Journal of Ayurveda and Integrative Medicine 14 (2023) 100751

Contents lists available at ScienceDirect

Journal of Ayurveda and Integrative Medicine

journal homepage: http://elsevier.com/locate/jaim

Original Research Article

Comparision of traditional and laboratory methods of sulphur processing

T.A.N.R. Gunaratna [a], P.K. Prajapati [b], K.M. Nalin de Silva [c], W. Rohini M. de Silva [c, *]

[a] Faculty of Graduate Studies, University of Colombo, Colombo, 00300, Sri Lanka
[b] Department of Rasashastra and Bhaishajyakalpana, All India Institute of Ayurveda, University of Delhi, New Delhi, India
[c] Centre for Advanced Materials and Devices (CAMD), Department of Chemistry, Faculty of Science, University of Colombo, Colombo, 00300, Sri Lanka

A R T I C L E I N F O

Article history:
Received 2 October 2020
Received in revised form
28 November 2022
Accepted 14 June 2023
Available online xxx

Keywords:
Sulphur
Ayurveda
Shodhana
Purification
Eclipta alba (L.) Hassak

A B S T R A C T

Background: Since ancient times, the essential element sulphur has played an important role in different medical fields. It is one of the main materials used in herbo-mineral pharmaceutics in Ayurveda. However, for Ayurvedic pharmaceutical preparations, the purity of sulphur is crucial in avoiding any harmful reactions and to enhance the medicinal quality. Therefore, it is subjected to a process called *'gandhaka shodhana'* using cow's milk, ghee or occasionally plant extracts. The plant, *Eclipta alba* (L.) Hassak, containing many bioactive compounds, is one of the extracts known to be used in the *'shodhana'* process of sulphur. However, in comparison to the laboratory purification method of sulphur neither the effect of this *'shodhana'* process in removing impurities from sulphur nor its effect on the structure and morphology of sulphur has been evaluated.

Objectives: This study identifies physical, morphological, and structural changes that occur in sulphur when it is subjected to the *'shodhana'* process compared to the changes that occur in sulphur obtained after simple laboratory purification.

Methodology: Both samples were characterized using Scanning Electron Microscopy, Energy Dispersive X-ray Spectroscopy, X-ray Diffraction, Differential Scanning Calorimetry, Thermogravimetric Analysis, Fourier Transform Infrared spectroscopy, and Raman spectroscopy. Observed physical changes such as colour, allotropic form, odour, hardness, transparency, and lustre of the samples were also determined using recommended techniques.

Results: Although the laboratory purification method separates the sulphur from physical and chemical impurities, Ayurveda *'shodhana'* process with *E. alba* converts the sulphur into a more pharmaceutically suitable form by making it more nebulous and introducing higher brittleness, FT-IR data shows removal of chemical impurities from sulphur during *'shodhana'* process in contrast to laboratory purified sample.

1. Introduction

Sulphur was used as a pharmaceutical material by ancient people around the world, owing to its wide spectrum of chemical and biological properties. In iatrochemistry *(rasashastra)* of the Ayurveda medical system, sulphur has been described as *gandhaka* and it has been classified as the second most useable material for preparing *rasa* medicines as it is considered the main material under *uparasa* in *rasashastra* classification [1]. However, in order to eliminate impurities from sulphur that are harmful to humans, it is important to cleanse it or subject it to preparations. In this regard, in Ayurveda, before pharmaceutical preparations, sulphur is exposed to a *'shodhana'* procedure [2] and there are multiple methods of *'shodhana'* as described in different classics [3]. The *'shodhana'* process described in Ayurveda is not merely a purification step, but also a mechanism of transforming the physical structure of a material into a drug by aiding it to adsorb organic and inorganic substances [4]. In the Indian context, the *'shodhana'* process with ghee and cow's milk is the most common method performed for sulphur. In the Sri Lankan context,

* Corresponding author.
E-mail addresses: nirashagunaratna@gmail.com, nirashag@kln.ac.lk (T.A.N.R. Gunaratna), prajapati.pradeep1@gmail.com (P.K. Prajapati), kmnd@chem.cmb.ac.lk (K.M.N. de Silva), rohini@chem.cmb.ac.lk (W.R.M. de Silva).

Peer review under responsibility of Transdisciplinary University, Bangalore.

https://doi.org/10.1016/j.jaim.2023.100751

there are several methods adopted by the herbo-mineral pharmaceutical manufacturers for the *'shodhana'* process of sulphur [5]. Apart from that *bringaraja* in Sanskrit (false daisy and *keekirindiya* in Sinhala) or *Eclipta alba* (L.) Hassak [6] of the Asteraceae family [7] (Synonym: *Eclipta prostrata* Linn.) [8] is also used for the *'shodhana'* process of sulphur [5]. In addition, some steps of the *'shodhana'* process have been modified with the knowledge of Sri Lankan traditional medical system known as *desheeya cikitsa* [9]. For instance, the *'shodhana'* process of sulphur with fresh *E. alba* plant extract. During this process, the Sri Lankan method uses *swedaniyantra* [10] or *vashpaswedana yantra* instead of *dolayantra* for the completion of the second step of the process [9]. This is a modified step which is merged with the term *'swedana'*, and the process used to perform *'swedana'* known as *'kadapimme tembima'*. When comparing with *dolayantra* method, this modified step can perform the *'swedana'* process for bulk amounts of sulphur time and cost effectively. This could be the main reasons for using this merged step of Ayurveda and Sri Lankan traditional medical systems in Sri Lankan Ayurveda pharmaceutical industry. Nonetheless, there are no reported studies that have been carried out to identify the physical, morphological, structural characteristics of this *'shodhana'* method. Moreover, laboratory purification of sulphur has been the focal point of sulphur research for many years [11] and no work has been carried out to compare laboratory sulphur purification with *'gandhaka shodhana'*. Hence, this study was carried out to identify and evaluate the physical, morphological, and structural changes of sulphur when subjected to the *'shodhana'* process in Ayurveda with fresh *E. alba* plant extract [5], as performed in Sri Lankan Ayurveda pharmaceutical industry by replacing the *dolayantra* method in the second step. At the same time these changes were compared with purified sulphur obtained using a laboratory purification method. For this purpose, sulphur was dissolved in xylene [12], as this medium was identified as less toxic and appropriate compared to other organic solvents [13]. The findings are useful to Sri Lankan manufactures for optimization or alteration of their *'gandhaka shodhana'* processes. The outcomes of this study will be beneficial to the international Ayurveda community to compare their findings and carry out further research.

