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Research Article

Morpho-Anatomy, Physiochemical and Phytochemical Standardization with HPTLC Fingerprinting of Aerial Parts of *Trichosanthes lobata* Roxb.

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ABSTRACT

To study the morpho-anatomy of the aerial parts of *Trichosanthes lobata* (Cucurbitaceae) to increase the knowledge and standardization parameters of these plants. Morpho-anatomical studies of leaves have been carried out by free hand. The different types of histochemical test were performed by using staining reagents. Phytochemical and quantitative estimation has been determined along with HPTLC fingerprinting. Leaves simple, reniform, or ovate, 5 lobbed and glabrous. Anomocytic stomata are present on lower epidermis. A single layer of elongated palisade cells are present below upper epidermis. Leaf bears covering trichomes unicellular to 3-4 celled long. The central region is occupied by arc shaped vascular bundles comprising of xylem vessels surrounded with phloem cells. Physicochemical paradigms such as; ash value, inorganic elements, moisture content and extractive values were determined to develop stringent Pharmacognostic standards. Qualitative, and Quantitative standardization and, HPTLC fingerprint study of alcoholic and aqueous extracts confirmed the presence of quercitin as biomarker polyphenolic compound. These studies provides referential information for correct identification, as well as assessment of purity, quality of this plant, which definitely gaining the relevance in plant drug research and establishment of plant monograph.

Keywords: *Trichosanthes lobata* Roxb, Morpho-anatomy, HPTLC fingerprinting, Quercitin, Physicochemical, Standardization.

INTRODUCTION

Natural products are a source of synthetic and traditional herbal medicines. They are the primary health care system in some parts of the world[1]. However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control of herbal drugs. Thus, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by morpho-anatomical, physicochemical and phytochemical studies of the plant material to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy[2]. *Trichosanthes lobata* Roxb (Family-Cucurbitaceae) known as Patola (Sanskrit) and wild snake gourd (English), is largely found in Maharashtra, India. Traditionally, the plant used as bitter tonic, laxative, depurative, digestive, cardiotonic, anthelmentic, and in treatment of jaundice[3]. Documented reports suggest the presence of various phytoconstituents viz; cucurbita-5, 24-dienol, α, β carotene, lycopene, lutein, vitamin C and β-sitosterol in *Trichosanthes lobata*[4]. In the present study, an attempt has been made for morpho-anatomical as well as physicochemical and phytochemical standardization of this plant for contribution in the quality control of herbal drug and increase in the knowledge of plant and its family.

MATERIALS AND METHODS

Plant material and extraction

The fresh and healthy plant material was collected in the month of August to September from the vicinity of Pune district, Maharashtra, and authenticated by Sr. taxonomist from Botanical Survey of India, Pune. The voucher specimen number is BSI/WC/Tech/2008/354-RRWTL-2. The herbarium was submitted at the department of

Figure 1: leaf of *Trichosanthes lobata plant.*

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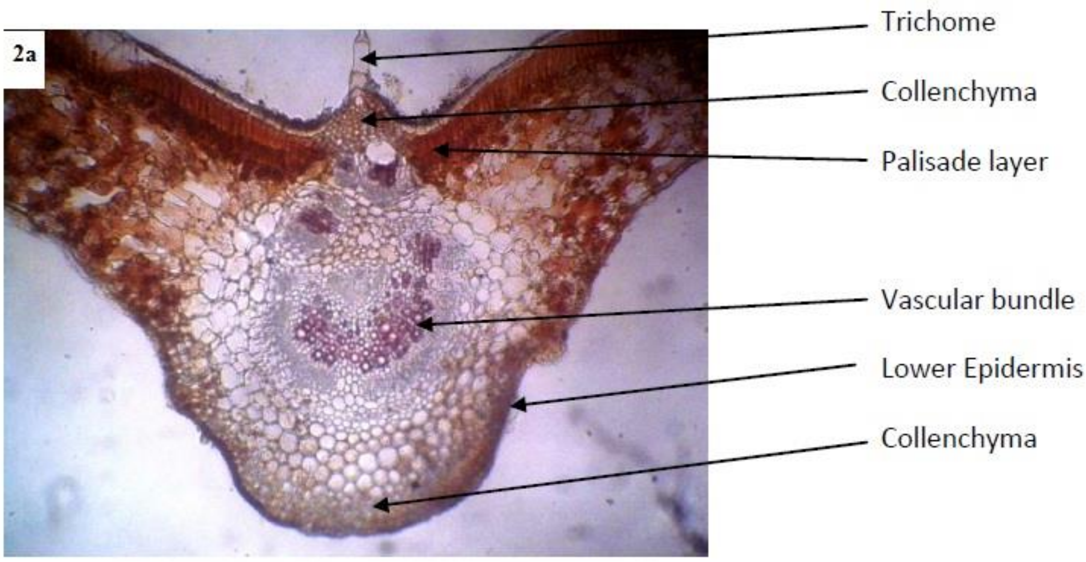


