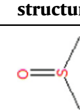
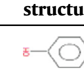
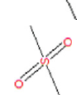
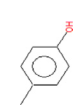
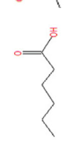
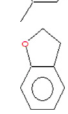
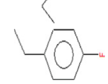
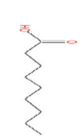
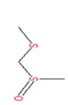
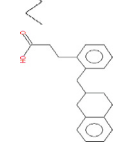
(B)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	16.5 AU	0.01 Rf	25.9 AU	3.06 %	0.03 Rf	0.0 AU	211.8 AU	0.52 %
2	0.24 Rf	1.7 AU	0.29 Rf	27.9 AU	3.30 %	0.29 Rf	23.0 AU	745.6 AU	1.83 %
3	0.33 Rf	25.1 AU	0.35 Rf	51.8 AU	6.12 %	0.38 Rf	19.2 AU	1341.3 AU	3.29 %
4	0.42 Rf	20.5 AU	0.45 Rf	33.1 AU	3.91 %	0.46 Rf	31.9 AU	849.8 AU	2.09 %
5	0.48 Rf	29.1 AU	0.50 Rf	47.1 AU	5.57 %	0.52 Rf	28.7 AU	1358.9 AU	3.34 %
6	0.62 Rf	29.6 AU	0.63 Rf	64.1 AU	7.57 %	0.65 Rf	33.1 AU	955.6 AU	2.35 %
7	0.67 Rf	51.7 AU	0.73 Rf	198.8 AU	23.48 %	0.75 Rf	13.8 AU	8136.7 AU	19.98 %
8	0.75 Rf	114.4 AU	0.81 Rf	180.0 AU	21.27 %	0.84 Rf	23.1 AU	10585.8 AU	26.00 %
9	0.84 Rf	123.4 AU	0.90 Rf	217.7 AU	25.72 %	1.00 Rf	0.0 AU	16533.9 AU	40.60 %

(C)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	5.6 AU	0.01 Rf	22.5 AU	0.66 %	0.03 Rf	2.3 AU	271.6 AU	0.15 %
2	0.03 Rf	2.6 AU	0.05 Rf	21.6 AU	0.63 %	0.06 Rf	0.2 AU	276.7 AU	0.16 %
3	0.16 Rf	13.5 AU	0.20 Rf	101.2 AU	2.97 %	0.24 Rf	17.4 AU	2674.7 AU	1.52 %
4	0.27 Rf	24.8 AU	0.32 Rf	64.6 AU	1.90 %	0.33 Rf	57.6 AU	2299.3 AU	1.31 %
5	0.34 Rf	70.9 AU	0.37 Rf	89.6 AU	2.63 %	0.37 Rf	85.7 AU	2367.3 AU	1.35 %
6	0.37 Rf	86.0 AU	0.40 Rf	155.5 AU	4.56 %	0.41 Rf	27.4 AU	3662.9 AU	2.09 %
7	0.41 Rf	127.4 AU	0.45 Rf	412.0 AU	12.09 %	0.48 Rf	47.5 AU	16709.2 AU	9.53 %
8	0.48 Rf	350.3 AU	0.50 Rf	473.6 AU	13.90 %	0.52 Rf	78.1 AU	12259.0 AU	6.99 %
9	0.52 Rf	178.8 AU	0.58 Rf	426.2 AU	12.51 %	0.60 Rf	95.5 AU	20492.7 AU	11.68 %
10	0.61 Rf	295.6 AU	0.71 Rf	636.6 AU	18.69 %	0.75 Rf	99.8 AU	59109.9 AU	33.70 %
11	0.75 Rf	401.4 AU	0.80 Rf	545.8 AU	16.02 %	0.87 Rf	65.6 AU	38723.0 AU	22.07 %
12	0.88 Rf	165.8 AU	0.91 Rf	259.7 AU	7.62 %	0.94 Rf	41.2 AU	11375.2 AU	6.48 %
13	0.94 Rf	141.4 AU	0.97 Rf	198.1 AU	5.81 %	0.99 Rf	31.3 AU	5199.0 AU	2.96 %

Table 7
Indicates the respective list of molecules present in the samples MT and MMRT-derived compounds and their structure.