2. Materials and methods

Amalasara gandhaka which is available in yellow crystal form is considered suitable for medicinal purposes [3]. Since *gandhaka* and most of the other *rasa* materials are not available in Sri Lanka, they are imported mainly from India. Hence, after careful market survey, crude sulphur *(amalasara gandhaka)* was bought from a local Ayurveda material supplier, Karunarathna and Company in Mulleriyawa Fresh *E. alba* plants were collected from a non-polluted area in Gampaha district of Western province. This plant was authenticated by the National Herbarium, Royal Botanical Gardensin Peradeniya, Sri Lanka. Xylene AR (99.5%, Research Lab-India) was purchased from Organic Trading (Pvt) Ltd, Sri Lanka. Experiments were carried out according to the reference Ayurveda and laboratory methods [12]. Ayurveda *'gandhaka shodhana'* concept was adopted from the *'shodhana'* processes mentioned in *Rasa Jala Nidhi* textbook (volume II) of *rasashastra* literature under the sulphur section (second process) [5] as it has been used by Sri Lankan Ayurveda *rasa* medicine manufacturers [9]. The adopted method consists of three steps: *dhalana* as the first step, *swedana* as the second step and once again *dhalana* as the final step. According to the *Rasa Jala Nidhi*, second step should be carried out in *dolayantra* [5] for a moment, but in Sri Lankan context this step is performed [9] in *swedaniyantra* [14] or *vashpaswedana yantra* [15]. Therefore, in this research *swedaniyantra* was used instead of *dolayantra*. Mild flame was also provided for a time period of 3h(one *yama*) to an appropriate environment inside the steamer and increase the reaction rate of *E. alba* with sulphur. In addition, ghee was not added. Generally, ghee is used in most of the sulphur *'shodhana'* procedures to avoid the overheating while melting and it also smeared on cloth to facilitate filteration without adhering to the cloth. As this was not mentioned in the *Rasa Jala Nidhi* Sanskrit verse [5], it was not done in the first and final steps. Further, in order to determine the accuracy of the procedure, total sulphur amount taken in each step was divided into equal portions such as 10 portions in the first step, 2 portions in the second step and 8 portions in the final step and the same procedure was carried out for all the samples to obtain purified sulphur. Although the division of the samples in each step was not uniform, uniform conditions were maintained at every step. Each step was completed on the same day as that of collection of *E.alba* plant material and preparation of extract according to the concept of *swarasa* [16]. The mechanical extraction of each batch of *E. alba* extract (1kg) with support of industrial bench top was done by adding 50.00 mL of distilled water for blending. Each of this sample was characterised to see if they had any similarities. After completion of each step, the samples were labelled and stored in airtight polypropylene containers. Additionally, laboratory purification of sulphur was also performed using xylene as the solvent. Sample containers were kept at the university laboratory until further analyses.

2.1. *'Gandhaka shodhana'*

Initially, fresh *E. alba* plants were collected, physical impurities removed and washed thoroughly with water followed by washing with distilled water two times (5 L x 2). Cleaned *E. alba plants* were cut into small pieces and blended using an industrial bench top blender (10,000 rpm). The extract was squeezed through a clean cotton cloth into a stainless-steel vessel (Fig. 1). For this mechanical extraction, 1.00 kg of cleaned *E. alba* were blended for 5 min with 50.00 mL of distilled water and 749.00 mL of *E. alba* extract was obtained. Crude sulphur (5 kg) was crushed using an iron mortar and pestle and divided into portions weighing 500.00 g each. *E. alba* plant extract (1 L of extract for each vessel) was placed in ten separate stainless-steel vessels (vessel diameter- 12 inches and depth- 10 inches) and mouth of each vessel was closed with a clean cotton cloth and tied using a twine thread. Afterwards, each 500.00 g samples of powdered sulphur were heated under mild flame (using separate stainless-steel vessel) with continuous stirring until the sulphur completely melted. The molten sulphur sample was then poured through the cotton cloth into the vessel containing 1 L of plant extract. This was cooled for 10 min. The solidified sulphur was washed three times using 1.5 L of distilled water (room temperature) and then dried in a dryer at 27 °C (Fig. 2). Same procedure was repeated for the remaining nine sulphur portions.

The second step, *'shodhana'* using the steam of the plant extract, was carried out for the sulphur obtained from the previous step. For this purpose, 3 L of fresh *E. alba* extract was placed in a stainless-steel steamer (10 L capacity). Then the mouth of the steamer was covered with a cotton cloth and tied to keep it in place. A second steamer pot was also prepared in the same manner. Dried sulphur (2.28 kg for each pot) obtained from the first step was placed on top of the cotton cloth and covered with a lid. This was subjected to steam under mild flame for 3 h followed by cooling down to room temperature (Fig. 3). Finally, steamed sulphur solids were dried in a dryer at 27 °C (Fig. 4). The final step of the *'gandhaka shodhana'* was performed in the same way as the first step. For this purpose, 1 L of *E. alba* extract was used for each 564.5 ± 0.00 g of steamed sulphur portion. After the completion of final step, solidified sulphur granules were washed and dried in the dryer at 27 °C. This was then cooled down for 10 mins.

Fig. 1. Preparation of *E. alba* extract (a) cleaned plant (b) extract *of E.alba* (c) vessel with extract.

Fig. 2. First step of *'gandhaka shodhana'* (a) crude sulphur (b) melted sulphur powder (c) melted sulphur poured into the prepared vessel.

Fig. 3. Second step of *'gandhaka shodhana'* (a) preparation of the vessels with sulphur obtained after first step (b) sulphur placed in steamer for 3 h (c) steamed sulphur.

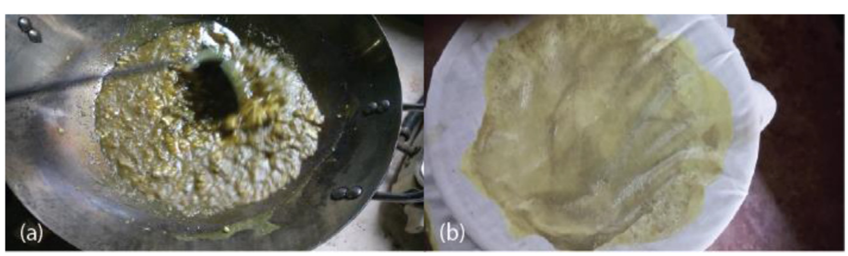

Fig. 4. Final step of *'gandhaka shodhana'* (a) sulphur obtained after second step melted (b) melted sulphur poured into prepared vessel

2.2. Laboratory sulphur purification

Recrystallization of the crude sulphur was adopted for the traditional laboratory purification method [12]. A 40.00 g portion of crude powdered sulphur was heated with 400 mL xylene under continuous stirring until the sulphur was completely dissolved. The mixture was then filtered through a clean cotton cloth and cooled slowly to room temperature allowing the formation of crystals. The resultant crystals were separated using filtration and dried at 27 °C (Fig. 5).

2.3. Characterization methods of sulphur

Sulphur samples, obtained from *'gandhaka shodhana'* procedure and laboratory recrystallization procedure along with un-purified crude sulphur, were analysed initially by observing physical parameters such as, colour, phase, allotropic form, odour, solubility, hardness, transparency, and lustre. The detailed surface morphology of sulphur samples was characterised under different magnification using Scanning Electron Microscopy (SEM, Hitachi SU6600). The relative chemical abundance was analysed using energy-dispersive X-ray spectroscopy (EDS, Hitachi SU6600 SEM). Internal structures of prepared sulphur samples were analysed using X-ray diffraction patterns (XRD) on Rigaku SmartLab SE X-ray powder diffractometer supplied with Cu Kα radiation (=0.154 nm) over a 2 theta range of 0−80, with a step size of 0.02 and a step time of 1s. Thermal degradation was determined using Differential Scanning Calorimetry (DSC) analysis for each sample using a Netzsch DSC 204F1 Phoenix instrument. Samples (12−15 mg) were loaded into Alumina crucibles with pierced lid and heated from 30 °C to 300 °C at 10 °C/min under a flow of nitrogen (60 mL/min). Thermogravimetric studies (TGA) were performed in PerkinElmer

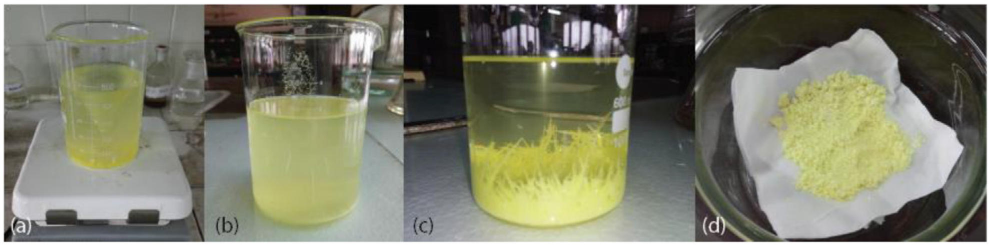

Fig. 5. Sulphur laboratory purification (a) crude sulphur dissolved in xylene (b) filtered solution (c) recrystallization of sulphur (d) dried purified sulphur.

