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Analytical characterization and quality assessment of Tayir cuntic curanam using chemical markers

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Abstract

In Indian traditional medicine systems metals and minerals along with herbs or herbal juices are included in some formulations, viz., parpam, centuram, chunnam, kulampu, ilagam, curanam, tailam, etc., in Siddha and bhasma, racayana, etc., in Ayurveda. The quality control of these drugs involved chemical analysis techniques to determine their identity, purity, potency and safety. The main purpose of this study is to authenticate the mineral ingredients of Tayir cuntic curanam (TCC) viz., Induppu (Rock salt), Valaiyaluppu (Sandevere/Glass salt), Punir (Alkaline salt), Corruppu (Common salt) and Kalluppu (sochal salt/rock salt) through powder X-ray diffractometer (PXRD) and to assess the quality of TCC using the phytomarkers 6-gingerol and stigmasterol. Proximate analysis and mineral identification test were carried out using standard methods. Herbal drug was identified by high performance thin layer chromatography (HPTLC) and the quantification of bioactive compounds 6-gingerol and stigmasterol was completed by high pressure liquid chromatography (HPLC). The mineral drugs were analysed by powder X-ray diffractometer analysis (PXRD). Results revealed the composition, purity, crystal phases of the minerals identified by PXRD method and physico-chemical parameters. High performance thin layer chromatography was run to check the presence of the chemical markers of the herbal ingredient (*Zingiber officinale* rhizome) in TCC and quantified them by HPLC. The PXRD identified the minerals as halite, sylvine, trona, etc. The results of this study positively can be used as base for the identification of above salts and physicochemical standards for the quality assessment of TCC.

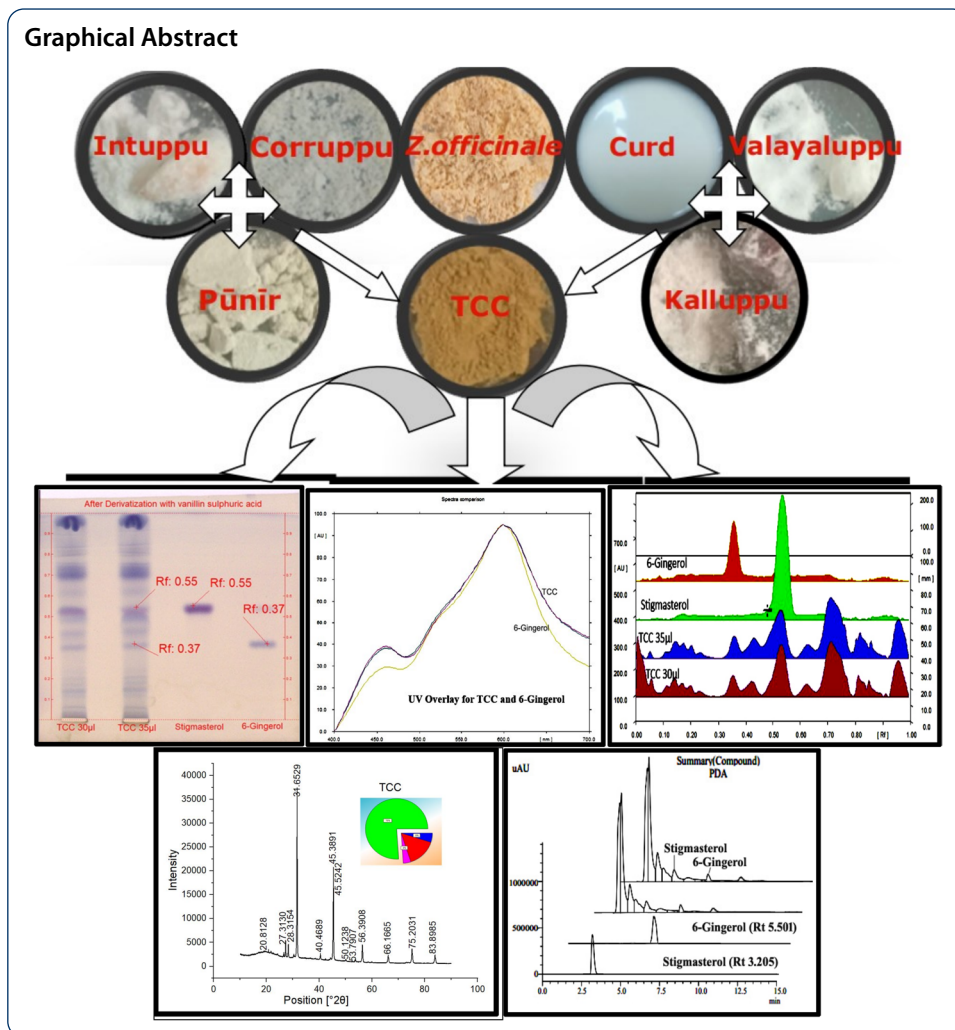
Keywords Sāmudralavana, Sochal salt, Sauvarcala lavana, Saindava lavana, Stigmasterol, 6-gingerol, herbo-mineral formulation, HPTLC, HPLC, PXRD

1 Introduction

The Siddha system of medicine is one of the oldest Indian traditional systems. Many of herbo-mineral, metal and/or mineral drugs are used in the treatment of gastric ulcer, indigestion, blood diseases, nerves syphilis, poisonous bite, skin diseases, muscle cramp in trunk, vaginal diseases, delirium, cataract, polydipsia, asthma (dyspnea), haemorrhoids, pain due to vatha dosha, throbbing pain, etc. [1, 2]. Tayir cuntic cūṛaṇam is a



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herbomineral Siddha formulation used as a probiotic to improve gut health; it cures indigestion and diarrhea [3]. In TCC, five minerals viz., induppu (rock salt), valaiyaluppu (sandever/glass salt), punir (alkaline salt), corruppu (common salt) and kalluppu (rock salt/sochal salt) are the mineral ingredients. Many Siddha and Ayurveda formulations are available with above minerals as ingredients which are presented in the Table 1.

Therapeutic values of induppu (rock salt) are chancroid ulcer, derangement of three humors, dyspepsia, gastric ulcer, pain, abdominal lump, diseases of abdomen, haemorrhoids, indigestion, inflammation, impaired digestive fire, polydipsia, spermatorrhoea, skin diseases, oral ulcer due to bites, vaginal diseases, etc. [5, 8].

