Original Research Article (Experimental)

Chemical characterization of an Ayurvedic herbo-mineral preparation - Mahalaxmivilas Rasa

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Abstract

Background: To protect the massive trust of patient in Ayurveda, a need aroused for the researches to ascertain the quality, safety & efficacy of herbo-mineral preparations on scientific lines. The rasa-aushadhis are having qualities such as instant effectiveness, requirement in very small dosage and ample therapeutic utility. Mahalaxmi Vilas Rasa [AFI, 20:27] has been used for treatment of a variety of ailments since time immemorial.

Objective: To prepare Mahalaxmi Vilas Rasa as per standard operating procedures (SoPs) mentioned in classical text and to characterize it chemically using modern analytical techniques.

Materials and Methods: The drug (Mahalaxmi Vilas Rasa) in three batches was prepared in GMP certified pharmacy. Physico-chemical analysis, HPTLC, Assay of elements by AAS & ICP-AES were carried out as per Ayurvedic Pharmacopoeia of India. Powder X-ray diffraction (XRD) was conducted using Rigaku Ultima-IV X-ray diffractometer.

Results: The elemental analysis shown the presence of Mercury, Sulphur, Calcium, Copper, Gold, Iron & Tin etc. and HPTLC revealed presence of organic constituents from plant material. The XRD had indicated that prepared drug contained free sulphur, cinnabar (mercury sulphide added as Kajjali), cassiterite (tin oxide, Vanga Bhasma), orpiment (Hartal, arsenic III sulphide) and mica (Leucite / Zeolite, Abhrak Bhasma). The drug was also tested for residual pesticide and microbiological contamination which were found within permissible limits.

Conclusion: Classical pharmaceutical procedures of Mahalaxmi Vilas Rasa showed converting the macro elements into therapeutically effective medicines of micro form. Standards laid down in this study certainly utilized as an important tool for standardization and quality assurance of this herbo-mineral formulation. © 2018 Transdisciplinary University, Bangalore and World Ayurveda Foundation. Publishing Services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

A specialized branch in Ayurveda, which is “Rasa Shastra” having literal meaning as “Science of Mercury” deals with materials known as ‘Rasa dravyas’. Rasa denotes mainly Parada (Mercury). Formulations made by mercury and incinerated metals and minerals are known as Rasa-aushadhis (Herbo-mineral-metallic preparations).

The rasa-aushadhis are having qualities such as instant effectiveness, requirement in very small dosage and ample therapeutic utility. There are four methods of preparation of these formulations i.e. Khalviya Rasayana, Parpati Rasayana, Kupipakawa Rasyana, Pretali Rasayana. Mahalaxmivilas Rasa is a herbo-mineral-metallic preparation comes under the Khalviya Rasayana.

It is well known for its use in Urdhwa Jatragat rogases (Upper Respiratory Disorders) and Amavata (Rheumatoid arthritis). Important therapeutic uses are in Kasa (Cough), Pinasa (Chronic rhinitis/sinusitis), Rajayakshma (Tuberculosis), Amavata (Rheumatism), Vajikarana (Aphrodisiac), Gula Roga (Diseases of throat), lymphadenopathy, Antra Vriddhi (Hernia), Kushtha (Diseases of skin), non-healing wounds, Atisara (Diarrhoea), Prameha (Urinary disorders), Shlipada (Filariasis), Vrana (Ulcer), Nadivrana (Fistula), Bhagandara (Fistula-in-ano), Arsha (Hemorrhoids), Udarasa (Diseases of abdomen/enlargement of abdomen), Raktabikara (Disorders of blood), Stri roga (Gynaecological disorders), Tvagroga (Skin disease),
Nasa Roga (Disease of nose), Netraroga (Eye disorder), Mukha Roga (Disease of mouth) and Shula (Colicky Pain) [1].

The adjuvant or anupan with which medicine should be taken depends on diseases to be treated (Table 1). It is used in Vati/tablet form. As it is an important and commonly used formulation, the documentation on chemical characterization and safety/toxicity studies is essential for its global acceptance. Hence, this study was conducted under Golden Triangle Partnership programme with the objectives to evolve physicochemical profile along with characterization. This paper is dealt only with preparation and chemical characterization.

