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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY AND CHARACTERIZATION OF GREEN SYNTHESIZED SILVER NANOPARTICLES FROM *BLEPHARIS MADERASPATENSIS (L) HYNE EX. ROTH*

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ABSTRACT

The morphology of the silver nanoparticles synthesized from aqueous extract through bioreduction method shows spherical morphology. Silvernanoparticles are randomly distributed with average diameter >100nm. The biological way of synthesizing silver nanoparticles has advantages like cost effectiveness and eco-friendly. Biosynthesized nanoparticles showed maximum antimicrobial activity against *k.pneumonia* (15mm), *bacillus subtilis* (15mm), *bacillus pumillus*(13mm), *E.coli* (15mm) followed by *Candida albicans* (17mm), *aspergillus flavus*(13mm).The silver nanoparticles were studied using UV-Vis absorption spectroscopy, FTIR, XRD, scanning electron microscopy (SEM), Zeta potential analysis. This was the first work to report on sliver nanoparticles synthesis from the leaf extract of blepharis maderaspatensis hyne Ex.roth. This investigation may be used in future direction as alternative therapeutic agents for the treatment of human diseases.

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INTRODUCTION

Nanotechnology is one of the most dynamic areas of research in modern materials science and technology. Nanomedicine an imperative applications of nanotechnology which uses nanomaterials to pioneer into human body to perform cellular repairs in the molecular level. Today, clinical trials have been investigated by nanostructures. The main purpose of this paper is to evaluate the antimicrobial activity, characterization and synthesis of silver nanoparticles from the aqueous leaf extract from *Blepharis maderaspatensis hyne ex Roth.* In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. It is beneficial. Bioreduction agents are rich in plant extract which reduce silver ions to silver nanoparticles (Alam *et al*., 2011; Khan *et al*., 2010). The main advantages of biosynthesis of silver nanoparticles from plants are they are non toxic and cost effective.

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The rate of synthesis is rapid and the reducing form is more stable in plant extracts. *Blepharis maderaspatensis (L.) Heyne ex Roth.* The clearance mechanism of nano synthesized drugs which can alter the particle surface structures to either suppress macrophage detection or to control self aggregation of the particle. In the era of Ag NP preparation, plant-mediated green biomimetic synthesis of silver nanoparticles is considered a widely acceptable technology for rapid production of silver nanoparticles. Already Existing literature also reports the successful synthesis of silver nanoparticles through a green route where the reducing and capping agent selected was the latex obtained from Jatropha curcas. Ag NPs were also obtained using Aloe vera, Acalypha indica, and Garcinia mangostana leaf extracts. Not only Crataegus douglasii fruit extract and also various other plants extracts. It has been a well documented fact that since ancient times silver metal is known to have antimicrobial activities. AgNPs have been reported for their applications as antimicrobial agents, catalysis, bimolecular detection and therapeutics. Although there have been number of studies on antibacterial activity of AgNPs the actual mechanism has not yet been exactly studied.

Shahverdi *et al*. reported that because of its small size and high surface area the AgNPs might have been selected for its enhanced antimicrobial activity. (Nahrstedt and Butterweck, 2010; Rojas *et al*., 2003). The plant *Blepharis maderaspatensis hyne ex Roth* pertains to the family Acanthaceae. Its uses are more in traditional medicines. However for the best of our knowledge no studies have been done on this plant in nanotechnology platform which has enormous abundance. Phytochemical screening of the leaf extract was done to discover the phytochemical constituents in the various solvents. It is commonly named as creeping blepharis and seen commonly on slopes, among rocks, poor gravelly soil. Blepharis (Acanthaceae) is an Afro-asiatic genus comprising 129 species which occur in arid and semi-arid habitats .*Blepharis Maderaspatensis* is used for headache. Seeds are used as dysuria, diseases of nervous system, diuretic, aphrodisiac. It is used to cure cuts and wounds, juice extracted from leaf is heated with gingelly oil and applied on affected places to heal wound. Dry seeds of this plant contain steroids and the plant is used for brain disorders like Parkinson's disease. (Habbal *et al*., 2011) In the present investigation we report the highly stable bio synthesized nanoparticles and the evaluation of antimicrobial activity and the study also rely on the characterization of green synthesized nanoparticles by UV-Visible spectrophotometer, scanning electron microscope, Fourier transform infra red, XRD spectral analysis and zeta potential.