Pūnir (trona) is an impure form of sodium bicarbonate and it is a natural preservative agent [3, 4] and is being used as detergent since primordial times. It is obtained from the soil of alkaline earth metal [11]. Nahcolite (sodium + bicarbonate + mineral) is formed by the reaction of carbon dioxide with the mineral “Trona” in evaporated lake basins [12].

Common salt (sea salt) is prescribed for anuria/oliguria, abdominal lump, dyspepsia, flatulence, inflammation, impaired digestive fire, liver disease, neck tumour, 8 types of painful gastro intestinal disorders and children's indigestion [5]. Common salt comes under broad classification of alkali halides. It occurs in association with gypsum, polyhalite, anhydrite, clay, sand stone, calcite, etc. Tuticorin in Tamilnadu, South India.,

Cambay in Gujarat and Sambar Lake in Rajasthan are the notable commercial salt pans producing sites of common salt [13].

Kalluppu (black salt) is used to treat tastelessness, intestinal colic, mal absorption syndrome, abdominal dump, agnimāndya (digestive impairment and vitiation of kapha dosha) [6, 7].

All these salts are used for painful gastro intestinal disorders with indigestion, skin, liver disease which are now threatening in India [14].

Dried ginger (Cukku) is prescribed for indigestion, cough, ulcer, headache, etc. in Siddha medical system. In TCC, one of the herbal ingredient is the dried rhizome of *Zingiber officinale* Rosc. (Cukku) It is suggested for the major therapeutic claims like colic, haemorrhoids, diseases of throat and inflammation [15].

The *Z. officinale* rhizome is reported with several bioactive phytomarkers viz., 6-zingerine, 8-zingerine, 10-zingerine, 6-gingerol, 8-gingerol and 10-gingerol [16]; stigmasterol [17]; seven new diarylheptanoids [18]; 6-shogaol, zingiberol, β -phellandrene, α -zingiberene, ar-curcumen, β -bisabolene, α -santalol, β -eudesmol, nerolidol, farnesol, elemol, neral, geranial, α,β -pinene, camphene, sabinene, myrcene, limonene, 1,8-cineole, aliphatic alkanes, alcohols, aldehydes, ketones, sulfides [19–23]. The pharmacology reported with ginger are cardiogenic and antiplatelet action of gingerol. Ginger powder when given orally relieves the migraine headache [24–26]. The present study was aimed to investigate the chemical composition of mineral ingredients, their purity, their crystal phase identification using PXRD, determine its physico-chemical parameters as part of standardization using High-Performance Thin-Layer Chromatography (HPTLC) for identification and High Pressure Liquid Chromatography (HPLC) for quantification of phytochemicals.

2 Materials and methods

2.1 Collection of materials and chemicals

The minerals and dried rhizome of *Zingiber officinale* (cukku) were collected from Indian Medical Practitioners Co-operative Pharmacy and Stores Limited (IMPCOPS), Chennai, Tamil Nadu. The dried ginger was authenticated by the Research Officer (Pharmacognosy) of this Institute (Voucher No SCRI-PCOG-GRZ23). All the solvents were of Analytical Grade (Merck). 6-Gingerol (Batch no.0000466745, purity 99% by HPLC) and stigmasterol (Batch no.0000231517, purity 97.4% by GC) were procured Sigma-Aldrich Chemicals Private Limited, Bangalore.

2.2 Purification of minerals

2.2.1 Induppu

Soaked in vinegar for 3 days, dried in sunlight for use. Pūnir: Dissolved 1 part in 4 parts of water, filtered and dried completely in sunlight. This process was repeated for ten times. Valayaluppu: Soaked in vinegar for 90 min, washed then dried in sunlight. Corruppu: Dissolved in water, filtered and dried in sunlight [3].

2.3 Method of preparation and formulation composition

For the preparation of TCC, Induppu—Purified (1 part), Valayaluppu—Purified (1 part), Pūnir—purified (1 part), Corruppu—purified (1 part), Kalluppu—Purified (1 part), Cukku (Scraped) (5 part) were soaked in Pulitha thayir/Curd (40—Parts) and kept in sun light

Table 1 List of ingredients and their formulations

Common name	Other names in Siddha/Ayurveda	Name of the formulation in which the minerals are ingredients	Native Indian system
Common salt/Sea salt	Corruppu/kariyuppu/kataluppu/veettuppu/lavanam/samudra lavanam	Aya kantac centuram, Cankat Tiravakam, Iñci vaṭakam, Mayaṇat tailam, Nanti meḷuku, Kuṇma kuṭōri meḷuku, Nava uppu meḷuku, Pañca lavaṇa paṇpam, Pāvaṇak kaṭṭukkāy mātṭirai, Tamarākak kuṭinīr cūraṇam, Tayir cuṇṭi cūraṇam, Ulōka maṇṭūra centūram, Uppuc centūram [3]	Siddha
	Sāmudra/Sāmudraka	Tēṇkāy kuḷampu, Cintu vallāti meḷuku, Piranṭai uppu, Maṇṭūrac centūram, Māltēvic centūram [4]	
		Lavana Bhāskara cūrna, Sāmudrādya cūrna, Nārāyana cūrna, Vajra Ksara, etc. [5]	Ayurveda
Sandevera/ Glass salt	Valaiyal uppu/Maṭavār karattuppu	Kuṇma kuṭōri meḷuku, Nava uppu meḷuku, Noccit Tailam, Tayir cuṇṭi cūraṇam, Ulōka maṇṭūra centūram [3]	Siddha
Rock salt	Kalluppu/Kaṭarkuruvi	Kāḷamēka nārāyaṇa centūram, Kuṇma kuṭōri meḷuku, Nava uppu meḷuku, Noccit Tailam, Pañca lavaṇa paṇpam, Tayir cuṇṭi cūraṇam [3]	Siddha
		Uppuc centūram-l, Māltēvic centūram [4]	
	Sochal salt/Vida lavana	Bhaskaralavana Curna [6]	Ayurveda
		Citrakādi Curna [7]	Ayurveda

Table 1 (continued)