Figure 2a: Transverse section of *Trichosanthes lobata* leaf.

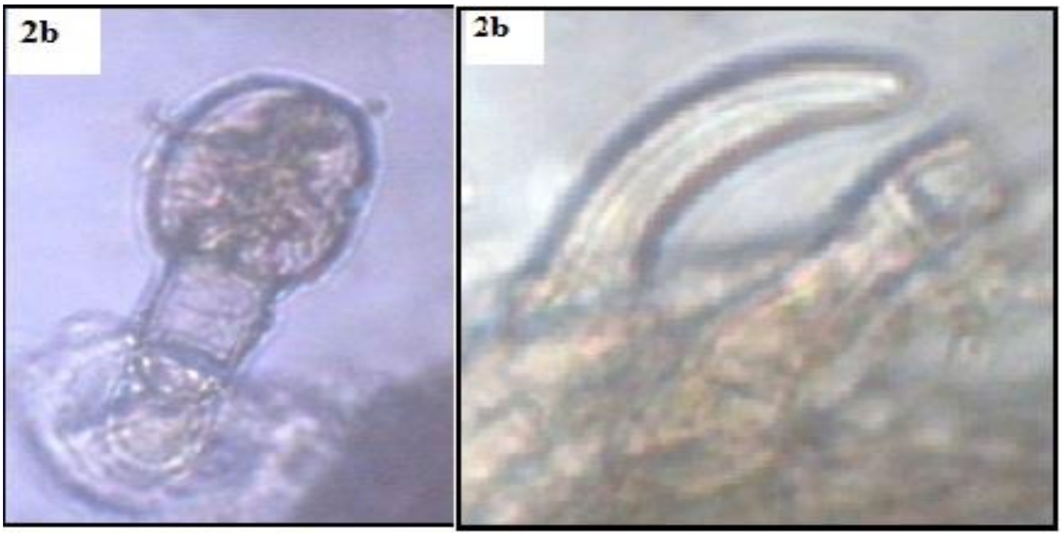


Figure 2b: Covering and Glandular trichomes.

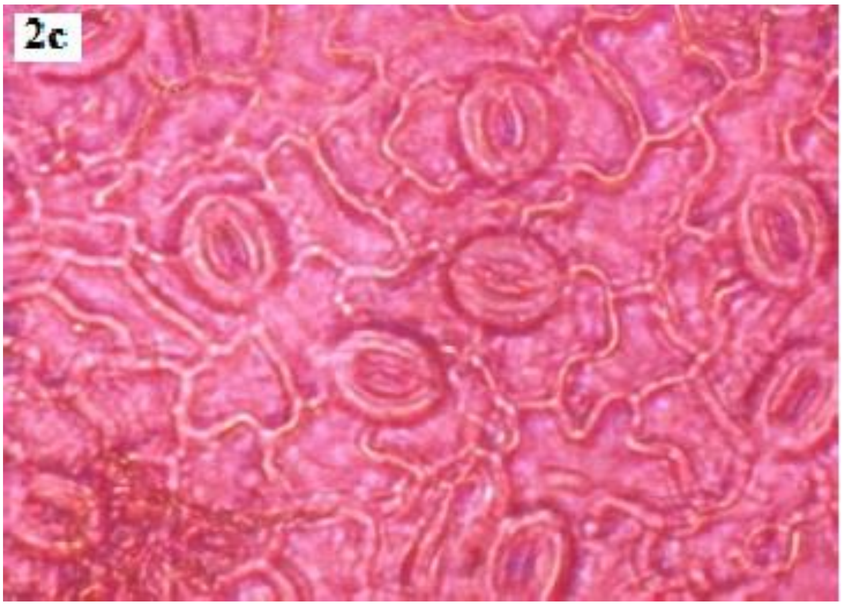


Figure 2c: Anomocytic Stomata.