MATERIALS AND METHODS

Collection of plant material

Blepharis maderaspatensis hyne ex Roth leaves were collected freshly and healthy, washed thoroughly thrice with tap water and twice with distilled water .they were shade dried and powdered finely using electric blender (Nahrstedt and Butterweck, 2010; Rojas *et al*., 2003). The fine powder of the stem and leaf was extracted at 47^{o}C by using Soxhlet apparatus using aqueous as a solvent. After extraction, they were stored properly for further studies. The medicinal plant selected for the study was collected from Megamalai, Theni, India.

Preparation of Extract

By using different solvents in Soxhlet apparatus the shade dried powder was percolated. Distillation method using Soxhlet apparatus the phytochemicals present in the plant material was extracted. There are three different solvents used in the separation process (aqueous, ethanol and chloroform). The leaves were washed with sterile water and then chopped into fine fragments. The materials were then shade dried at ambient temperature (32^{o} C) for 14 days and to avoid chemical changes the drying process was carried under proper conditions. Then it is crushed into fine powder using an electric blender and then the extract was prepared by boiling in Soxhlet apparatus using aqueous, ethanol and chloroform as solvents to study the phytochemical constituent. After extraction, the extract was stored in proper control conditions for further studies.(Shankar *et al*., 2003)

Preliminary screening of phytochemicals

The aqueous, ethanolic and chloroformed extracts were screened for the presence of phytochemical substances which reveal the characteristic nature of the extract. The bioactive substance such as terpenoids, phlobatannins, reducing sugar, flavonoids, phenols, glycosides, starch, proteins, peptide, amino acids, tannins, anthroquinones, sterols, steroids, coumarins, quinines, saponins and alkaloids were screened (Habbal *et al*., 2011; Shahverdi *et al*., 2007)

Biosynthesis of silver nanoparticles

Aqueous solution (5mM) of silver nitrate was prepared for the synthesis of silver nanoparticles. From the blepharis maderaspatensis hyne ex. Roth extract 10ml of leaf extract was taken and added with 95ml of aqueous solution of 5Mm silver nitrate to reduce into silver ions. The reduction of silver nitrate to silver ions was confirmed by the colour changes from yellow to brownish black (Harekrishna *et al*., 2009; Dorman and Deans, 2000) By using spectrophotometer the formation of silver nanoparticles have been determined. For 15min at 10,000 rpm the reduced solution was centrifuged, the supernatant was discarded. The pellet was redispersed in deionized water thrice dried powdered and stored as silver nanoparticles. The same protocol was repeated for chloroform and ethanol.

Anti –bacterial efficacy by well diffusion method

To evaluate the anti- bacterial efficacy of aqueous extracts bacterial cultures such as *k.pneumonia*, *bacillus subtilis*, *bacillus pumillus*, *E.coli* followed by *Candida albicans*, *aspergillus flavus* were used. The silver nanoparticles synthesized from *Blepharis maderaspatensis hyne ex Roth* as positive control and plain extract as negative control. Freshly prepared 1ml of the organism cultured in liquid broth was taken and was poured in the Petri plate and was spread evenly using L-rod. On the top of it, Nutrient agar medium was poured and was allowed to solidify. Once it is been solidified, three wells were punched using cork borer. One well was left empty and was used as a control, the next well was filled with aqueous extract and the last one was filled with silver nano particle. Then the plate was incubated in an incubator for 24hours. The agar well diffusion method was used to determine the growth inhibition (Jha *et al*., 2009; Khandelwal *et al*., 2010)

Characterizations of biosynthesized silvernanoparticles

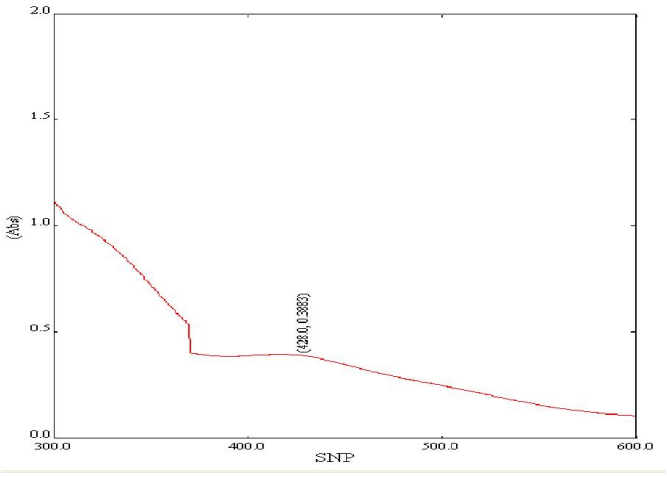

Fig. 1. UV-VIS spectral analysis

UV- Vis studies

The reduction of silver nitrate into silver ions was estimated by measuring the UV-Vis spectrum. The absorbance was recorded at a resolution of 1nm at 300-600 nm using UV-Vis spectrophotometer.

