



Evaluation of bacteriostatic and antioxidant activities of various extracts from aerial part of *Piper nigrum* grown in Gulf countries traditionally used for the treatment of various infectious diseases

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Abstract

Piper nigrum (*P. nigrum*) is a tropical medicinal plant widely available in Southeast Asian countries including Oman. In Asian It has a long history of usage in places such as Oman for treating of rheumatism, colds, and various infections. Based on the medicinal uses, the purpose of this research is to create diverse fractions using increasing solvent polarity from the aerial part of the collected plant species and evaluate their antibacterial and antioxidant activities by using established bioassays. The aerial part was collected from the Southern part of Oman and processed using the standard method. The processed materials were recovered using the maceration method using methanol, and the methanol raw extracts was further fractionated with water extraction, butanol, ethyl acetate (ETA), hexane, and dichloromethane are produced using different liquid polarities. Each of the six plant extracts was tested for antioxidant and antibacterial potential at different concentrations against DPPH (1, 1-diphenyl-2-picrylhydrazyl) and agar diffusion methods. All of the fractions at varied concentrations shown promising antioxidant activity with DPPH against the gallic acid. The maximum activity was observed in hexane extract, while the smallest potential was noticed in butanol extract, in the following order: hexane > chloroform > water > methanol > ethyl acetate > butanol extract. The antibacterial efficacy of six different polarities extracts gives moderate activity against two Gram-positive bacteria strains namely *S. saprophyticus* and *S. aureus* and three Gram-negative namely *P. vulgaris*, *K. senegalensis* and *E. coli*. Among the six extracts, hexane extract had the most antibacterial activity, whereas butanol extract had the smallest activity. The remaining four extracts, chloroform, ethyl acetate, methanol, and water, all had comparable activity. In conclusion, the extracts with the greatest activity from the chosen plant types might be employed as natural antioxidants to treat infectious disorders.

Keywords *Piper nigrum* · Infectious diseases · Maceration method · Various polarities extracts · Antibacterial and antioxidant activities

1 Introduction

Plants play an essential role in treating many diseases throughout history. Since ancient civilizations, plants have been employed as safe alternative therapy to address a range of illnesses. Hindu, Egyptian and other civilizations, Unani and various other traditional healing systems prefer plants as

an effective medication used to cure illnesses [1]. However, over time these systems have changed, and modern plant-based pharmaceutical medicine has been used for the treatment of different illnesses. Currently, as an urgent human need synthetic medicine emerged to treat human diseases and the use of synthetic medicine has increased tremendously [2]. Medicinal plants are traditionally utilised for illness therapy therefore currently medicinal plants play a important part in the uncovering of new medications. More than 7000 compounds were isolated from medicinal plants and the majority of those compounds are currently used in modern healing systems as prescription drugs. Most of these prescription drugs have been used for a few decades as natural healers and more than \$100 billion per annum is spent to buy medicinal plants and formulated products [2].

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In addition, plant-based medicines with significant pharmacological activity are used to treat chronic diseases, therefore due to safety and efficacy the use of plant-based medicine has continued to be explored. Since the last century, the eating and drinking habits of humans have changed drastically and it creates the development of several chronic diseases and incurable diseases. Currently, majority of the artificial medications on the market today have their efficacy declining as well as increasing adverse effects as compared to natural drugs [2]. Therefore, learning of the antioxidant action of the plant extracts might perform the important part of their prevention plus cure the diseases.