Thermal Analysis. Samples (7–10 mg) were scanned from 30 °C to 300 °C at a heating rate of 10 °C/min in nitrogen environment. Infrared spectra of the samples were recorded by Attenuated Total Reflection Fourier Transformed Spectroscopy technique (ATR-FTIR), using a Spectrum Two between 4500 cm^{-1} and 500 cm^{-1} range. Raman spectra were recorded on a Bruker Senterra Raman microscope spectrophotometer under the magnification 10x and laser of wavelength 532 nm was used as the excitation source with an average power of 10 mW.

2.4. Analysis of data

Origin software was used for the relevant areas and Mean ± SD was calculated at each step in *'gandhaka shodhana'* with multiple portions (10 portions in first step, 2 portions in second step and 8 portions in final step) to maintain uniform conditions within the each step.

3. Results and discussion

3.1. Sample preparation

The first step of the *'gandhaka shodhana'* method is known as the melting and pouring *(dhalana)* [17] process in which liquefied sulphur was poured into a fresh herbal extract through a clean cloth. During the melting, mild heat was used, resulting in the complete melting of each sulphur sample within 5 mins. The freshly prepared *E. alba* extract was dark bluish green at the beginning and the colour changed to dark brownish green after the process. During the drying, there was a colour transformation

Table 1
Summary of the *'gandhaka shodhana'* process with one portion of sulphur taken for each step.

Step	Av. weight of sulphur taken in each step (initial) (g)	Temp. (°C) inside the vessel	Temp. (°C) of the media		Av. weight of sulphur (final) in each step	
			Before	After (mean)	Av. weight after the step completed (g)	Analysis amount (g)
First	500.00 ± 0.00	115.3 ± 0.6	28	60.7 ± 2.64	483.30 ± 0.90	50.0
Second	2280.00 ± 0.00	–	28	90.0 ± 0.00	2270.0 ± 0.03	50.0
Final	564.50 ± 0.00	117.4 ± 0.6	28	60.5 ± 0.64	520.50 ± 0.70	50.0

* values were expressed as mean ± SD (first step n-10, second step n-2 and third step n-8).

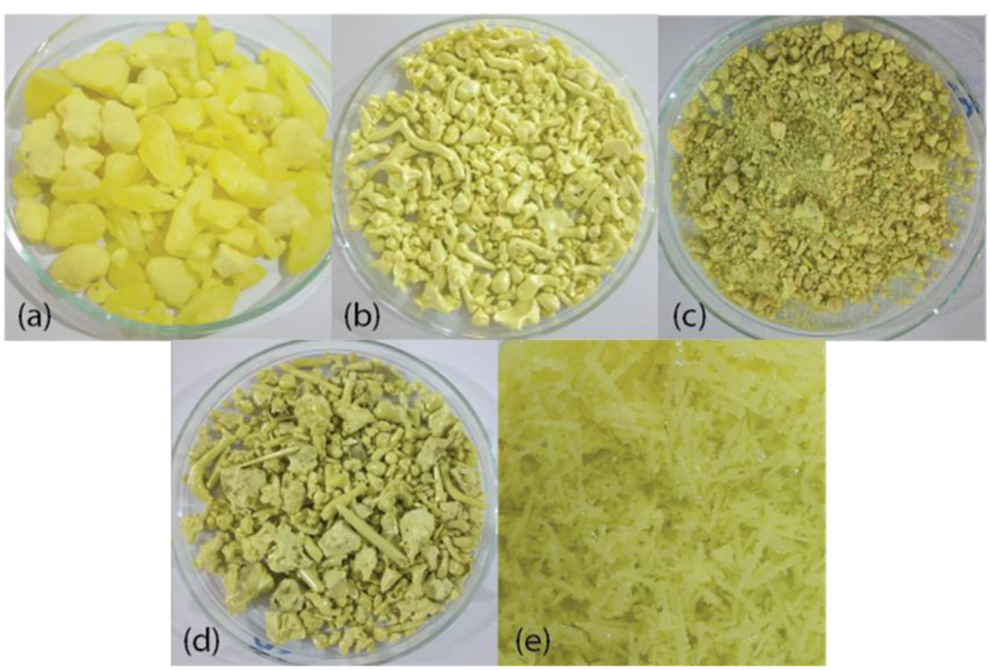

Fig. 6. Appearances of sulphur samples (a) crude sulphur (b) sulphur obtained after 1st step (c) sulphur obtained after 2nd step (d) sulphur obtained after shodhana process (e) sulphur obtained fater purification by xylene.

Table 2
Summary of the single purification process of sulphur with xylene.

Description	Information related to description
Weight of crude sulphur taken for the process	40.00 g
Volume of Xylene taken for the process	400 mL
Inside temperature at the beginning	27 °C
Time taken for total dissolving procedure	75 min
Inside temperature at the end	88 °C
Time taken for recrystallization	120 min
Inside temperature at the end of recrystallization	27 °C
Final volume of xylene after separating the recrystallized xylene	396 mL
Weight of the recrystallized sulphur	30.27 g
Weight loss of sulphur during the process	9.73 g

in the samples from dark yellow to light yellow. The average yield of sulphur portions taken from the first step was 483.3 ± 0.9 g and average wastage was 16.7 ± 0.9 g. Physical impurities were visible on the filter cloth i.e. stones after pouring into the vessel. Further, there was an orange-black glue-like residue on the melting vessel that gets converted to a rubber like structure (plastic sulphur). There was a very small amount of this residue in the concave stainless-steel vessel, despite the temperature remaining at 115.3 °C. This could have been caused by the bottom of the vessel being in contact with the controlled flame. This portion was not collected. The second step was a steaming *(swedana)* process with the *E. alba* extract. At the end of this step ,the colour of the sulphur samples changed slightly to brownish yellow. The temperature inside the steamer could not be monitored as the process occurred in a closed system, but the external temperature varied between 88 °C and 90 °C. The average yield obtained was 2.27 ± 0.03 kg and the average wastage was found to be 0.01 ± 0 g. During this step there was no considerable change in the average yield of sulphur compared to the first step as this stage does not involve any transferring steps. Additionally, this steaming procedure converts dried sulphur samples to a moist texture, as a result, some of the sulphur particles adhere to the cloth. The final step was a melting and solidification step similar to the first step and the time taken for melting of 564.5 g was 7 min. The average yield in this step was 520.5 ± 0.70 g and the average wastage was 44 ± 0.70 g. Overall observations in each step of the procedure are tabulated in Table 1, 3.

The reduction in the weight of sulphur (Table 1) was mainly due to wastages during heating, liquefying and pouring procedures. The yield of sulphur from the first, second and third steps were 92.32%, 99.64% and 92.55% respectively. It is clear that the main loss of the weight occurred at the first step. This was mainly due to the separation of sulphur from the physical impurities such as sand, stones [5] and mud as shown in Fig. 2c along with the elimination of other bonded volatile, impurities from sulphur samples [18]. When comparing the wastage of first step with the wastage of final step, slightly higher wastage was observed in the final step. During the separation of resolidified sample from the *E. alba* extract, fine sulphur particles remained within the extract and they were observed again during the washing and drying of sulphur obtained

Table 3
Physical changes in sulphur samples.

