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RESEARCH ARTICLE

Comparative Instrumental Analysis of Unpurified and Purified *Krishnappiragam* (Black Mica) Through FTIR, ICP-OES, XRD and NMR

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Abstract

Background: *Krishnappiragam* (Black Mica/Biotite) is a key mineral in Siddha medicine, requiring purification to enhance its therapeutic properties and eliminate toxins. **Aim:** This study compares the physicochemical, biochemical, and structural properties of unpurified (A1) and purified (A2, A3) *Krishnappiragam* using advanced techniques like FTIR, ICP-OES, XRD, and NMR. **Methods:** *Krishnappiragam* was purified using two traditional Siddha methods and analysed for organoleptic, physicochemical, and biochemical properties. FTIR identified functional groups, ICP-OES assessed heavy metal content, XRD analysed crystallinity, and NMR evaluated molecular structure and particle size. **Results:** Organoleptic analysis showed variations in colour and texture. Physicochemical analysis revealed differences in ash values, moisture content, and extractive values, with A3 showing the highest water-soluble extractive. Biochemical analysis confirmed changes in mineral composition post-purification. FTIR detected functional groups related to amines, alkanes, and carboxylic acids. XRD indicated enhanced crystallinity in A3, while NMR revealed A2 had the smallest and most uniform particle size. However, ICP-OES detected toxic heavy metals, including aluminium, nickel, and mercury, exceeding WHO and FDA limits. **Conclusion:** Purification alters *Krishnappiragam*'s chemical and structural properties, improving crystallinity and refining composition. However, high heavy metal concentrations raise safety concerns, highlighting the need for improved purification techniques. Future research should focus on optimising purification protocols and quality control to minimise toxicity while maintaining medicinal efficacy.

Major Findings: Purification of *Krishnappiragam* significantly enhances its crystallinity and biochemical composition, with A3 exhibiting the highest water-soluble extractive and A2 showing the smallest, most uniform particle size. However, the presence of toxic heavy metals exceeding WHO and FDA limits raises safety concerns, emphasising the need for improved purification techniques to ensure medicinal safety.

Keywords: Black Mica, Heavy Metals, *Krishnappiragam*, Purification, *Siddha* Medicine, Standardisation

Abbreviation: A1: Impure or raw *Krishnappiragam*; A2: *Krishnappirgam* purified by Method I; A3: *Krishnappirgam* purified by Method II; HCl: Hydrochloric acid; ICP-OES: Inductively Coupled Plasma - Optical Emission Spectroscopy; FTIR: Fourier Transform Infrared; XRD: X-ray Powder Diffraction; NMR: Nuclear Magnetic Resonance.

1. Introduction

The Siddha medical system is the ancient Dravidian system of medicine, otherwise known as Tamil medicine, which is practised predominantly in South India. In this medicinal practice, first, we have to purify the raw drugs, which not only removes the impurities, and enhances the properties of the drugs. Purification

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methods of raw materials include heating, firing, soaking, boiling, quenching, and certain techniques like *putam* (incineration) and steaming, for the removal of impurities in a plant source, animal source, metals and minerals. There are a lot of methods for a single drug purification[1].

Even after purification, it is essential to standardise the drug's quality and properties. A fundamental principle of the Siddha system is the purification of raw materials before incorporating them into medicines. These purification techniques are highly scientific and distinct from those in other medicinal systems[2].

The term *Suddhi* signifies "eliminating impurities." *Suddhi* (purification) of raw materials serves a dual purpose: removing contaminants and enhancing the drug's efficacy. This process improves brittleness, amplifies synergistic effects, increases potency, and refines therapeutic properties. Additionally, it converts inedible substances into consumable forms. Some toxic metals and minerals, after undergoing purification, are also utilised in the preparation of various medicines. The process typically involves cleaning, frying, soaking, grinding, and fermenting with herbal extracts or decoctions until all impurities are eliminated. No medicinal formulation should be prepared before undergoing purification. This procedure ensures that raw materials, particularly metals, minerals, and animal-derived substances, lose their harmful or undesirable effects, making them safer and more effective for therapeutic use[3].

Accordingly, the Siddhars used *chathru* and *mithru* processes to neutralise the poisonous effects. The process of purification transforms the unpurified raw materials into purified ones, which is helpful in the preparation of the safest drug form devoid of toxic substances by altering its unique and unfavourable physicochemical properties[4].

Nowadays, most medical practitioners are cautious about our Siddha medicine, due to the extensive use of heavy metals and minerals like mercury and lead in the preparation of higher-order medicines. Even though they think of minerals also, we have to break the unbearable thought among them, so standardisation for the raw drug after the purification process to rule out what elements are present before and after purification[5].

According to the text Bogar 700, *Krishnappiragam* (Black Mica/ Biotite) is a type of *appiragam* otherwise known as *Vajra Appiragam*-black in colour, which is one of the *uparasam*(natural minerals). After purification, it is used extensively in various Siddha medicine formulations in the form of *parpam, chendooram, chunnam* or *sathu* with great therapeutic significance. If it's impure, it will cause severe toxicity. So, it is a major need for us to prove the importance of the purification of poisonous natural minerals scientifically before going to drug preparation by that unpurified raw drug[6].

Appirham (*Mica*) is traditionally used in the form of *Appiraga parpam, Appiraha chenduram,* etc., in the treatment of *vatha, pitha* and *kapha* diseases, diabetes mellitus, impotency and venereal diseases. As per *Gunapadam Thathu Jeeva Vaguppu*- textbook, *Appiragam* has the action and properties of an astringent, alternative, tonic, aphrodisiac and diuretic. It is frequently used in the day-to-day practice of Siddha Physicians for predominantly treating diabetics and impotence[7].

There is a significant need to evaluate the chemical transformations occurring during both purification methods of *Krishnappiragam.*

2. Materials and Methods

2.1 Source of the Raw Materials

The unprocessed medicinal substances required for purification, along with the raw *Krishnappiragam*, were procured from a well-established country store named *M. Gopala Asan Naatu Marunthu Kadai* in Nagercoil, Tamil Nadu, India. Cow's milk and urine were obtained from a local livestock farm, while vinegar was sourced from a reputed grocery store.

2.2 Authentication of the Raw Drug

The identification and validation of the raw *Krishnappiragam* were carried out by *Dr P. Sathiyarajeswaran*, Assistant Director (I/c) at the *Siddha Central Research Institute, Arumbakkam, Chennai.* The herbal raw materials involved in the purification process, namely *Terminalia chebula* (Retz.), *Terminalia bellirica* (Gaertn.) Roxb., *Phyllanthus emblica* (Linn.), and *Zizyphus mauritiana* (Lamk.), were verified and authenticated by Dr Sunilkumar Narayanan, PhD, Research Officer (Pharmacognosy) at the Central Council for Research in Siddha, Chennai.

2.3 Purification of the Raw Drug[7]

The raw *Krishnappiragam* (referred to as A1) underwent purification using two distinct methods, following the standardised procedures detailed in the classical *Siddha* text, *Anuboga Vaidya Navaneetham* Part-2.

2.4 General Requirements

Small mud pots, measuring cylinder/ jar, stove for heating, tongs and weighing machine (electronic). These are basic things required for the purification of minerals and metals, handled with care in the minimum standard operating procedure.