2. Materials and methods

2.1. Preparation of Mahalaxmivilas Rasa

All the raw materials were procured by M/s. Maharishi Ayurveda Pharmacy, Noida and authenticated by Dravyaguna expert/Botanist, while the metal/minerals were certified by Rasa Shashtra expert. The composition of Mahalaxmi Vilas Rasa is provided in the Table 2. The process involves following steps viz. Preparation of Bhasmas (Abhraka/Swarna/Vanga/Tamra), Preparation of Kajjali, Purification of Haritika, Preparation of Dhatura Bij, Preparation of Nagavalli Swarasa, Bhavana with Nagavalli Swarasa.

2.1.1. Preparation of Bhasmas (Abhraka/Swarna/Vanga/Tamra)

2.1.1.1. Preparation of Abhraka bhasma [2]. The Vajrabhraka was heated to red hot in an iron pan and immersed in Triphala kwatha. This process was repeated for seven times. The processed Abhraka was bundled in a jute bag with 1/4th quantity of paddy and immersed in kanjika for 3 days. Thereafter, the bundle was rubbed thoroughly and squeezed in the liquid itself so that only fine abhraka particles can escape through the holes of the bag. Bag was removed from the kanjika and the contents were allowed to settle down. The supernatant liquid layers were separated carefully to collect fine particles of Dhanyabhbraka [3] that were settled down in the container.

Dhanyabhbraka (fine Biotite mica obtained through above process) was levigated with Arka isheera (latex of Calotropis procera (Ait) R. Br.) for one day; chakrikas (thin, flat cakes) were made, and dried in sun rays. These chakrikas were placed in a sharara samputa (earthen plates) and the junctions were sealed properly. This sealed apparatus was subjected to Gaja puta (method of heating with specific temperature pattern). The material thus obtained at the end of the puta, was processed in the similar way for six more times. At the end of 7th puta; abhraka was levigated with Nyagrodha-mula kwatha (Decoction of root of Ficus bengalensis Linn.), dried and three Gaja putas were given. The Abhraka was next levigated with Kadali rasa (juice of rhizome of Musa paradisica Linn.) and 07 Gaja putas were given. After completion of putas it was ground and preserved in air tight glass jar or porcelain containers [4].

2.1.1.2. Preparation of Tamra bhasma. Thin patras (plate of shodhita Tamra [5,6], (processed copper) were boiled in Nimbu swarasa for 3 days. One fourth quantity of shuddha parađa (processed mercury) was mixed with Tamra patra and ground well for 3 h in presence of Nimbu swarasa (lemon juice). Shuddha gandhaka (processed sulphur) was added to this, levigated by adding Nimbu swarasa and made into bolus (golaka). This bolus was covered carefully with the paste of Punarnava root (Boerahvia diffusa Linn.) and allowed to dry completely. This dried bolus was placed in sharara (earthen saucer), covered by another sharara and junction was sealed carefully with clay smeared cloth. This sharara was then placed in heating device and heated in kramavridadi agni (gradually increased heat) for 12 h. When cooled, the sharara was removed from the pot. The product thus obtained was ground with juice of Surana kanda (corn of Amorphophallus campanulatus Blume) for a day. A bolus of this was prepared by adding half part of Gandhaka and a little quantity of cow ghee. This was placed in Sharara and after sealing the joint of two Suranas heated in Gajaputa. This procedure was repeated for two times more [7].

2.1.1.3. Amrutikarana of Tamra bhasma [8]. The process was performed to remove the remnant impurities present in the tamra bhasma and to enhance the therapeutic efficacy by inducing nectar like properties [9]. For this, Shuddha gandhaka was added to tamra bhasma and levigated well with Nimbu rasa for 3 h. This was made into a bolus and placed into S. kanda scooped in the middle to accommodate the bolus. The upper portion was covered with S. kanda and wrapped with clay smeared cloth and dried. This was