Common name	Other names in Siddha/Ayurveda	Name of the formulation in which the minerals are ingredients	Native Indian system
Rock salt (Impure)	Induppu/Chandiranuppu/MathiKoormai/Mathiuppu	Akattiyar kuḷampu, Ārumukac centūram, Ātātōtai ney, Attātic cūraṇam, Aya kānta centūram, Caṅkat tirāvakam, Cilōtari maṇi mātṭirai, Cittati Enney, Cukkut Tailam, Iḷanīr kuḷampu, Kaucikar Kuḷampu, Kuṇma kuṭōri meḷuku, Mekanatak Kulikai, Nava uppu meḷuku, Pañca lavaṇa paṇpam, Pāvaṇak kaṭukkāy mātṭirai, Piṇacāṭ tailam, Pirami Ney, Piraṇṭai vaṭakam Tayir cuṇṭi cūraṇam, Ulōka maṇṭūra centūram, Uḷuntu tailam, Viṣak kuḷampu (Karuṇ kuḷampu) [3]	Siddha
	Saindava lavana/Sindūttha	Amara mātṭirai, Karppa viyātikku eṇṇey, Kalikka mai nacyam, Naṅkarantai iḷakam, Nāyuruvi ney, Paṭalaṅkaḷukku ney, Pīnaca ney, Poṭutalaik kuḷampu, Māltēvic centūram, Viḷi ney, Veṭiyupput tirāvakam [4]	Siddha
Trona	Pūnīr/Pūvalalai	Ṣaḍbindu taila, nārāyaṇa taila, kāsīsādi taila, tutthādi lepa, pathyādi lepa, nārikela lavaṇa, intuppukāṇa cūrṇa, hutabhugādi cūrṇa [8]	Ayurveda
	Svarjikṣāra/Sajjikshara/Sajji	Āmai ōṭu paṇpam, Aya kānta centūram, Caṅkat tirāvakam, Kāḷamēka nārāyaṇa centūram, Kuṇma kuṭōri meḷuku, Nava uppu meḷuku, Noccit Tailam, Tayir cuṇṭi cūraṇam, Nanti meḷuku, Naṇṭukkal paṇpam [3]; Māltēvic centūram [4]	Siddha
		Citrakadi Curna [7, 9]	Ayurveda

Table 1 (continued)

Common name	Other names in Siddha/Ayurveda	Name of the formulation in which the minerals are ingredients	Native Indian system
Dried ginger	Cukku	Ēlāti cūraṇam, Nilavākai cūraṇam, Pañca tīpākkini cūraṇam, Pāvāṇa kaṭukkāy cūraṇam, Tirikaṭuku cūraṇam [3]; Akkini cañcivi mātṭirai, Amara mātṭirai, Iñci ilakam, Ilavañkāti cūraṇam, Katalippū racāyaṇam, Kapāṭa ilakam, Kāya ilakam, Cantāṇa ilakam, Cuvāca kuṭōri cūraṇam, Caupākkiya cuṇṭi ilakam, Tontacurak kuṭinīr, Namaṇāca meḷuku, Naracimma ilakam, Naṛkarantai ilakam, Mahāvilvāti ilakam, Muruñkaippū ilakam, Parañkicckaica cūraṇam, Peruñkāya ilakam [4]	Siddha
	Sunthi	Punarnavāsava, Madhusnuhi Rasāyana, Nārasimha Cūrṇa, Pañcatikta Guggulu ghrta [6]; Savubhāgya sunḥṭi lēkiyam, Tirikaṭukkuc cūraṇam, Savubhāgya vati, Vaiśvānara, etc. [10]	Ayurveda

till it becomes dry. Then it was powdered finely, sieved through 355 μm IS Sieve (sieve no. 44) and stored in an air tight glass bottle [27].

2.4 Physico-chemical analysis

The following physico-chemical parameters namely loss on drying, total ash, water soluble ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value and pH were carried out by following standard procedures (Supplementary File S1). Three replicates were carried out and the mean values were computed [28, 29].

2.5 Qualitative minerals analysis

The qualitative inorganic test carried out for detection of cations and anions with reference to spot tests using inorganic semi qualitative analysis methods [30].

2.6 Preparation of sample solution

One gram of TCC was taken in a 25 ml conical flask with 10 ml of methanol solvent and sonicated for 30 min. The sonicated ethanol solution was filtered using Whatman filter paper no. 42 and made upto 10 ml in a standard flask concentration of sample 100 $\mu\text{g/ml}$ solution in refrigerator for further use.

2.7 Preparation of standard solutions

Stigmasterol and 6-gingerol (10 mg each) were weighed and dissolved in 10 ml methanol in 10 ml volumetric flasks separately. From these stock solutions, 20, 40, 60, 80, 100 and 120 μL were micropipetted out and made up to 1 ml by adding 980, 960, 940, 920, 900, 880 μL of methanol in sample vials to get the working standards of 20, 40, 60, 80, 100 and 120 $\mu\text{g}/\text{ml}$ using micropipettes.

2.8 Instrument specifications

The HPTLC system included sample applicator (ATS4), developing chamber (twin-trough chambers (10 $\times$ 10 cm), TLC plate heater III, Visualizer and densitometric scanner (TLC Scanner 4), winCATS software (version 1.4.2) were from CAMAG, Switzerland; aluminium TLC plate silica gel 60F₂₅₄ (0.2 mm thickness) from Merck (Germany) was used in the current study. Ultrasonic Sonicator (Soltec Sowzioni Tecnologiche, Italy, Sonica 2200MH S3, SN 2017014314) was used for sonication of sample for 15 min.

Analytical cum preparative HPLC (SHIMADZU Corporation, Kyoto, Japan) LC 20AP equipped with SPD-M20A detector was used for analysis of phytomarkers.

Aeris Malvern Panalytical X B.V The Netherlands (DY926) high resolution Powder X-Ray Diffractometer (CuK α radiation, $\lambda = 1.54443 \text{ \AA}$) with the software data viewer version 1.9a and high score version 4.8 was utilized for the analysis of mineral samples. The diffractometer was equipped with divergence, scattering slits size 0.4584° and position sensitive detector (PSD) length $5.54^\circ 2\theta$.

2.9 HPTLC condition

HPTLC analysis was carried out on a 10 $\times$ 10 cm sized precoated aluminum plate. The extract of the sample was applied in the volumes of 30, 35 μL and that of standards in 10 μL as a 9 mm width band by leaving a distance of 20 mm between the tracks at an application position of 10 mm in Y axis and 15 mm in X axis. *Toluene* and *ethyl acetate* mixture with the ratio of 8: 2, v/v was chosen as the mobile phase as thermo stable solvents are preferred to avoid variation in the ratio of mobile phase during development. The mobile phase was saturated for 30 min. Then TLC plate was developed up to a distance of 80 mm at temperature of $27 \pm 1^\circ\text{C}$. After chromatographic development, the TLC plate was dried, dipped in vanillin sulphuric acid reagent, heated at $110 \pm 2^\circ\text{C}$ till the development of color of spots and scanned at 520 nm in absorption mode using tungsten lamp with fixed slit dimension (6 $\times$ 0.45 mm) and at a scanning speed of 100 nm/s in scanner 4 [31–33].