2.5 *Krishnappiragam* - Purification Method 1

2.5.1 Step 1 of Purification Method 1

2.5.1.1 Required Materials

- Raw *Krishnappirgam* - 50 gm
- Cow's milk- 400 ml

2.5.1.2 Method

The raw *Krishnappirgam* weighing 50 gm was taken; the cow's milk was taken for boiling and quenching, which may be eight times as *Krishnappirgam* weight, 400 ml. First, the raw ore was heated in the mud pot or furnace using the smithy, whether it had to be heated or came red hot; otherwise, the raw material had to withstand the heat, and sound produced from *Krishnappirgam*. After heating, the *Krishnappirgam* was boiled in milk, and the amount of milk was reduced to half of the total amount. This process was repeated seven times; sometimes the heated raw material was quenched in milk in a small mud pot. For every time of step, fresh milk was used. After the heat was reduced in the quenching process, the milk was siphoned or drained, and the remains of *Krishnappirgam* were taken.

2.5.2 Step 2 of Purification Method 1

2.5.2.1 Required Materials

- Remains of *Krishnappirgam* collected from step I,
- Decoction powder of *Muppalai Kudineer* - 400g (*Terminalia chebula* (Retz.), *Terminalia bellirica* (Gaertn.) Roxb., *Phyllanthus embilica* (Linn.)
- Water -3.2 litres

2.5.2.2 Method

For the preparation of *Muppalai* decoction, 400 g of decoction powder was taken first in a vessel. 3.2 litres of water were added to the vessel, then it was heated and boiled to it reduced to one-fourth of its total amount, thus the decoction was prepared. The remains of *Krishnappirgam* collected from step I were heated and fried in the mud pot until a cracking sound appeared. After that it was transferred to another mud pot then the decoction was poured up to the *Krishnappirgam* submerged,, it was left aside for the quenching process, the heat was reduced, then the decoction was siphoned/ drained from the pot, remaining *Krishnappirgam* was allowed to cool and the then transferred to first mud pot used for frying, like this every time the *Krishnappirgam* that colled was heated again and quenching in decoction, this should be repeated for seven times, for every step had to use fresh decoction. Finally, the remains of *Krishnappirgam* were taken.

2.5.3 Step 3 of Purification Method 1

2.5.3.1 Required Materials

- Remains of *Krishnappirgam* collected from step II,
- Vinegar - 900 ml

2.5.3.2 Method

The remains of *Krishnappirgam* collected from step II were heated and fried in the mud pot until a cracking sound appeared. After that it was transferred to another mud pot then the vinegar was poured up to the *Krishnappirgam* submerged, it was left aside for the quenching process, the heat was reduced, then the vinegar was siphoned/drained from the pot, remaining *Krishnappirgam* was allowed to cool and the then transferred to first mud pot used for frying, like this every time the *Krishnappirgam* that colled was heated again and quenching in vinegar, this should be repeated for seven times, for every step had to use fresh vinegar. Finally, the remains of *Krishnappirgam* were taken.

2.5.4 Step 4 of Purification Method 1

2.5.4.1 Required Materials

- Remains of *Krishnappirgam* collected from step III,
- Cow's urine- 900 ml

2.5.4.2 Method

The remains of *Krishnappirgam* collected from step III were heated and fried in the mud pot until a cracking sound appeared. After that it was transferred to another mud pot then the cow's urine was poured up to the *Krishnappirgam*

submerged, it was left aside for the quenching process, the heat was reduced, and then the cow's urine was siphoned/drained from the pot, remaining *Krishnappirgam* was allowed to cool and the then transferred to first mud pot used for frying, like this every time the *Krishnappirgam* that colled was heated again and quenching in vinegar, this should be repeated for seven times, for every step had to use fresh vinegar. Finally, the remains of *Krishnappirgam* were taken, washed with fresh water and dried. At least it was grounded in the mortar with the help of a pestle, then the *Krishnappirgam,* which was purified by Method I (named as A2), was broken into powder, easily brittle.

2.6 *Krishnappiragam* - Purification Method 2

2.6.1 Required Materials

- *Zizyphus marutiana* (Lamk.) root - 450 gm
- Raw *Krishna Appiragam*-50gm
- Water - 3.6 litre preparation of *illanthai* root decoction

2.6.2 Method

For the preparation of *illanthai* root decoction, 450 g of *illanthai* root was taken first in a vessel. 3.6 litres of water were added to the vessel, then it was heated and boiled to it reduced to one-fourth of its total amount, thus the decoction was prepared. The raw *Krishnappirgam* was heated and fried in the mud pot until a cracking sound appeared. After that it was transferred to another mud pot then prepared *illanthai* root decoction was poured up to the *Krishnappirgam* submerged, it was left aside for the quenching process, the heat was reduced, and then the *illanthai* root decoction was siphoned/drained from the pot, remaining *Krishnappirgam* was allowed to cool and the then transferred to first mud pot used for frying, like this every time the *Krishnappirgam* that colled was heated again and quenching in *illanthai* root decoction, this should be repeated for seven times, for every step had to use fresh *illanthai* root decoction. Finally, the remains of *Krishnappirgam* were taken, washed with fresh water and dried. At least it was grounded in the mortar with the help of a pestle, then the *Krishnappirgam,* which was purified by Method II (named as A3), was broken into powder, easily brittle.

2.7 Organoleptic Parameters – Assessment[8]

Organoleptic parameters of A1, A2 and A3, such as colour, odour and nature, were examined manually by visual and sensory assessment and depicted in Figure 1.

2.7.1 Colour

Samples of A1, A2, and A3 were collected, and their colour was observed under natural sunlight. The observations were recorded.

2.7.2 Odour

A small quantity of A1, A2, and A3 was taken, and the aroma was assessed by slowly and repeatedly inhaling the air above the sample. The findings were documented.

2.8 Physicochemical Parameters - Assessment[8]

The unprocessed *Krishnappiragam* (A1), *Krishnappiragam* purified using Method I (A2), and *Krishnappiragam* purified using Method II (A3) underwent physicochemical analysis. This study was carried out at the Centre for Plant Biotechnology, Department of Botany, St. Xavier's College (Autonomous), Tirunelveli.

2.8.1 Total Ash Value

Approximately 3 g of air-dried powdered samples of A1, A2, and A3 were accurately weighed and placed in a pre-weighed silica crucible. The samples were incinerated in a furnace, gradually increasing the temperature until reaching a dull red heat at 600°C, ensuring complete carbon elimination. After cooling, the crucible was reweighed, and the process was repeated until a constant weight was achieved. The total ash percentage was then calculated relative to the air-dried sample.

2.8.2 Acid-Insoluble Ash Value

The ash obtained from A1, A2, and A3 samples was used to determine the acid-insoluble ash content. The ash was boiled with 25 ml of 2N HCl for 5 minutes. The resulting insoluble material was collected on ash-free filter paper, washed with hot water, dried, ignited, and then weighed. The percentage of acid-insoluble ash was calculated relative to the air-dried sample.

2.8.3 Water-Soluble Ash Value

The total ash from the A1, A2, and A3 samples was boiled with 25 ml of water for 5 minutes. The insoluble material was collected on ash-free filter paper, washed with hot water, dried, and ignited at a temperature

not exceeding 105°C for 15 minutes. The weight of the insoluble portion was subtracted from the total ash weight. The resulting difference represented the water-soluble ash, and its percentage was determined concerning the air-dried sample.