This plant is available the majority of tropical nations, involving India plus the Middle East. *Piper nigrum* (*P. nigrum*) is considered a flowering plant. It is known as black pepper, belongs to the Piperaceae family and is commonly found on the east coast of India and in tropical regions of Asian countries. In many cultures, this spice is used as a condiment. The height of this selected plant is about 10 m or more. The plant is a perennial vine that grows very well with the help of other trees or poles. The plant has approximately 10–20 primary and secondary roots that start from the base of the mature stem. The selected plant leaves are 2–5 cm long, simple, and alternate. Petiole notched, leaf length and width variable, 8–20 cm and 4–12 cm respectively [3]. Normally, after 2 to 3 years of cultivation, the plant gives flowers from May to July each year [4]. The length of the fruit varies by 3–15 cm. After 10–15 days, the prickly appearance, the first flower appears on the top then it has completed approximately 6–10 days later. The fruit is bright in color. The ripe fruits are about 5 mm in diameter with a spherical shape [3–6]. The harvested fruits are dried in the sunlight for later it is ready to use. Nowadays, the world populations are suffering several chronic diseases such as diabetes, cancer, hypertension etc. However, drugs in order for relieve those persistent illnesses are not sufficient. There are some synthetic drugs accessible on international sectors to be cure or management of those chronic illnesses, but they are costly and have significant adverse effects [2]. Therefore, at present, a good number of research have been focused on plant and other natural resources to find a new medicine that are more effective than synthetic medicines. Based on the geographical distribution Oman, has a good number of plant species that are traditionally used to treat various ailments. The climate of Oman is favorable for the growing all these rare plants. The question is there any documentation that antioxidants and antimicrobial substances are present in the plant of *P. nigrum*. The plant and its related food products are the main sources of biologically active ingredients such as phenols and their derivatives, flavonoids, terpenes and sterols and their derivatives. All these plant ingredients have been evaluated for biological and pharmacological activities. The major ingredient of the selected plant

species is about 2–7% piperine [6]. The plant volatile oil contains piperamides, β -caryophyllene and nerolidol as major ingredients that exhibited insecticidal activities [7–9]. The other ingredient β -caryophyllene has narcotic properties [9]. Piperine is a plant product, and it has been used in perfumes, cosmetics and other related products. The selected plant contains about 0.4–7% of the dry weight of essential oil and it is potent for rheumatism, common cold, fatigue, muscle pain and disorders and various infections [7–12]. In traditional medicine and alternative systems, the selected plant was well known to have gastrointestinal activity, loss of appetite, cough, bronchitis, and common cold, sore throat, breathing problems, dysentery, worms and piles [13–16]. In particular, this plant is used to treat types of diabetes including digestive problems, loss of appetite, cough, bronchitis, colds, sore throat, breathing problems, dysentery, worms and hemorrhoids. Therefore, the goal is for identify an antimicrobial and antioxidative properties for the selected plant plus ascertain whether it will act as an antibacterial and be used as an antibiotic or not. Then the ingredient responsible for antioxidants and antimicrobials will be isolated from the important extract. Through this research, its outputs and components will be contributed to help the Omani and global community prepare a medicine that contains high efficiency against microbes and antioxidants such as Antibiotics from this plant, as it has also been proven to contain antidiabetic substances. Based on the importance, this study aims in order to make the high portion obtained from the selected plant species and determine their antimicrobial, antioxidant, and photochemistry study by using the usual method.

2 Material and methods

2.1 Chemicals

Altogether seven solvents namely hexane, chloroform, ethyl acetate, butanol, methanol, water and acetone employed to that were purchased of the Sigma-Aldrich Company (Germany). The purity of the above-mentioned solvents is in the range of 95 to 97.45%. Dimethyl sulphoxide (DMSO), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), and levofloxacin were obtained from British Drug Houses (BDH, UK).

2.2 Bacterial strains

A total of five bacterial strains namely *Staphylococcus saprophyticus* (*S. saprophyticus*), *Staphylococcus aureus* (*S. aureus*), *Proteus vulgaris* (*P. vulgaris*), (*K. senegalensis*), and *Escherichia coli* (*E. coli*) were collected from Nizwa Hospital, Nizwa on end of September 2023. All of them were cultured bacteria

strains. Among the bacterial strains two Gram positive namely *S. saprophyticus* and *S. aureus* and three Gram negative namely *S. proteus*, *S. klebsiella*, and *E. coli*. After collecting the strains, all bacterial strains were kept in the incubator until the conduct of the antibacterial experiment.

2.3 Sample collection

The aerial parts of plant samples were gathered from Salalah in Oman's the Southeast region. The samples were collected on March 9, 2023, at around 10 a.m. The samples were semi-dried under the shade instantly and packed in the polyethylene bag. The sample was carried by bus from Salalah to Nizwa. The semi-dried plant samples were washed again and dried under shade for 1 week [17]. The dried samples (500 gm) were made into course powder by using a ball machine (Panasonic, Japan). The specimen of harsh powder (427.43 gm) was saved in a colored flask and stored for extraction.