2.10 HPLC condition

The data was acquired on the Lab solution administrator data system and column shim-pack GIST C₁₈, 5 μm , 150 mm $\times$ 4.6 mm was used. Injection of 20 μL was carried out using a 25 μL Hamilton syringe and the flow rate was monitored at 0.5 ml/min. Detection of stigmasterol was performed at 205 nm and 6-gingerol was performed at 280 nm with 15 min running time. The mobile phase *acetonitrile: water* (95: 5, v/v) was filtered through Nylon 6,6 membrane 0.2 μm (47 mm) filter and degassed by sonication for 10 min [34, 35].

2.11 PXRD condition

The sample was scanned from 7.0129 to $89.9749^{\circ}2\theta$ for the experiments using generator setting voltage and current 8 mA , 40 kV settings with a step size of $0.0220^{\circ}2\theta$ and under constant temperature $25 \pm 1^{\circ}\text{C}$ [36–40].

3 Results

3.1 Proximate analysis

The physicochemical parameters of TCC were analyzed to check their quality by various parameters such as loss on drying, total ash, acid-insoluble ash, water-soluble ash, water soluble extractives, alcohol soluble extractives and the results are presented in the Table 2. The total ash content was estimated as 36.59% which is directly indicating the content of ingredient minerals of TCC. The amount of acid-insoluble ash in the TCC was found to be 5.35% which indicates the silica/quartz identified from pūnīr. The water-soluble extractive was found to be 43.91% which again directly indicates the content of mineral ingredients and the organic glycosides, tannins, saponins, etc. from the *Z. officinale* rhizome. Alcohol soluble extractive value was calculated as 34.82% which indicates presence of phytoconstituents such as terpenoids, flavonoids, steroids, tannins, saponins, etc. from the rhizome and the soluble mineral ingredients such as induppu, valayaluppu, corruppu and kalluppu. The pH value of 10% of solution of sample 5.75 indicates the acidity of the drug TCC showing probability of more shelf life.

3.2 Mineral analysis

Qualitative inorganic salt analysis of the drug readily answered yellow color in the flame test for sodium ion and iron. Yellow flame dominated the lilac color of potassium ion. Silver nitrate test confirmed the presence of chloride. Few drops of silver nitrate solution when added to the TCC sample dissolved in water, the chloride ion forms white precipitate of silver chloride which is insoluble in water and nitric acid.

3.3 Phytomarker identification by HPTLC

The TLC of derivatized plate (Fig. 1) showed spots at R_f 0.05 , 0.15 , 0.18 , 0.22 , 0.36 (6-gingerol), 0.43 , 0.53 (stigmaterol), 0.70 , 0.80 (all purple). The HPTLC analysis of alcoholic extract of TCC when scanned at 520 nm after derivatization with vanillin sulfuric acid (Fig. 2) showed various peaks at R_0 0.05 , 0.15 , 0.17 , 0.20 , 0.23 , 0.36 (6-gingerol), 0.43 , 0.53 (stigmaterol), 0.63 , 0.71 , 0.82 , 0.86 and 0.96 . The similarity of stigmaterol and 6-gingerol with their corresponding spots in TCC were spectral scanned and the UV overlay spectra are presented in Fig. 3.

Table 2 Physicochemical parameters of TCC

S. No.	Parameter	I	II	III	Mean	Standard deviation
	Loss on drying, % w/w	14.32	12.64	14.50	13.82	± 0.133
	Total ash, % w/w	35.26	40.21	34.30	36.59	± 0.68
	Water soluble ash, % w/w	30.98	31.03	29.64	30.55	± 0.95
	Acid insoluble ash, % w/w	5.44	5.41	5.20	5.35	± 0.17
	Water soluble extractive value, % w/w	43.88	42.98	44.87	43.91	± 0.70
	Alcohol soluble extractive value, % w/w	35.80	35.26	33.40	34.82	± 1.70
	pH (10% solution)	5.73	5.75	5.77	5.75	± 0.03

