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# Chemical Characterization of Siddha Poly Herbal Formulation *Amurthathi Chooranam* by Using Modern Analytical Techniques

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**KEYWORDS**

Amurthathi Chooranam, Siddha Poly Herbal formulation, Instrumental Analysis.

**ABSTRACT:**

Background: Amurthathi Chooranam(AMC) is a Siddha poly herbal formulation mentioned in the Siddha text "AnubavaVaithiya Deva Ragasiyam" which is indicated for Urolithiasis. The drug Amurthathi Chooranam was prepared as per the standard operative procedure and it exposed to several studies to reveal for its effectiveness. Standardization of Siddha poly herbal formulations is mandatory to assess the quality of the drugs for treatment procedures.

Aim: The aim of the study was to standardize the AMC by modern instrumental analytical techniques such as Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES), Scanning Electron Microscope (SEM) and Fourier Transform Infra-Red Spectroscopy (FTIR).

Materials and methods: FTIR and ICP-OES analysis was performed at IIT, Madras as per the standard procedure. SEM analysis was carried out as per the standard procedure at Anna University, Crystal Growth Centre, Chennai.

Results and Discussion: ICP-OES reveals high concentration of P in AMC (146.341 mg/L). It also has physiologically important minerals like Ca, Fe, K, Mg, Na, S and Zn. The heavy metals such as Mercury, Arsenic, Lead, Cadmium,and Aluminium were observed as Below Detectable Limit.The chemical finger print was exhibited by using modern analytical techniques like Fourier Transform Infra-Red Spectroscopy (FTIR).The stretches and bonds present in the FTIR analysis indicated the presence of functional groups Amide, Phenols, Alcohols, Alkanes, Aldehyde, Amine, Alkenes, Alkanes, Ester, Ether, Alkyne and Halo compounds which may be the reason for the therapeutic potency of the trial drugAMCand the SEM picture indicated the existence of microparticles and its bio availability. Based on the results, Amurthathi Chooranam is safe, preferably non-toxic to human for its therapeutic dose.

## INTRODUCTION

Standardization of Siddha formulations is avitalpart for the institution of a regular biological activity and chemical profile or just a top quality assurance program for production and producing of Siddha formulations. It also indicatesan evaluation of its identity, quality and purity ofover all phases of its cycle i.e. shelf-life, storage, absorption, metabolism, distribution and elimination.[1]

The currentresearch deals with the Standardization of Siddha poly herbal formulation *AmurthathiChooranam* (*AMC*) mentioned in the Siddha literature

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"*AnubavaVaithiya Deva Ragasiyam*". [2]*Chooranam* is one of the 32 types of internal medicines of Siddha medicine.*AmurthathiChooranam* is indicated for Urolithiasis. The extract of *Amurthavalli*(*Seenthil*) is the prime ingredient of *AmurthathiChooranam*. So the drug is named as *AmurthathiChooranam*.The formulation was evaluated by using modern instrumental techniques such as FTIR, ICPOES and SEM proved the refinement of the classical literature.

## MATERIALS AND METHODS

### Selection of Drug

The trial drug *AMC* was selected from the classical Siddha literature, "*Anubava Vaithiya Deva Ragasiyam*". The ingredients of *Amurthathi Chooranam* (*AMC*) are

**Table No 1: Ingredients of *Amurthathi Chooranam* [2]**

| ***S.No*** | ***Tamil Name*** | ***Botanical Name*** | **Part used** | **Quantity** |
|---|---|---|---|---|
| *1.* | *Seenthil* | *Tinospora cordifolia* | Stem | 35 Gram |
| *2.* | *Jaathikkai* | *Myristica fragrans* | Fruit | 5.1Gram |
| *3.* | *Jaathipathiri* | *Myristica fragrans* | Aril | 5.1Gram |
| *4.* | *Vaal milagu* | *Piper cubeba* | Fruit | 5.1Gram |
| *5.* | *Elam* | *Elettaria cardamomum* | Seed | 5.1Gram |
| *6.* | *Kirambu* | *Syzygium aromaticum* | Flower bud | 5.1Gram |
| *7.* | *Kasakasa* | *Papaver somniferum* | Seed | 5.1Gram |
| *8.* | *Thalisapaththiri* | *Taxus baccata* | Leaf | 5.1Gram |
| *9.* | *Maasikkai* | *Quercus infectoria* | Fruit | 5.1Gram |
| *10.* | *Sarkarai* | *Saccharum officinarum* | Sugar (Extract) | 75.8Gram |