2.4 Extraction

All coarse powder samples (427.43 gm) were transferred into the three-liter capacity bottle and 1.5 L of methanol was then kept in the bottle for a few days with intermittent shaking. After three days, the sample mixture was filtered by a Buchner funnel. Again, 1 L of methanol was added to the bottle and kept the bottle for 24 h then filtered. The filtered methanol samples were transferred to the rotary round bottom flask and evaporated by a rotary evaporator until dryness. The weight of the methanol crude extract was 62.78 gm. From there 61.78 gm of methanol extract was dissolved in aqueous solvent (200 ml) and fractioned successively with hexane, chloroform, ethyl acetate, and butanol solvent [18]. This separatory funnel was filled with the entire combination. And added 30 ml of hexane then shake by hand for 20 min. The funnel was kept for 30 min to separate into two layers. The upper layer, the hexane layer, was collected in a 100 ml conical flask. Again 20 ml of hexane had been put into a funnel for separation. Containing mixture and shaken the funnel for 10 min then kept for thirty minutes. It was obtained two layers, the upper layer means the hexane layer was collected and both upper layers were added to a conical flask. Then the hexane extract obtained was produced by evaporating hexane (14.61 gm). Exactly, the same way prepared chloroform, ethyl acetate, and butanol extract to produce the appropriate chloroform (6.34 gm), ethyl acetate (8.66 gm), and butanol (2 gm) correspondingly. After fractionation, the water portion also evaporated the same way to give water extract (31.17 gm). All the prepared different polarities organic crude extracts

had been employed in order to determine antibacterial and antioxidative potential.

2.5 Antioxidant activity

Each polarity organic crude extract at five different concentrations was assessed on their anti-oxidative properties versus 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method [19]. Initially, ten milliliters of methanol had been used to melt mg of every extract. The prepared concentration was 100 ppm. From there 50, 25, 12.5 and 6.25 ppm were prepared by adding methanol with the sequential dilution method. Similarly, 3.3 mg of DPPH was dissolved in 100 ml volumes the flask containing 100 ml of methanol. As a standard, gallic acid was used in this experiment. Three microliters (3 μ l) of each polarity organic extract at different concentrations were taken separately in a 5 ml capacity clean test tube. Then 1.2 ml of DMSO of each test tube and finally added 2.7 ml of DPPH of each test tube. After adding DPPH, the solution had been mixed well as kept at room humidity in dark area for sixty minutes. This total absorbency of samples that had been incubating were evaluated at 517 nm in opposition to DPPH and methanol. Utilizing the following equation, the absorbance for every extract at various concentration was calculated:

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{extract}}}{A_{\text{control}}} \times 100$$

2.6 Bacteriostatic activity

That antimicrobial behavior for various polarities for organic extracts obtained at four concentrations against two Grams (+) and three Grams (-) bacterial strains was assessed using the disc dispersion technique, via a minor alteration [20]. Four distinct concentrations of the chosen plant's crude extract for each polarity such as 2, 1, 0.5 and 0.25 mg/ml were used for the assessment of the antibacterial study. Levofloxacin, a wide-ranging antibiotic, and DMSO solvents served as positive and negative controls. For thirty minutes, filter paper discs measuring six millimeters had been submerged in every concentration of the organic extract for every polarity. After that, the sample-containing disks were put on the ready-made agar plates and left in the chamber for a full day at 37 degrees Celsius. The five applied Gram (+ and -) bacterial strains were used to measure the inhibition of the incubated plates at each concentration of plant extracts.

2.7 Statistical analysis

Three replicates of each experiment were conducted. Each experiment was repeated at least two times. The control and treatment groups were compared using the *t* test. *P* values ≤ 0.05 were considered significant.

3 Results

3.1 Preparation of crude extract

People had used greenery during which ages ago and plant-derived medications for relieve various illnesses. Some plant drugs and their products are highly significant against diseases. Among the plants, *P. nigrum* is one of the medicinal plants available in Southeast Asian countries including Oman. Originally, the chosen plant kind has been utilized to relieve rheumatism, cold and infectious diseases. Based on the medicinal uses, the selected plant was undertaken for the assessment of antioxidative and antimicrobial activities. Various polarity extracts were prepared from the coarse powder of the aerial part of the plant by using methanol solvent by the solvent extraction technique the prepared different crude extracts and their percentage of yield are presented in Table 1.

