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Chemical characterization of *Deepalinga chenduram*(DLC)- A Siddha Herbo-mineral formulation.

Yasiga.S[1] Sudha Revathy.S[2*]

[1]PG scholar, Department of Gunapadam, National Institute of Siddha, Tambaram Sanatorium, Chennai – 47, Tamil Nadu, India
[2]Assistant Professor, Department of Gunapadam, National Institute of Siddha, Tambaram Sanatorium, Chennai – 47, Tamil Nadu, India

Correspondence to Author: Dr. S. Sudha Revathy
Assistant Professor,Department of Gunapadam, National Institute of Siddha, Chennai – 600047, Tamil Nadu, India.E-mail: dr.sudharevathy@gmail.com

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Abstract
Siddha system of medicine has a collection of formulations written in Tamil language for various ailments. The Siddha medicinal formulations are made using herbal products dried or fresh, animal by products, metals, minerals and marine products. *Deepalinga Chenduram,* is one such Siddha Herbo mineral formulation from Siddha literature *Anuboga Vaithya Navaneedham.* In this study the chemical characterisation of DLC is done by analysing its physio chemical properties, HPTLC and ICP-OES. This study is done to provide scientific validation for the Siddha medicinal formulation. With the physio chemical characterisation, the quality and consistency were analysed. The HPTLC analysis revealed distinct phytochemical profiles that is essential for standardization. Concurrently, ICPOES was employed to quantify mercury and ensure the safety of the formulation by detecting any potential toxic metals. The results from these analytical techniques provide comprehensive data on the chemical composition and elemental content of *Deepalinga Chenduram,* supporting its therapeutic efficacy and safety. This study highlights the importance of modern analytical methods in validating traditional medicines, ensuring their quality, and promoting their integration into contemporary health care practices.
Key words: Physio chemical, Siddha medicine, *Deepalinga Chenduram,* characterization.

Introduction

The Siddha system of medicine is one of the oldest medical systems and still in use, and it is particularly prevalent in southern India. Though Siddha system of medicine is known for the medicinal formulations prepared using herbs and its by products, the area of specialisation are

the formulations made using metals and minerals. The characteristic feature of the medicinal formulations containing metals and minerals is that they show higher efficacy with minimal quantity.

This study is carried out to elucidate the physio chemical properties, develop HPTLC fingerprinting, analyse the phytoconstituent and quantify the amount of mercury present in the Siddha herbo mineral formulation *Deepalinga Chenduram* (DLC). This preparation is from Siddha classical literature called *Anuboga Vaithya Navaneedham*[1]. The ingredients used in this formulation is Cinnabar, *Piper longum, Piper nigrum, Zingiber offficinale, Styrax benzoin* and *Cinnamomum camphor.* The major ingredient of this Siddha Herbo mineral formulation is Cinnabar[1].According to the Siddha literature the Tamil name of Cinnabar is *Lingam* or *Saathi Lingam*[2] and it has been used to cure diseases like fever, non-healing ulcers, urticaria, eczema, polyuria, respiratory tract infection and leprosy. It has been said that Cinnabar is a vital drug against syphilis. It also has alterative and analgesic properties[2].

Cinnabar is the ore of mercury, it is a hydrothermal mineral formed in the regions of volcanoes and hot springs, these are formed in between the rocks in the form of granular crusts[3]. The chemical name of Cinnabar is Mercuric sulphide since it is produced by combining sulphur and mercury under high temperature. Cinnabar is often known as vermillion, because of the bright red colour. In the ancient times it is found that this mineral has been used by the Chinese, Greeks and Romans in various artifacts[4]. The artificial Cinnabar can be prepared using mercury, sulphur and potassium nitrate as per Siddha literature[2].

Piper nigrum belongs to Piperaceae family, the Tamil name is *Milagu.* It is a perennial climber with adventitious root. It also has got good aroma. The fruits are indehiscent seeded berry. The chemical constituents are piperene, piperolene, gunineesnine, feruprine, pipercide etc., and it grows in regions of heavy rainfall[5]. The Siddha literature states that it has acrid, carminative, anti-periodic, stimulant, rubefacient, resolvent activities[7].

Piper longum also belongs to Piperacaea family, The Tamil name is*Thippili.* This plant is a slender climber distributed in the warmer regions of the country. The fruits are small, ovoid, sunken structure. The phytoconstituents present are piperine, piperlongumine, piperlonguminine[6.] The fruits of *Piper longum* has stimulant and carminative activity[7].

Zingiber officinale belongs to Zingeberaceae family, The dried form of ginger is used in this formulation and it is known as *Chukku.* On fracture they are short and fibrous. The chemical

constituents are Shogaol, Gingerol, Zingibernol, β-phelladrene, α-zingeberene, β-biasbolene[5]. It has stimulant, stomachic and carminative activity[7].

Cinnamomum camphora belongs to Lauraceae family. The whole plant is used in preparing camphor. It is insoluble in water and it is volatile in nature. It has expectorant, soporific activity. The disease of head and ear can be cured with the medicines made from camphor[2].

Styrax benzoin belongs to Styraceae family. This is a kind of resin collected by scraping the cambium of the tree. It contains benzoic acid. It is greyish brown in colour. It is used in treating renal calculi and it also has expectorant, anti-septic and stimulant activity[8].

Materials and methods

Procurement and identification of drugs

The raw materials required for the preparation of the medicine are Cinnabar, *Piper longum, Piper nigrum, Zingiber offficinale, Styrax benzoin* and *Cinnamomum camphora,* they were procured from the reputed from traditional trader, Chennai. The botanist and faculty members of the Gunapadam Department at the National Institute of Siddha, Tambaram Sanatorium in Chennai, confirmed the authenticity of the raw drugs.

