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PHYTOCHEMICAL SCREENING AND BACTERICIDAL POTENCY OF JUSTICIA ADHATODA AGAINST CLINICAL PATHOGENS

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ABSTRACT

Objective: Increasing prevalence of bacterial resistance has made an important public health issue in the modern world. Nowadays, many research efforts have been directed toward the development of drugs from medicinal plants for the treatment of diseases caused by microorganisms. Hence, the present attempt has been made to evaluate the bactericidal potency of traditional medicinal plant, *Justicia adhatoda* against selected clinical pathogens.

Methods: Leaf extracts of *J. adhatoda* were subjected to standard phytochemical analyses by qualitative color tests to confirm the presence of active Phytoconstituents. Bactericidal potency of the leaf extracts of *J. adhatoda* was assessed by growth inhibition zone against *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus*. Sensitivity of the tested bacterial strains was also checked with standard reference antibiotic vancomycin.

Results: Qualitative phytochemical analyses revealed the presence of alkaloids, terpenoids, flavonoids, tannins, steroids, anthraquinones, saponins, resins, glycosides, and phenols. Fourier-transform infrared spectrum confirmed the presence of phytochemicals belong to various functional groups such as alkenes, alkanes, aliphatic and aromatic amines, aldehydes, esters, alcohols, and phenols. Results on bactericidal efficacy demonstrated that solvents used in the present experiment showed higher activity in the order ethanol >ethyl acetate >chloroform >diethyl ether >methanol >acetone >water. Leaf extracts of *J. adhatoda* showed the higher activity for different clinical pathogens in the order of *P. aeruginosa >B. cereus >E. coli >K. pneumoniae >S. aureus*. Among the tested bacterial pathogens, *B. cereus* exhibited maximum susceptibility (18.33±0.94 mm) toward ethyl acetate extract. The maximum activity index (2.67±0.42) and fold area increase (6.31±2.24) were also observed against *B. cereus* for ethyl acetate extract.

Conclusion: The present study revealed that *J. adhatoda* has broad spectrum of antibacterial activity and a potential source of antibacterial agents that could be useful for the control of infectious diseases.

Keywords: Justicia adhatoda, Phytochemical screening, Bactericidal potency, Activity index, Fold area increase.

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INTRODUCTION

Increased resistance of microorganisms to conventional drugs has necessitated the search for new efficient and cost effective ways for the control of infectious diseases [1]. This would trigger immense interest in the search for new antimicrobial drugs of plant origin [2]. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives offering profound therapeutic benefits and more affordable treatment [3]. It is also expected that plant extracts showing target sites on microorganisms other than those used by antibiotics. Therefore, it will be active against drug-resistant microbial pathogens [4]. Moreover, the drugs obtained from plants are less toxic: side effects are scanty and also cost effective. For medicinal purposes, the antimicrobial activity of substances derived from plant extracts has been recognized and widely studied for many years [5,6]. The medicinal values attributed to plants are a function of the bioactive phytochemical constituents. Phytochemical constituents are natural bioactive compounds present in plants that are used as food and medicine to protect against illness and to maintain human health [7]. Phytochemicals have antioxidant or hormone like effect which helps in fighting against many diseases including cancer, heart diseases, diabetes, high blood pressure, and preventing the formation of carcinogens on their target tissues [8].

Justicia adhatoda is a medicinally important plant belonging to the family Acanthaceae that is native of South Asia. It is commonly known as Vasaka or Malabar nut. It is a highly valuable Ayurvedic medicinal

plant used to treat cold, cough, asthma, and tuberculosis [9]. This medicinal plant is exclusively studied for its active components such as quinazoline, alkaloids, vasicine, vasicinone, deoxyvasicine, oxyvasicine, maiontone, and other essential oils [10]. The major alkaloids of the plant, vasicine, and vasicinone have been found to be biologically active. The source of the drug "Vasaka" is well known in the indigenous system of medicine for its beneficial effects, particularly in bronchitis [11]. Leaves and roots of this plant are efficacious in coughs, arthritis, diarrhoea, and dysentery as well as having the best chemostatic quality. Leaves are anti-inflammatory, analgesic effective in skin disorders [12]. Because of many medicinal properties possessed by *J. adhatoda*, the main objective of this study was to evaluate the bactericidal potency of the leaf extracts of *J. adhatoda* against clinical pathogens.