Pharmacognosy, K.L.E University's College of Pharmacy, Belagavi. After authentication, the leaves of *Trichosanthes lobata* were dried under the shade until they were free from moisture and subjected to morpho-anatomical and physicochemical studies. The powder material of *Trichosanthes lobata* extracted successively by petroleum ether, chloroform and alcohol solvent. Whereas, chloroform water IP 1996 was used for preparation of aqueous extract. The extracts were concentrated under reduced pressure (17 mm Hg, 45º C, 10 -20 min) depending on the solvents. The extracts were stored at cool place in dark until use. Each time before extraction with next solvents, the powder material was dried in hot air oven below 50 º C.

Chemicals and instruments:

Staining reagents such as phloroglycinol, hydrochloric acid, 5 % Iodine, Sudan red and all other chemicals used in the study were of analytical grade. Instruments: microscope (Make- Zeiss Company with Axis Vision Ac Rel 4.5 Software), Microtome (Make: Thermo Electron Corporation and Model Shandon Finesse) and CAMAG HPTLC system scanner 3, Reprostar 3 and WIN CATS-4 software.

Organoletic evaluation

The fresh leaves of *Trichosanthes lobata* were subjected to morphological studies composed of organoletic characteristics viz, color, odour, taste, shape, texture were examined as per standard WHO guidelines[5].

Microscopical evaluation

For microscopical studies, the required plant sample was cut and removed from healthy plant and washed with water. After proper washing the killing and fixing of the specimen was carried out using solution of (90 ml 70 % ethanol + 5 ml of glacial acetic acid + 5 ml of formaldehyde) for one week. Further, dehydration of the tissues was done with the help of different grades of tertiary butyl alcohol. Thereafter, the process of infiltration was followed by filling the cells with increasing order of paraffin. Furthermore, thin transverse sections was taken using microtome (Make: Thermo Electron Corporation and Model Shandon Finesse) and histochemical tests were carried out using staining reagents such as phloroglycinol + hydrochloric acid (1:1) for (lignified cells), 5 % Iodine for (starch grains), Sudan red for (stone cells). Photomicrographs of the microscopical sections were captured with the help of microscope (Make- Zeiss Company with Axis Vision Ac Rel 4.5 Software)[6].

Leaf constants and physicochemical investigation

For establishing standardization parameters various leaf constants, palisade ratio, vein islet number, vein termination number and stomatal index evaluated. The shade dried leaves were subjected to size reduction to get fine powder (# 40 size mesh) and then evaluated for ash value, inorganic substance identification, extractive value and moisture content as per literature[7].

Qualitative and quantitative physicochemical investigation

The preliminary qualitative phytochemical identification has been carried out by using phytochemical test[8]. Whereas, the alcoholic and aqueous extracts were subjected for estimation of total phenolic and total flavonoids content[9,10].

HPTLC fingerprinting

HPTLC study was carried out on aqueous and alcoholic extract by the method of Harborne and Wagner et al[11].

Development of solvent system

A number of solvent were tried individually as well in combination for separation and identification of quercitin from the respective extracts but the satisfactory resolution was obtained in the solvent system Ethyl acetate: Formic acid: glacial acetic acid: Water (99:12:11:27 v/v/v).