Purification of the raw drugs[2]

. The purification of raw drugs is carried out by the process mentioned in classical Siddha literature and are as follows. The required quantity of Cinnabar was taken and placed on the earthen plate. It is then heated on low flame. Equal quantity of freshly prepared lemon juice, extract from *Acalypha indica* and cow's milk are poured on the Cinnabar drop by drop, then the drug is washed and stored. The outer layer of *Zingiber officinale* is peeled off and the dust particles from the *Piper nigrum and Piper longum* are removed

Preparation of the medicine[1]

The *Styrax benzoin* and *Cinnamomum camphora* was ground together for three hours. Then, the mixture was divided into ten equal portions. The divided portion of the mixture was smeared on thin separate clothes and they were used to cover the Cinnabar. It was well packed and closed tightly, the Cinnabar was burnt. This procedure was repeated for ten times. Later the Cinnabar was cleaned gently and ground to fine powder. The other drugs namely *Piper*

longum, Piper nigrum, Zingiber offficinale were purified and made into fine powder. Finally the powder of Cinnabar and the other drugs were ground together for one hour. The final drug was stored in an air tight container.

Dosage

260-520 mg. The adjuvant used for DLC is honey

Chemical parameters[9]

Loss on drying

Five grams of the trial medicine DLC was measured precisely and placed in a dish that was tared to evaporate. For five hours, the test medication was heated at 105˚C in an oven. The process was carried out again until the disparity between two successive procedures does not exceed 0.25 percent. Next, the sample's percentage moisture content was determined in comparison to that of the shade-dried drug.

Total ash

On a tared silica dish, 2g of the test drug was placed. The test drug was put in the furnace and burned at 450˚C until it released no carbon. After allowing the sample to cool, it is weighed. The proportion of total ash is computed in relation to the medication that has been air-dried.

Water-soluble ash

After 25 minutes of boiling 2 grams of ash in 25 millilitres of water, the insoluble material was gathered in a crucible The collected material is washed with hot water and burned for five minutes at 450 ˚C. The weight of the insoluble matter was subtracted from the weight of ash. The water-soluble ash was represented by this, and the percentage of water-soluble ash was computed in relation to the drug's air-dried ash.

Acid insoluble ash

After adding 25 millilitres of hydrochloric acid to the ash, the insoluble material was obtained. Hot water was used to wash it. Following that, the sample was heated to a consistent weight on a hot plate and dried. After 30 minutes of cooling in a desiccator, it is weighed. subsequently, the acid insoluble ash was computed using the drug that had been air dried.

TLC and HPTLC analysis[10, 11]

Thin layer chromatography is a chromatographic method that distinguishes the components in the sample with two phases: the stationary phase and the mobile phase. Alumina or silica gel are the most widely utilized stationary phases. There is a volatile organic solvent or a combination of solvents in the mobile phase. A small patch of the sample solution containing a combination of chemicals is put to the adsorbent material near one edge. The TLC plate is positioned vertically with the spot applied edge down in a closed container (developing chamber). The mixture of compounds is separated as a result of the solvent, which is at the bottom of the container, moving the compounds up the plate at varying rates as it passes over the spot and up the layer of adsorbent by capillary action

Instrument: HPTLC CAMAG

The sample were placed in five tracks 3.0µl, 6.0 µl, 9.0 µl, 12.0 µl and 15.0 µl

Stationary phase: Merck, HPTLC Silica gel 60 F254

Plate format: 100 x 100 mm

Application Position Y: 8.0 mm, length: 8.0 mm, width: 0.0 mm

Track First position X: 15.0 mm, distance: 17.4 mm

Solvent front position 80 mm

Tank: TTC 10x10

Mobile phase: Toluene: Ethyl acetate: Formic acid (5:4:1) v/v

Saturation time: 20 min

The developed fingerprints were analysed at 254nm, 366nm.

Inductively coupled plasma optical emission spectroscopy (ICP-OES)[12]

The amount of light emitted at each wavelength is measured by the ICP-OES method, which then uses this data to estimate the mercury concentration in the sample.
An ICP-OES was calibrated by measuring solutions with known concentrations of each element. A calibration curve is generated using this data. The calibration curve establishes the correlation between the concentration of the element in the solution and the intensity of light emitted at a certain wavelength.

The sample for ICP-OES was prepared with 0.6233g of sample diluted upto 50ml with mili-Q water. The RF power was 1.20KW, the sample depth was 8 mm. The carrier gas flow was 0.72/min, 12RPM was used in the nebulizer pump and the plasma gas flow was 12L/min. Later the sample was analysed for the concentration of mercury present.

Results

Table 1: Physiochemical analysis

Parameter	Unit	Instrument	Result
Colour and odour	-	Chemically	Maroon and characteristic
Particle size (80-100 mesh)	-	Chemically	99.10
Loss on drying at 105˚c	%w/w	Hot air oven	3.60
Total ash	%w/w	Chemically	2.45
Acid insoluble ash	%w/w	Chemically	0.13
Water soluble ash	%w/w	Chemically	1.40