MATERIALS AND METHODS

Materials

Pure and analytical grade chemicals were used in the current study. Acetone, ethanol, methanol, chloroform, ethyl acetate, diethyl ether, nutrient agar, nutrient broth, Mueller-Hinton agar, agar-agar, and standard antibiotic disk Vancomycin were purchased from HiMedia (P) Ltd, Mumbai, India. It was used as starting materials without further purification.

Methods

Collection and preparation of leaf materials

Healthy, disease free and mature *J. adhatoda* leaves were collected from V. V. Vanniaperumal College for Women campus. The leaves were washed

thoroughly with running tap water and then with sterile distilled water to remove the dirt before drying process. Then, the leaves were dried in shade at room temperature for a week to remove moisture content. The leaves were regularly examined to check any fungal growth or rotting. The dried leaf materials were powdered using mixer grinder to obtain fine particle size. Finally, the powdered sample was stored at room temperature for further studies.

Preparation of leaf extracts

2.5 g of powdered leaf sample was taken in air tight bottles. To this, add 50 ml of different solvents such as acetone, ethanol, methanol, chloroform, ethyl acetate, diethyl ether, and distilled water. After 2 days, the contents were stirred well thoroughly and filtered through Whatman no.1 filter paper. Then, the filtrate was collected and stored in sterile bottles at 4°C until further use.

Phytochemical screening of J. adhatoda leaf extracts

Freshly prepared leaf extracts of *J. adhatoda* were subjected to standard phytochemical analyses [13,14] to find out the presence of various phytochemical constituents such as alkaloids, terpenoids, flavonoids, tannins, steroids, anthraquinones, saponins, resins, glycosides, and phenols.

Test for alkaloids (Mayer's test)

1 ml of *J. adhatoda* leaf extract was added with 2–3 drops of Mayer's reagent (dissolve 1.36 g of mercuric chloride in 60 ml water and pour into the solution containing 5 g of potassium iodide in 100 ml water). The appearance of cream color or pale yellow color precipitate indicated the presence of alkaloids.

Test for terpenoids (Knollar's test)

1 ml of leaf extract was mixed with 2 ml of chloroform. To this, add 1 ml of conc. sulfuric acid carefully to form a layer. Formation of reddish brown coloration at the interface indicated positive results for the presence of terpenoids.

Test for flavonoids (sodium hydroxide test)

1 ml of leaf extract was dissolved in diluted sodium hydroxide and then hydrochloric acid was added. The solution color changed from yellow to colorless indicated the presence of flavonoids.

Test for tannins (lead acetate test)

To 1 ml of leaf extract, few drops of 1% lead acetate solution were added. Formation of yellowish precipitate indicated the presence of tannins.

Test for steroids (chloroform test)

1 ml of leaf extract was dissolved in 10 ml of chloroform, and then add equal volume of conc. sulfuric acid by sides of the test tube. The upper layer turned red and sulfuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids in the leaf extracts.

Test for anthroquinone (free anthraquinones test)

0.5 ml of leaf extract was mixed with 10 ml of benzene and then the content was filtered using Whatman no.1 filter paper. To this filtrate, 5 ml of 10% ammonia solution was added and shaken well. Formation of pink, red or violet color indicated the presence of anthraquinone derivative.

Test for saponins (foam test)

3 ml of leaf extract was diluted with 20 ml of distilled water and shaken well for about 15 min. Formation of stable foam suggested the presence of saponins.

Test for resins (sodium hydroxide test)

0.5 ml of leaf extract was treated with caustic soda (NaOH). Development of red color indicated the presence of resins.

Test for glycosides (Keller-Kilian's test)

To 1 ml of leaf extract, add 2 ml of glacial acetic acid containing 2 drops of 2% ferric chloride solution. The mixture was poured into another test tube containing 2 ml of conc. sulfuric acid. A brown ring was seen at the interphase indicated the presence of glycosides.