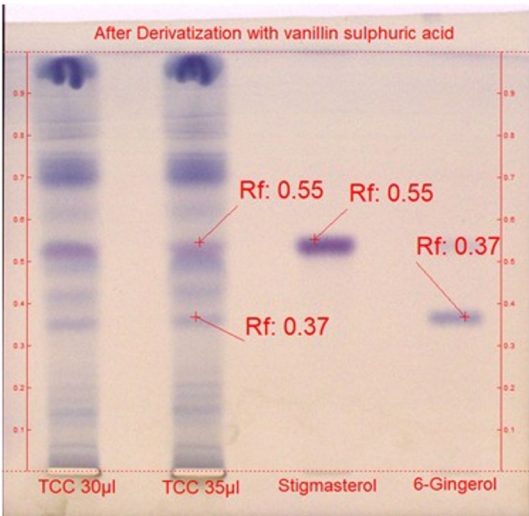


Fig. 1 TLC photo documentation of TCC with 6-gingerol and stigmasterol

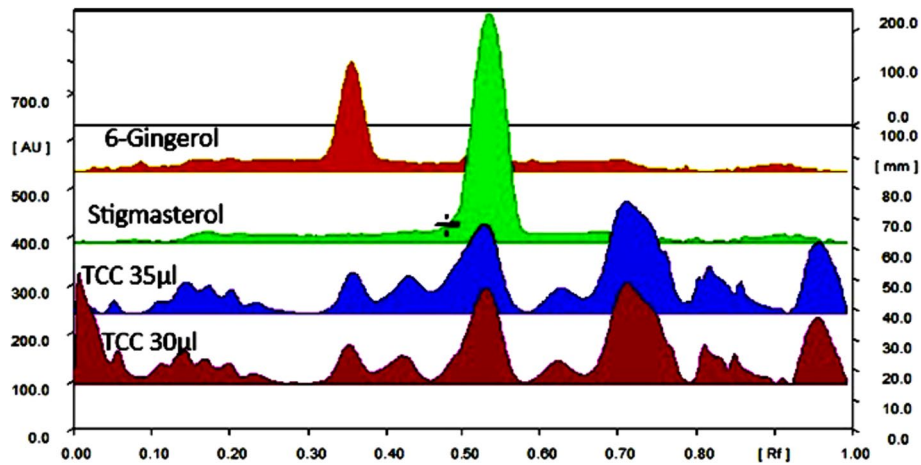


Fig. 2 HPTLC 3D chromatogram of TCC with 6-gingerol and stigmasterol

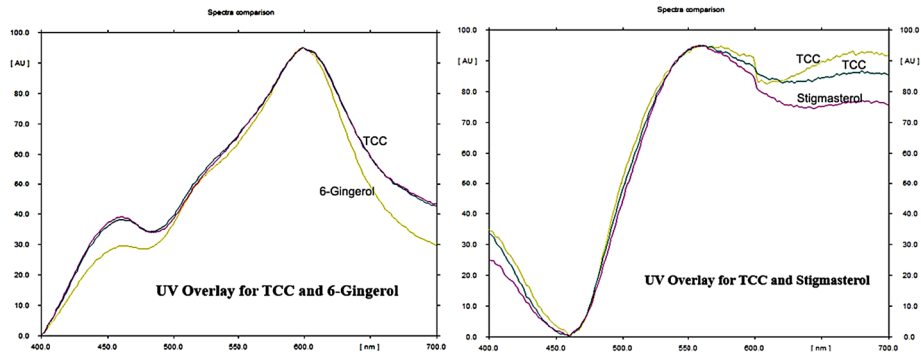
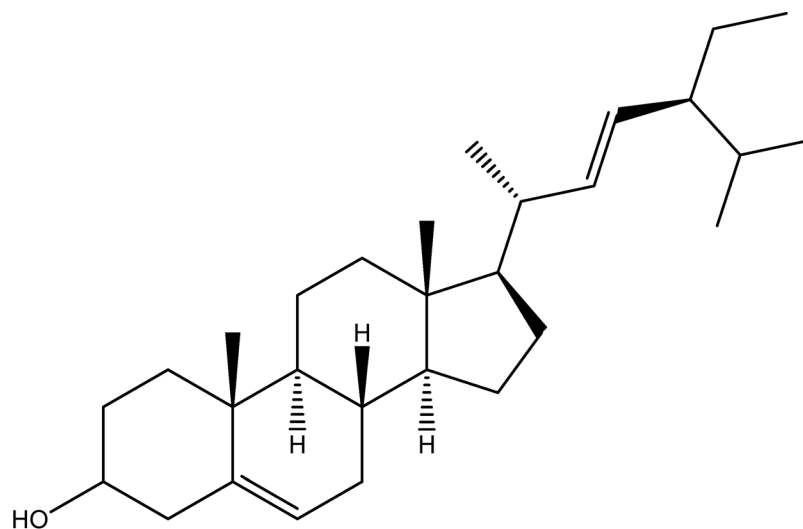
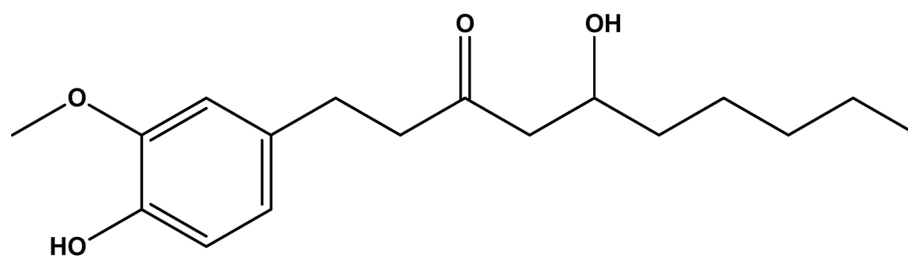


Fig. 3 UV overlay spectra of 6-gingerol and stigmasterol



(3 β ,22E)-Stigmasta-5,22-dien-3-ol (Stigmasterol)



5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one (6-Gingerol)

3.4 Phytomarkers quantification by HPLC

The phytomarkers stigmasterol and 6-gingerol were estimated by HPLC and the observed values of retention time, accuracy, precision, specificity, linear regression, correlation coefficient, standard deviation, limit of detection, limit of quantification, resolution and symmetry/tailing factor are presented in the Table 3. The content of stigmasterol was calculated as 0.189% (w/w) and that of 6-gingerol was estimated as 0.002%

Table 3 Quantification parameters of stigmasterol and 6-gingerol by HPLC

S. No.	Quantification parameters	Stigmasterol	6-Gingerol
1.	Linearity range ($\mu\text{g/ml}$)	20–120	20–120
2.	Wavelength (λ)	205	280
3.	Accuracy (%)	100.1–102.5	98.3–103.4
4.	Precision (%)	0.7699	3.407
5.	Specificity	No interference	No interference
6.	Retention time (min.)	3.205	5.501
7.	Linear regression (y)	57123x – 20,393	57158x – 10,788
8.	Correlation coefficient (R^2)	0.998	0.994
9.	Standard deviation (%)	0.759	3.296
10.	Limit of detection (μg)	0.0019	0.0058
11.	Limit of quantification (μg)	0.03	0.08
12.	Resolution (R_s)	1.232	0.939
13.	Symmetry/Tailing factor (S)	1.809	1.912

(w/w). The calibration curves of stigmasterol and 6-gingerol are presented in Fig. 4. The HPLC 3D chromatogram is presented as Fig. 5.

3.5 Minerals analysis for crystal structure and phase identification

PXRD analysis identified the crystal structure and the phases of minerals of the ingredients and TCC. The results are given in Table 4.

PXRD data of induppu (Fig. 6a) showed peaks with 2θ values at 27.3152, 31.6640, 45.3942, 56.4127, 66.1773, 75.2270, 83.9190 °C which corresponds to halite form of sodium chloride. Valaiyaluppu (Fig. 6b) is composed of 97% of halite form of sodium chloride with 2θ values at 27.3405, 31.6741, 45.3839, 56.4089, 66.1734, 75.2177, 83.9197 °C and 3% of thenardite form of sodium sulphate with 2θ values at 19.0231, 32.1092, 45.5256, 53.8125, 66.3693, 75.4475 °C. The composition of punir (Fig. 6c) is mainly trona (65%) quartz high (35%). The 2θ values of trona are 17.9593, 28.9606, 31.6139, 33.7529, 45.3433, 56.3546, 66.1215, 75.1874, 83.8871 °C and that of quartz are 20.7831, 26.5526, 39.8720 °C. In the PXRD data of corruppu (Fig. 6d), peaks were observed at 27.3380, 31.6696, 45.4173, 56.4317, 66.1848, 75.2419 °C and 83.9318 °C which is identified with halite form of sodium chloride. The composition of kalluppu (Fig. 6e) is halite (60%) and potassium iron III oxide (40%). The present study using PXRD identified the phases and crystal structures of TCC (Fig. 6f) as 76% halite (NaCl), 5% sylvine (KCl), 4% quartz low and 15% potassium carbonate.