HPTLC

Fig 1: HPTLC plate at 254nm

Fig 2: HPTLC plate at 366nm

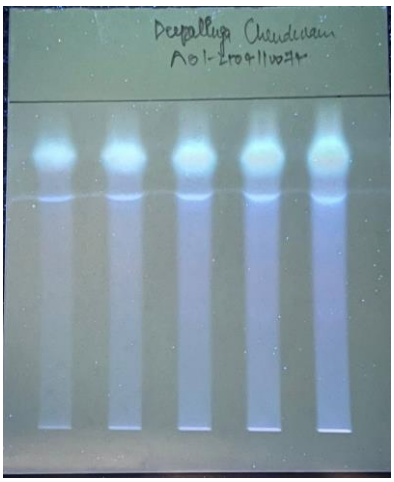
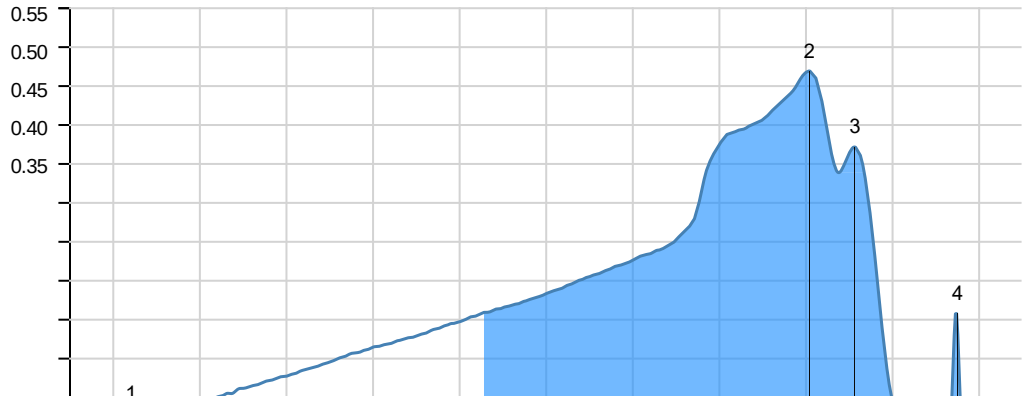


Fig 3: 3.0 µl of DLC scanned at 254nm



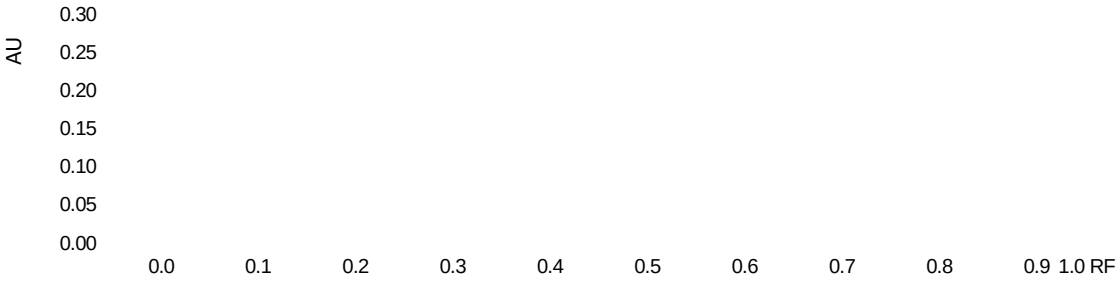


Table 2: Rf value of DLC at 3.0 µl at 254 nm

Peak	Start		Max			End		Area	
#	Rf	H	Rf	H	%	Rf	H	A	%
1	0.006	0.0000	0.021	0.0278	2.72	0.031	0.0170	0.00039	0.28
2	0.425	0.1561	0.804	0.4680	45.75	0.839	0.3374	0.11720	86.19
3	0.839	0.3374	0.857	0.3705	36.22	0.929	0.0103	0.01713	12.60
4	0.965	0.0073	0.975	0.1566	15.31	0.989	0.0000	0.00126	0.92

Fig 4: 6.0 µl scanned at 254nm

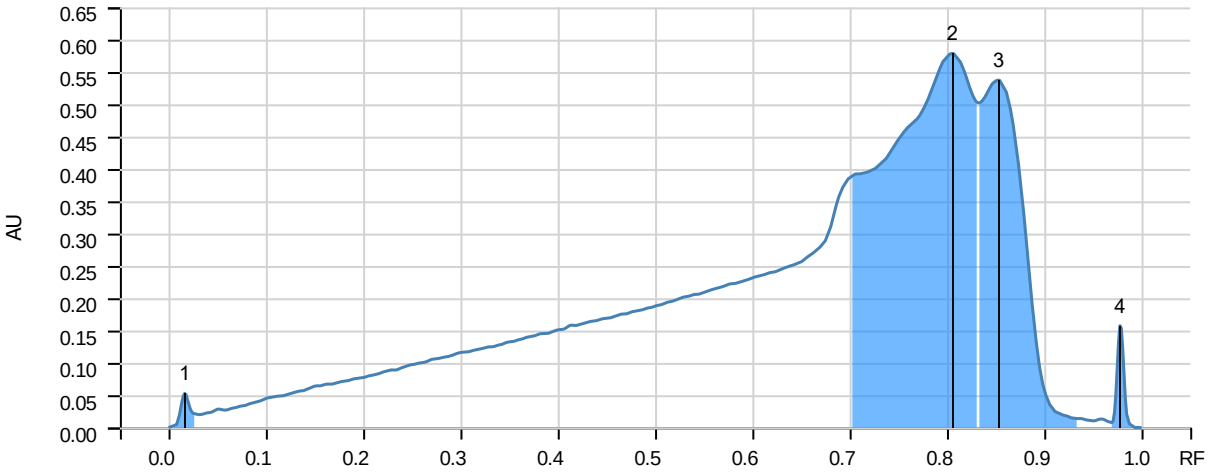
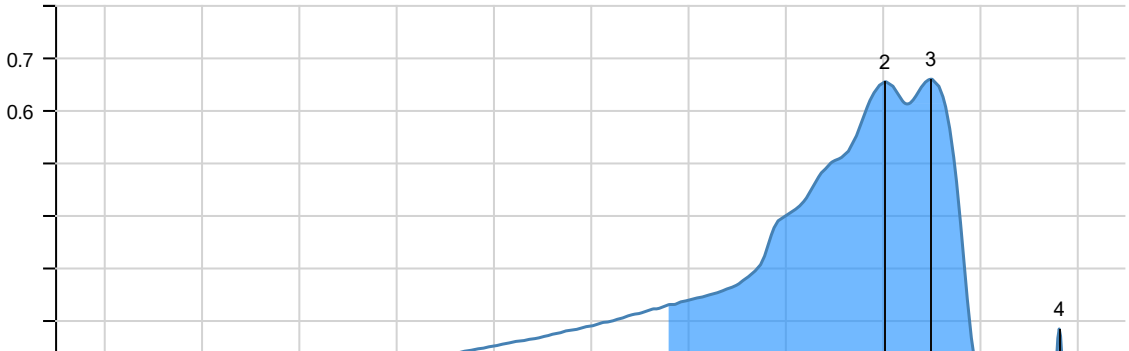