Sample application and development of chromatogram

The aqueous and alcoholic extracts of *Trichosanthes lobata*, was dissolved in respective HPTLC grade ethanol and water which were used for sample application on precoated silica gel GF 254 aluminium sheets (Made-

Table 1: Fluorescence analysis of powdered leaves of *Trichosanthes lobate.*

Sr.no	Treatment	Observation under		
		Ordinary light	UV light	
			254 nm	366 nm
01	Powder as such	Dark green	Green	Dark green
02	Powder + nitrocellulose	Dark green	Light green	Dark green
03	Powder + 1N NaOH in methanol	Light yellow	Yellow	Dark Yellow
04	Powder + 1N NaOH in methanol + nitrocellulose in amyl acetate	Florescent	Light Florescent	Dark Florescent
05	Powder + 1N HCl	Light brown	Light brown	Brown
06	Powder + 1N HCl + nitrocellulose in amyl acetate	Brown	Light brown	Dark brown
07	Powder + 1N NaOH in water	Yellow	Light green	Light green
08	Powder + 1N NaOH in water, dried and mounted in nitrocellulose in amyl acetate	Yellowish brown	Light yellowish brown	Dark yellowish brown
09	Powder +HNO_3 (1:1)	Brown	Brown	Brown
10	Powder + H_2SO_4 (1:1)	Light black	Black	Black

Table 2: Physicochemical evaluation of powder leaves of *Trichosanthes lobate.*

Parameters		% w/w (Mean [a]± SEM)
Ash Values	Total ash	4.39 ± 0.31
	Acid insoluble ash	0.42 ± 0.02
	Water soluble ash	1.01 ± 0.02
Extractive Values	Alcohol	20.21 ± 0.22
	Water	22. 19 ± 0.21
Moisture content		8.1 ± 0.88

[a]Mean value of three readings.

Merck). The samples (5 µL) were spotted in the form of bands of width 6 mm with a 100 µL sample using a Hamilton syringe on silica gel which was precoated on aluminium plate GF-254 plates (20 cm X 10 cm) with the help of Lineman 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software. Prepared plates were developed in previously saturated twin trough chamber (20 cm X 10 cm) in the linear ascending direction.

Detection of spots

The developed plates were dried by hot air to evaporate solvents from the plate. The developed plates were sprayed with 5 % Ferric chloride as spray reagent and dried at 100 ºC in hot air oven for 3 min. The plates were kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under UV light at 254 nm. The R_f values and finger print data were recorded by WIN CATS software.

RESULTS

Morphological evaluation

Morphologically leaves are simple green in color, broader than long, orbicular-reniform or broadly ovate in shape, more or less deeply 3-lobed, distantly denticulate somewhat glabrous (Figure 1) and bitter in taste.

Microscopical and Histochemical evaluation

Study of midrib

Epidermis layer showed presence thin cuticle on both surfaces shows presence of glandular and covering trichomes particularly on upper surface. Upper surface also shows the presence of number glands occupying part of palisade layer. A single layer of elongated palisade cells are present below upper epidermis. Mesophyll consists of spongy parenchyma with air spaces and brown pigment. The midrib region shows collenchymatous cells beneath both epidermal layers. The central region is occupied by arc shaped vascular bundles comprising of xylem vessels surrounded with phloem cells (Figure 2a). Leaf bears covering trichomes unicellular to 3-4 celled long and conical and glandular trichomes with unicellular to multicellular gland and unicellular to multicellular stalk (Figure 2b). Upper epidermal cells are about 60-80 micron long while stomata are about 22-25 micron in diameter. Epidermis layer showed presence of anomocytic stomata (Figure 2c).

Powdered drug analysis

The powder was dark green in colour, with characteristic odour, and bitter taste. After shaking the powder with water in test tube, no persistent foam was formed indicating absence of saponins. Powdered drug under ultra-violet and ordinary light when treated with different reagent emitted various colour radiations which help in identifying the drug in powder form. Behaviour of powder with different chemical reagents is summarized in (Table 1).

Quantitative microscopy and physicochemical evaluation

In leaf constants, stomatal index and palisade ratio were found to be 15-20 and 7-8 respectively. Whereas vein islet and vein termination number were ascertained as 20-24 and 50-55 respectively. The ash content of drug also showed presence of calcium, magnesium and sulphate while absence of sodium, potassium and phosphate types of inorganic compounds. The physicochemical parameters such as ash value, extractive value and moisture content were important to determine purity of the drug summarised in (Table 2).