Physical characteristics	Crude sulphur	*'Gandhaka shodhana'* method			Laboratory method
		1st step sulphur	2nd step sulphur	Final step sulphur	
Colour	Lemon yellow	Greenish yellow	Brownish yellow	Brown-green mixed yellow	Bright lemon yellow
Phase	Solid	Solid	Solid	Solid	Solid
Allotropic form	Crystalline lumps	Granular aggregates	Granular aggregates	Granular aggregates	Crystalline particles
Odour	Typical sulphur odour	Characteristic	Characteristic	Characteristic	Xylene odour
Solubility in water	Not soluble	Not soluble	Not soluble	Not soluble	Not soluble
Hardness	Brittle +	Brittle ++	Brittle ++	Brittle +++	Brittle +++
Transparency	Translucent	Opaque	Opaque	Opaque	Translucent
Lustre	Resinous	Pearly	Dull	Pearly	Vitreous

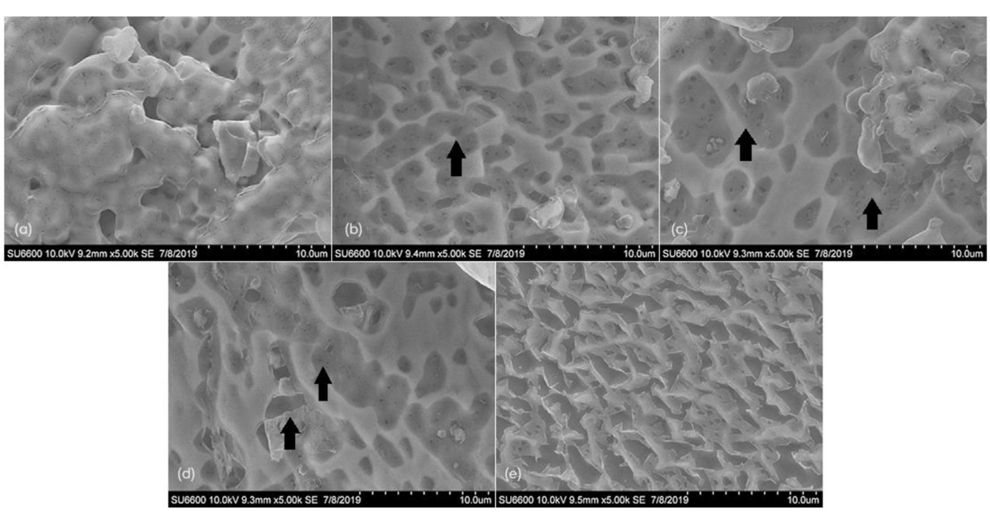

Fig. 7. SEM images of sulphur samples related to *'gandhaka shodhana'* with *E. alba* and laboratory purification with xylene (a) crude sulphur (b) sulphur obtained after 1st step(c) sulphur obtained after 2nd step (d) 'sulphur obtained after 'shodhana' process (e) sulphur obtained after purification by xylene.

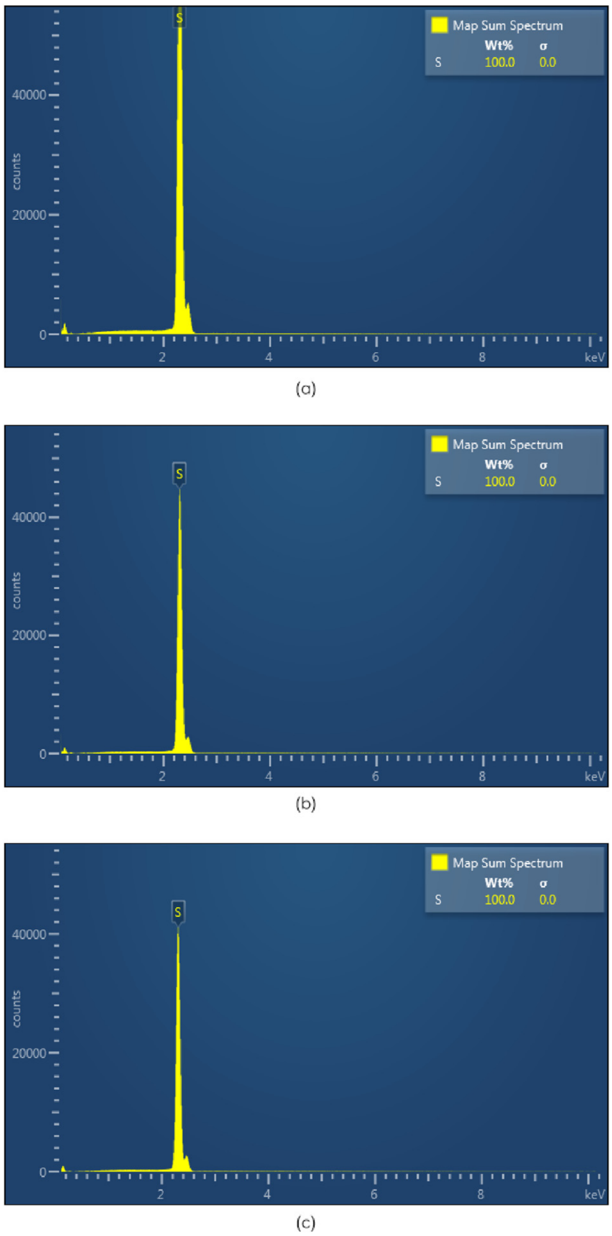

Fig. 8. EDS of sulphur samples (a) crude sulphur (b) sulphur obtained after 'shodhana process' (c) purified sulphur by xylene.

after *'shodhana'* process. This is mainly due to the enhancement of sulphur fragility with the *'shodhana'* process [19]. Enrichment of the sulphur sample with plant materials was evident from the brownish colour patch on the cotton cloth after filtration and this is supposed to be beneficial for further processes [20].

On the other hand, in the laboratory purification of sulphur with xylene, sulphur was deposited on the bed of the beaker at the beginning and with increasing temperature, sulphur started to dissolve in xylene as shown in Fig. 5b. After the sulphur completely dissolveed the colour of the xylene turns slightly yellow. When the mixture is filtered through a clean cotton cloth, dark coloured solid particles with some residual sulphur were observed over the cotton filter cloth. The recrystallization of sulphur started at the the base of the beaker, and it spread upwards. Recrystallized sulphur appeared as shiny crystals. Despite the low level of impurities on the filter cloth, 9.73 g of waste was generated. This is likely due to some sulphur remaining in the xylene used for the procedure.The evidence for this was found in the colour of the used xylene and in the occurrence of sulphur crystals over time. Observations are tabulated in Table 2, 3.

3.2. Physical analysis

The physical changes such as colour, allotropic form, odour, hardness, transparency and lustre of the crude sulphur, first step completed sulphur, second step completed sulphur, third step completed sulphur by *E. alba* and laboratory purified sulphur with xylene are given in Table 3 and Fig. 6.

The initial lemon colour of sulphur was converted to a greenish yellow hue during the first step due to the stable bluish green dye of the *E. alba* plant extract. This colour further changed into a brownish yellow and a brown-green mixed yellow after second and third stages of *'gandhaka shodhana'* respectively. This implies that there are physical and chemical interactions between sulphur and functional groups present in *E. alba* plant extract which caused the sulphur to obtain a more suitable texture as a pharmaceutical material. In the laboratory sulphur purification method, there was no visual colour change as the xylene only acts as a solvent to dissolve sulphur and there is no other interaction. Mohs hardness range of crude sulphur is 1.5–2.5 [21] and therefore hardness of each sample was tested using fingernail and found that brittleness of samples increased with the *'shodhana'* steps. This could be due to the *dhalana* procedure during the *'shodhana'*. Also, crude sulphur's lustre became pearly with the first step of the *'shodhana'* process, but then became dull with the *'swedana'* stage. However, upon