**Figure No: 1 Ingredients of *AmurthathiChooranam***

**Figure No: 1A** ***Tinosporacordifolia***

**Figure No: 1B** ***Myristicafragrans***

**Figure No: 1C** ***Myristicafragrans***

**Figure No: 1D** ***Piper cubeba***

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**Figure No: 1E**
***Elettaria cardamomum***

**Figure No: 1F**
***Syzygium aromaticum***

**Figure No: 1G**
***Papaver somniferum***

**Figure No: 1H**
***Taxus baccata***

**Figure No: 1I**
***Quercus infectoria***

**Figure No: 1J**
***Saccharum officinarum***

**Collection of the drugs:**

The ingredients of above mentioned A*murthathi Chooranam* was procured from authenticated country drug store, Chennai, Tamilnadu.

**Identificationauthentication:**

Identification and Authentication of all the ingredients of *Amurthathi Chooranam* were recognized and certified by Department of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai - 600106. Specimen sample of all the above ingredients was labelled and were kept in the same as earlier mentioned for future consideration.

**Process for Purification and Preparation:**

**Purification of the drugs:**

All the drugs mentioned here were purified as per the Siddha literature *Sarakku Sutthimuraigal*.[3]

- *Seenthil – Tinospora cordifolia* (Stem) was washed thoroughly and gently remove the outer skin.
- *Jaathikkai – Myristica fragrans* (Fruit) were cleaned without any dust and impurities.
- *Jaathipathiri - Myristica fragrans* (Aril) were cleaned without any dust
- *Vaal milagu - Piper cubeba*(Fruit) were cleaned and fried in few minutes.
- *Elam - Elettaria cardamomum* (Seed) dried and cleaned well and seeds are isolated.
- *Kirambu – Syzygium aromaticum* (Flower bud) were cleaned and fried within few minutes.
- *Kasakasa - Papaver somniferum* (Seed) were cleaned without any dust and impurities.
- *Thalisapaththiri- Taxus baccata* (Leaf) were cleaned well and fried within few minutes.
- *Maasikkai – Quercus infectoria* (Fruit) were cleaned without any dust.
- *Sarkarai – Saccharum officinarum* (Extract) were cleaned well without any dust.

**Preparation of *Seenthil Sarkarai* [4]**

*Seenthil* stem (*Tinospora cordifolia*) is cleaned and crushed well. Crushed *Seenthil* stem is soaked in water and stirred well. On next day the coarse part of the *Seenthil* is removed from the water. Then the water in the vessel is placed under the sunlight for 2-3 hours until sedimentation is obtained. Poured out the clear supernatant water and collected the sediment. Added some more water to this and removed the remaining coarse powder. Allowed the starch to settle down and collected it and then made it to dry.

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**Figure No: 2. *Seenthil Sarkarai***

**Preparation of the drug *Amurthathi Chooranam*:**

All the above mentioned ingredients were purified as per Siddha classical literature and taken it into an anhydrous form poundwell and grounded it in an iron mortar separately.

**Purification of the *Chooranam*:**

**Steaming process (*Pittaviyal murai*):**

The *Chooranam* was purified by steam cooking with milk (*Pittaviyal* process) as per Siddha literature. A pot was taken and it was half filled by milk and half filled by pure water. The mouth of the pot was sealed by a cloth. Then this *Chooranam* was placed over the cloth and the pot was heated. Later the same drug was dried and powdered then sieved again. Then added equal quantity of sugar to the above mixture and grinded well. The powder was sieved through a mesh (80 -100) particle size.