Qualitative and quantitative phytochemical investigation

Successive soxhlet extractive values colour and consistency of extracts of leaves of *Trichosanthes lobata* was found to be: Pet ether (2.8% w/w, dark green, sticky mass); Chloroform (4.9% w/w, bottle green, sticky mass); alcohol (6.6 % w/w, dark green, sticky mass) and water (9.9 % w/w, light, semi-solid), respectively.

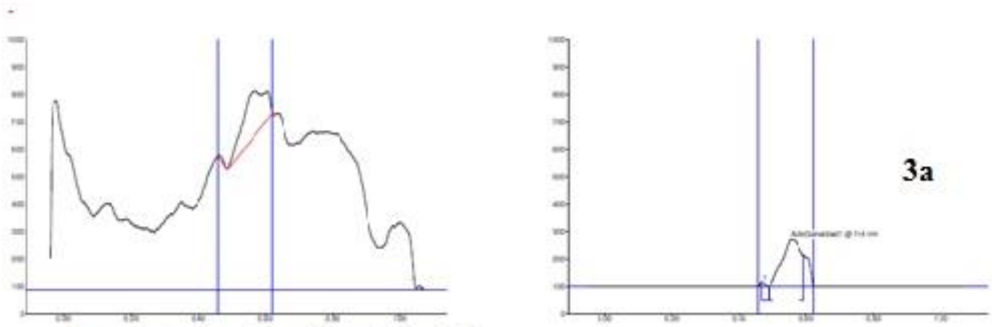
Preliminary phytochemical analysis of alcoholic extract revealed presence of alkaloids, tannins, flavonoids whereas, aqueous extract showed presence of polyphenolic compounds, flavonoids, tannins and glycosides. The quantitative estimation of total phenolic content in alcohol and aqueous extract was found to be 211 ± 2.5 and 213 ± 1.9 mg/g of extract and the quantitative estimation of total flavonoid content in alcohol and aqueous extract was found to be 38 ± 3.1and 64 ± 5.5 mg/g of extract.

HPTLC analysis

The HPTLC chromatogram at 214 nm showed presence of quercitin and exhibited blackish (visible) band in the Rf range of 0.47 to 0.52. According to the literature of flavonoids the Rf range are found to be 0.4 to 0.6. Therefore, the Chromatogram fingerprint suggests the presence of quercitin in aqueous and alcoholic extract of *Trichosanthes lobata* shown in Figure 3a-3c and 4.

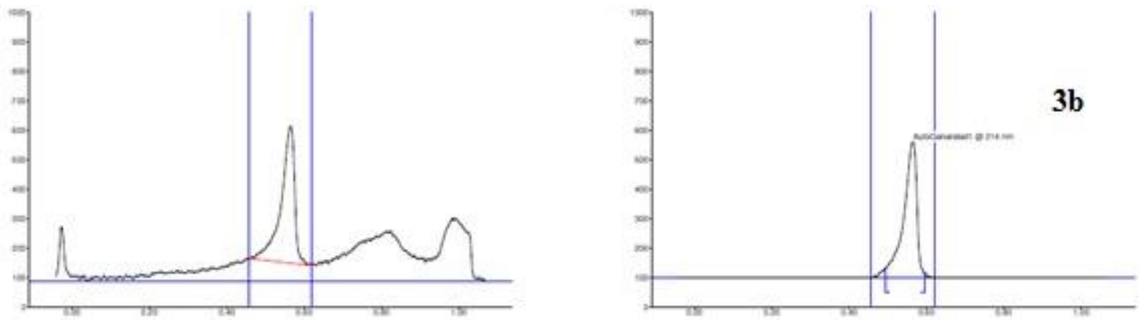
DISCUSSION

The present study was undertaken with the aim of developing the stringent morpho-anatomical, physicochemical and phytochemical standards of *Trichosanthes lobata*. Some of the important diagnostic features of leaf are its bitter taste, presence of anomocytic stomata on lower epidermis, elongated palisade cells, spongy parenchyma with air spaces, glandular trichomes with unicellular to multicellular gland and stalk. Pharmacognostical parameters including HPTLC are helpful for the future identification and authentication of