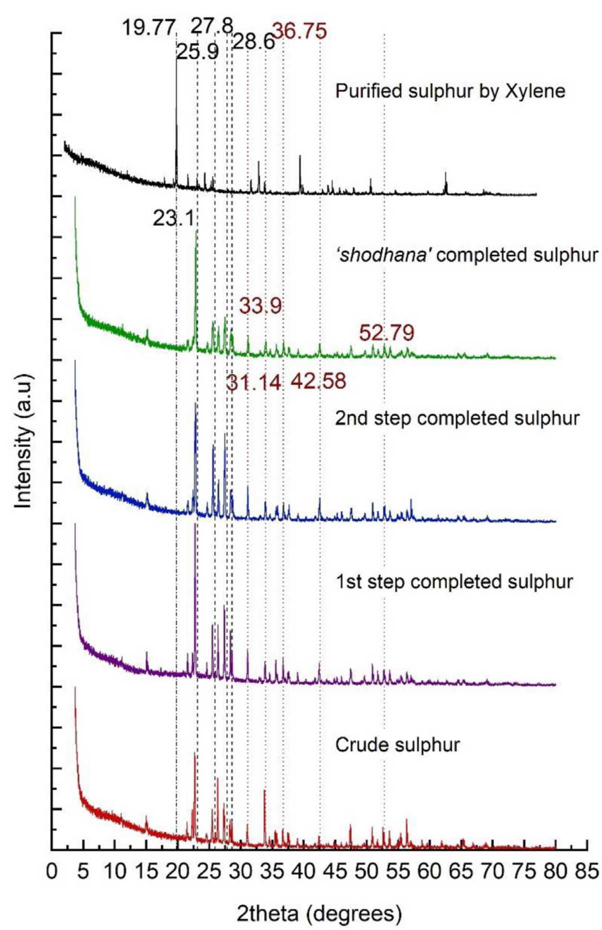

Fig. 9. XRD patterns of sulphur samples.

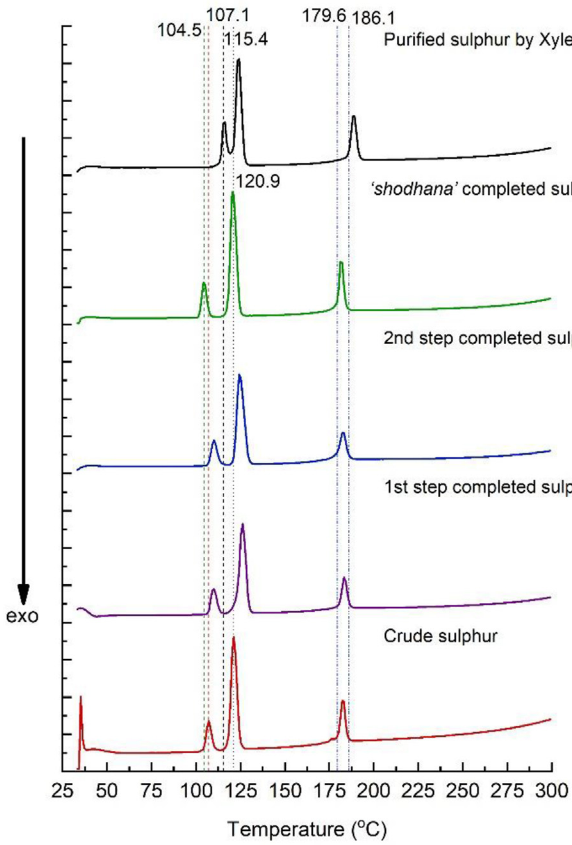

Fig. 10. DSC thermograms of sulphur samples.

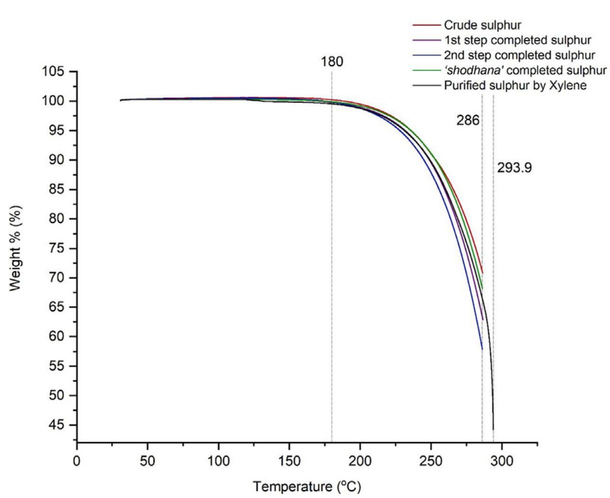

Fig. 11. TGA curves of sulphur samples.

completion of the *'shodhana'* process, sulphur regained its pearly appearance as the final step also engaged with the *dhalana* process. On the other hand, laboratory purified sulphur showed vitreous lustre with its recrystallization process. As a simple molecular structure, sulphur atoms combine with covalent bonds to form molecules which are held together by intermolecular bonds [22] to form crystalline sulphur that consists of puckered S_8 rings in the shape of crowns [23]. During the heating process in the first step of Ayurveda method, crude sulphur melts at 115.3 ± 0.62 °C breaking down the intermolecular bonds and to form a mobile, amber liquid containing S_8 rings. Usually this liquid contains S_8 orthorhombic and monoclinic [24] sulphur forms. During this process sulphur comes in contact with plant extract and, the physical and chemical interactions with the natural products in the plant extract, cause the sulphur to distort from its original forms by forming aggregated granules as shown in Fig. 6 (b, c and d). After the completion of this step, sulphur was dried under room temperature and this process changes the amorphous sulphur to orthorhombic form (supported by XRD data) and during this transition, the solid sulphur dries into a more brittle nature by the formation of many cracks [24]. All other characteristic physical changes such as transparency and lustre exhibited by sulphur samples can also be attributed to this Ayurveda *'shodhana'* method of sulphur. On the other hand, in the laboratory purification method, crude sulphur was dissolved in xylene at 88 °C and the recrystallization of sulphur occurs at room temperature under gradual cooling. During this process, the stable rhombic form is formed below 96 °C [25] with better transparency and lustre of pure crystalline sulphur [26]. The differences of physical allotropic form, size, and shape of the samples in *'shodhana'* process steps and recrystalized sulphur are mainly due to the differences in the steps. There were different sizes and shapes of granular sulphur particles in the samples of *'shodhana'* process steps and this was due to the filtration through the fabric during the first and final step of *'shodhana'* process. This was further augmented with the conjugation of melted sulphur with resolidified sulphur. On the other hand, size, and shape of the recrystallized sulphur during the laboratory purification also varied as that recrystallization formed chained sulphur crystals.

3.3. Scanning Electron Microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDS) analysis

SEM images obtained for different stages give morphological information during the *'shodhana'* and laboratory purification processes (Fig. 7). Samples showed an irregular surface. The porous structure of the sulphur was observed to have increased with the thermal changes during the steps involved in Ayurveda procedure. The porosity of laboratory purified sulphur had also increased giving regularly arranged small pores with good crystallinity even after a single step. Nevertheless, careful observation of SEM images of obtained sulphur through Ayurveda *'shodhana'* process clearly reveal its increased porous nature going from first step (Fig. 7b) to the second step (Fig. 7c) and to the third step (Fig. 7d) compared to the crude sulphur sample (Fig. 7a). According to the images, sulphur obtained after the third step shows very large interconnected pores [27] this can be credited to its high brittleness as observed in Table 3 [28]. This brittleness and porosity is very important to enhance its suitability as a pharmaceutical material [29]. Further, EDS of each sample showed the presence of sulphur as the only significant or major chemical element (100 wt%) within the ppm level (minimum is mass fraction of C = 0.0001 or 100 ppm) [30] according to the mass concentration (Fig. 8). Despite the fact that the other elements could have been present in the crude sulphur or in the *'shodhana'* process involved and in laboratory purified sulphur samples, their detectability was far below the practical limit for EDS of bulk specimens [30]. Hence, the EDS related to the sulphur samples showed only sulphur (100 wt%) as the major element.