SEM analysis

The structure and the size of the nano particles were analyzed using scanning electron microscope. The sample was prepared as a 1µg thin film and coated in a copper grid by dropping method and the excess amount of solution in the grid is wiped out using blotting paper and it is allowed to dry under a Mercury lamp for five minute to get fine results. The images of nanoparticles were studied using SEM (JPEG, model JFC - 1600). (*Singhal et al.,2011).*

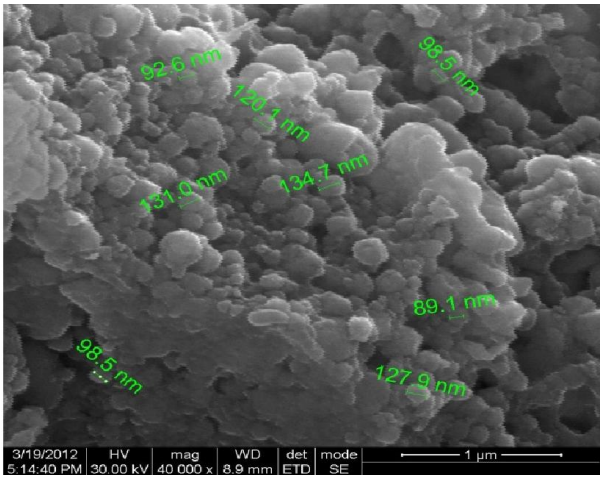

Fig. 2. SEM analysis

FTIR analysis

Perkin-Elmer spectrometer FTIR Spectrum in the range 4000-400 cm−1 at a resolution of 4 cm−1 was used for the analysis. The sample was mixed with KBr crystals. Thin sample disc was prepared by pressing with the disc preparing machine and placed in Fourier Transform Infrared (FTIR) for the analysis of the nanoparticles as well as for the leaf extract.(Shankar *et al*., 2004)

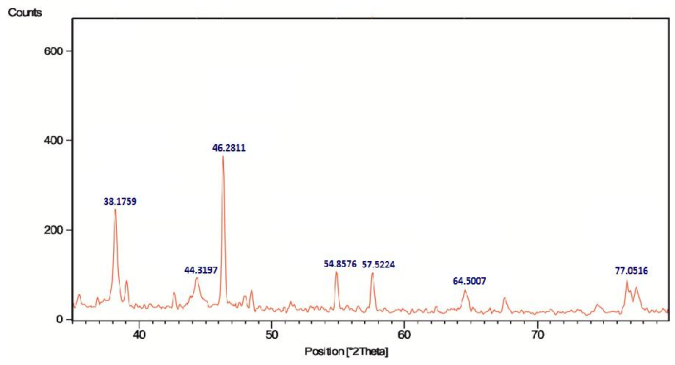

Fig. 3. XRD analysis of biosynthesized silvernanoparticles from blepharis maderaspatensis hyne Ex.roth

XRD analysis

The dried pellet was purified and the silver nanoparticles were further analyzed with X-ray diffractrometer. The data obtained for silver as matched with the joint committee on powder diffraction standards JCPDS. The grain size of the silver nanoparticles formed in the process was estimated from the debye-scherrer equation. The quality of the dried mixture was analyzed by XRD technique (PAN analytical BV, Netherlands) operated at a voltage of 40kv, and a current of 30mA, with CuKa radiation in an h-2-h configuration.(Siddique *et al*., 2008)

Zeta potential analysis

Zeta potential of Ag NPs was measured according to Tantra, et.al with the help of Zetasizer Nano ZS (Malvern) and a titrator MPT-2. Before the measurement, aqueous suspension of silver nanoparticles was filtered through a 0.45 µm PTFE membrane. The zeta potential was calculated by the help of the software. (Elumalai *et al*., 2010)

RESULTS AND DISCUSSION

Phytochemical analysis

The preliminary phytoscreening studies revealed that the leaf extract of *Blepharis maderaspatensis hyne ex. Roth* have lot of bioactive substances namely terpenoids, phlobatannins, reducing sugar, flavonoids, phenols, glycosides, starch, proteins, peptide, amino acids, tannins, anthroquinones, sterols, steroids, coumarins, quinines, saponins and alkaloids. (Table 1)