**Figure No: 3A** **Figure No: 3B**

**Figure No: 3 Steaming process (*Pittaviyalmurai*)**

**Storage of the drug:**

**Figure No: 4*AmurthathiChooranam***

The prepared test drug was stored in a clean airtight container. It was labeled as " *Amurthathi Chooranam"(AMC)*. The *Chooranam* was examined repeatedly to avoid moisture.

**Administration of the drug:**

**Form of the medicine:** *Chooranam*

**Route of Administration:** Oral

**Vehicle:** water

**Dose:** 1gm, twice a day

**Duration:** 24 days.

**Indication:** *Mehaneer* (Urinary disorders), *Prameham* (Urological disorders - Renal stone), *Madhumeham* (Diabetes), *Vellai* (Leucorrhoea), *Vettai* (Gonorrhoea).

**Sophisticated Instrumental Analysis**

**ICP Optical Emission Spectrometry Principle(ICP-OES)[5]**

ICP-OES analysis was done at IIT, Madras.ICP, abbreviation for Inductively Coupled Plasma, is one method of optical emission spectrometry. When plasma energy is given to an analysis sample from outside, the component elements (atoms) is

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excited. When the excited atoms return to low energy position, emission rays (spectrum rays) are released and the emission rays that correspond to the photon wavelength are measured.

The element type is determined based on the position of the photon rays and the content of each element is determined based on the ray's intensity. To generate plasma, first, argon gas is supplied to torch coil and high frequency electric current is applied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas is ionized and plasma is generated. This plasma has high electron density and temperature (10000K) and this energy is used in the excitation-emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the center of the torch tube.

**Equipment**

Equipment for ICP optical emission spectrometry consists of a light source unit, a spectrophotometer, a detector and a data processing unit. There are several types of equipment based on differences in the Spectrophotometer and the detector. The most common type is shown in Figure 5.

**1) Sequential type**

A spectrophotometer with a Czerny-Turner monochrometor, and a detector with a photomultiplier is most common for this type. With this equipment, programmed wavelength of the spectrophotometer is consecutively varied to measure multiple elements. This causes rather long measuring time, however, with its high resolution spectrophotometers, it is favorable for measurement of high-matrix samples.

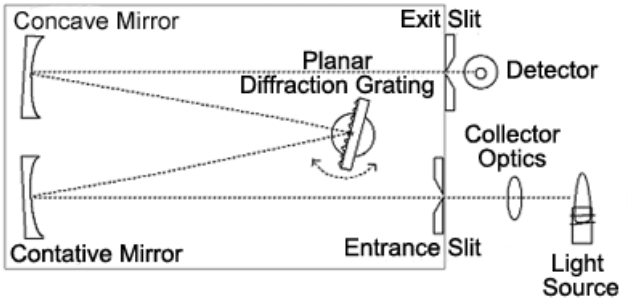


**Figure No: 5 Sequential Type ICP-OES**

**2) Simultaneous Type**

This type typically uses an echelle cross disperser in spectrophotometers and semi-conductor detector such as CCD for the detector. Echelle cross disperser disperses light of measurable wavelength range two-dimensionally by combining prism and echelle diffraction grating. Combination of echelle cross disperser and a CCD detector enables multi-element measurement at any wavelength. The most notable feature of this equipment is the high-speed measurement, providing information on all 72 measurable elements in measurements of 1 to 2 minutes normally.

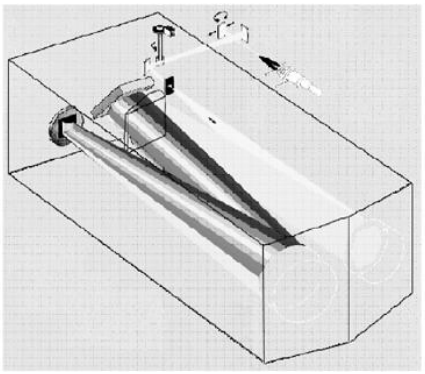

**Figure No: 6 Simultaneous ICP-OES**

**Application:**

***AmurthathiChooranam* analysis**

Trial drug *AmurthathiChooranam*has attracted attention because it is thought to contain a person's health history on some level and is thought to act as an excretory organ for heavy metal in the body. However, there are problems because there are few usable samples and knowledge about multiple elements is required. With simultaneous analysis equipment, we can collect useful information with a small amount of sample.