Table 3: Rf value of 6.0 µl scanned at 254nm

Peak	Start		Max			End		Area	
#	Rf	H	Rf	H	%	Rf	H	A	%
1	0.004	0.0000	0.017	0.0528	3.98	0.031	0.0201	0.00068	0.75
2	0.703	0.3901	0.806	0.5788	43.63	0.832	0.5023	0.06184	68.14
3	0.833	0.5023	0.853	0.5375	40.51	0.936	0.0139	0.02695	29.70
4	0.969	0.0075	0.978	0.1577	11.88	0.994	0.0000	0.00128	1.41

Fig 5: 9.0 µl scanned at 254nm



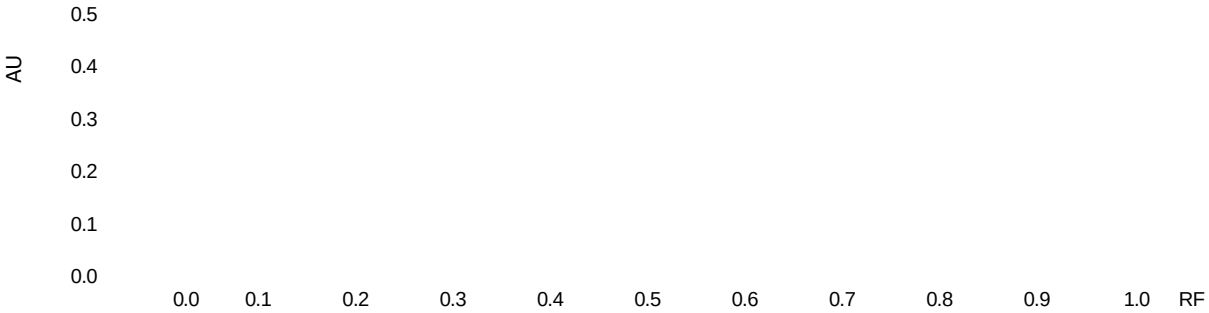


Table 4: Rf value of 9.0 µl scanned at 254nm

Peak	Start		Max			End		Area	
#	Rf	H	Rf	H	%	Rf	H	A	%
1	0.000	0.0000	0.014	0.0809	5.13	0.025	0.0199	0.00085	0.60
2	0.578	0.2274	0.803	0.6535	41.47	0.826	0.6114	0.10120	71.26
3	0.826	0.6114	0.850	0.6585	41.78	0.969	0.0105	0.03844	27.07
4	0.974	0.0086	0.982	0.1830	11.61	1.000	0.0000	0.00152	1.07

Fig 6:12.0 µl scanned at 254nm

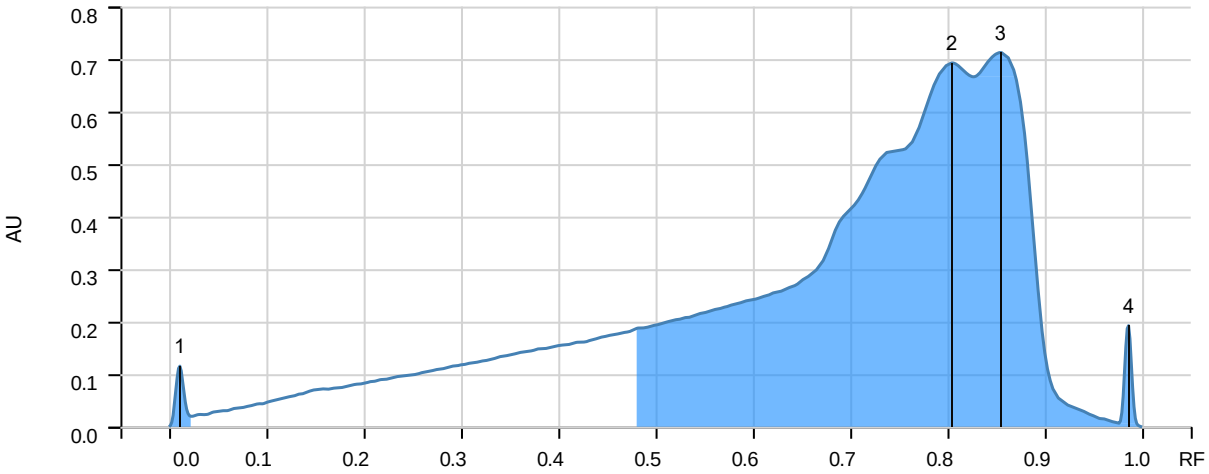
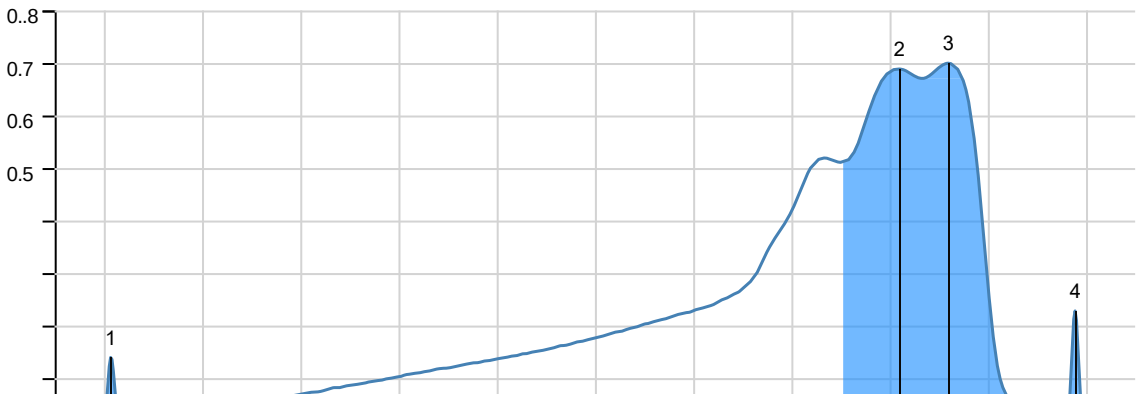