Antibacterial efficacy

The silver nanoparticles of *Blepharis maderaspatensis hyne Ex.roth* showed the maximum zone of inhibition with microbial species like k.pneumonia (15mm) followed by E.coli and bacillus subtilis but the minimum zone of inhibition was observed in bacillus pumillus . The antifungal properties show maximum zone of inhibition in Candida albicans (17mm) and minimum in aspergillus flavus (13mm). The aqueous extract shows nil results when compared to biosynthesized nanoparticles. Moreover, the present study also proved to have potential antimicrobial activities with the *Blepharis maderaspatensis* leaf extract synthesized silver nanoparticles .the mechanism behind this is the denaturation of bacterial cell wall, destabilization of the outer membrane, stopping bacterial respiration and depletion of ATP. The gram positive and gram negative bacteria's have different sensible characters which attribute the membrane permeability. Instead of this barrier the biosynthesized silvernanoparticles exhibited the strong inhibition with gram negative bacterial strains (Kumar *et al*., 2011; Jayaseelan *et al*., 2010) The present study concluded that, the biosynthesized silver nanoparticles from the aqueous leaf extract of *Blepharis maderaspatensis hyne Ex.roth* revealed active antibacterial activity with various bacterial pathogens which could be further used as an effective antibacterial agents. (Table 2).

Table 1. Phytochemical analysis of blepharis maderaspatensis

Solvent extracts	alkaloids	glycosides	Reducing sugar	flavonoids	terpenoids	phenols	Amino acids	sterols
aqueous	+	+	+	+	+	+	+	_
ethanol	_	_	_	_	+	_	+	+
Chloroform	_	_	+	_	+	+	+	+

Table 2. in vitro antibacterial potential of biosynthesized silver nanoparticles using blepharis maderaspatensis hyne ex Roth leaf extract (well diffusion method)

Microbial pathogens	Leaf extract of b. maderaspatensis	Biosynthesized silver nanoparticles of b. maderaspatensis
Bacillus subtilis	nil	15mm
Bacillus pumillus	nil	13mm
Klebisiella pneumonia	nil	15mm
Escherichia coli	nil	15mm
Candida albicans	nil	17mm
Aspergillus flavus	nil	13mm

Characterizations of biosynthesized silver nanoparticles

UV-VIS spectro analysis

UV-VIS spectrophotometer measurements were performed after the extraction of silver nanoparticles. The aqueous and silvernanoparticles are the two samples undergone the measurements. The silver nanoparticles in the solution could be correlated with the respective UV-VIS spectrum. It exhibited a strong absorption between 300nm and 600nm.

SEM analysis

SEM indicates the presence of nanoparticles. It revealed that the nanoparticles of silver are spherical in shape and also cubic too. The average size of the nanoparticles was studied, from the image it can be seen that the spherical morphology of silver nanoparticles is randomly distributed with average diameter >100nm. The morphology of the silver nanoparticles was determined by using scanning electron microscopy.

XRD analysis

Silver nanoparticles showed the diffraction peaks which indicated the metallic face centered cubic silver phase. In addition AgNps had a similar diffraction profile (Ag XRD ref No. 01-087-0719), and XRD peaks at 2θ of 38.18°, 44.32°, 57.52°, 64.50° and 77.05° could be attributed to the 111, 200, 103, 220 and 311 crystallographic planes of the face-centered cubic silver crystals, respectively. The XRD pattern thus clearly illustrated that the AgNps formed in this study is crystalline in nature. The results are corresponds to the Braggs reflection. The yellowish brown colour formation of the silvernanoparticles from the leaf extract may due to surface plasmon excitation. The sharp bands of Braggs peaks may be due to the stable capacity of biosynthesized silvernanoparticles.

FTIR analysis

The FTIR spectrum of silvernanoparticles is shown in the table. The band at 3947cm-1 is assigned as O-H stretching of H-bonded alcohols and phenols. The band at 3,851cm-1 is O-H stretching of carboxylic acids.

The band at 3,712cm-1 corresponds to N-H bonding of primary amines.1,652 –1384 cm-1 are corresponding to C-C stretching alcohols, carboxylic acids, esters and ethers. The FTIR analysis revealed the presence of functional groups of alcohols, carboxylic acids, esters and ethers which are the binding metals to form silvernanop articles and prevents its agglomeration.