2.8.4 Water-Soluble Extractive Value

A weighed amount (5 g) of the powdered drug was placed in a stoppered conical flask, followed by the addition of 100 mL of water. The flask was continuously shaken for 6 hours using an electric shaker and left undisturbed overnight for maceration. The mixture was then carefully filtered, and the filtrate was evaporated to dryness. The weight of the extractive was recorded, and its percentage was calculated concerning the air-dried sample.

2.8.5 Alcohol-Soluble Extractive Value

A 5 g sample of powdered drug was placed in a stoppered conical flask, followed by the addition of 100 ml of 90% alcohol. The flask was shaken continuously for 6 hours in an electric shaker and allowed to stand overnight for maceration. The solution was then filtered carefully, and the filtrate was evaporated to dryness. The extractive weight was recorded, and its percentage was calculated relative to the air-dried sample.

2.8.6 Moisture Content

The moisture content was determined using the Karl Fischer titration method. This technique relies on the Bunsen reaction between iodine and sulfur dioxide in an aqueous medium. Methanol serves as the solvent, while pyridine acts as the buffering agent. Initially, the water factor was determined, indicating the moisture present in methanol and the Karl Fischer reagent. A 2 g sample was weighed and added to methanol, then titrated with Karl Fischer reagent using an automatic titrator to identify the endpoint. The moisture content was calculated using the formula:

Water factor = (Weight of water taken (mL) / Burette reading) Moisture content = (Burette reading × Water factor × 100) / Sample weight (mg)

2.8.7 Loss on Drying

A 2 g powdered drug sample was placed in a pre-weighed crucible that had been dried at 105°C in a hot air oven. The sample was then kept in the oven at a constant temperature of 105°C for 2 hours. The percentage loss on drying was calculated from the difference in weight, considering the air-dried sample, and the results were recorded.

2.9 ICP-OES Analysis[8]

The RF frequency was set at 40 MHz, with a detection range spanning 165–782 nm. The detection limit extended to ppm levels using an SCD detector. Solid samples required 10–20 mg, whereas liquid samples needed approximately 25 ml. All samples had to be non-explosive and non-corrosive to ensure safe analysis.

2.9.1 Sample Preparation

A 0.25 g test sample was placed in the instrument's liner. Nitric acid or sulfuric acid (9 ml) was added gradually, ensuring no sample residue remained on the slide. The mixture was stirred and allowed to react for a few minutes. The liner was positioned inside the inner jacket, and the screw cap was hand-tightened. The vessel was sealed and inserted into the microwave rotor, where the temperature was maintained at 180°C for at least 10 minutes. After cooling to below 60°C internally and below 50°C externally, the rotor was removed. The digested sample was diluted to 100 mL using Millipore water. If undissolved particles remained, the solution was filtered through Whatman filter paper and transferred to labelled plastic containers.

2.10 Fourier Transform Infrared (FTIR) Spectroscopy Analysis[8]

FTIR spectra were obtained using a Perkin Elmer Spectrum One with an ATR-FTIR unit. A few milligrams of the A3 sub-fraction were placed on the ATR head. The spectra were recorded with a resolution of 4 cm^{-1} and 16 co-added scans within a 450–4000 cm^{-1} wavelength range. Each spectrum was obtained by averaging 16 scans, and the results were analysed using Spectrum software (Perkin Elmer).

2.11 X-ray Powder Diffraction (XRD) Analysis[8]

A PXRD instrument was used for analysis. When X-rays interacted with a crystal, they produced diffraction patterns unique to its structure. These patterns were plotted as intensity versus detector angle (2θ). Crystalline materials exhibited diffraction, while

amorphous substances like glass lacked long-range order and did not generate significant patterns. A 25 g sample was required.

2.11.1 Sample Preparation

A 1 g portion was set aside as a reference, while 5 g was prepared for analysis, and the remainder was used for further fractionation. The sample was mixed in hot distilled water using a Waring blender until fully dispersed. It was centrifuged, and the wash water was discarded. The sample was dried and manually disaggregated using a mortar and pestle. Coarse material was ground to silt size, followed by further grinding under butanol for 2 hours to prevent overheating. After evaporation of butanol, the sample was treated with trihexylamine acetate and pressed into a sample holder.

2.12 Nuclear Magnetic Resonance (NMR) Spectroscopy[8]

NMR spectroscopy was conducted to analyse the local magnetic environment of atomic nuclei. The technique is based on the absorption of radiofrequency radiation (4–900 MHz) in the presence of a strong magnetic field. Nuclei with a magnetic moment undergo energy transitions, generating resonance signals that provide insights into molecular structure, chemical environment, and dynamics.

2.12.1 Sample Preparation

For solid-state NMR, 20–50 mg of finely powdered solid samples was prepared using an agate mortar and pestle to ensure uniformity. The sample was packed into a rotor, sealed to prevent contamination, labelled appropriately, and placed in the spectrometer for analysis.

3. Results

3.1 Results of Organoleptic Analysis of A1, A2 and A3

The organoleptic characteristics of A1, A2, and A3, including colour, odour, and texture, are summarised in Table 1.

3.2 Results of Physicochemical Analysis of A1, A2 and A3

The physicochemical properties, such as appearance, total ash (%), acid insoluble ash (%), water soluble ash (%), water soluble extractive, alcohol soluble extractive, moisture content (%), pH, and Loss of weight, were analysed, and the results are presented in Table 2.

3.3 Results of Qualitative Biochemical Analysis

The biochemical composition of A1, A2, and A3 was assessed, revealing variations in mineral and organic compound presence, as detailed in Table 3.

Table 1. Results of organoleptic analysis of A1, A2 and A3

Sl. No.	Parameters	Observations		
		A1	A2	A3
1	Colour	Black	Silver	Brown
2	Odour	No specific odour	No specific odour	No specific odour
3	Texture	Fragmented thick sheet	Fragmented thin sheet	Powdered and crystalline

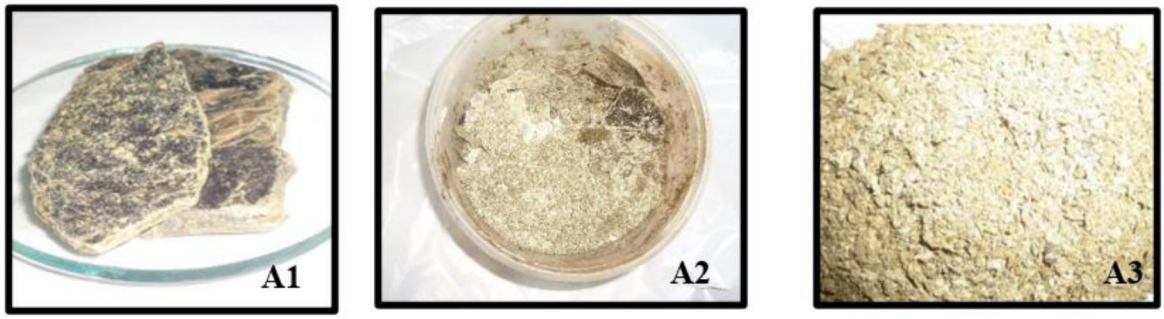


Figure 1. Appearance of samples - A1, A2 and A3.