Test for phenols (ferric chloride test)

To 1 ml of leaf extract, add 2 ml of 2% ferric chloride solution. Formation of blue or green color indicated the presence of phenols.

Fourier-transform infrared (FT-IR) analysis

To identify the functional groups present in the compounds of *J. adhatoda* leaves, IR-spectral analysis was performed using Shimadzu FT-IR 8300 instrument. The spectra were recorded from 500 to 4500 cm^{-1} .

Antibacterial screening of J. adhatoda leaf extracts

Culture and maintenance of the test organisms

The following bacterial cultures of *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus* were procured from the Department of Microbiology, V. V. Vanniaperumal College for Women, Virudhunagar. The procured cultures were reconfirmed by Gram staining and biochemical testing methods. After confirmation, all the bacterial strains were grown on nutrient agar plates and maintained in the nutrient agar slants at 4°C. The bacterial strains were inoculated in nutrient broth and incubated overnight at room temperature for antibacterial screening.

Assay to evaluate bactericidal potency

The antibacterial potency of *J. adhatoda* leaf extracts was evaluated using agar-well diffusion method. Approximately 20 ml of sterile Muller-Hinton agar was poured into sterile Petri plates and allowed to set. Plates were then seeded with 0.5 ml of overnight incubated bacterial culture using a sterile glass rod (L-rod). The plates were allowed to dry. For doing agar well diffusion method, wells were made on the plate with the aid of a sterile hole puncture (6 mm diameter). $50 \,\mu$ l of *J. adhatoda* leaf extracts were poured into the respective wells. The plates thus prepared were left at room temperature for 10 min, allowing the diffusion of the extracts into the agar. Then, the plates were placed in the incubator at 37°C for 24 h. The antibacterial activity of the leaf extracts was assessed by growth inhibition zone surrounding the well. The zone of inhibition was measured and expressed in millimeters (mm). The assay was performed in triplicates.

Assessment of activity index and fold area increase

Activity index [15,16] and fold area increase [17] were calculated by comparing the resultant inhibition zones of leaf extracts with the standard reference antibiotic using the following formula:

Activity index $(AI) = \frac{\text{Inhibitory zone of leaf extracts}}{\text{Inhibitory zone of reference antibiotic}}$

Fold area increase (FI) = $b^2 - a^2/a^2$

Where, a and b refer to the inhibitory zone of reference antibiotic and leaf extracts, respectively.

RESULTS AND DISCUSSION

Phytochemical screening of bioactive compounds in leaf extracts of *J. adhatoda*

The leaf extracts of *J. adhatoda* were screened for the presence of various bioactive compounds by qualitative color tests and the results are given in Table 1.

Successful prediction of bioactive compounds from plant materials is highly depends on the type of solvents used for extraction. The yield

Compounds	Acetone extract	Ethanol extract	Methanol extract	Chloroform extract	Ethyl acetate extract	Diethyl ether extract	Aqueous extract
Alkaloids	-	+	-	+	-	-	+
Terpenoids	-	+	+	-	-	+	+
Flavonoids	+	+	-	-	-	+	+
Tannins	-	+	-	-	+	-	+
Steroids	+	+	+	+	-	-	+
Anthraquinones	+	-	-	+	+	-	-
Saponins	+	+	+	+	+	+	+
Resins	+	-	-	+	-	-	-
Glycosides	-	+	-	-	+	-	+
Phenols	-	+	+	+	-	-	-