3.4. X-ray diffraction (XRD) analysis

The powder XRD data indicates the nature of the internal structure (crystal character) of a substance. All sulphur samples were subjected to XRD studies and XRD patterns obtained are given in Fig. 9. The orthorhombic form (alpha sulphur) was present in all samples by the typical characteristic diffraction peaks at $2\theta = 23.1°$, 25.9°, 27.8° and 28.6° and it is in agreement with the international centre for diffraction data, mineral database and previous sulphur diffraction data [27,31]. There were peaks related to the monoclinic form [28] (beta sulphur) (between 2θ, 20°−40°) as well. This can be attributed to the rapid heat changes in the Ayurveda procedure and the lower intensity of these peaks are related to their instability at room temperature [28]. The most important observation of this study is the change of crystalline nature from crude sulphur to *'shodhana'* process completed sulphur. As the *'shodhana'* steps proceed, sulphur turns more nebulous agreeing with both SEM and brittleness observations. This nebulous nature of sulphur is more suitable for pharmaceutical preparations as it can easily blended and homogenized with other ingredients. In contrast, laboratory purified sulphur clearly highlights a diffraction peak at $2\theta = 19.77°$ when comparing with the *'shodhana'* process steps. This could be due to the recrystallization process using xylene as it induces high crystallinity of sulphur. With this highly crystalline nature (high rigidity) and extremely sharp peaks, it shows that laboratory purified sulphur is inappropriate for pharmaceutics.

3.5. Differential Scanning Calorimetry (DSC) analysis

All sulphur samples shows three transitions. As given in Fig. 10, at the beginning sulphur undergoes solid–solid transition from orthorombic crystal to monoclinic crystal observed at at 107.1 °C in crude sulphur which decreased to 104.5 °C in the final purified sampleafter undergoing three *'shodhana'* steps. However, in the laboratory purified sulphur the temperature increased to 113.0 °C. With further heating, all the suphur samples undergoes melting at ~120.9 °C. Third endothermic step happened at 182.7 °C for crude sulphur and it slightly dropped to ~178.1 °C for sulphur samples after *'shodhana'* process and increased to 186.1 °C for laboratory purified sulphur sample. In general sulphur undergoes ring

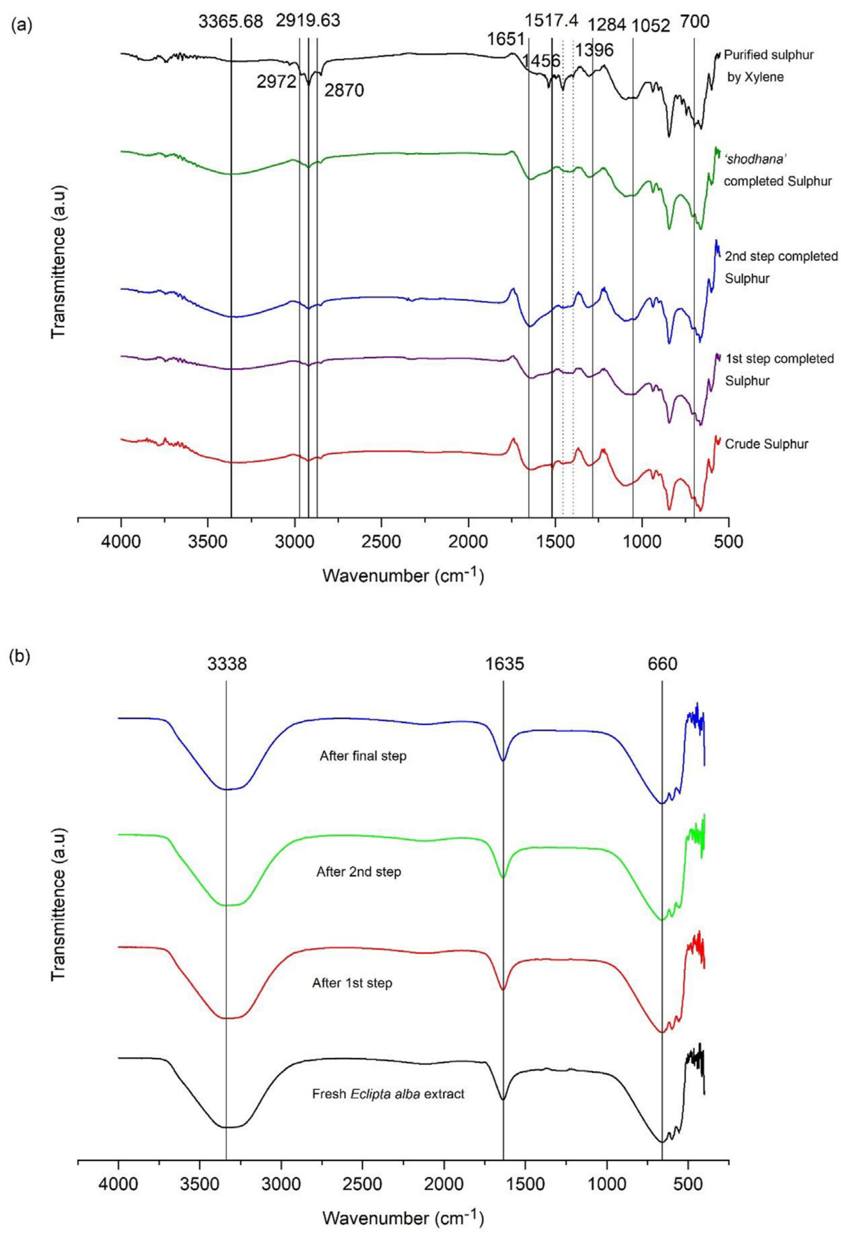

Fig. 12. (a) FT-IR of sulphur samples taken after the completion of each step in *'gandhaka shodhana'* and laboratory method (b) FT-IR of *E. alba* extract.

opening polymerization with the heating as discussed in the previous reports [32]. According to Jena et al., elemental sulphur exhibits rich allotropy: S2, S3, S4 ….Sx and most stable form is eight membered ring which is known as cyclooctasulphur or S8. This type forms orthorhombic or alpha sulphur at room temperature and pressure. When it is heated, it undergoes several transitions; first solid to solid transition from orthorhombic to monoclinic or beta sulphur at ~95 °C and secondly, with further heating, solid liquid transition at ~115 °C upon further heating, sulphur undergoes free radical ring opening at ~159 °C and above. This polymeric form of sulphur is not stable at room temperature and pressure, therefore it reverts to S8 form [33]. During the *'shodhana'* and purification procedures of this research, the temperature did not exceed ~120 °C and therefore DSC was only limited to 0–300 °C temperature range (Fig. 10).

3.6. Thermo-gravimetric analysis (TGA) analysis

Thermo-gravimetric analysis was used to determine thermal stability of sulphur and fractions of its volatile components with each *'shodhana'* step in the Ayurveda method and the laboratory purification process with xylene compared to the crude sulphur (Fig. 11). According to the results, all sulphur samples start their degradation around ~180 °C. Major weight loss in all sulphur samples starts at ~286.4 °C. However, all samples do not show the same reduction at this temperature. This is likely due to combination with other compounds during the *'shodhana'* process. Overall, all the sulphur samples decompose thermally in the range of 100 °C–500 °C and this is in agreement with previous studies.

3.7. Fourier transformed infrared spectroscopy (FT-IR) analysis

IR spectra of sulphur samples are shown in Fig. 12a. Crude sulphur shows a weak broad band at 3365.68 cm^{-1} which is related to –OH stretching and later with the *'shodhana'* steps from Ayurveda method, this band becomes much more prominent. This broad band is missing in the laboratory purified sulphur sample and –CH stretching vibrations at 2972 cm^{-1}, 2919.63 cm^{-1} and 2870 cm^{-1} [28] are prominent in this sample. Stretching vibration of –C=O at 1651 cm^{-1} appears in all the samples except in laboratory purified sulphur sample. The most prominent observation is the absence of $-NO_2$ stretching band at 1516.61 cm^{-1} for sulphur samples after the Ayurveda *'shodhana'* process. This could be due to the chelation process [34] caused by the elements such as Fe, Zn available in the *E. alba* extract [35] supported by the removal of nitro compounds. Considering the toxicity issues of nitro compounds in medicines [36], this removal enhances the medicinal quality of the *'shodhana'* process completed *gandhaka.* This band is clearly present for crude and laboratory synthesized sulphur samples. Bands at 1396 cm^{-1} and 1456 cm^{-1} in laboratory purified sulphur can be related to –CH bending vibration [37]. Absorption bands at 1284 cm^{-1} and 1052 cm^{-1} could be assigned to –CO stretching vibrations [37].