Table 2. Results of physicochemical analysis of A1, A2 and A3

Sl. No.	Parameters	Observation		
		A1	A2	A3
1	Appearance	Fragmented thick sheet	Thin sheet with a powdered nature	Powder with tiny sheet
2	Total ash (%)	0.16 g	0.12 g	0.18 g
3	Acid insoluble ash (%)	0.95	0.89	0.70
4	Water soluble ash (%)	0.19	0.21	0.27
5	Water-soluble extractive	0.09	0.11	0.15
6	Alcohol soluble extractive	0.03	0.07	0.18
7	Moisture content (%)	0.15	0. 11	0.05
8	pH	5.28	5.34	5.19
9	Loss of weight	0.08 g	0.06 g	0.1 g

Table 3. Results of biochemical analysis of A1, A2 and A3

Sample	Presence	Absence
A1 (Unpurified Black Mica)	Calcium, Sulphate, Chloride, Ferric Iron, Ferrous Iron, Amino Acid	Carbonate, Starch, Phosphate, Albumin, Tannic Acid, Unsaturated Compound, Reducing Sugar, Zinc
A2 (Black mica purified by method 1)	Calcium, Sulphate, Chloride, Ferric Iron, Ferrous Iron	Carbonate, Starch, Phosphate, Albumin, Tannic Acid, Unsaturated Compound, Reducing Sugar, Amino Acid, Zinc
A3 (Black mica purified by method 2)	Calcium, Chloride, Ferric Iron, Ferrous Iron, Amino Acid	Sulphate, Carbonate, Starch, Phosphate, Albumin, Tannic Acid, Unsaturated Compound, Reducing Sugar, Zinc

Table 4. Results of FTIR chromatogram analysis of A1, A2 and A3

Sample	Sl. No	Peak	Class	Structure	Assignments
A1	1	591.60	Alkyl halides	R-Br	C-Br stretch
	2	1635.05	Amides	RCONH2	NH out of Plane
	3	2114.48	Misc	Si-H silane	Si-H silane
	4	3310.33	Amines	R2NH	NH stretch
A2	1	1014	Amines	R2NH	C-N Stretch
	2	1112	Amines	RNH2	C-N Stretch
	3	1450	Alkanes	RCH2CH3	CH stretch
	4	1639	Alkanes	3 Ring	C=C Stretch
	5	2842	Carboxylic acid	RCO-OH	Dimer C=O
	6	3312	Amines	R2NH	NH stretch
A3	1	668	Alkyl halides	R-Br	C-Br strength
	2	750	Aromatic	Monosubst	C-H out of plane
	3	1214	Misc	P O Phosphoramide	PO
	4	1378	Alkanes	C-H rock	C-H rock
	5	1465	Alkanes	CH2	CH2
	6	2853	Alkanes	RCH2CH3	CH strength
	7	2924	Alkanes	CH2	CH2

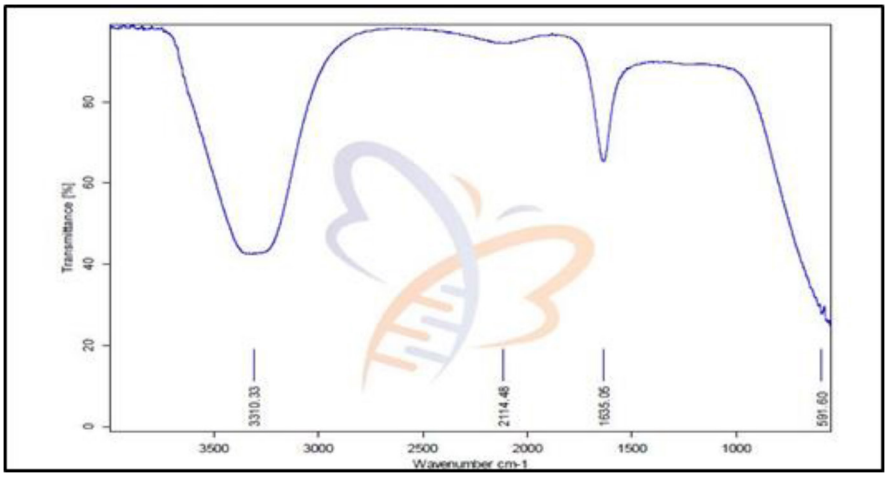


Figure 2. FTIR chromatogram results of A1.

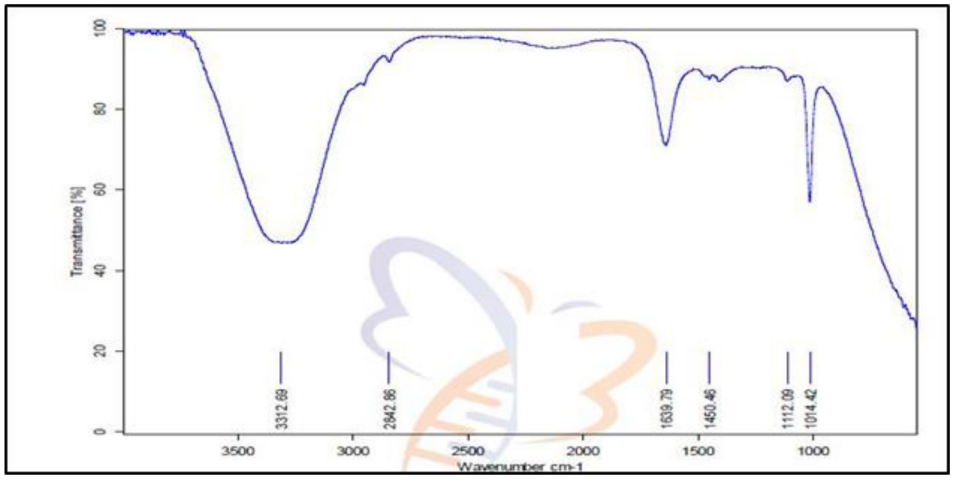


Figure 3. FTIR chromatogram results of A2.

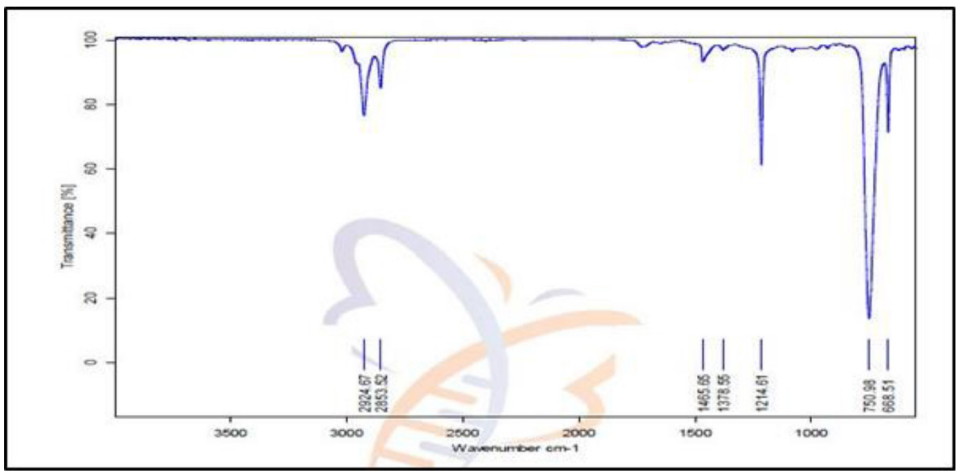


Figure 4. FTIR chromatogram results of A3.