S. No.	MT-derived compounds	Chemical formula	MT-derived compound's structure	MMRT -derived compounds	Chemical formula	MMRT -derived compound's structure
1.	Dimethyl sulfoxide	C2H6OS		Phenol	C6H6O	
2.	Dimethyl sulfone	C2H6O2S		*p*-Cresol	C7H8O	
3.	Hexanoic acid	C6H12O2		Benzofuran, 2,3-dihydro	C8H8O	
4.	*p*-Fluoroethylbenzene	C8H9F		Nonanoic acid	C9H18O2	
5.	FAMSO	C3H8OS2		Hydrocinnamic acid, o-[(1,2,3,4-tetrahydro-2-naphthyl)methyl]	C20H22O2	

(continued on next page)

Table 7 (*continued*)

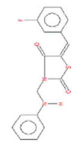
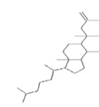
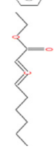
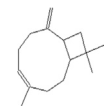
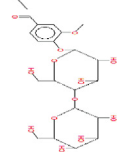
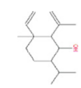
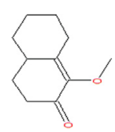
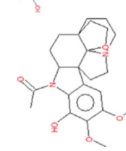
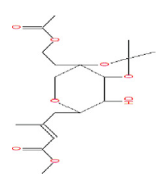
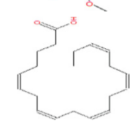
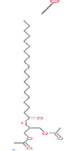
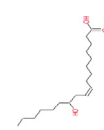
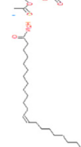
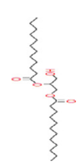
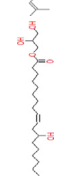
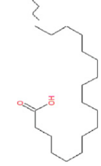
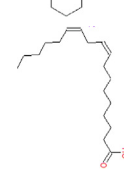
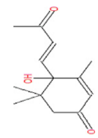
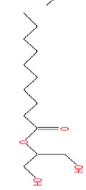
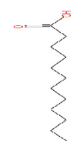
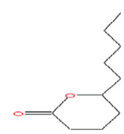
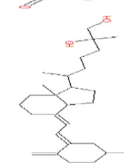
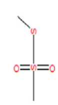
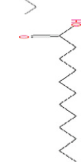
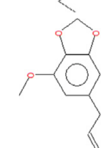
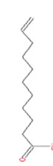
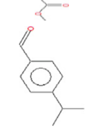
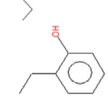
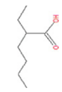
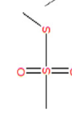
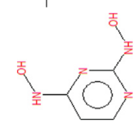
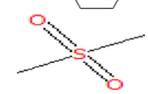
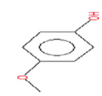
S. No.	MT-derived compounds	Chemical formula	MT-derived compound's structure	MMRT -derived compounds	Chemical formula	MMRT -derived compound's structure
6.	3-Anilinomethyl-5-(3-fluorobenzylidene)-2,4-thiazolidinedione	C17H13FN2O2S		Cholestan-3-ol,2-methylene-(3β,5α)-	C28H48O	
7.	Nona-2,3-dienoic acid, ethyl ester	C11H18O2		Caryophyllene	C15H24	
8.	Vanillin lactoside	C20H28O3		6-epi-shyobunol	C15H26O	
9.	Silane, Chloro(chloromethyl)dimethyl-	C3H8Cl2Si		Cis-Vaccenic acid	C18H34O2	
10.	2(3H)-Naphthalenone,4,4a,5,6,7,8-hexahydro-1-methoxy	C11H16O2		Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy	C23H30N2O5	
11.	d-Allo-dec-2-enonic acid,5,8-anydro-2,3,4,9-tetradeoxy-8C-(hydroxymethyl)-3-methyl-7,8-O-(1-methylethylidene)-metyl ester	C18H28O8		Cis-5,8,11,14,17-Eicosapentaenoic acid	C20H30O2	
12.	Eicosanoic acid,2-(acetyloxy)-1[(acetyloxy) methylethyl ester	C27H50O6		Ricinoleic acid	C18H34O3	
13.	13-Docosenamide, (Z)	C22H43NO		Squalene	C30H50	
14.	Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	C27H52O5		1-Glyceryl ricinoleate	C21H40O5	
15.	Dodecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	C15H30O4		Octadecanoic acid	C18H36O2	
16.	n-Hexadecanoic acid	C16H32O2		9,12-Octadecadienoic acid (Z,Z)	C18H32O2	