| Table 1 | List of adjuvant or anupan to be taken for various diseases. |
|---------|------------------------------------------------------------|
| No.     | Diseases/Roga | Anupan                  |
| 1       | Heart palpitation, heart pain | Arjuna bark Kwath or Arjunaarishtta |
| 2       | Sinusitis      | Triphala decoction      |
| 3       | Chronic Coryza | Mulli powder ghee, honey mixed unequal quantity |
| 4       | Tuberculosiss/Kshaya roga | Chausath Prahi Pippal + Honey |
| 5       | Prameha, Impotency, | Shilajita + Milk |
| 6       | Vitiation of Tridosha | Betel leaf juice |
| 7       | Rheumatism     | Dashmoola Kwath |
| 8       | Diseases of abdomen | Punarnava Ras |
| 9       | Diarrhoea, Dysentery | Dry ginger powder + honey |
| 10      | Cold, corzya   | Betel leaf juice |
| 11      | Fever due to cough | Ginger juice + Honey |

| Table 2 | Composition and proportion of each ingredients of the formulation [1]. |
|---------|---------------------------------------------------------------------|
| S. No.  | Materials used | Botanical/scientific names | Part/form used | Proportion |
| 1       | Abhraka          | Biotite mica              | Bhasma (Incinerated powder) | 16 parts |
| 2       | Gandhaka        | Sulphur                  | Processed powder           | 8 parts  |
| 3       | Vanga           | Tin                      | Bhasma (Incinerated powder) | 4 parts  |
| 4       | Vridhdhuru      | Argyrea nervosa (Burm£ Bojer) | Dried seeds | 4 parts  |
| 5       | Dhatura         | Datura metel Linn.       | Processed seeds            | 4 parts  |
| 6       | Paroda          | Mercury                  | Processed Mercury          | 2 parts  |
| 7       | Haritika        | Orpinent                 | Processed powder           | 2 parts  |
| 8       | Karpura         | Cinnamonum camphora Linn. | Sublimated Extract         | 2 parts  |
| 9       | Jatikosa        | Myristica fragrnai Houtt. | Dried Aril                | 2 parts  |
| 10      | Jatikosa        | Myristica fragrnai Houtt. | Dried Seeds               | 2 parts  |
| 11      | Swarna          | Gold                     | Bhasma (Incinerated powder) | 1 part   |
| 12      | Tamra           | Copper                   | Bhasma (Incinerated powder) | 1 part   |
| 13      | Nagavalli       | Piper betel Linn.        | Leaf juice                | Q.S.(for bhavana) |
subjected to gaja puta. On cooling, the ashes were removed and tāmra was collected, powdered and preserved.

2.1.1.4. Preparation of Vanga Bhasma [10]. Vanga was taken in a metallic spoon and heated and given till it was melted. The melted Vanga was immersed in a pot containing churnodaka. After cooling, the vanga was collected from the pot and the above process was repeated again for 6 times to obtain processed Vanga. The Processed Vanga was kept in an iron pan and heated. While it was melting, powders of cincha and Asvattha twak were sprinkled in small quantities and stirred with loha darvi (iron spatula). This process was continued till the melted vanga was reduced to powder form called Jarita Vanga [11]. Jarita vanga was mixed with haritala churna and ground well in nimbu swaras. Small thin cakrikas were prepared, dried and placed in sarava samputa and artha gaja puta was given. This process was repeated for 10 times. One fourth of haratala is added to vanga from the 2nd puta onwards. On cooling, the ashes were removed and vanga was collected, powdered and preserved. Fig. 1

2.1.1.5. Preparation of Swarna Bhasma [12]. The Swarna patras were heated to red hot state and immersed thrice in each of the liquids viz. Tila-taila (tulsi oil), takra (butter milk), kanjika, gomutra (cow urine) and Kulatha kashaya (decoction of horse gram) consecutively [13]. The Processed patras so obtained were used for further processing. In suddha parada, sodhita svarna was added and levigated to make a bolus. Half the quantity of gandhaka was kept in a sarava and swarna golaka was kept on it. The golaka was covered with another sarava. Sandhi lepa of sarava samputa was done and dried in sun rays. Puta with 30 vanyopalas was given adding gandhaka each time in 1 part and the process was repeated 14 times. On cooling, the ashes were removed and swarna was collected, powdered and preserved [14].

2.1.2. Preparation of Kajjali (black sulphide of mercury) [15]

Hingulotha Parada [16] (Mercury obtained from Cinnabar) and shuddha Gandhaka [17] (processed sulphur) were taken in equal quantities and stirred with cincha vanga each was kept in a pot containing tin oil, was kept on it. The vanga was collected from the pot and the above process was repeated again for 6 times to obtain processed vanga. The Processed Vanga. The Processed Vanga was immersed in a pot containing tamra and then they were washed with water and dried in the sun and subjected to svedana in a dolu yantra containing juice of Kusmanda (Benincasa hispida Thunb.) for 3 h.