3.4 Results of FTIR Chromatogram Analysis of A1, A2 and A3

The FTIR spectral data of the samples indicate the presence of key functional groups, as shown in Table 4 and depicted in Figures 2, 3 and 4.

3.5 Results of ICP-OES - Elements Analysis of A1, A2 and A3

ICP-OES is an analytical technique used for the detection of trace metals. The intensity of this emission is indicative of the concentration of the elements within the drug. Specification of Siddha, Ayurveda and Unani products intended for exports:

BDL- Below Detecting Limit, 1% = 10000 ppm, (1 ppm = 1/1000000 (or) 1 ppm = 0.0001%)

The WHO and FDA permissible limits for toxic heavy metals are outlined in Table 5, serving as a reference for the contamination levels detected in the samples.

The concentration of toxic heavy metals in A1, A2, and A3, analysed using ICP-OES, is detailed in Table 6, highlighting significant deviations from permissible limits.

Table 5. Toxic heavy metals and the permissible limits as per the WHO and the FDA

Sl. No.	Heavy metals	WHO and FDA permissible limits	
		In ppm	In mg/kg
1	Aluminium (Al)	1.5 ppm	1.5 mg/kg
2	Arsenic (As)	10 ppm	10 mg/kg
3	Lead (Pb)	10 ppm	10 mg/kg
4	Nickel (Ni)	1 ppm	1 mg/kg
5	Mercury (Hg)	1 ppm	1 mg/kg
6	Iron (Fe)	50 ppm	50 mg/kg

3.6 Results of XRD Analysis of A1, A2 and A3

The XRD analysis revealed distinct crystalline structures in the samples, as illustrated in Table 7, and as well as depicted in Figures 5, 6 and 7. This demonstrates the impact of purification methods on crystallinity.

3.7 Results of NMR Analysis of A1, A2, A3

The particle size distribution and molecular characteristics of A1, A2, and A3 were further assessed

Table 6. Results of toxic heavy metals in A1, A2 and A3 by ICP-OES analysis

Sl. No.	Test Parameters	Instrument Used	Results		
			A1 (mg/kg)	A2 (mg/kg)	A3 (mg/kg)
1	Aluminium (Al)	ICP-OES	58919	49375	57894
2	Arsenic (As)	ICP-MS	BDL (DL: 0.05)	1.51	2.31
3	Lead (Pb)	ICP-MS	0.61	0.40	1.71
4	Nickel (Ni)	ICP-OES	31.5	31.2	35.5
5	Mercury (Hg)	ICP-MS	BDL (DL: 0.1)	5.07	9.81
6	Iron (Fe)	ICP-OES	42561	37162	45942
BDL: Below Detection Limit; DL: Detection Limit					

Table 7. Results of XRD analysis of A1, A2 and A3

Sample A1		Sample A2		Sample A3	
Angle	Intensity	Angle	Intensity	Angle	Intensity
2 Theta	Count	2 Theta	Count	2 Theta	Count
10.9113	459	10.0221	579	14.4077	356
27.5444	416	27.9890	321	38.2155	1443
31.9301	1769	38.2761	2515	44.3594	525
45.5518	699	44.5211	813	64.4889	304
56.6069	469	54.8283	159	77.4432	207
66.4089	113	64.7516	583		
75.3216	120	77.5852	519		

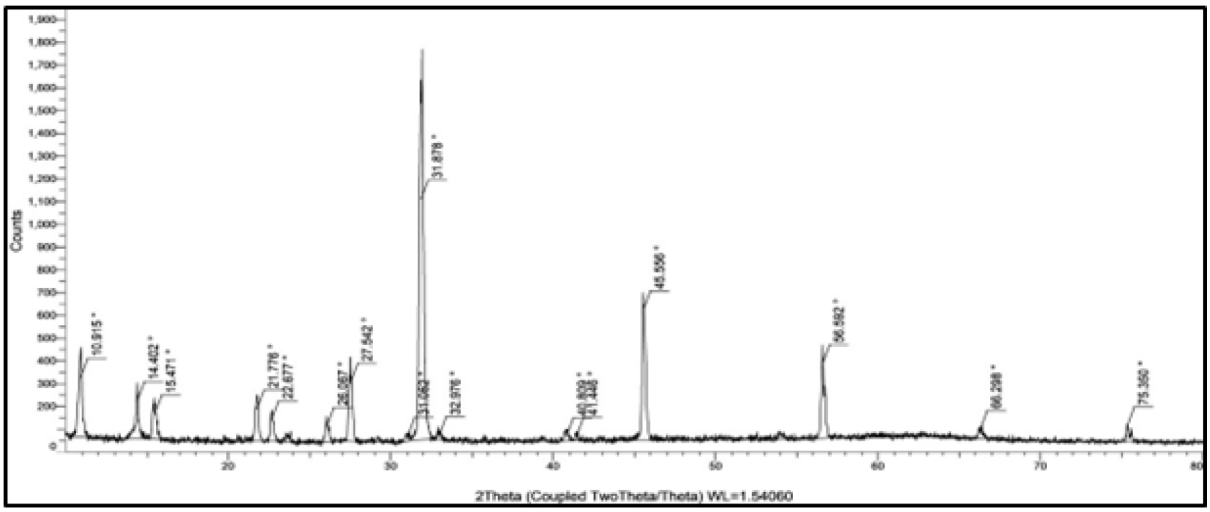


Figure 5. XRD analysis of A1.

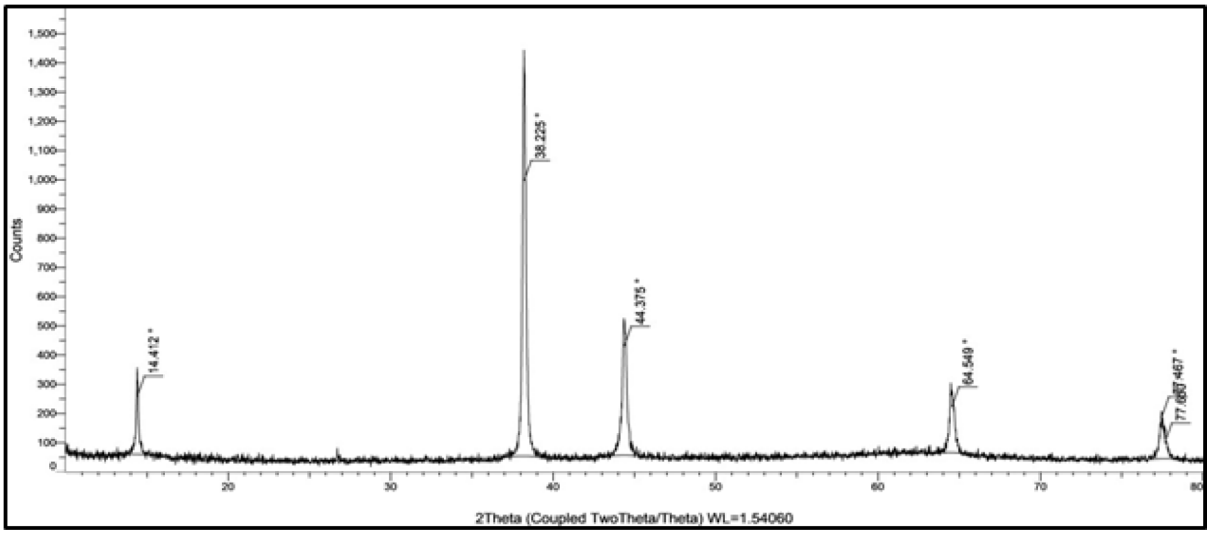


Figure 6. XRD analysis of A2.