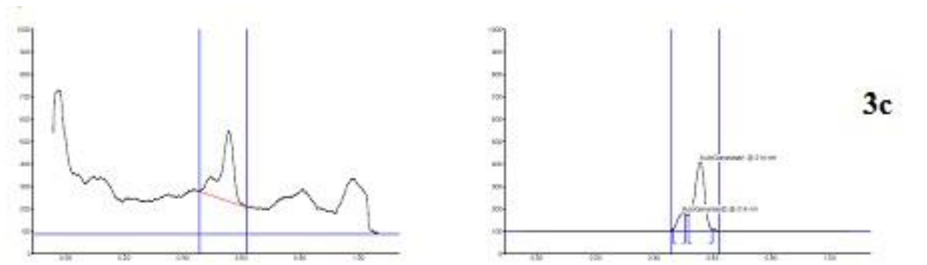
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.47	13.3	0.47	15.5	8.27	0.49	0.3	134.8	1.67	unknown *
2	0.49	0.0	0.55	172.3	91.73	0.59	110.7	7957.2	98.33	AutoGenerated1

Figure 3a: HPTLC chromatogram of alcoholic extract of *Trichosanthes lobate.*



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.50	35.7	0.57	464.3	100.00	0.60	16.5	13125.6	100.00	AutoGenerated1

Figure 3b: HPTLC chromatogram of standard quercitin.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.47	12.9	0.50	83.8	21.21	0.51	74.7	1805.0	16.30	AutoGenerated3
2	0.52	72.4	0.56	311.5	78.79	0.60	6.5	9269.5	83.70	AutoGenerated1

Figure 3c: HPTLC chromatogram of aqueous extract of *Trichosanthes lobate.*

this plant in the herbal industry. The stomatal index, palisade ratio, vein islet and vein termination number was determined in the quantitative microscopy and they can be used to differentiate closely related other *Trichosanthes* species. The estimation of moisture content of the drug is essential requirements in evaluation, as it supports bacteria, fungi or yeast growth. Also determination of ash value and acid-insoluble value has equal importance in the evaluation and identification of inorganic impurities in crude drugs[12]. Plant considered as the richest source for the biosynthesis of major secondary metabolites such as polyphenolic compounds that exhibits significant physiological effects. Presence of important plant secondary metabolites such as tannin, phenolic substances, steroids, glycosides in *Trichosanthes lobata*, could make the plant useful for treating different ailments of living organism because therapeutic efficiency of any plant is usually trace by their chemical compounds[13]. Thus preliminary screening test may be useful in the detection of bioactive principles. HPTLC results indicate the presence of constituents and further facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compound[14]. The leaf has shown presence of remarkable amount of polyphenolic present in alcoholic and aqueous extracts. An important

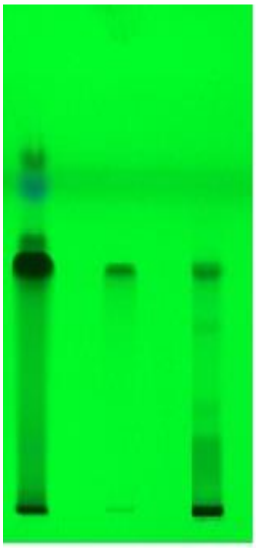


Figure 4: HPTLC profile of alcoholic extract of *Trichosanthes lobata (TLAL); Quercitin and* aqueous extract of *Trichosanthes lobata (TLAQ).*

observation from Phytochemistry point of view is presence of quercitin in the leaf quantified by using HPTLC.
Since the plant, *Trichosanthes lobata* is useful in traditional medicine for the treatment of various ailments; it is need of time to standardize the plant for development of quality control parameters. The pharmacognostic constants of this plant and diagnostic microscopic features reported in this work could be useful for the compilation of a suitable monograph and proper identification as well as distinguishing between closely related species.

CONFLICT OF INTEREST STATEMENT
We declare that we have no conflict of interest.

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