**Equipment:** Simultaneous ICP-OES, **PERKIN ELMER OPTIMA 5300 DV**

**Sample:** 0.5gm of *Amurthathi Chooranam* is measured and then dissolved in a decomposition vessel with nitric acid into 10ml solution. Partial spectral profile and analysis were done.

ICP optical emission spectrometry is now highly rated as a multipurpose analysis technique.It is well regarded as an environmental measurement technique, along with atomic absorption spectrometry

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and ICP mass spectrometry, and its use is expected to expand even further in the future.

**Scanningelectron microscope (SEM)** [6]

SEM analysis was done at Anna University, Chennai. In scanning electron microscope high-energy electron beam was focused through aprobe towards the sample material. Variety of signals was produced on interaction withthe surface of the sample. This results in the emission of electrons or photons and it wascollectedbyanappropriatedetector.

The types of signal produced by a scanning electron microscope include

- ❖ Secondary electrons
- ❖ backscattered electrons
- ❖ characteristicx-rays, light
- ❖ specimen current
- ❖ Transmitted electrons.

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample.

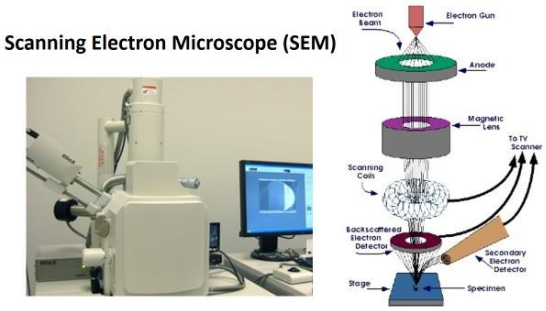


**Figure No: 7ScanningElectron Microscope**

**FT-IR (Fourier Transform Infra-Red Spectroscopy)** [7]

FTIR analysis was performed at IIT, Madras. FT-IR is an important and more advanced technique to identify the functional group. The spectrum that appears denotes the molecular absorption and transmission. It formed the molecular fingerprint of the sample. Like the finger print there was no two unique molecular structures producing the same infrared spectrum. It was recorded as the wavenumber and the peaks were seen in the spectrum indicates the amount of material present.

**Details regarding the FT-IR analysis**

The Perkine Elmer Spectrum One Fourier Transform Infrared (FTIR) Spectrometer was used to derive the FTIR Spectrum of *Amurthathi Chooranam* placed in Potassium Bromide (KBr) discs with scan rate of 5 scan per minute at the resolution 4cm-1 in the wave number 4000-500 were recorded the FT- IR Spectrum under Standard condition.FT- IR Spectra were used to determine the presence of the functional groups and bands in the trial drug *Amurthathi Chooranam*. The recorded spectrum shows in figures. The standard table of FTIR is given in Table-1.

FT-Ir is the most advanced and the major advantage is its

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated

**Figure No: 8 FT-IR (Fourier Transform Infra-Red Spectroscopy)**

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## RESULTS AND DISCUSSION

### Sophisticated Instrumental Analysis

### ICP-OES (Inductively Coupled Plasma Optic Emission Spectrometry)

**Table No: 2 ICP-OES Results of *AMC***

| S.No | Elements Symbol | Wavelength (nm) | *AMC* Concentration (Wt. 0.334570 g) |
|---|---|---|---|
| 1. | Hg | 253.652 | BDL |
| 2. | As | 188.979 | BDL |
| 3. | Pb | 220.353 | BDL |
| 4. | Cd | 228.802 | BDL |
| 5. | Cu | 327.393 | BDL |
| 6. | Al | 396.152 | BDL |
| 7. | Fe | 238.204 | 01.258 mg/L |
| 8. | Ca | 315.807 | 45.180 mg/L |
| 9. | K | 766.491 | 23.821 mg/L |
| 10. | Mg | 279.077 | 01.104 mg/L |
| 11. | Na | 589.592 | 24.345 mg/L |
| 12. | C | 193.030 | 154.123 mg/L |
| 13. | P | 213.617 | 146.341 mg/L |
| 14. | S | 180.731 | 01.254 mg/L |
| 15. | Zn | 206.200 | 01.018 mg/L |