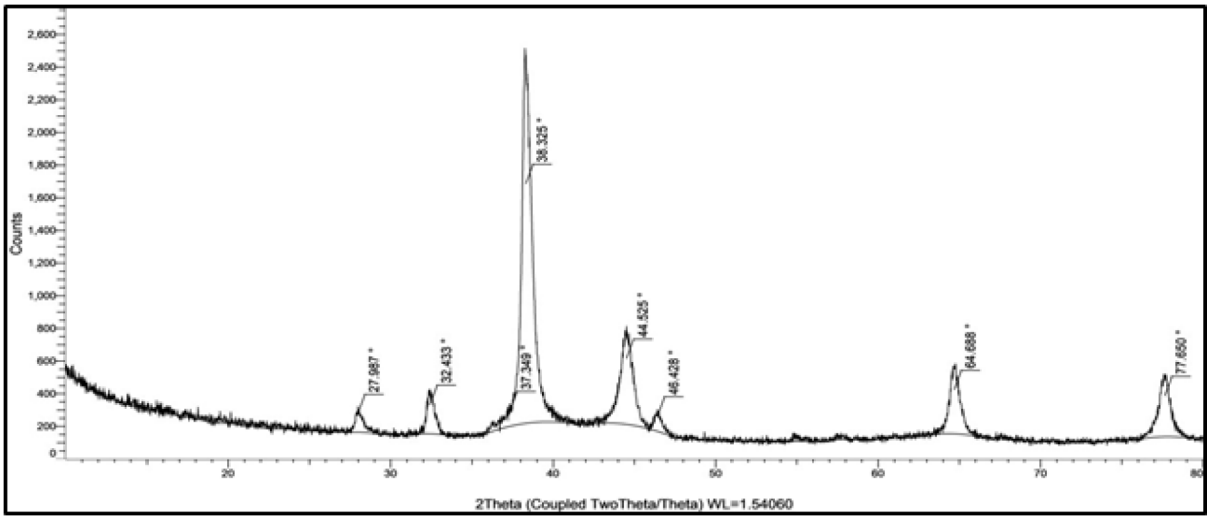


Figure 7. XRD analysis of A3.

Table 8. Results of NMR analysis of A1, A2, A3

Sample	Sample A1	Sample A2	Sample A3
Particle Size in nm	117.12 nm	111.89 nm	113.9 nm

through NMR analysis, with the results summarised in Table 8.

4. Discussion

4.1 Organoleptic Analysis of A1, A2, and A3

The results of the organoleptic analysis of A1, A2, and A3 are summarised in Table 1. The colour, odour, and nature of the samples varied, with A1 being black, A2 silver, and A3 brown. All samples showed no specific odour. The nature of the samples differed as well: A1 was observed to be a fragmented thick sheet, A2 a fragmented thin sheet, and A3 was in a powdered and crystalline form.

4.2 Physiochemical Analysis of A1, A2, and A3

The physicochemical analysis of A1, A2, and A3, as shown in Table 2, revealed several key differences. A1 was a fragmented thick sheet, A2 was a thin sheet with a powdered nature, and A3 was a powder with tiny sheet-like structures. The total ash content was relatively similar across all samples, with A1 showing 0.16 g, A2 0.12 g, and A3 0.18 g. Acid-insoluble ash, water-soluble ash, and extractive values varied slightly, with A3 exhibiting the highest water-soluble extractive and alcohol soluble extractive. The moisture content was lowest in A3 (0.05%), while A1 and A2 showed slightly higher values (0.15% and 0.11%, respectively). The pH values were in a similar range, indicating a slightly acidic nature for all three samples.

4.3 Biochemical Analysis of A1, A2, and A3

The biochemical analysis revealed important qualitative findings. Unpurified Black mica (A1) contained calcium, sulfate, chloride, ferric and ferrous iron, and amino acids, but lacked carbonate, starch, phosphate, albumin, tannic acid, unsaturated compounds, reducing sugars, and zinc. Purified Black mica (Method 1, A2) retained calcium, sulfate, chloride, ferric and ferrous iron but lacked other compounds such as starch, phosphate, albumin, and amino acids. Further purification using Method 2 (A3) resulted in the presence of calcium, chloride, ferric and ferrous iron, and amino acids, while sulfate, carbonate, starch, and other compounds were absent. These results suggest that the purification methods significantly affected the biochemical composition, with Method 2 (A3) leading to a more refined composition compared to the unpurified A1 and Method 1 (A2). The observed variations in physicochemical and biochemical properties may have implications for the potential applications of these materials in medicinal or industrial fields.

4.4 FTIR Chromatogram Analysis

4.4.1 FTIR Chromatogram Analysis of A1

Sample A1 exhibited distinct peaks in the FTIR spectrum, revealing the presence of various functional groups. The peak at 591.60 cm^{-1} is indicative of alkyl halides, specifically the C-Br stretch, suggesting that bromine is incorporated into the sample. The 1635.05 cm^{-1} peak corresponds to amides, with an NH out-of-plane vibration, pointing to the presence of amide groups in the structure. The 2114.48 cm^{-1} peak corresponds to Si-H stretching vibrations, indicating the presence of silane compounds in A1. Additionally, the 3310.33 cm^{-1} peak is associated with amines, confirming the presence of amine groups through the NH stretch. These findings suggest a complex mixture of organic compounds, including alkyl halides, amides, silanes, and amines, in Sample A1.

4.4.2 FTIR Chromatogram Analysis of A2

Sample A2 displayed a different profile in its FTIR spectrum. The 1014 cm^{-1} and 1112 cm^{-1} peaks suggest the presence of amines, with C-N stretches characteristic of secondary and primary amines, respectively. The 1450 cm^{-1} peak is associated with alkanes, indicating the presence of alkyl groups. A significant peak at 1639 cm^{-1} corresponds to a C=C stretch in alkenes, suggesting cyclic structures within the sample. Additionally, the 2842 cm^{-1} peak, characteristic of a carboxyl group stretch, points to the presence of carboxylic acids, further supported by its dimeric form. The 3312 cm^{-1} peak confirms the presence of amines, similar to A1, via the NH stretch. These findings suggest that A2 is a more complex sample, incorporating amines, alkanes, carboxylic acids, and cyclic compounds.