2.1.3. Purification of Haritala [18]

Small pieces of haritala were bundled in a piece of cloth and subjected to svedana in Dola yantra containing juice of Kusmanda (Benincasa hispida Thunb.) for 3 h.

2.1.4. Purification of Dhatura Beeja [19]

Dhatura seeds were soaked in go-mutra (cow urine) for 12 h and then they were washed with water and dried in the sun and subjected to svedana in a dolu yantra containing go-dugdha (cow milk) for 3 h.

2.1.5. Preparation of Nagavalli Swaras

Nagavalli patra (leaves of Nagavalli) were collected from the local market, washed under tap water and made into small pieces. The pieces of leaves were grinded and a fine paste was prepared. The paste was strained in to a stainless steel vessel through a clean cloth. Total 1700 ml of juice was collected. This swarasa was utilized for bhavana.

2.1.6. Bhavana with Nagavalli Swaras [preparation of finished product (Tablet)]

This liquid (nagavalli swarasa) was added to the ground material in the end runner and trituration process [Bhavana] was carried out. The trituration was continued till the material dries completely. At the end of all levigation process, the material is removed from the

Table 3

| S. No. | Parameter tested | Observations of three batch analysis |
|-------|------------------|--------------------------------------|
| 1.    | Organoleptic characters |                                      |
|       | Colour           | Brownish Black                       |
|       | Taste            | Slightly bitter                      |
|       | Odour            | Aromatic                             |
|       | Appearance       | Tablet                               |
| 2.    | Physico-chemical parameters |           |
|       | Identification   | Yields the reaction characteristics of Sulphur, Mercury, Gold, Copper, Tin, Arsenic & Iron |
|       | Loss on drying   | 2.5–4.0                              |
|       | Organic and volatile matter | 40.0–41.0                             |
|       | Total Ash %w/w  | 52.0–55.0                            |
|       | Acid Insoluble Ash %w/w | 22.0–27.0                               |
|       | Water soluble extractive | 3.0–6.0                             |
|       | Alcohol (90%) soluble extractive | 3.0–5.0                             |
|       | pH of aqueous extract | 6.0–6.5                               |
|       | Specific gravity | 0.9999 ± 0.0004                      |
|       | Particle size distribution |                                      |
|       | 10%,             | 3.46–15.29 μm                        |
|       | 50%,             | 25.35–64.27 μm                       |
|       | 90%              | 119.37–210.50 μm                     |
| 3.    | Assay of Elements |                                      |
|       | Mercury (%w/w)  | 4.0–5.0                              |
|       | Gold (%w/w)     | 1.5–2.0                              |
|       | Copper (%w/w)   | 1.5–2.0                              |
|       | Tin (%w/w)      | 5.0–6.0                              |
|       | Arsenic (%w/w)  | 1.0–2.0                              |
|       | Iron (%w/w)     | 7.0–8.0                              |
|       | Sulphur (%w/w)  | 18.0–20.0                            |
|       | Calcium (%w/w)  | 2.0–4.0                              |
|       | Silica (%w/w)   | 1.0–4.0                              |
|       | Magnesium (%w/w)| 1.0–1.50                             |
|       | Lead (%w/w)     | 0.50–1.50                            |
|       | Boron (%w/w)    | 0.10–0.20                            |
|       | Manganese (%w/w)| 0.05–0.10                            |
|       | Aluminium (%w/w)| 0.10–2.0                            |
|       | Chromium (%w/w) | 0.02–0.04                            |
|       | Cadmium (ppm)   | 0.10–0.20                            |
| 4.    | Residual pesticide (mg/kg) |         |
|       | Alpha and beta HCH | Not detected                        |
|       | Gamma HCH       | Not detected                        |
|       | Delta HCH       | Not detected                        |
|       | DDT and metabolites | Not detected                      |
| 5.    | D.T.            | 16–17 min                           |
|       | Hardness        | 1.5 kg/cm²                          |
|       | Friability      | 0.60–0.70%                          |
|       | Average wt.     | 125.0–125.30 mg/tab                 |
| 6.    | Microbiological examination |                 |
|       | Total aerobic count | 8000–20 000                        |
|       | Coliform        | Not detected                        |
|       | Ecoli           | Not detected                        |
|       | Salmonella sp.  | Not detected                        |
|       | Staphylococcus aureus | Not detected             |
|       | Yeasts          | Not detected                        |
|       | Moulds          | Not detected                        |
|       | Pseudomonas aeruginosa | Absent                 |