**BDL - Below Detection Limit.**

**Discussion:**

- ICP-OES reveals high concentration of P in *AMC* (146.341 mg/L). It also has physiologically important minerals like Ca, Fe, K, Mg, Na, S and Zn.
- From the above results, the heavy metals such as Mercury, Arsenic, Lead, Cadmium,and Aluminium were observed as BDL and those are within the WHO permissible limits. Hence the safety of the drug *AMC* is ensured for Pre-clinical use.[8]
- ICP optical emission spectrometry is now highly rated as a multipurpose analysis technique. It is well regarded as an environmental measurement technique, along with atomic absorption spectrometry and ICP mass spectrometry and its use is expected to expand even further in the future.

**Scanning electron microscope analysis (SEM)**

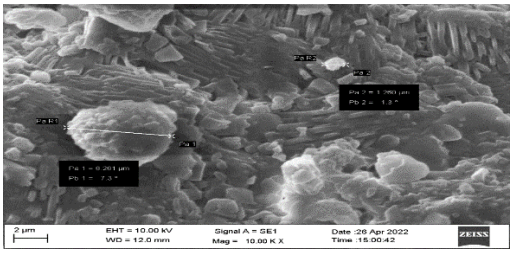

**Figure No: 9 - SEM Picture of *AMC*- granules, stacks**

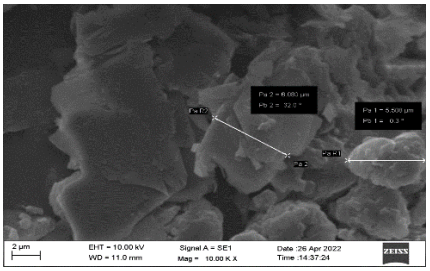

**Figure No: 10 – SEM Picture of *AMC*- Zooming of the stacks**

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**SEM Results of *AMC***

SEM analysis means the surfaces of materials, particles and fibers so that fine details can be measured and analyzed via image analysis. Also SEM analysis attested the morphological changes in the trial drug *AMC*.

The above SEM studies, (Fig 9 and Fig 10) the morphology of*AMC* showednumerus rods, plates like structures as if stacked one on the top and the other in the bottom like nature. Micro scale study at the size of 2 micrometer and magnification at 10.00KX was used as the basic study tool. It showed objects of sizes ranging from 6.261µm to 1.260µm. The surface of the sample*AMC*granules is uniformly arranged in stacks. They are micro particles ranging from 6.261 µm, 6.080µm, 5.508 µm, 1.260 µm

**Discussion for SEM**

**(Micro particles-significance)**

- Microparticles are definedas particulate dispersion orsolid particles with a size intherangeof1-1000µmindiameter.
- Size and surface of micro particles can be easily manipulated to achieve bothpassiveand active drugtargeting.
- They control and sustain the release of drug during the transportation and at thesite of localization, alter drug distribution in the body and subsequent clearanceof the drug so as to achieve increased drug therapeutic efficacy thereby bio-availabilityandreducedsideeffects. [8]