Table 1: Qualitative phytochemical analyses of J. adhatoda leaf extracts

+: Present, -: Absent, J. adhatoda: Justicia adhatoda

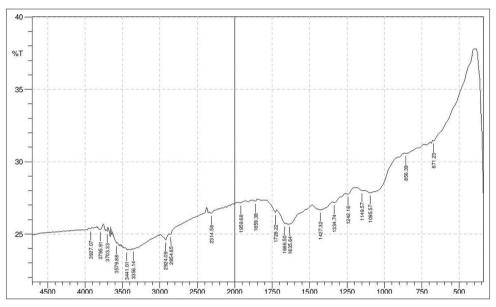


Fig. 1: Fourier-transform infrared spectrum of Justicia adhatoda leaves

of extraction mainly depended on solvents, time, and temperature of extraction as well as chemical nature of the sample [18]. Solvents such as aqueous mixture of methanol, ethanol, and acetone are the most recommended and effective one [19,20]. In similar to this, the solvents selected for the extract preparation in the present study are good and effective. The results on phytochemical screening showed the presence of various bioactive compounds such as alkaloids, terpenoids, flavonoids, tannins, steroids, anthraquinones, saponins, resins, glycosides, and phenols in some of the extracts. The ethanolic and the aqueous extracts were rich in alkaloids, terpenoids, flavonoids, tannins, steroids, saponins, glycosides, and phenols than in other extracts. While the diethyl ether extract was poor in their phytochemical contents, since it contains only terpenoids, flavonoids, and saponins. Similar type of bioactive components were identified [21] in the leaf extract of J. adhatoda and their results revealed the presence of various components such as alkaloids, anthraquinones, flavonoids, saponins, phytosterols, triterpenoids, and poly-phenols. The diversity of phytochemicals found in the J. adhatoda leaf extract suggested that the selected medicinal plant could be served as a source of useful drug.

FT-IR analysis

Fig. 1 illustrates the FT-IR spectrum of *J. adhatoda* leaves. The spectrum revealed the presence of several absorption bands for various functional groups ranging from 500 to 4500 cm⁻¹. The main bioactive compounds found in the *adhatoda* plants are vasicine, vasicinone, vasicol, vasicinol, vasicinolone, etc. [22].

The resultant functional groups attributed to absorption bands are listed in Table 2. The characteristic peak appeared at 671.23 cm⁻¹ and 856.39 cm⁻¹ is due to alkene C-H bending vibrations. The bands observed at 1095.57 cm $^{\text{-1}}$, 1149.57 cm $^{\text{-1}}$, and 1246.16 cm $^{\text{-1}}$ are ascribed to C-N stretching frequencies of aliphatic amines. The presence of aromatic amines and aromatics showed the stretching bands at 1334.74 cm⁻¹ and 1427.32 cm⁻¹, respectively. The peaks at 1666.50 cm⁻¹ and 1728.32 cm⁻¹ indicated the presence of C=C and C=O stretching vibrations. The functional groups of esters and allene showed characteristic bands at 1859.38 cm⁻¹, 2314.58 cm⁻¹, and 1959.68 cm⁻¹. The absorption bands at 2584.65 cm⁻¹ and 2924.09 cm⁻¹ indicated the presence of alkanes containing C-H stretching vibrations. The IR bands noticed at 3356.14 cm⁻¹, 3441.01 cm⁻¹, and 3579.88 cm⁻¹ are due to O-H stretching frequencies of alcohols and phenols. The peaks seen at 3703.33 cm⁻¹, 3795.91 cm⁻¹, and 3927.07 cm⁻¹ are attributed to aromatic CH group/heterogram containing C-C group.

Bactericidal potency of J. adhatoda leaf extract

The antibacterial effect of various leaf extracts (acetone, ethanol, methanol, chloroform, ethyl acetate, diethyl ether, and aqueous extracts) of *J. adhatoda* was determined by the presence of inhibitory zone. The antibacterial activity of the leaf extracts varied according to various organisms tested. Similarly, Tilak *et al.* [23] found out that different extracts of same plant exhibit different antibacterial activity against the similar bacterium. The inhibition zone ranged between 6.00 ± 0.00 mm and 18.33 ± 0.94 mm (Table 3).