Table 5: Rf value of 12.0 µl scanned at 254nm

Peak	Start		Max			End		Area	
#	Rf	H	Rf	H	%	Rf	H	A	%
1	0.000	0.0000	0.011	0.1154	6.74	0.024	0.0199	0.00123	0.70
2	0.479	0.1861	0.804	0.6927	40.43	0.826	0.6663	0.12749	72.37
3	0.826	0.6663	0.854	0.7125	41.59	0.974	0.0079	0.04583	26.01
4	0.976	0.0067	0.986	0.1925	11.24	1.000	0.0000	0.00162	0.92

Fig7: 15.0 µl scanned at 254nm



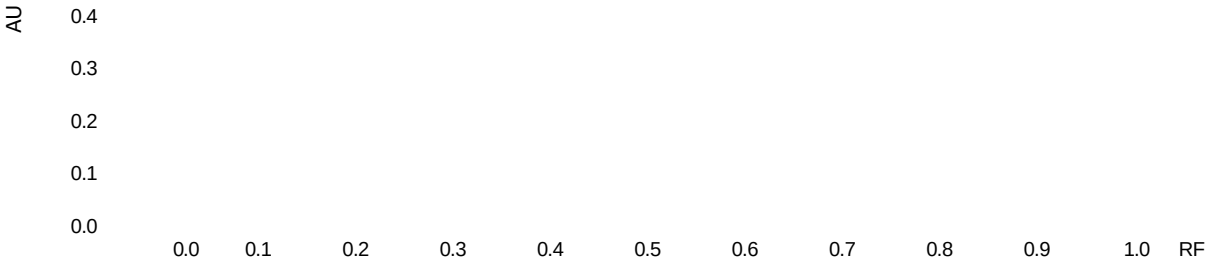


Table 6: Rf value of 15.0 µl scanned at 254nm

Peak	Start		Max			End		Area	
#	Rf	H	Rf	H	%	Rf	H	A	%
1	0.000	0.0000	0.007	0.1394	7.94	0.021	0.0047	0.00122	1.21
2	0.751	0.5107	0.810	0.6885	39.22	0.833	0.6705	0.05148	51.08
3	0.833	0.6705	0.860	0.6996	39.86	0.976	0.0079	0.04612	45.76
4	0.979	0.0055	0.989	0.2280	12.99	1.000	0.0000	0.00196	1.95