4.4.3 FTIR Chromatogram Analysis of A3

Sample A3 exhibited several key peaks in its FTIR spectrum. The 668 cm^{-1} peak indicates the presence of alkyl halides, particularly C-Br stretches. The 750 cm^{-1} peak suggests monosubstituted aromatic compounds with C-H out-of-plane bending vibrations. The 1214 cm^{-1} peak corresponds to phosphoramide, confirming the presence of phosphoric acid derivatives in A3. The 1378 cm^{-1} and 1465 cm^{-1} peaks are associated with alkanes, indicating the presence of aliphatic hydrocarbons. Furthermore, the 2853 cm^{-1} and 2924 cm^{-1} peaks confirm the presence of alkyl groups, further suggesting that A3 is composed of a mixture of alkyl halides, aromatic compounds, and phosphoramide derivatives.

4.4.4 Comparative Analysis of FTIR Chromatogram

When comparing the FTIR spectra of Samples A1, A2, and A3, notable differences emerge in the functional groups present. While all three samples share the presence of alkyl halides (C-Br stretch), A1 and A3 show clear indications of amides and silane compounds, respectively, whereas A2 stands out for its more complex mixture of amines, carboxylic acids, and cyclic structures. A1 and A3 primarily feature alkyl and amine groups, with A1 also showing silane characteristics. In contrast, A2 exhibits a broader range of functional groups, including cyclic alkenes and carboxylic acids, pointing to a more intricate molecular structure. These differences highlight the structural diversity across the samples, suggesting distinct chemical compositions that may influence their properties and potential applications.

4.5 ICP-OES Analysis of Heavy Metals A1, A2, and A3

The ICP-OES analysis of heavy metals in samples A1, A2, and A3 revealed concerning levels of toxic elements, particularly aluminium, nickel, and mercury. The concentration of aluminium in all three samples (A1: 58,919 mg/kg, A2: 49,375 mg/kg, A3: 57,894 mg/kg) significantly exceeded the permissible limit of 1.5 mg/kg set by WHO and FDA. This elevated concentration of aluminium suggests contamination levels that could pose long-term health risks, particularly to the nervous and renal systems.

Similarly, the nickel levels in the samples (A1: 31.5 mg/kg, A2: 31.2 mg/kg, A3: 35.5 mg/kg) were far above the acceptable limit of 1 mg/kg, raising concerns about potential adverse effects, such as allergic reactions and respiratory issues. Arsenic was detected in A2 (1.51 mg/kg) and A3 (2.31 mg/kg), exceeding the WHO/FDA limit of 10 mg/kg but remaining below levels that would pose immediate danger. However, chronic exposure to arsenic, even at low levels, can lead to serious health problems such as cancer and organ damage.

Lead was found in all samples, but in quantities (A1: 0.61 mg/kg, A2: 0.40 mg/kg, A3: 1.71 mg/kg) below the WHO and FDA permissible limit of 10 mg/kg, suggesting that while the presence of lead is a concern, it is not at hazardous levels in these samples. On the other hand, mercury was detected in A2 (5.07 mg/kg) and A3 (9.81 mg/kg), surpassing the permissible limit of 1 mg/kg. Mercury is highly toxic, and its presence in these samples is a significant concern, particularly since it can cause neurological and organ toxicity.

Iron levels in all three samples were extremely high (A1: 42,561 mg/kg, A2: 37,162 mg/kg, A3: 45,942 mg/kg), significantly surpassing the 50 mg/kg limit set by WHO/FDA. While iron is an essential element for the body, excessive intake can lead to serious health complications, such as organ damage. The high concentration of iron, coupled with elevated levels of aluminium and nickel, calls for further examination and control of heavy metal content in these products. The overall findings highlight the need for rigorous quality control and safety protocols to ensure that these products are safe for human consumption, particularly when intended for export.

4.6 XRD Analysis

4.6.1 XRD Analysis of A1

For Sample A1, significant peaks were observed at 2θ values of 10.91°, 27.54°, 31.93°, 45.55°, 56.61°, and 66.41°, with corresponding intensities of 459, 416, 1769, 699, 469, and 113, respectively. The strong intensity at 31.93° (1769 counts) suggests a dominant crystalline phase with high symmetry and periodicity. This peak may correspond to a well-ordered crystalline phase in the sample. Other peaks, such as those at 27.54° and 45.55°, also exhibit significant intensities, indicating the presence of additional crystalline components or phases within the sample.

4.6.2 XRD Analysis of A2

For Sample A2, the diffraction pattern revealed peaks at 2θ values of 10.02°, 27.99°, 38.28°, 44.52°, 54.83°,

64.75°, and 77.59°, with corresponding intensities of 579, 321, 2515, 813, 159, 583, and 519, respectively. The most prominent peak at 38.28° (2515 counts) suggests that Sample A2 may have undergone some degree of purification, as the intensity is notably higher than that of A1. The presence of sharp, distinct peaks indicates the formation of well-crystallised phases in the sample. Additionally, the peak at 44.52° (813 counts) also points to a significant crystalline phase, albeit with slightly lower intensity compared to A2's dominant peak at 38.28°.

4.6.3 XRD Analysis of A3

For Sample A3, key peaks were observed at 2θ values of 14.41°, 38.22°, 44.36°, 64.49°, and 77.44°, with corresponding intensities of 356, 1443, 525, 304, and 207, respectively. The most intense peak at 38.22° (1443 counts) is similar to the peak observed in A2 but with a higher intensity, indicating a more crystalline or purified structure. The presence of peaks at higher angles, such as 64.49° and 77.44°, suggests that A3 may exhibit more compact or closely packed crystallographic arrangements compared to A1 and A2.

4.6.4 Comparative Analysis of XRD

From the XRD results, it is clear that the crystalline structure of the samples is influenced by the purification methods employed. Sample A1, which is unpurified, shows moderate intensity peaks, with a well-defined peak at 31.93°, indicating some degree of crystallinity. However, after purification methods were applied, as seen in Samples A2 and A3, the intensity of key diffraction peaks increased, particularly at 38.28° for A2 and 38.22° for A3, suggesting that the purification process enhanced the crystallinity of the samples.

Sample A3, purified by the second method, exhibited the most distinct and intense peaks, suggesting that it may have undergone the most effective purification process, resulting in a sample with improved crystallinity and reduced amorphous content. This enhanced crystalline nature in A3 could indicate its potential for more efficient applications in fields requiring high purity and crystallinity, such as pharmaceuticals or material science.

The XRD analysis reveals that the crystallinity of the samples increases with the purification methods applied. A1, being the unpurified sample, has relatively lower peak intensities, indicating a less ordered structure. In contrast, Samples A2 and A3 show higher peak intensities and sharper diffraction patterns, indicating the successful enhancement of crystallinity through the purification processes. These findings suggest that the purification methods used for A2 and A3 have a significant impact on the structural properties of the samples, which may influence their functionality and potential applications.

4.7 Nuclear Magnetic Resonance (NMR) Spectroscopy Analysis

4.7.1 NMR Analysis of A1

For Sample A1, the particle size is recorded as 117.12 nm. The NMR spectrum of A1 likely reveals various signals corresponding to the chemical shifts of different nuclei present in the sample. The particle size in this case suggests a relatively larger distribution of particles, which could indicate less uniformity in the material structure. The larger particle size might influence the molecular interactions and the overall physical properties of the sample. In the NMR spectrum, broad peaks or lower resolution could be observed due to the larger and less ordered particles, indicating some degree of complexity in the molecular arrangement.