Plants produce a wide diversity of secondary metabolites which serve them as defence or signal compounds and therefore they exhibit a wide array of biological and pharmacological properties [38]. This work used *E. alba* which is known to be rich in phenolic and flavonoid [39] compounds and which reflects its ability to act as a detoxifying [40] and chelating agent [34]. Comparison of IR bands of sulphur samples obtained after completion of 'shodhana process' are given in Fig. 12b No differences were seen between the samples confirming the presence of active principles which such as coumestans, alkaloids, flavonoids, glycosides, polyacetylenes, triterpenoids of *E. alba* [41] within the sulphur samples. Additionally, it is also confirmed that the *'shodhana'* process with *E. alba* extract did not facilitate the inclusion of functional groups during the process but did enhance the medicinal properties by modulating the sulphur nature.

3.8. Raman analysis

Raman spectroscopy was used to examine low frequency vibrational modes and homo-nuclear molecular bonds of the sulphur samples. Elemental sulphur could be easily identified by the typical Raman bands at 84 cm^{-1}, 115 cm^{-1}, 150 cm^{-1}, 184 cm^{-1}, 219 cm^{-1}, 245 cm^{-1}, 435 cm^{-1} and 472 cm^{-1} [33]. As presented in Fig. 13, research samples clearly show the Raman bands at 82 cm^{-1}, 152 cm^{-1}, 217 cm^{-1}, 245 cm^{-1}, 435cm^{-1} , and 471 cm^{-1} within the spectral range 100 cm^{-1}–600 cm^{-1}. These bands represent homonuclear sulphur bonds (S–S and S–S–S) [42], but no bands are present within the 500 cm^{-1}–1500 cm^{-1} range in agreement with reports in the literature [33].

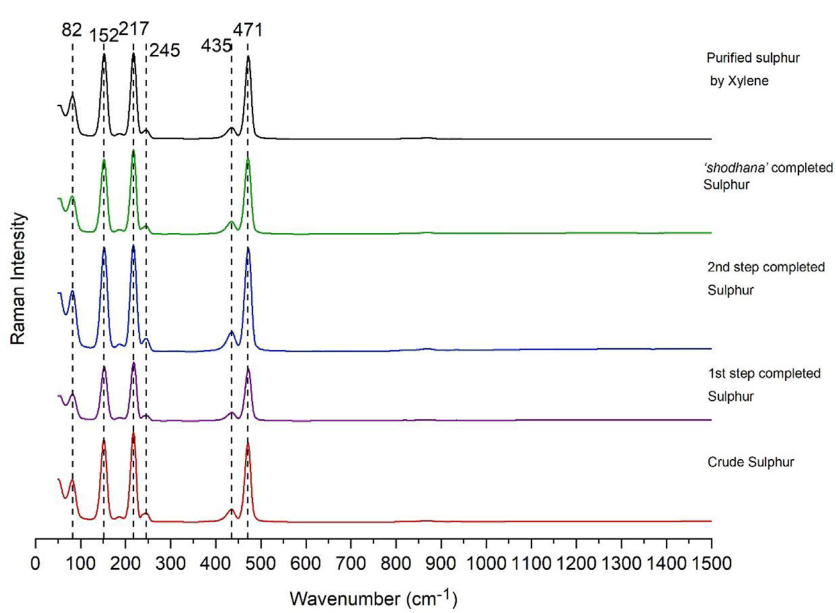

Fig. 13. Raman spectra of sulphur samples.

4. Conclusions

This research focuses on the physical, morphological, and structural changes in sulphur when subjected to '*gandhaka shodhana*' and laboratory purification processes. This was confirmed by the SEM, EDS, XRD, DSC, TGA, FT-IR and Raman analyses. SEM showed that the porous structure of the sulphur increases during the *gandhaka shodhana*' steps. Large interconnected pores were visible in the '*gandhaka shodhana*' processed samples using *E. alba* extract in comparison to laboratory purified sulphur. This increased porosity was correlated to the brittleness, and this can be considered beneficial as sulphur can be mixed more efficiently with other ingredients during Ayurveda pharmaceutical preparations. Additionally, XRD data further supported the nebulous nature of Ayurveda processed sulphur compared to sulphur purified using xylene. The FT-IR spectra indicated the removal of nitro compound even after the first step in the '*gandhaka shodhana*' method. Further, when comparing the change in E. alba extract with the change in sulphur samples after the '*shodhana*' process, it was shown that active principles in plant extracts are not always absorbed or adsorbable, but that the overall process aids in disintegrating the samples. As a result, it is evident that the concept of '*shodhana*' has a strong scientific basis in Ayurveda. On the other hand, DSC, TGA, Raman, and EDX data also indicate sulphur is retained as it is, as evidenced by the presence of S-S and other sulphur-related bonds after *gandhaka shodhana*'. Although the laboratory purification method separates the sulphur from chemical impurities, the Ayurveda '*shodhana*' process converts the sulphur into a state where it is more suitable as a pharmaceutical material by enhancing porosity, fragility as these qualities support to disintegration and blend with other materials during the pharmaceutical manufacturing processes. Therefore, mixing with herbal extract, *E. alba*, enhances the utility of sulphur as a therapeutic while performing detoxification as well.

Sources of funding

This work was supported by the University Grants Commission, Sri Lanka [UGC/VC/DRIC/PG2018(I)/KLN/01].

CRediT author statement

T.A.N.R. Gunaratna: Conceptualization, Methodology, Investigation, Formal analysis, Writing - Original Draft. **P.K. Prajapati**: Conceptualization, Methodology, Supervision. **W.R.M. de Silva**: Conceptualization, Methodology, Writing - Review & Editing, Supervision. **K.M.N. de Silva**: Conceptualization, Methodology, Supervision.

Declaration of competing interest

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

Acknowledgment

Authors are thankful to Techno Solution Private Limited, Nugegoda, Sri Lanka for their assistance to conduct FT-IR analysis.