3.2 Antioxidant activity

Utilizing the improved DPPH technique, determine the antioxidative properties of six polarities extracted from the aerial part of *P. nigrum* samples [19]. The antioxidant activity as a percentage inhibition (%) was determined for different polarities of organic extracts at five varied concentrations by using the established formula. Among the six crude extracts of the plant, the highest activity was found in hexane extract and the lowest was in butanol and followed the discerning order hexane < chloroform < water < methanol < ethyl acetate < butanol extract.

Table 1 Yield of crude extracts of aerial parts of *P. nigrum*

Crude extracts	Yield (gm)	Ratio of yield (%)
Hexane	14.61	23.64
Chloroform	6.34	10.26
Ethyl acetate	8.66	14.01
Butanol	2	3.23
Water	30.17	48.83
Methanol	62.78 from 427.43	14.68

Table 2 Antioxidant potential of hexane, ethyl acetate, chloroform, butanol, methanol and water crude extracts of *P. nigrum*

Crude Extracts	Concentration $\mu\text{g/ml}$	Absorption of DPPH	Absorption of samples	% Inhibition
Methanol	100	1.787	0.093	94.79 \pm 0.23
	50		0.187	89.50 \pm 0.17
	25		0.170	90.48 \pm 0.08
	12.5		0.136	92.38 \pm 0.16
	6.25		0.657	63.23 \pm 0.27
Hexane	100	1.787	0.128	92.83 \pm 0.11
	50		0.191	89.31 \pm 0.98
	25		0.108	93.95 \pm 0.20
	12.5		0.164	90.82 \pm 0.14
	6.25		0.242	86.45 \pm 0.23
Chloroform	100	1.787	0.300	83.21 \pm 0.14
	50		0.241	86.51 \pm 0.10
	25		0.119	93.34 \pm 0.76
	12.5		0.382	78.62 \pm 0.33
	6.25		0.353	80.24 \pm 0.12
Ethyl acetate	100	1.787	0.161	90.99 \pm 0.55
	50		0.202	88.69 \pm 0.21
	25		0.150	91.60 \pm 0.16
	12.5		0.230	87.12 \pm 0.12
	6.25		0.266	85.11 \pm 0.10
Butanol	100	1.787	0.364	80.00 \pm 0.56
	50		0.422	76.38 \pm 0.13
	25		0.226	87.35 \pm 0.29
	12.5		0.263	85.28 \pm 0.44
	6.25		0.156	91.27 \pm 0.17
Water	100	1.787	0.133	92.55 \pm 0.20
	50		0.207	88.41 \pm 0.15
	25		0.357	80.02 \pm 0.28
	12.5		0.406	77.28 \pm 0.11
	6.25		0.100	74.40 \pm 0.09

Each value is a means of three replicates

The activity shows the restriction to be a percentage of all prepared extracts at different concentration presented in Table 2.

3.3 Bacteriostatic activity

In the present study, two Gram-positive microbes' strains namely two Gram-positive (*S. saprophyticus* and *S. aureus*) and three Gram-negative (*P. vulgaris*, *S. klebsiella*, and *E. coli*) were utilized to evaluate microbial activity at different four concentrations of each polarity organic extract by using the dispersion technique using agar discs [20]. Levofloxacin and DMSO were utilized in the experiment as positive and negative control respectively. Every single prepared concentration of each

polarity organic extract showed moderate tableau three displays the behavior as well as the findings of the current investigation.

4 Discussion

Natural products from various sources are vital information during their creation of novel medications with potential therapeutic properties in the world. Natural sources for new drugs are plants, animals, and various organisms. Among the sources, plants and their derived products are considered primary sources due to reliability, efficacy, and safety [1–5]. One of the primary problems for the health care system is plants and plant products which are used in some areas of the globe. Due to its importance and safety, in the past few decades, scientists have been working on plants as a major primary source to invent bioactive agents that could be given a new and safe drug to treat diseases [6, 21].