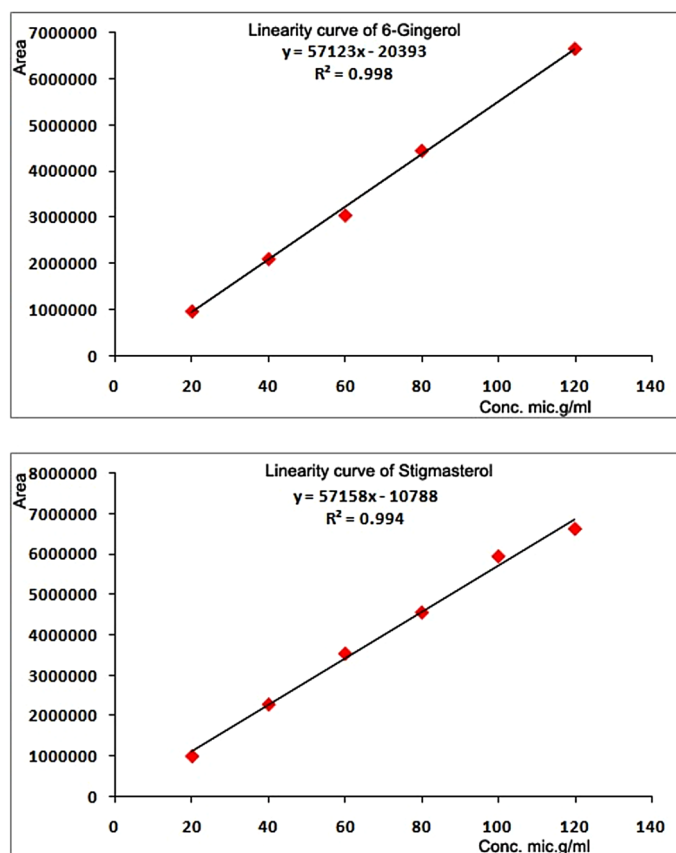


Fig. 4 Calibration curve of 6-gingerol and stigmasterol

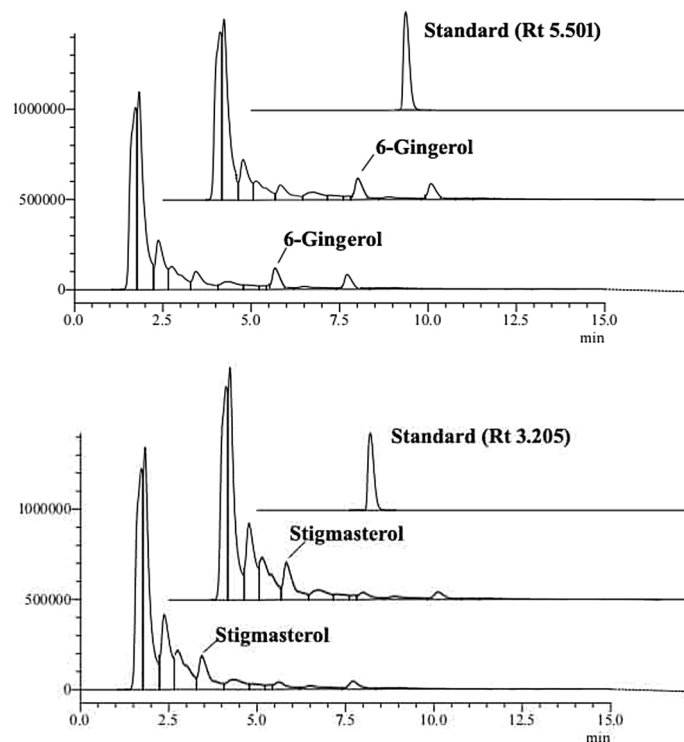


Fig. 5 HPLC chromatogram of TCC with 6-gingerol and stigmasterol

4 Discussion

The physiochemical study can be used as a quality check parameters of the TCC drug. The physicochemical parameters relevant to curanam viz., loss on drying, total ash, acid-insoluble ash, water-soluble ash, water soluble extractives, alcohol soluble extractives and pH were carried out. The result of loss on drying was found to be 13.82% which is in higher side compared to other curanam of herbal [41], herbomineral origins [42]. TLC and HPTLC methods are selective, sensitive and rapid. It provides chromatographic finger print profiles of phytomarkers which is desirable for confirming the identity and purity of herbal medicine.

Stigmasterol from *Z. officinale* rhizome [17] possesses many pharmacological effects such as antioxidant, antibacterial, antifungal, anticancer, anti-inflammatory, anti-osteoarthritis, antiparasitic, anti-diabetic, immunomodulatory and neuroprotective properties [43]. In a previous report, stigmasterol has been eluted with the mobile phase *methanol, water* and *acetic acid* in the ratio of 7:3:0.1, v/v/v and detected at 254 nm [44].

6-Gingerol has been reported in another study with the eluting solvent mixture of methanol and 0.05% ortho phosphoric acid in water (3:2, v/v) and detection at 280 nm [45]. In the present study, the selected mobile phase, *acetonitrile: water* (95:5, v/v) separated the peaks with respect to stigmasterol and 6-gingerol in an acceptable difference in the retention time.

Accuracy of any method is the closeness of the obtained value to the true value. In the present study, accuracy value of stigmasterol was 100.1–102.5% and that of 6-gingerol was 98.3–103.4%. The acceptable value of precision is 2–5% and <2% is of highest precision. For stigmasterol estimation, the precision value was 0.7699% revealing the highest precision while that of 6-gingerol was found to be 3.407% which is in acceptable range.