The given results clearly depicted that all the tested leaf extracts showed profound antibacterial efficacy against the selected pathogenic strains. The maximum zone of inhibition of 18.33±0.94 mm was noticed for ethyl acetate extracts against B. cereus, whereas, the minimum inhibition zone (6.00±0.00 mm) was seen against K. pneumoniae for methanolic leaf extract. Overall, the ethanolic extract of J. adhatoda had strong growth inhibitory effects on all tested pathogens, followed by ethyl acetate, chloroform, diethyl ether, methanol, acetone, and aqueous extracts. The findings from the present study indicated that the antimicrobial activity may vary with the nature of the solvents used for extraction [24]. The presence of different phytochemicals such as alkaloids, terpenoids, flavonoids, tannins, steroids, saponins, glycosides, and phenols in the leaf extracts could be related to the broad spectrum activity. The antibacterial activity possessed by the leaf extracts is due to the presence of bioactive compounds in the plants [25]. Sankar et al. [18] reported that the antibacterial activity of the plant extracts might be attributed due to the presence of bioactive

Table 2: Functional groups present in the compounds of *J. adhatoda* leaves

Wavenumber (cm ⁻¹)	Functional group	Name of the functional group
671.23, 856.39	C-H	Alkene
1095.57, 1149.57, 1242.16	C-N	Aliphatic amines
1334.74	C-N	Aromatic amines
1427.32	C-C	Aromatics
1635.64	N-H	Amines
1666.50	C=C	Alkenes
1728.22	C=0	Aldehydes
1859.38, 2314.58	0-C=0	Esters
1959.68	C=C=C	Allene
2584.65, 2924.09	C-H	Alkanes
3356.14, 3441.01, 3579.88	0-H	Alcohols, phenols

compounds such as tannins, phenolic compounds, polyphenols, and flavonoids. Among these bioactive compounds, phenolics were the most important active compounds against bacteria [26]. The results obtained from bactericidal effect of *J. adhatoda* leaf extracts were compared with the standard reference antibiotic disk vancomycin. It revealed that the calculated activity value of leaf extracts was higher than 1 (AI >1), which means that all the tested bacterial pathogens in the present experiment showed very strong inhibitory action than the reference antibiotic (Table 4). The maximum activity index value (2.67±0.42) was obtained against *B. cereus* using ethyl acetate extracts. Inhibitory potency of the tested extracts could be mostly considered as important when comparing the antibacterial activity of the tested clinical pathogens with the reference antibiotics [2].

In accordance to this, bactericidal effect exhibited by J. adhatoda leaf extracts with the standard reference antibiotic vancomycin in the present experiment demonstrated the inhibitory potency of the tested leaf extracts as the calculated activity value was higher than 1. Fold area increase of *I. adhatoda* leaf extract with reference to standard antibiotic against selected clinical pathogens as noticed by an increase in diameter of inhibition zone and the results are given in Table 5. Among the tested bacterial pathogens, B. cereus showed highest increase in fold area (6.31±2.24) using ethyl acetate extract, followed by K. pneumoniae (4.05±1.55) and E. coli (3.45±1.05) using ethanolic extracts. S. aureus and P. aeruginosa exhibited maximum fold area increase for the ethanolic (1.53±0.85) and chloroform (1.30±0.87) leaf extracts, respectively. Overall results on bactericidal potency exhibited by Ladhatoda leaf extracts demonstrated that Gram-negative bacterial strains were more susceptible to all the extracts used than Grampositive pathogens. This could be attributed to its cell wall structure. The exact mechanism of antimicrobial activity of plant compounds is not clearly defined. However, multiple dose-dependent pathways have been postulated. These include damage of membrane at bactericidal concentrations of plant compounds as well as loss of energy production which finally lead to cell death [27].

J. adhatoda: Justicia adhatoda

Table 3: Zone of inhibition of leaf extracts of J. adhatoda against selected clinical bacterial pathogens