The southern region of Oman namely Dhofar governorate including almost all desert areas has amusing medicinal plant resources. Most of the potential plants with therapeutic values are available in those areas. Based on the statistics showed that more than two-thirds of total medicinal plants used in Oman by the different ethnic communities come from the Dhofar Governorate. A good number of medicinal plants in those areas with significant medicinal values were documented.

4.1 Antioxidant activity

The DPPH method is the most popular and established method used to assess antioxidant activity [19]. In our experiment, five varied concentrations of each polarity organic extract the chosen plant kind were ascertained through an application during a DPPH technique. [19]. All the working samples were incubated and the absorbance of each concentration of each polarity of extract of the plant was measured with UV method. The ratio for every extract's restriction at five different concentrations was calculated by through the application of the predetermined equation. The standard used is gallic acid. According to Table 2, butanol had the least antioxidants properties while hexane had their largest, with a determining ranking of hexane, chloroform, water, methanol, ethyl acetate, and butanol extract following. From the literature, it showed that the plant contains several biologically active compounds namely different fatty acids derivatives, vitamins A and K, oxalic acid, saturated and unsaturated polyaromatic hydrocarbons, minerals potassium, calcium and complex starch, tannin acid and derivatives, cardiac

glycosides derivatives, polycyclic phenolic derivates that are directly or indirectly responsible for biological activities [13, 14, 16]. The above-mentioned active ingredients from the plant extract might be capable of reducing DPPH color by donating protons [3]. Our experiment results Table 2, showed that hexane extract at all five concentrations gave the highest activity compared to gallic acid standard and other herbal obtain that have been chosen. The highest level of oxidative capacity indicates that many bioactive compounds, mainly nonpolar, are biological activity materials in the hexane extract. Additionally, a biological activity substance found in the hexane extract might be their concentration was very high therefore the obtains containing hexane had the most active extract. However, butanol obtains had the least active extract because of the number and concentration of bioactive compounds present in the butanol extract. The other extracts namely chloroform, water, methanol, ethyl acetate, and water extracts at five varied concentrations also gave potential antioxidant activities. It is indicated that all those extracts from the selected plant also contain the maximum number and concentration of biologically active compounds that are directly involved in their activity. The variation of activity among the extracts could be due to varied polarities of extracts, number and percentage of active compounds, harvesting of plant and drying of plant samples. In addition, it could affect or damage the volatile compounds due to the drying and evaporation of the plant samples. For the above reason, the antioxidant activity results are different in different polarities extracts. Similar results and relationships were obtained between the antioxidant activity of crude extracts and active ingredients as has previously been reported by several authors [13, 14, 16].

4.2 Bacteriostatic action

Six different polarities organic raw obtains were ready at five varied concentrations of the aerial parts the disk dispersion technique was used to evaluate the chosen extracted plants in opposition to two Gram-positive (*S. saprophyticus* and *S. aureus*) and three Gram-negative (*P. vulgaris*, *K. senegalensis*, and *E. coli*). The antibacterial activity was exhibited activity against applied bacterial strains, at varied concentrations within the range of 0–15 mm. The highest activity was obtained in hexane crude extract at a concentration of 2 mg/ml against *E. coli* in comparison to *S. aureus*, the obtain of methanol at a rate of 0.25 mg/ml had their least amount of behavior. All the six prepared aerial plant crude extracts at varied concentrations gave antibacterial activity comparatively low against the applied Gram-positive bacterial strains namely *S. saprophyticus* and *S. aureus*. On the other hand, all six organic extracts from selected plant species at varied

Table 3 Antibacterial behavior of raw extracts of *P. nigrum* high parts with varying polarities opposed to the chosen bacterial strain