Table 4 The crystal structure parameters of mineral ingredients (Induppu, Valayaluppu, Pūnir, Corruppu, Kalluppu and TCC)

Parameters	Induppu	Valayaluppu	Pūnir	Corruppu	Kalluppu	TCC
Phase	Halite	Halite	Trona	Quartz high	Halite	Halite
Molecular formula	NaCl	NaCl	Na ₂ CO ₃ ·NaHCO ₃ ·2H ₂ O	SiO ₂	NaCl	NaCl
Composition	100	97	65	35	60	76
Crystal structure	Cubic	Cubic	Monoclinic	Hexagonal	cubic	Cubic
Crystal size (nm)	40.4685	41.9916	31.1261		32.01944	18.2610
Space group/group no	Fm ^{−3} m (225)	Fm ^{−3} m (225)	C12-C1 (15)	P6222 (180)	Fm ^{−3} m (225)	Fm ^{−3} m (225)
Lattice volume	179.60	179.60	760.51	113.58	179.60	179.60
Lattice parameter a(Å ^o)	5.6440	5.6440	20.4220	4.9210	5.6440	5.6440
Lattice parameter b(Å ^o)	5.6440	5.6440	3.4910	4.9210	5.6440	5.6440
Lattice parameter c(Å ^o)	5.6440	5.6440	10.3330	5.4160	5.6440	5.6440
Z	4.00	4.00	4.00	3.00	4.00	4.00

No interference were recorded with both phytomarkers which conveys the specificity of the method. The correlation co-efficient (R^2) values were 0.998 and 0.994 respectively for stigmasterol and 6-gingerol which are near to 1. Like precision, standard deviation values also within the highly acceptable (0.759% for stigmasterol) and acceptable range (3.296% for 6-gingerol). Resolution factors of stigmasterol and 6-gingerol were 1.232 and 0.939 respectively which are within the recommended acceptable value of <1.5 . The symmetry value between 0.8 – 1.8 is acceptable and >2 is unacceptable while both phytomarker's symmetry values of 1.809 and 1.912 are near to the acceptable limit [46].

6-Gingerol is the most abundant phenolic compound found in ginger among other phenolic acids such as 8-gingerol, 10-gingerol, methyl-6-gingerol, 6-gingerdiol, diacetoxy-8-gingerdiol, 1-dehydro-10-gingerdione and 6-shogaol [47].

6-Gingerol has an optimal lipophilicity with a partition coefficient between *n*-octanol and water value of 3.13. It has high gastrointestinal absorption and also possesses good blood brain barrier permeation may be responsible for the therapeutic use of TCC in digestive problems [48].

Induppu is the halite form of sodium chloride. Valaiyaluppu is composed of 97% of halite form of sodium chloride and 3% of thenardite form of sodium sulphate. Trona is the main composition of punir (65%) and another crystal structure identified is quartz high (35%). Corruppu is identified with halite form of sodium chloride. The crystal composition in kalluppu is halite (60%) and potassium iron III oxide (40%). All these mineral ingredients are explored with the exact chemical composition using PXRD. The present study using PXRD identified the phases and crystal structures of minerals in TCC as 76% halite (NaCl), 5% sylvine (KCl), 4% quartz low and 15% potassium carbonate; sylvine and potassium carbonate are not detected in the mineral raw drugs. Potassium Iron (III) Oxide (KFeO_2) which is detected in kalluppu has undergone chemical change and the potassium is substituted with carbonate and chloride in TCC.

Rock salt is a natural supplement that can provide health benefits [9]. It is used as preservative in foods and also in herbal drugs to increase the shelf life [49]. Induppu or rock salt or common salt or Himalayan salt, is the pure form of the mineral halite (molecular formula—NaCl; molecular weight—58.44 g/mol). It is coarsely crystalline, sedimentary rock containing massive, fibrous or granular aggregates. Rock salt is dominantly halite however other minerals such as calcite, dolomite, anhydrite, gypsum and potash minerals may also present in it. In National Mineral Inventory, halite with $>60\%$ pure sodium chloride (NaCl) content is certified as Grade I and $<60\%$ NaCl content is certified as Grade II. The rock salt distributed in Mandi district, Himachal Pradesh, India is placed under Grade-I category [9]. There are two types of salts, one is known as Himalayan black and other is known as Himalayan pink salt which were already reported with the structural characterization as black salt [50].

A recent study on trona (sajjikshara) reports its composition as impure sodium bicarbonate [51]. The composition of trona has been reported as 46% sodium carbonate and 36% sodium bicarbonate [$\text{Na}_3\text{H}(\text{CO}_3)_2 \cdot 2\text{H}_2\text{O}$]. It is reported as stable at $<57^\circ\text{C}$ in withered condition and decomposes to sodium carbonate at $>160^\circ\text{C}$ [52].

Valaiyaluppu (Sandever/Glass salt) [3] is a glass salt. But major composition of Valaiyaluppu is sodium chloride with sulfate, silicate and clay. Kalluppu (sochal salt/rock salt) is composed mainly of sodium chloride and iron oxide.