Fig. 1. Images of finished drug of Mahalaxmivilas rasa.
Moreover, the total ash of a crude drug also represents an important criterion to analyse the identity and purity of crude drugs. Ash values are important quantitative standards for the authentication of the raw drugs and provide information about correct identification, correct formulation and may help in preventing its adulteration. Ash values obtained using water and alcohol are useful for the evaluation of quality, standardization and safety of Ayurvedic drugs.

### 2.2. Experiments & results of chemical analysis

#### 2.2.1. Physico-chemical analysis

Physicochemical analysis is the important characteristics to evaluate the quality, standardization and safety of Ayurvedic drugs and provide information about correct identification and authentication of the raw drugs & formulations and may help in preventing its adulteration. Ash values are important quantitative standards and criterion to analyse the identity and purity of crude drugs. Moreover, the total ash of a crude drug also reflects the care taken in drug preservation, and the purity of crude and the prepared drug. Acid insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Extractive values obtained using water and alcohol are useful for the evaluation of a crude drug as it gives an idea about the nature of chemical constituents present in it and is useful for estimation of chemical constituents, soluble in that particular solvent used for extraction. The pH conventionally represents the acidity and alkalinity.

Physicochemical parameters, viz. description, estimation of Loss on drying, Ash content, Acid insoluble ash, Water/Alcohol soluble extractive, pH, etc., qualitative/quantitative elemental testing, Residual pesticide, Microbiological examination and tablet parameters viz. Hardness, Friability, Average wt., Dissolution time, etc. were carried out by following standard methods as per Ayurvedic Pharmacopoeia of India (API) [20–25] guidelines. The quantitative estimation of Heavy Metals viz. Hg, As, & Cd was carried out by Atomic Absorption Spectrometer (Perkin Elmer (USA) Analyst 400) and the other elements viz. Mg, Pb, Ca, B, Mn, Al, Cr were analysed on ICP-AES (THERMO ELECTRON Corporation’s model IRIS INTREPID II XDL). However, Sulphur, Silica, Sn, Au, Fe & Cu were quantified by using conventional methods [25]. The results of physico-chemical analysis are mentioned in below Table 3.

#### 2.2.2. HPTLC analysis

**Sample preparation:** 2 g powder each of three batches of MLV were soaked overnight separately in 20 ml of methanol. The solutions were continuously stirred for 6 h and kept for next 18 h and then filtered the samples, dried and made 10% solution.

High Performance Thin Layer Chromatography was performed on TLC plates pre-coated with 0.25 μm thin layers of silica gel 60 F254 (E. Merck). 10 μl methanolic solution of formulation (three batches) were applied on the plates as bands 8.0 mm wide by use of a Linomat-IV applicator (CAMAG, Switzerland) fitted with a 100 μl syringe (Hamilton, Switzerland). The application positions X and Y were both 10 mm, to avoid edge effects. Linear ascending development to a distance of 80 mm with Toluene: Ethyl acetate: formic acid 10: 3:1 (v/v/v) as mobile phase was performed in a twin-trough glass chamber previously saturated with vapors of mobile phase for 20 min. The plates were dried in air and visualized under 254 nm and 366 nm for ultra violet detection and taken the fingerprints as evident. The same TLC plate was also derivatized with anisaldehyde-sulphuric acid agent and visualized in white light. The HPTLC profile along with the Rf values are shown in the mentioned Fig. 2 and Table 4.

**2.2.3. X-ray diffraction study**

Powder X-ray diffraction (XRD) analysis of Mahalaxmivilas Rasa was carried out using Rigaku Ultima-IV X-ray diffractometer with CuKz radiation (λ = 1.54 A˚) operating at 40 kV and 30 mA. Pattern was recorded for angle (2θ) ranging from 10 to 100° at a scanning rate of 1°/second and scan step of 0.1°. Sample identification was done by matching d-spacing with the standard database. The detailed XRD data and XRD pattern are given in below mentioned Table 4 and Figs.3 and 4.