Fig 8: 3.0 µl scanned at 366nm

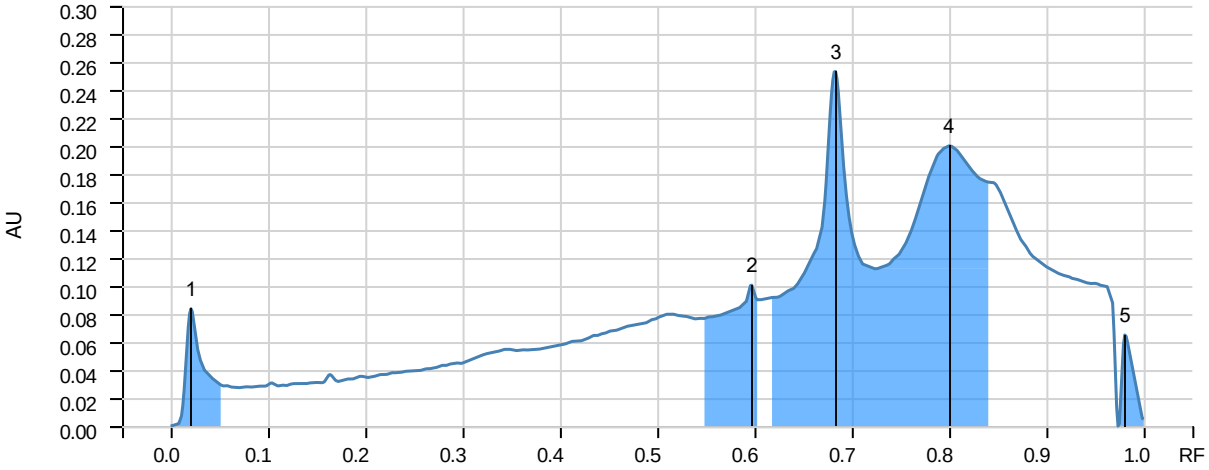
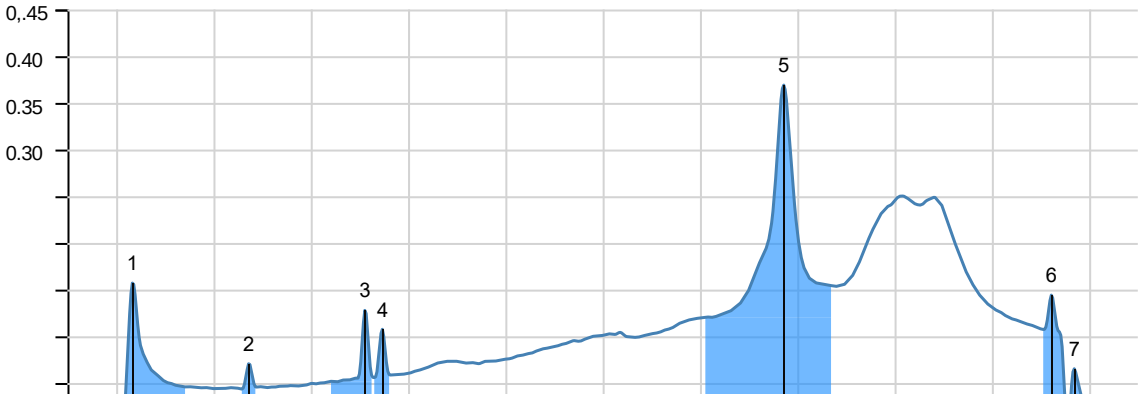


Table 7: Rf value of 3.0 µl scanned at 366nm

Peak	Start		Max			End		Area	
#	Rf	H	Rf	H	%	Rf	H	A	%
1	0.000	0.0000	0.021	0.0839	11.93	0.054	0.0287	0.00195	4.72
2	0.549	0.0768	0.597	0.1006	14.32	0.604	0.0901	0.00468	11.36
3	0.615	0.0913	0.683	0.2532	36.02	0.724	0.1123	0.01453	35.26
4	0.724	0.1123	0.800	0.2001	28.47	0.842	0.1742	0.01916	46.48
5	0.974	0.0000	0.981	0.0651	9.26	1.000	0.0050	0.00090	2.19

Fig 9: 6.0 µl scanned at 366nm



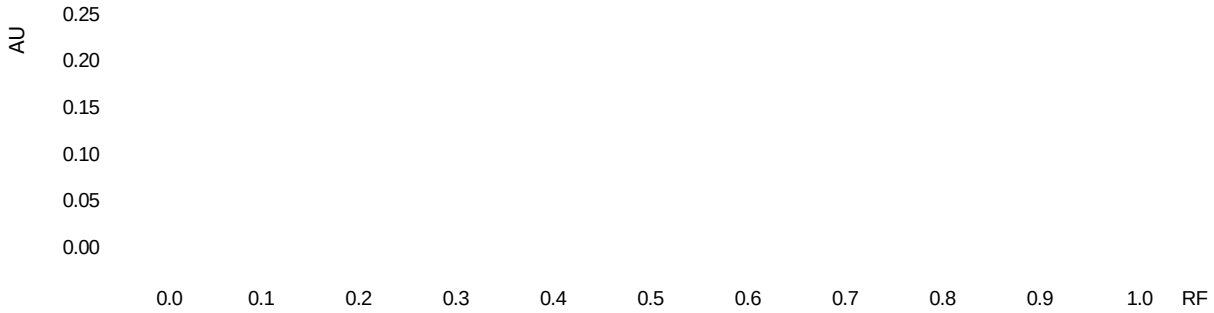


Table 8: Rf value of 6.0 µl scanned at 366nm

Peak	Start		Max			End		Area	
#	Rf	H	Rf	H	%	Rf	H	A	%
1	0.000	0.0000	0.0017	0.1570	15.08	0.072	0.0458	0.00458	12.25
2	0.129	0.0435	0.136	0.0706	6.78	0.144	0.456	0.00085	2.28
3	0.217	0.0509	0.256	0.1278	12.27	0.264	0.0560	0.00303	8.10
4	0.265	0.0558	0.274	0.1075	10.33	0.282	0.0585	0.00129	3.46
5	0.604	0.1200	0.686	0.3691	35.44	0.739	0.1537	0.02434	65.14
6	0.953	0.1073	0.961	0.1441	13.84	0.978	0.0000	0.00252	6.74
7	0.978	0.0000	0.0985	0.0653	6.27	1.0000	0.0061	0.00076	2.04