Table 7 (*continued*)

S. No.	MT-derived compounds	Chemical formula	MT-derived compound's structure	MMRT -derived compounds	Chemical formula	MMRT -derived compound's structure
17.	2-Cyclohexan-1-one,4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	C13H18O3		Dodecanoic acid, 2,3-dihydroxypropyl ester	C15H30O4	
18.	Tetradecanoic acid	C14H28O2		Decanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	C13H26O4	
19.	Dodecanoic acid	C12H2402		Tetradecanoic acid	C14H28O2	
20.	2H-Pyran-2-one,tetrahydro-6-pentyl-	C10H18O2		9,10-Secocholesta-5,7,10 (19)-triene-3,25,26-triol, (3β,5Z,7E)	C27H44O3	
21.	Octanoic acid	C8H16O2		Hexadecanoic acid, ethyl ester	C18H36O2	
22.	S-Methyl methanethiosulphonate	C2H6O2S2		Dodecanoic acid	C12H2402	
23.				1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)	C11H12O3	
24.				Undecylenic acid	C11H20O2	
25.				8,11-Octadecadiynoic acid, methyl ester	C19H30O2	
26.				Benzaldehyde, 4-(1-methylethyl)	C10H12O	
27.				Octanoic acid	C8H16O2	
28.				Phenol, 2-ethyl	C8H10O	

(*continued on next page*)

Table 7 (*continued*)

S. No.	MT-derived compounds	Chemical formula	MT-derived compound's structure	MMRT -derived compounds	Chemical formula	MMRT -derived compound's structure
29.				Hexanoic acid, 2-ethyl	C8H16O2	
30.				Heptadecanoic acid	C17H34O2	
31.				S-Methyl methanethiosulphonate	C2H6O2S2	
32.				2,4-Bis (hydroxylamino) pyrimidine	C4H6N4O2	
33.				Dimethyl sulfone	C2H6O2S	
34.				Mequinol	C7H8O2	

types of skin wound healing and the related tissue approximation in humans is amazing. However, to date, the chemical constituents present in MMRT have not been studied and are not available in the public domain. The phyto, physio-chemical, antioxidant, HPTLC and GC–MS analyses support the properties shown by the tailams. From GC–MS analysis, it has been evidenced that more than 50% of constituents of MT and MMRT are fatty acids and fatty esters. Specific molecules like Squalene, methylsulfanyl, Caryophyllene, Vaccenic acid, and Ricinoleic acid in addition to fatty esters and fatty acids accountable for the healing property evidenced upon using MT and MMRT. Thus, the present study explores the actual constituents of MMRT and MT elaborately. Further, the method of preparations of MT and MMRT and their successful and sustainable use in the limited patient community as per the prescription given by our ancestors and the current vaithiyars were kept dormant in textbooks for various reasons on the timeline.

5. Conclusions

SSM is the oldest documented medical system in the world. In the modern era, SSM is not gaining proper popularity because of a lack of understanding about metallic preparations and principles. Though the effectiveness of the Siddha drugs has been realized at a greater level, the acceptance of the drug by the wide community needs scientific validation or fingerprinting. Since the preparation of each drug is unique, the chemistry behind the transformation of plant extracts and other natural molecules in the presence of oil and heat provided during the preparation needs to be explored and add value to the use of Siddha drugs. It is the right time to explore the potency of the promising Siddha medicines through scientific methods and applied to the modern concept of medicine which might help to treat unresolved medical issues.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Contributions of authors

The authors contributed to the research structure, data analysis, graphical presentations, and manuscript drafting. The authors wrote, reviewed, and endorsed the manuscript for the main final version.

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