Extracts	Conc. (mg/ml)	<i>S. saprophyticus</i>	<i>S. aureus</i>	<i>S. proteus</i>	<i>S. klebsiella</i>	<i>E. coli</i>
Methanol	2	10±0.21	12±0.09	12±0.19	9±0.15	13±0.18
	1	9±0.13	8±0.19	11±0.13	8±0.11	11±0.42
	0.5	9±0.09	7±0.35	11±0.25	8±0.52	11±0.09
	0.25	8±0.17	6±0.13	10±0.18	8±0.10	9±0.25
	S	16±0.44	27±0.15	29±0.32	11±0.19	12±0.13
Hexane	2	11±0.13	10±0.14	14±0.12	12±0.44	15±0.18
	1	10±0.21	9±0.29	12±0.17	10±0.15	11±0.69
	0.5	10±0.24	8±0.16	12±0.09	9±0.08	9±0.78
	0.25	9±0.12	8±0.13	10±0.39	8±0.11	9±0.09
	S	18±0.15	26±0.26	31±0.18	11±0.17	12±0.18
Chloroform	2	11±0.55	10±0.16	11±0.09	10±0.24	12±0.21
	1	9±0.16	8±0.18	10±0.11	10±0.29	11±0.37
	0.5	9±0.13	7±0.58	9±0.08	9±0.14	9±0.17
	0.25	8±0.42	6±0.20	9±0.16	8±0.23	9±0.21
	S	17±0.29	28±0.34	32±0.61	11±0.10	11±0.14
Ethyl acetate	2	12±0.09	15±0.16	10±0.25	10±0.09	11±0.90
	1	10±0.12	11±0.08	9±0.29	9±0.17	9±0.49
	0.5	10±0.59	10±0.09	9±0.18	8±0.13	9±0.10
	0.25	9±0.55	9±0.19	8±0.14	8±0.20	8±0.24
	S	18±0.18	25±0.11	29±0.45	11±0.50	11±0.08
Butanol	2	11±0.11	8±0.51	9±0.79	10±0.10	11.5±0.56
	1	11±0.18	7±0.13	8±0.15	9±0.67	10±0.11
	0.5	9±0.16	6±0.20	7±0.22	9±0.17	9±0.15
	0.25	9±0.40	6±0.31	7±0.45	8±0.38	9±0.46
	S	17±0.11	26±0.10	31±0.12	10±0.12	11±0.13
Water	2	10±0.34	9±0.77	10±0.14	10±0.18	10±0.09
	1	9±0.09	9±0.19	10±0.11	9±0.54	10±0.16
	0.5	8±0.14	7±0.12	8±0.18	8±0.27	9±0.15
	0.25	8±0.20	7±0.23	8±0.16	8±0.14	8±0.28
	S	17±0.11	27±0.45	30±0.08	10±0.25	12±0.11

S Positive control; Each value is a mean of three replicates

concentrations gave high antibacterial activity against three Gram-negative namely *P. vulgaris*, *K. senegalensis*, and *E. coli* bacterial strains compared to Gram-positive bacterial strains. From Table 3, it is indicated that all polarities organic crude extracts at varied concentrations gave almost similar moderate activity. It also indicates that its antibacterial capacity is not greatly varied among the varied polarities of organic crude extracts at varied concentrations. It could be implied that the bioactive secondary metabolic compounds have strong antimicrobial activity [6, 12, 19]. In previous studies done by different authors on the same species and their results in their antioxidant and antibacterial activities are not similar [22, 23]. This variation might be due to the synonyms, harvest of plant, drying process, extraction procedure, fractional process, evaporation of the selected plant samples.

5 Conclusion

In this present study, all prepared aerial parts organic crude extracts of the selected plant species at varied concentrations were assessed for their antioxidant, and antibacterial activities against DPPH, and Gram (positive and negative) bacterial strains. Our results showed that all the extracts gave moderate antioxidant and antimicrobial activity. Hexane extract gave among each of the six extractions, there are encouraging antibacterial and antioxidants properties. Of the six organic extractions, butanol extract had the lowest levels of antioxidants and antibacterial activity. In conclusion, the promising activity of organic crude extract namely hexane obtain might have applied as a natural organic antioxidant as well as antibacterial agent to treat infectious diseases.

5.1 Recommendations

Further studies will be designed on the isolation constituents from the active compounds and to confirm their antioxidant, and antibacterial activity and in vivo studies are also needed for the preparation of new drugs.

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Author contributions DIMA: Sample collection, data generation, and data analysis. SSJT: Overall supervision and edit draft manuscript. MAH: Planning, data analysis, and preparing manuscript.

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Data availability All necessary data of the current study are available.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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