References

[1] Satpute AD. Rasaratna samuccaya (English translation), uparasa and sadharana rasa: chapter 3, verse 1. Delhi: Chaukhamba Sanskrit Pratishthan; 2012. p. 53.
[2] Vagbhata Shree. Hindi commentary by siddhinandan mishra on rasaratna samuccaya, uparasa varga: chapter 3, verse 23. Rasaratna samuccaya. 1st ed. Varanasi: Chaukhambha Orientalia; 2011. p. 64.
[3] Sharma S. English commentary by ravindra angadi on rasatarangani; gandhakavignaniya taranga: chapte 7, verse 7-31. Varanasi. 1st ed. 2015. p. 120. 3.
[4] Murulidhar N, Mohan Kumar BN. A unique process : concept of shodhana. World J Pharm Pharmaceut Sci 2016;5:657–63. https://doi.org/10.20959/wjpps201611-8035.
[5] Mookerjee B. Rasajalanidhi-volume II; gandhaka: chapter 2, verse 2. 4th ed. Varanasi: Chaukhambha Publishers; 2004. p. 132.
[6] Jahan R, Al-Nahain A, Majumder S, Rahmatullah M. Ethnopharmacological significance of Eclipta alba (L.) hassk. (Asteraceae). Int Sch Res Notices 2014: 1–22. https://doi.org/10.1155/2014/385969.
[7] Thakur VD, Mengi SA. Neuropharmacological profile of Eclipta alba (Linn.) hassk. J Ethnopharmacol 2005;102:23–31. https://doi.org/10.1016/j.jep.2005.05.037.
[8] Kamble VM, Pawar SG. Characterization of Eclipta prostrata (L.) L . leaves eaves by FTIR spectroscopy method , CHNS and ICP ICP-MS MS analysis techniques. Int Res J Biol Sci 2017;6:30–5.
[9] Gunaratna TANR, Jayasinghe JMPRK, Hewavithana T, Weerasooriya WMB, Jayakody JTR. Current status of provincial Ayurveda pharmaceutical industry in Sri Lanka. Int. Conf. Multidiscip. Approaches, Faculty of Graduate Studies. University of Sri Jayawardenapura; 2015. p. 56.
[10] Vagbhata Shree. Hindi commentary by siddhinandan mishra on rasaratna samuccaya, yantrani: chapter 9, verse 2. Rasaratna samuccaya. 1st ed. Varanasi: Chaukhambha Ayurveda Prathisthan; 2011. p. 226.
[11] Susman S, Rowland SC, Volin KJ. The purification of elemental sulfur. J Mater Res 1992;7:1526–33. https://doi.org/10.1557/JMR.1992.1526.
[12] Wermink WN, Spinu D, Versteeg GF. Sulfur solubilities in toluene, o-xylene, m-xylene and p-xylene at temperatures ranging from 303.15 K to 363.15 K. J Nat Gas Eng 2019;3:71–95. https://doi.org/10.7569/jnge.2018.692504.
[13] Kandyala R, Raghavendra SP, Rajasekharan S. Xylene: an overview of its health hazards and preventive measures. J Oral Maxillofac Pathol 2010;14:1. https://doi.org/10.4103/0973-029x.64299.
[14] Vagbhatta. Hindi commentary by siddhinandan mishra on rasaratna samuccaya, yantrani: chapter 9, verse 5. Rasaratna samuccaya. 1st ed. Varanasi: Chaukhambha Orientalia; 2011. p. 226.
[15] Sharma S. English commentary by ravindra angadi on rasatarangani; yantravignaniya taranga: chapte 4, verse 35-38. Rasa Tarangani Sri Sadananda Sharma. 1st ed. Varanasi: Chaukhamba Subharati Prakashan; 2015. p. 40.
[16] Sarangadhacharya Shree. Hindi commentary by parasurama satri on sarangadhara samhita; swarasadhyaya-madhyamakhanda: chapter 1, verse 2. 2nd ed. Bombay: Pandurang Yawaji; 1931. p. 137.
[17] Kalaskar MG. Concept of ayurvedic shodhana process - not mere purification. J Nat Ayurvedic Med 2018;2:1–3.
[18] Sawant RS, Lagad CE, Bhoyar MB, Wadodkar DS. Comparison of physicochemical properties of raw and purified sulphur. J Biol Sci Opin 2014;2:42–4. https://doi.org/10.7897/2321-6328.02110.
[19] Patil S, Prasasd PVNR. Concept of shodhana - a review with reference to rasashastra. AyurbubCom 2017;II:632. 9.
[20] Yadav PR. Evaluation of different shodhana processes of Gandhaka and their effect on certain Kshudra kustha. Gujarat Ayurved University; 2014.
[21] Zeng X, Xiao Y, Ji X, Wang G. Mineral identification based on deep learning that combines image and mohs hardness. Minerals 2021;11:506. https://doi.org/10.3390/min11050506.
[22] Meyer B. Elemental sulfur. Chem Rev 1976;76:367–88. https://doi.org/10.1007/BF02026365.
[23] Taylor P, Trofimov BA, Sinegovskaya LM, Gusarova NK. J Sulfur Chem Vibrations of the S – S bond in elemental sulfur and organic polysulfides: a structural guide n.d.:37–41.
[24] Mokhatab S, Poe WA, Mak JY. Sulfur recovery and handling. 2019. https://doi.org/10.1016/b978-0-12-815817-3.00008-3.
[25] Černošek Z, Holubová J, Černošková E, Růžička A. Sulfur-a new information on this seemingly well-known element. J Non-Oxide Glas 2009;1:38–42.
[26] Gómez RW, Pérez JLM, Marquina V, Ridaura R, Marquina ML. Polymerization of commercial Mexican sulfur. Rev Mexic Fisica 2007;53:30–3.
[27] Rajalakshmy MR, Sruthi CV, Maniyan AK, Vendamirtham S, Neeraj PT, Sindhu A. Physicochemical transformation of sulfur during pharmaceutical processing in traditional Indian medicine. Indian J Pharmaceut Sci 2017;79: 794–800. https://doi.org/10.4172/pharmaceutical-sciences.1000293.
[28] Lau GE, Cosmidis J, Grasby SE, Trivedi CB, Spear JR, Templeton AS. Low-temperature formation and stabilization of rare allotropes of cyclooctasulfur (β-S8 and γ-S8) in the presence of organic carbon at a sulfur-rich glacial site in the Canadian High Arctic. Geochem Cosmochim Acta 2017;200:218–31. https://doi.org/10.1016/j.gca.2016.11.036.
[29] Joshi D. Concept of ayurvedic sodhana method and its effects with reference to sulpher. Ancient Sci Life 1982;1:214–22. ttps://doi.org/ASL-1-229 [pii].
[30] Goldstein JI, Newbury DE, Michael JR, Ritchie NWM, Scott JHJ, Joy DC. Trace analysis by SEM/EDS. Scanning Electron microsc. X-ray microanal. New York, NY: Springer New York; 2018. p. 341–57. https://doi.org/10.1007/978-1-4939-6676-9_21.
[31] Downs RT, Hall-Wallace M. American mineralogist crystal structure database. Am Mineral 2003;88:247–50.
[32] Lv C, Wu H, Lin W, Illerup JB, Karcz AP, Ye S, et al. Characterization of elemental sulfur in chalcopyrite leach residues using simultaneous thermal

analysis. Hydrometallurgy 2019;188:22–30. https://doi.org/10.1016/j.hydromet.2019.05.020.

[33] Jena KK, Alhassan SM. Melt processed elemental sulfur reinforced polyethylene composites. J Appl Polym Sci 2016;133:43060–74. https://doi.org/10.1002/app.43060.

[34] Sears ME. Chelation : harnessing and enhancing heavy metal detoxification — a review2013; 2013.

[35] Hussain I, Khan H. Investigation of heavy metals content in medicinal plant, Eclipta alba L. J Chem Soc Pakistan 2010;32:28–33.

[36] Nepali K, Lee H-Y, Liou J-P. Nitro-group-containing drugs. J Med Chem 2019;62:2851–93. https://doi.org/10.1021/acs.jmedchem.8b00147.

[37] Richardson J. Table of characteristic IR absorptions. Univ Color Boulder, Chem Biochem Dep 2011;3610:1.

[38] Wink M. Modes of action of herbal medicines and plant secondary metabolites. Medicine (Baltim) 2015;2:251–86. https://doi.org/10.3390/medicines2030251.

[39] Patel M, Verma R, Srivastav P. Antioxidant activity of Eclipta alba extract. J Med Plants Stud 2016;4:92–8.

[40] Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. Medicine (Baltim) 2018;5:93. https://doi.org/10.3390/medicines5030093.

[41] Saraswat VK, Verma S, Musale SV, Jaiswal ML. A review on traditional and folklore uses , phyto-chemistry. Int Ayurvedic 2015;3:2462–9.

[42] Selvaraj H, Chandrasekaran K, Gopalkrishnan R. Recovery of solid sulfur from hydrogen sulfide gas by an electrochemical membrane cell. RSC Adv 2016;6: 3735–41. https://doi.org/10.1039/c5ra19116e.