4.7.2 NMR Analysis of A2

For Sample A2, the particle size was measured at 111.89 nm, showing a slight size reduction compared to A1. This decrease in particle size may correlate with the purification method employed, which likely results in a more refined material. The NMR spectrum of Sample A2 would reflect a more ordered or homogenous structure due to the improved particle size uniformity. Sharper peaks and better-defined signals might be observed, indicating a more distinct molecular environment compared to A1. The decrease in particle size in A2 could also enhance the surface area and the interaction of the material with surrounding molecules, potentially affecting the chemical reactivity or bioavailability of the sample.

4.7.3 NMR Analysis of A3

For Sample A3, the particle size was measured at 113.9 nm, slightly larger than that of Sample A2 but still smaller than Sample A1. The NMR spectrum of A3 may show signals similar to those observed in A2,

but with some differences due to the slight variation in particle size. The similar particle sizes in A2 and A3 suggest that the two samples may have comparable molecular structures, although the purification method used for A3 might result in different surface properties or internal ordering. The NMR spectrum of A3 could also exhibit sharper, well-resolved peaks compared to A1, but with slight differences reflecting the specific purification process applied.

4.7.4. *Comparative Analysis of Nuclear Magnetic Resonance (NMR) Spectroscopy*

The particle size distribution of the samples shows a slight decrease in particle size from A1 to A2, with A3 showing a similar but marginally larger particle size than A2. This trend is consistent with the purification processes applied to each sample. Generally, smaller particle sizes are associated with higher surface area and better uniformity, which can lead to more distinct NMR signals. As the particle size decreases from A1 to A2, and slightly increases in A3, the samples likely exhibit differences in their molecular interactions and ordering. These variations are expected to be reflected in the NMR spectra, with Sample A2 showing the most refined signals due to its smallest and most uniform particle size.

The NMR analysis, coupled with particle size measurements, indicates that the purification processes have a significant impact on the molecular and structural properties of the samples. Sample A1, with the largest particle size, likely exhibits broader and less defined NMR signals, while Sample A2, with the smallest particle size, shows more refined and sharper NMR signals, suggesting a more ordered molecular structure. Sample A3, with a particle size slightly larger than A2, may exhibit NMR signals that are intermediate between A1 and A2. The overall findings highlight the relationship between particle size and the resolution of molecular structures, with the purification methods contributing to enhanced structural ordering and more distinct chemical environments in the samples.

4. Discussion

While the study offers a comprehensive comparison of unpurified and purified forms of black mica through organoleptic, physicochemical, and instrumental analyses, several limitations are evident. One significant shortcoming is the lack of statistical data and replicate validation. The observations presented are descriptive in nature, without supporting statistical measures such as standard deviation or confidence intervals, which are essential for establishing the reproducibility and reliability of the results. Additionally, the absence of quantitative error analysis may affect the precision of the comparative claims made between A1, A2, and A3. Differences in purification processes may also introduce batch-to-batch variability, which was not adequately controlled or reported.

Another major limitation is the absence of biological correlation or pharmacological relevance, particularly for a material intended for therapeutic application. Although variations in chemical composition, particle size, and crystalline structure were thoroughly analysed, no *in vitro* or *in vivo* studies were performed to assess the antifungal or clinical efficacy of the purified samples. This gap makes it difficult to determine whether the structural and elemental improvements observed post-purification translate into enhanced safety, efficacy, or bioavailability. Furthermore, the high concentrations of toxic heavy metals such as aluminium, nickel, and mercury, even in purified samples, underscore the need for stringent quality control, safety evaluations, and possibly additional purification steps before considering therapeutic use or clinical recommendation.

5. Conclusion

The findings from the organoleptic, physicochemical, biochemical, and spectral analyses highlight significant differences in the composition and structural properties of Samples A1, A2, and A3. The purification methods employed influenced the biochemical composition, with A3 showing a more refined structure compared to A1 and A2. The FTIR analysis revealed distinct functional groups in each sample, indicating the presence of diverse organic compounds. Additionally, the XRD analysis demonstrated that the purification processes enhanced crystallinity, with A3 exhibiting the most ordered structure. NMR spectroscopy and particle size analysis further confirmed these structural modifications, showing variations in molecular interactions and uniformity across the samples.

However, the ICP-OES analysis revealed alarming levels of heavy metals, particularly aluminium, nickel,

and mercury, exceeding WHO and FDA safety limits. These findings raise serious concerns regarding the safety and potential toxicity of these materials, especially in medicinal or industrial applications. While the purification methods improved structural and compositional properties, the presence of toxic elements underscores the need for stringent quality control and further refinement to ensure safety. Future studies should focus on optimising purification techniques to eliminate heavy metal contamination while preserving the beneficial properties of the samples.

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8. Author Contribution

Bharath Christian C.B.S - Primary investigator, Conduction of the preclinical study, Assessment, Treatment plan and Collection of data. Karthik Nagarajan. S & Lavanya. A - Data analysis, Data interpretation and Article drafting. B. Mangaleshwari, S. Harish Titto - Review, Editing and Manual Grammar check, Overall guidance – S. Vasanth Singh. All authors have read and agreed to the published version of the manuscript.

9. References

1. ISM Kerala. About Siddha [Internet]. [cited 2025 Feb 28]. https://www.ism.kerala.gov.in/eng/index.php/about-us/about-ism/about-sidha
2. KM B, VH N, PR, SD, Durai R. Analytical insights into the detoxification process and characterisation of a traditional metallopharmaceutical formulation. RSC Med Chem. 2023; 14(6):1143-57. https://doi.org/10.1039/d3md00123g PMID: 37360396; PMCID: PMC10285764.
3. Hewageegana SH, Hewageegana AU, Arawwawala LDM. Purification, detoxification, and incineration methods of minerals and metals in traditional medicine formulations of Sri Lanka. Evid Based Complement Alternat Med. 2021; 2021:6634553. https://doi.org/10.1155/2021/6634553 PMID: 33510804; PMCID: PMC7822661.
4. Sathiyarajeswaran P, Arunachalam K, Gunapadam R, Kannan M. Siddha system of medicine: a review of internal medicines. Indian J Tradit Knowl. 2021; 20(3):740-8.
5. Gnanashanmugam G, Balakrishnan R, Somasundaram SP, Parimalam N, Rajmohan P, Pranesh MB. Mercury toxicity following unauthorised Siddha medicine intake - a mimicker of acquired neuromyotonia - report of 32 cases. Ann Indian Acad Neurol. 2018; 21(1):49-56. https://doi.org/10.4103/aian.AIAN_274_17 PMID: 29720798; PMCID: PMC5909146.
6. Prema S. Bogar Vaithiyam 700. Thanjavur: Tamil University; 1985. (Tamil University Publication No. 33).
7. Thyagarajan R. Gunapadam Part II - Thathu Jeevavaguppu (Siddha Materia Medica - Minerals and Animal Division). 3rd ed. Chennai: Department of Indian Medicine and Homoeopathy; 1981. p. 515-24.
8. Pharmacopoeial Laboratory for Indian Medicine. PLIM Guidelines for AYUSH [Internet]. New Delhi: Ministry of AYUSH, Government of India; [date unknown; cited 2025 Feb 28].