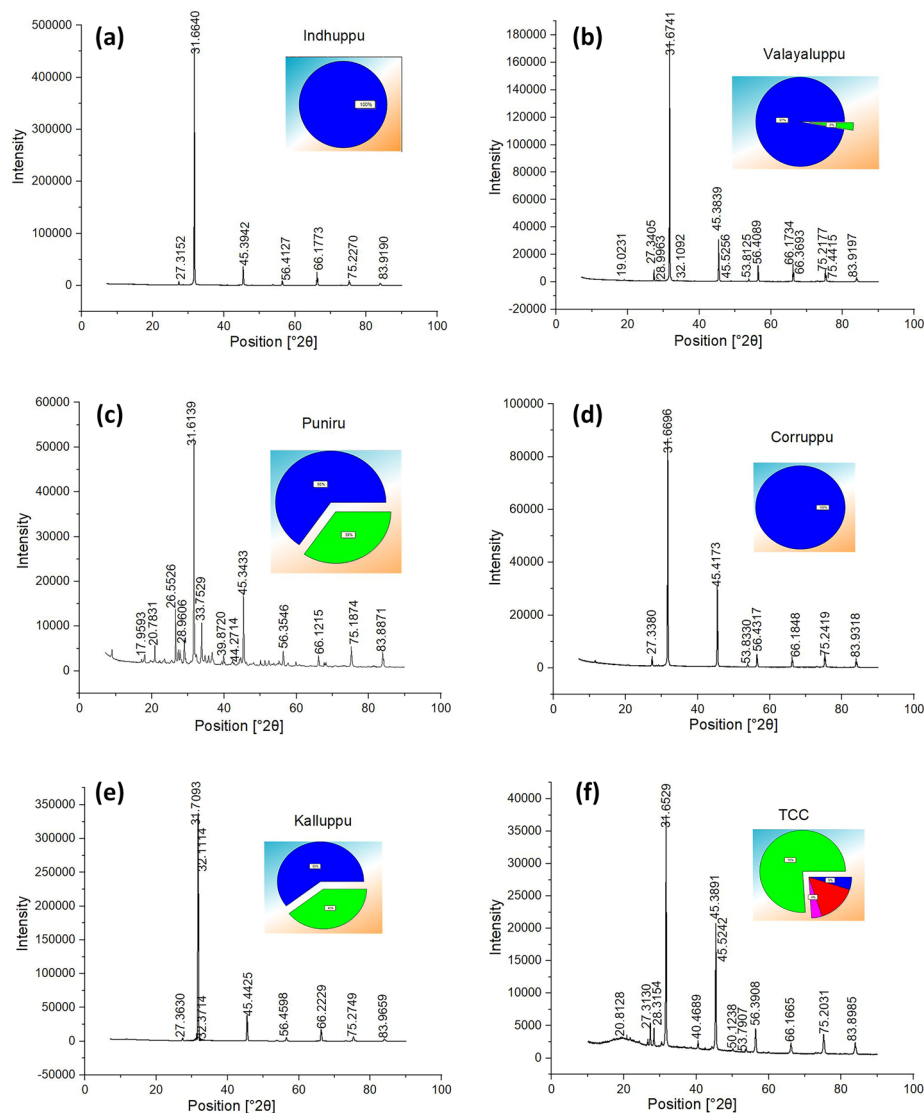


Fig. 6 Powder X-ray diffraction spectra. **a.** Indhuppu; **b.** Valayaluppu; **c.** Corruppu **d.** Pūnir; **e.** Kalluppu; **f.** TCC

In this study, the reference intensity ratio (RIR) method for the mineral analysis was followed [53, 54]. X-ray diffraction patterns of induppu, valayaluppu, samudra lavanam, pūnir, kalluppu and TCC and their respective search match standard references are given in Fig. 6a-f. The crystal structure parameters viz., phase, crystal structure, space group or group number, lattice volume and lattice are presented in Table 4.

The PXRD data of the salts used for the preparation of TCC revealed that pūnir salt is 65% trona ($\text{Na}_2\text{CO}_3 \cdot \text{NaHCO}_3 \cdot 2\text{H}_2\text{O}$) and 35% quartz high (SiO_2); induppu and corruppu are 100% halite (NaCl); kalluppu is composed with 60% halite (NaCl) and 40% potassium iron (III) oxide (KFeO_2) and valaiyaluppu contain 97% halite (NaCl) and 3% thenardite (Na_2SO_4) [55–58].

This PXRD study on TCC identified the phases of the crystals and their compositions. Halite (NaCl) in cubic shape, sylvine (potassium chloride— KCl) in cubic form, quartz low (SiO_2) in hexagonal structure and potassium carbonate (K_2CO_3) in hexagonal arrangement were identified. PXRD study evidently showed that TCC contains more of sodium

and chlorine rather than other identified elements such as potassium, carbon, oxygen and silicon.

The salt ingredients of TCC are composed of halite (NaCl) majorly; sodium in other forms as sulfate/bicarbonate/carbonate, potassium, iron and silicon as oxide. Salt plays an important role in the quality and safety of food by controlling undesirable microorganisms. The sodium chloride and potassium chloride reduces the growth of unwanted microorganisms. A study on the effect of NaCl and KCl on the propagation of 31 strains of lactic acid bacteria revealed the reduction of their growth and attenuating effect [59]. The subject drug TCC (one to two grams) with warm water after food is advised for the treatment of digestive disorder, gastro intestinal disorder and diarrhea [3]. The human friendly probiotic *Lactobacillus* bacteria is present in curd [60] which can attenuates the therapeutic efficacy of TCC [61–63]. Their further growth is prevented by sodium chloride and potassium chloride salts present in it. Further research is required to prove the biological role of TCC in improving gut health.

5 Conclusion

In conclusion, the identity of induppu (rock salt) and corruppu (common salt) were confirmed as halite while kalluppu (black salt) is found with 40% potassium iron (III) oxide (KFeO_2) in halite and valayaluppu is detected with 3% sodium sulphate in halite. The standardization of herbo-mineral drug is necessary for quality, safety and therapeutic efficacy, as well as to increase trust among health practitioners and consumers. The results of present study on physicochemical analysis, TLC/HPTLC and PXRD of Tayir cunṭic cūraṇam revealed can be referred for its quality assessment. The powder X-ray diffraction analysis confirmed the presence of crystalline structure, phases of the salts and composition of minerals and indicated the formation of potassium chloride and potassium carbonate which are not found in any of the mineral raw drugs.

Abbreviations

TLC	Thin Layer Chromatography
HPTLC	High Performance Thin Layer Chromatography
HPLC	High Pressure Liquid Chromatography
PXRD	Powder X-Ray Diffractometer
NaCl	Sodium chloride
KCl	Potassium chloride
Na_2CO_3	Sodium carbonate
NaHCO_3	Sodium bicarbonate
SiO_2	Silicon dioxide
KFeO_2	Potassium Iron Oxide
Na_2SO_4	Sodium sulphate
K_2CO_3	Potassium carbonate
RIR	Reference Intensity Ratio
μg	Microgram
μL	Microliter, μm - micrometer
mL	Milliliter
mg	Milligram
mm	Millimeter
nm	Nanometer
cm	Centimeter
min.	Minute
v/v	Volume by volume
Å	Angstrom
λ	Lambda
KV	Kilovolt
mA	Milliampere
Acetonitrile	Ethane nitrile
Toluene	Methylbenzene
Ethyl acetate	Ethyl ethanoate

Water Dihydrogen Oxide

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44371-026-00679-5>.

Supplementary file 1.

Acknowledgements

Authors are thankful to the Director General, Central Council for Research in Siddha for support and facilities

Author contributions

SM: Data curation, formal analysis, investigation, methodology, validation, writing draft. RS: Investigation, project administration, reviewing and editing. PT: Data curation, validation. AK: Conceptualization, NJM: Supervision.

Funding

This work was carried out without any funding.

Data availability

All the data are included in the manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 23 September 2025 / Accepted: 16 April 2026

Published online: 22 April 2026

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