Fig 10: 9.0 µl scanned at 366nm

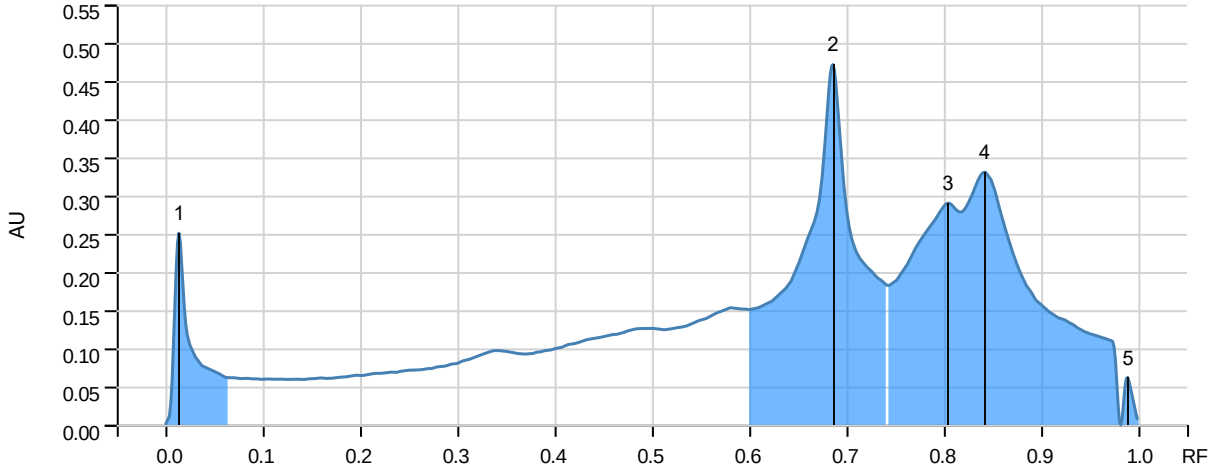
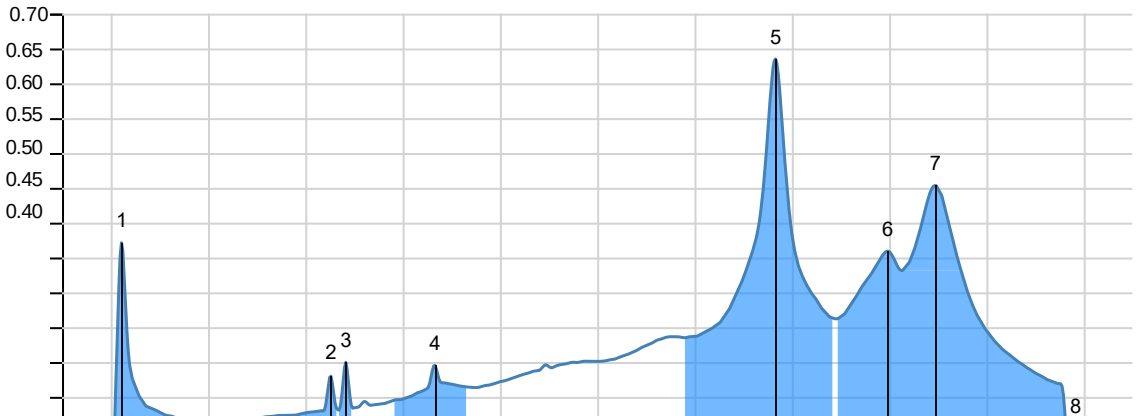


Table 9: Rf value of 9.0 µl scanned at 366nm

Peak	Start		Max			End		Area	
#	Rf	H	Rf	H	%	Rf	H	A	%
1	0.000	0.0000	0.014	0.2509	17.85	0.067	0.0614	0.00624	6.98
2	0.600	0.1508	0.686	0.4716	33.56	0.742	0.1827	0.03349	37.42
3	0.743	0.1823	0.804	0.2901	20.65	0.817	0.2784	0.01790	20.00
4	0.817	0.2784	0.842	0.3306	23.53	0.982	0.0000	0.03126	34.92
5	0.982	0.0000	0.989	0.0620	4.41	1.000	0.0078	0.00061	0.68

Fig 11: 12.0 µl scanned at 366nm



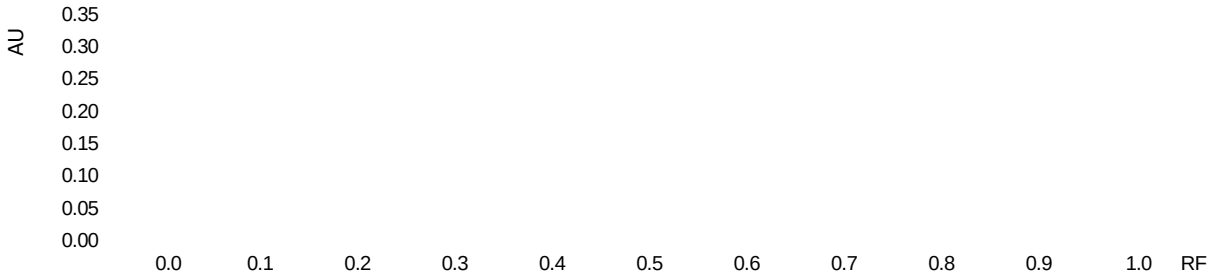


Table 10: Rf value of 12.0 µl scanned at 366nm

Peak	Start		Max			End		Area	
#	Rf	H	Rf	H	%	Rf	H	A	%
1	0.000	0.0000	0.011	0.3211	15.32	0.069	0.0698	0.00791	6.34
2	0.181	0.0732	0.226	0.1296	6.18	0.233	0.0814	0.00442	3.54
3	0.235	0.0809	0.242	0.1495	7.13	0.249	0.0837	0.00157	1.26
4	0.290	0.0944	0.333	0.1451	6.92	0.374	0.1132	0.00937	7.52
5	0.590	0.1845	0.683	0.5849	27.90	0.744	0.2118	0.04435	35.56
6	0.746	0.2118	0.799	0.3089	14.74	0.813	0.2813	0.01771	14.20
7	0.813	0.2813	0.847	0.4032	19.23	0.985	0.0000	0.03895	31.23
8	0.986	0.0000	0.992	0.0540	2.58	1.000	0.0104	0.00043	0.35