**FTIR**

**AMC has following functional groups**

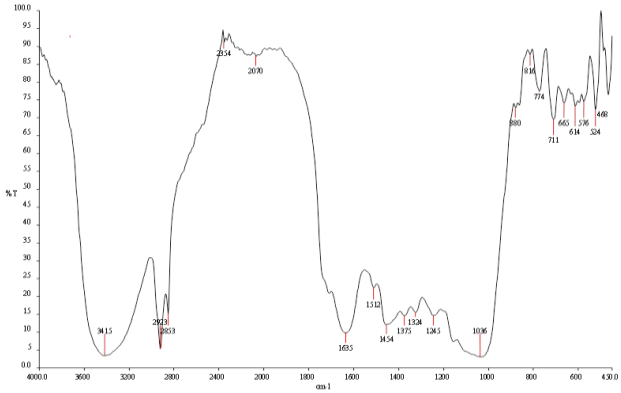

**Fig No: 11 FTIR**

**analysis graph of *AMC***

REF value from 4000 to 450

*Amurthathi Chooranam:* 3415, 2923, 2853, 2354, 2070, 1635, 1512, 1454, 1375, 1324,

1245, 1036, 880, 816, 774, 711, 665, 614, 576, 524, 468

The FTIR analysis graph has 21PEAKS

**Table No: 3 The FTIR result of the trial drug*AMC* is shown in table below.**

| Absorption peak $cm^{-1}$ | Stretch | Functional group |
|---|---|---|
| 3415 | Water OHstretch | Carbohydrates, proteins, and polyphenols, Alcohol group |
| 2923 | C-Hstretch | Alkane |
| 2853 | - C-Hstretch | Alkane |
| 2354 | -C=C Stretch | Conjugated Alkane |
| 2070 | N=C=S Stretch | Isothiocyanate |
| 1635 | C=C Stretch | Conjugated alkene |
| 1512 | N-O stretching | Nitro compound |
| 1454 | $CH_2$ bend | Alkane |
| 1375 | C-H bending | Alkane |
| 1324 | S=O stretch | Sulfone |
| 1245 | C-N stretching<br>C-O stretching | Amine<br>Alkyl aryl ether |

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| | | |
|---|---|---|
| 1036 | S=O stretching | Sulfoxide |
| 880 | C-H bending<br>C-Cl | 1,2,4-trisubstituted |
| 816 | C=C bending<br>C-Cl | Alkene, trisubstituted |
| 774 | C-H bending<br>C-Cl | 1,2,3-trisubstituted |
| 711 | C-H bending<br>C-Br | Monosubstituted<br>benzene derivative |
| 665 | C-Br stretching<br>C=C bending | Halo compound<br>Alkene disubstituted |
| 614 | C-Br stretching | Halo compound |
| 576 | C-Br stretching<br>C-I stretching | Halo compound<br>Halo compound |
| 524 | C-Br stretching<br>C-I stretching | Halo compound<br>Halo compound |
| 468 | C-I Stretching | Halo compound |

**Discussion of FTIR Spectrum**

FTIR instrument analysis was done. The test drug *Amurthathi Chooranam* was identified to have 21peaks.

**The below functional groups are presented in the trial drug *Amurthathi Chooranam*.**

The FTIR results shows the observed waterO-H stretch, O-H stretch, H-C-H stretch, C=O stretch, N-H stretch, C-C=C symmetric stretch, H-C-H bend, C-O stretch, C -H bend, C-C stretch which indicates that the presence of functional groups Amide, Phenols, Alcohols, Alkanes, Aldehyde, Amine, Alkenes, Alkanes, Ester, Ether, Alkyne and Halo compounds.

- OH group has higher potential towards inhibitory activity against microorganisms.[8]
- Phenols possess high Anti-Oxidant property which enhances the trial drug *AMC*effectagainsttheUrolithiasis.

## CONCLUSION

Based on the results, *AMC* is preferably non- toxic to human in its therapeutic dose. The standardization of the drug was evaluated by chemical characterization with heavy metal analysis, functional group analysis, elemental analysis and determination of particle size by ICP-OES, SEM and FTIR respectively. The FTIR result indicates the presence of some organic functional groups such as Amide, Phenols and alcohols, Alkanes, Aldehyde, Amine, Alkenes, Alkanes, Ester, Ether, Alkyne and Halo compounds. ICP-OES reveals that the safety of the drug. The SEM picture shows that *AMC* is a kind of micro medicine which favours the advantages of bio availability, better absorption and non-toxic with minimal dose level.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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