Name of the extract	Zone of inhibition (mm)							
	Tested organisms							
	B. cereus	E. coli	K. pneumoniae	P. aeruginosa	S. aureus			
Acetone	7.33±0.47	7.67±1.25	11.33±1.25	11.67±2.87	8.00±0.82			
Ethanol	10.33±1.25	14.67±1.70	13.33±2.05	12.33±2.49	13.33±1.25			
Methanol	8.00±0.82	12.00±1.63	6.00±0.00	10.33±2.05	11.00±2.16			
Chloroform	11.00±1.63	12.00±2.45	8.00±0.82	14.67±2.05	11.00±0.00			
Diethyl ether	12.67±2.05	10.00±1.63	9.00±2.16	11.00±1.63	9.00±1.63			
Ethyl acetate	18.33±0.94	12.33±2.05	10.67±2.49	12.00±0.82	6.67±0.94			
Aqueous	10.00±2.16	7.00±0.82	11.00±0.82	10.00±2.16	8.00±0.82			
Standard reference antibiotic vancomycin	7.00±0.82	7.00±0.00	6.00±0.00	10.00±1.63	8.67±0.94			

Each value is the mean±SD of triplicate analysis. B. cereus: Bacillus cereus, E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae, P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus, J. adhatoda: Justicia adhatoda

Name of the extract	Activity index							
	Tested organisms							
	B. cereus	E. coli	K. pneumoniae	P. aeruginosa	S. aureus			
Acetone	1.06±0.13	1.10±0.18	1.89±0.20	1.18±0.29	0.93±0.05			
Ethanol	1.52±0.36	2.10±0.24	2.22±0.35	1.29±0.39	1.57±0.28			
Methanol	1.15 ± 0.02	1.71±0.23	1.00 ± 0.00	1.03 ± 0.04	1.26±0.11			
Chloroform	1.57 ± 0.05	1.71±0.35	1.33±0.13	1.50±0.28	1.29±0.13			
Diethyl ether	1.88±0.51	1.43±0.23	1.50±0.36	1.12 ± 0.20	1.07±0.28			
Ethyl acetate	2.67±0.42	1.76±0.29	1.78±0.42	1.23±0.19	0.78±0.16			
Aqueous	1.42±0.20	1.00±0.11	1.83±0.13	0.99 ± 0.07	0.94±0.14			

Each value is the mean±SD of triplicate analysis. B. cereus: Bacillus cereus, E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae, P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus, J. adhatoda: Justicia adhatoda

Table 5: Fold area increase of leaf extracts of J. adhatoda against selected clinical bacterial pathogens

Name of the extract	Fold area increa	ase						
	Tested organisms							
	B. cereus	E. coli	K. pneumoniae	P. aeruginosa	S. aureus			
Acetone	0.30±0.05	0.41±0.17	0.56±0.35	0.56±0.35	0.14±0.10			
Ethanol	1.43±1.15	3.45±1.05	1.11±0.49	1.11±0.49	1.53±0.85			
Methanol	0.31±0.03	1.99±0.80	0.06±0.08	0.06 ± 0.08	0.60±0.28			
Chloroform	1.45±0.16	2.06±1.20	1.30±0.87	1.30 ± 0.87	0.66±0.32			
Diethyl ether	2.75±1.93	1.10 ± 0.67	0.42±0.33	0.42±0.33	0.56±0.26			
Ethyl acetate	6.31±2.24	2.19±1.06	0.54±0.50	0.54 ± 0.50	0.36±0.27			
Aqueous	1.07±0.56	0.19±0.14	0.12±0.09	0.12±0.09	0.29±0.05			

Each value is the mean±SD of triplicate analysis. B. cereus: Bacillus cereus, E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae, P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus, J. adhatoda: Justicia adhatoda

CONCLUSION

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide. It is becoming an important cause of morbidity and mortality in immune compromised patients particularly in developing countries like our India. Thus, the findings from the present study revealed that *J. adhatoda* leaf extracts showed varying degrees of antibacterial property against the selected clinical pathogens. Thus, the broad spectrum of antibacterial activity exhibited by *J. adhatoda* may help to discover new chemical classes of antibiotic substances that could serve as selective agents of infectious disease chemotherapy and control.

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AUTHOR'S CONTRIBUTIONS

The author Dr. M. Selvarani designed the experiment and determined the bactericidal potency of *J. adhatoda* leaf extracts. Dr. V. Jeyasimga prepared the leaf extracts of *J. adhatoda* and carried out phytochemical analyses. Both the authors prepared and accepted the manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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