Fig 12: 15.0 µl scanned at 366nm

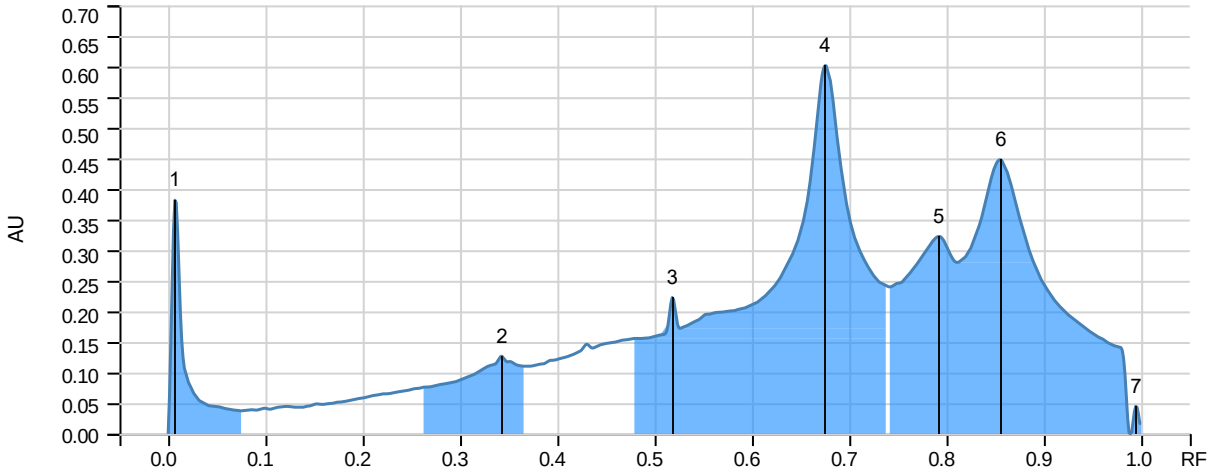


Table 11: Rf value of 15.0 µl scanned at 366nm

Peak	Start		Max			End		Area	
#	Rf	H	Rf	H	%	Rf	H	A	%
1	0.000	0.0000	0.007	0.3817	17.77	0.075	0.375	0.00674	4.40
2	0.260	0.0751	0.343	0.1267	5.90	0.367	0.1103	0.01046	6.82
3	0.476	0.1552	0.518	0.2227	10.37	0.526	0.1723	0.00842	5.50
4	0.526	0.01723	0.675	0.6015	28.01	0.740	0.2403	0.06179	40.33
5	0.742	0.2401	0.7920	0.3224	15.01	0.811	0.2800	0.01963	12.81
6	0.811	0.2800	0.856	0.4478	20.85	0.989	0.0000	0.4589	29.95
7	0.989	0.0000	0.994	0.0450	2.09	1.000	0.0169	0.00028	0.18

Table 12: Estimation of mercury by ICP-OES

Label	**Solution concentration**	**Unit**	**SD**	**%RSD**	**Intensity**	**Calculated concentration**
Hg(184.887nm)	0.471	Ppm	1.267	3.35	342.517	37.800

Discussion

In Siddha system of medicine internal medicine is provided in 32 various forms. One such internal medication method is called *chenduram* in which the metals or minerals are ground with the required plant extracts or salt derivatives for the required time and burnt or sautéed until it reaches its consistency which is the reddish colour. The end product will be very fine powder which will be highly effective in minimal quantity.

Since these medicines are prepared from metals, minerals, mercury its by products and various other salts which are known to have some toxic traits, the standardization process is required to show that the toxic characteristics of the medicines are minimalized by the procedures followed during the preparation and to provide scientific validation for the need of global acceptance of Siddha medicines.

In this study the colour of DLC is found to be maroon which is similar to the colour mentioned in the Siddha literature so the basic quality of this medicine to be *chenduram* is checked. The medicine's moisture content was assessed by the loss on drying technique and it is 3.60% which indicates that the moisture content is under permissible limits and hence it is less prone to microbial infection. The total ash[13] value determines the inorganic materials present after incineration; this helps in determining the mineral contents of the medicine and the total ash value of this medicine is 2.45%.The acid insoluble ash[14] was detected by treating the total ash content with dilute hydrochloric acid so that the compounds like calcium oxide and calcium carbonates which are soluble in acid was solubilized and the remaining ash was weighed to detect the earthy matters, and its value in DLC is 0.13%, lesser the acid insoluble ash lesser silica compounds in the medicine is observed. Water soluble ash is used to determine the contents which are dissolved in water, DLC has 1.40% of water-soluble ash. The HPTLC analysis was carried out to analyse the presence of phytoconstituents present. By comparing with the standard R_F value this Siddha formulation is found to have phytoconstituents like piperene, R_F: ~ 0.63[15] at 254nm and 6-gingerol: R_F ~ 0.02, geraniol:R_F ~ 0.13, citral:R_F ~ 0.33 at 366nm[16] and several other phytoconstituents with their Rf values have been found. The ICP-OES analysis was done for the mercury. 37.80 ppm of mercury was detected. Since it is a known fact that Cinnabar is a natural source of mercury it is very obvious that its content is high but the toxicity caused by the mercury will be very minimal because of the proper detoxification process and standard procedures followed during the preparation of medicines.

The further toxicity of the drug should be evaluated and confirmed by appropriate *in vitro* and *in vivo* studies and this analysis performed gives the frame work of the basic characteristics of *Deepalinga chenduram.*

Conclusion

This study provided the basic data about *Deepalinga chenduram* in terms of physio chemical characterisation, phytoconstituents and the quantity of mercury present. This will be used as a baseline information for the further studies to be carried using this Siddha Herbo mineral formulation.

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