### 3. Discussion

The organo-leptic observation shows that the prepared Mahalaxmivilas Rasa is in the form of Brownish Black coloured tablet having aromatic odour and slightly bitter taste. The qualitative analysis shows the positive test for the presence of Mercury, Sulphur, Calcium, Copper, Gold, Iron and Tin etc. The Chemical analysis revealed that it contains 4.34% of Mercury, 19.12% of Sulphur, 7.29% of Iron, 5.33% of Tin, 1.85% of Gold, 1.74% of Copper, 1.43% of Arsenic, 2.75% of Calcium, 1.3% of Magnesium, 2.5% of silica together with...
trace elements viz., Aluminium, Manganese, Lead, Boron, Chromium, Cadmium etc. which have been found in $<1.0\%$ range. Moisture content 3.70% was found when determined loss on drying at 105 °C. Total ash content (53.35%) representing the elemental composition is left after burning of organic/volatile matter (40.26%). The observations show that water soluble (4.67%) and alcohol soluble (4.12%) matter are also present in this formulation. The drug also tested as per API guideline for Residual pesticide and Microbiological examination which found in permissible limits.

The results from HPTLC profile as shown in Fig. 2 and Table 3 revealed the presence of few bands at different $R_f$ representing organic constituents. Generally, the Herbo-Mineral preparations

![Graph](image1)

Fig. 3. XRD pattern of *Mahalaxmivilas Rasa* (batch –I, II, III).

![Graph](image2)

Fig. 4. Overlay of XRD pattern of *Mahalaxmivilas Rasa* (batch –I, II, III).
are insoluble in common organic solvents such as Chloroform, Acetone, Methanol and water. In this case, the alcohol soluble extractive is very less (<5.0%). This soluble portion did not show any defined behaviour on TLC may be due to its non-homogeneous nature.

XRD pattern of Mahalaxmivilas Rasa (3 batches) as shown in spectra Figs. 3 and 4 and results mentioned in Table 5, have indicated that all the samples contained free sulphur, cinnabar (mercury sulphide added as Kaijali); cassiterite (tin oxide, Vanga Bhasma); orpiment (Hartal, arsenic III sulphide); and mica (Leucite/Zeolite; Abhraka Bhasma). Abhraka bhasma is the major inorganic constituent added by weight followed by free sulphur and Vanga bhasma, in the Mahalaxmivilas Rasa samples. No signature of tamra bhasma or Swarna Bhasma could be identified in these spectra, indicating that the methods used for their preparation, did not yield any crystalline products or their signature is buried under the strong lines of other constituents. But their presence in the sample could be identified from the chemical analysis showing the presence of gold, boron, copper etc. The crystalline form of abhraka bhasma remains inconclusive. It can be seen that the three XRD patterns on the samples are qualitatively same in the relative intensities of the peaks. Kaijali is meta-cinnabar and in its preparation elemental mercury observed to be absent. The presence of free sulphur ensures that the kaijali does not decompose into mercury and sulphur and also some partial conversion into mercury oxide due to exposure to air; the presence of free sulphur is confirmed by XRD. The assay of total ash content in these samples (52%–55%) almost matching the expected fraction of inorganic constituents by weight and the XRD results indicate that the inorganic contents have remained intact over time.

4. Conclusion

The inferences and the standards laid down in this study certainly utilized as an important tool for standardization and quality assurance of this herbo-mineral formulation. It may be useful for Researchers/Scientists/Academicians and also may be considered for laying down the pharmacopoeial standards of Mahalaxmivilas Rasa.

This study reveals that Mahalaxmivilas Rasa prepared by ancient classical pharmaceutical processes is safe and very effective in converting the macro elements into therapeutically effective medicines of micro form. Further, the sub-chronic oral toxicity studies in male and female rats was studied and found non toxic. As this paper is dealt only with preparation and chemical characterization of Mahalaxmivilas Rasa, the detailed data on safety/toxicity studies will be published separately.

Conflicts of interest

There are no conflicts of interest.

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