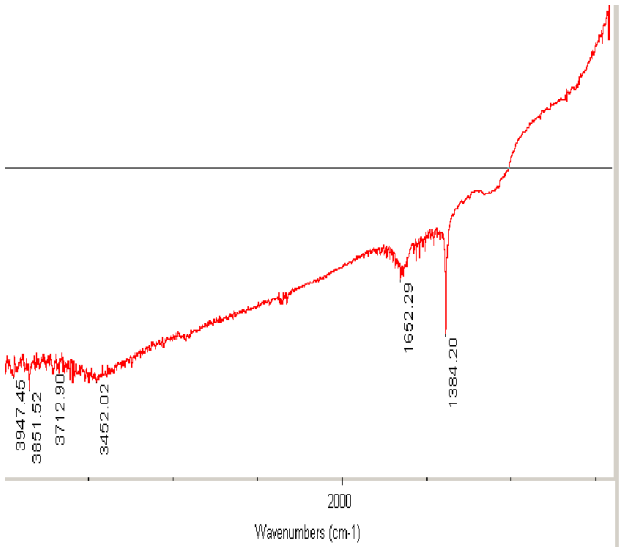

Fig 4. FTIR analysis of biosynthesized silvernanoparticles from blepharis maderaspatensis hyne Ex.roth

Zeta potential

Zeta potential of Ag NPs was measured according to Tantra, et.al with the help of Zetasizer Nano ZS (Malvern) and a titrator MPT-2. Before the measurement, aqueous suspension of silver nanoparticles was filtered through a 0.45 µm PTFE membrane. The zeta potential was calculated by the help of the software. Stability of AgNPs is determined by zeta potential measurement. Zeta potential value ±30 mv is considered as stable nano suspension.

Summary and Conclusion

Nanomedicine is the phenomena in which the nano products are used in medicine having some unique properties to cure diseases.

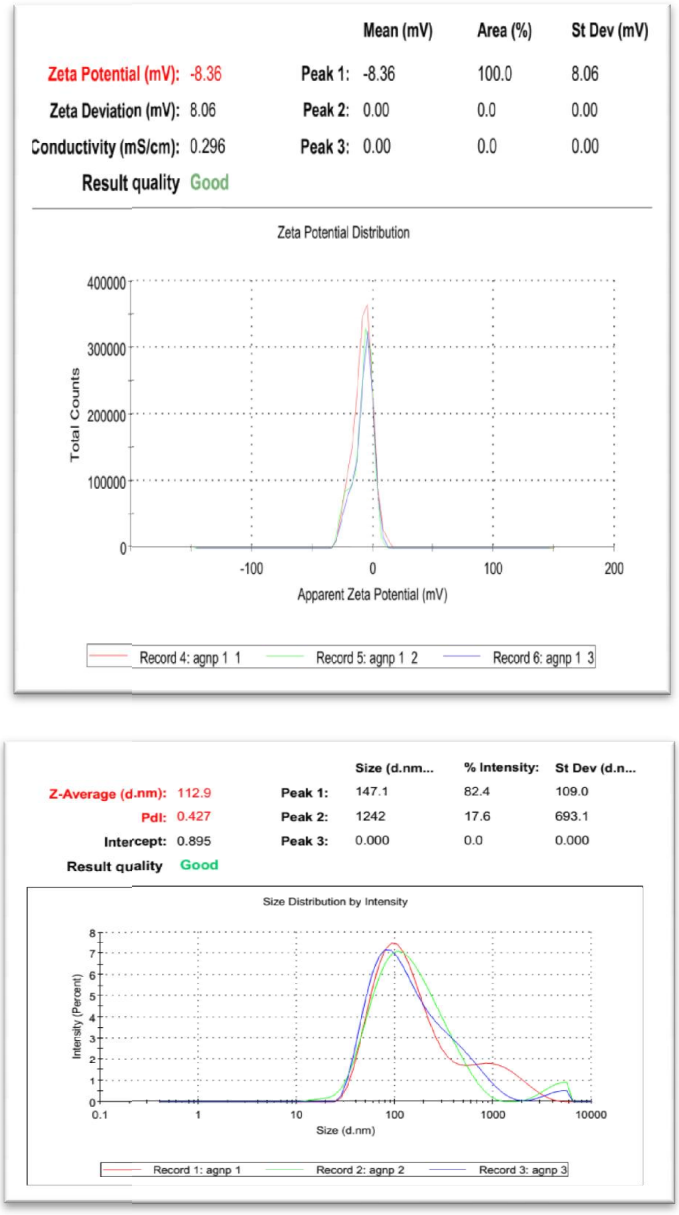

Fig 5. zeta potential analysis of biosynthesized silver nanoparticles from blepharis maderaspatensis leaf extract

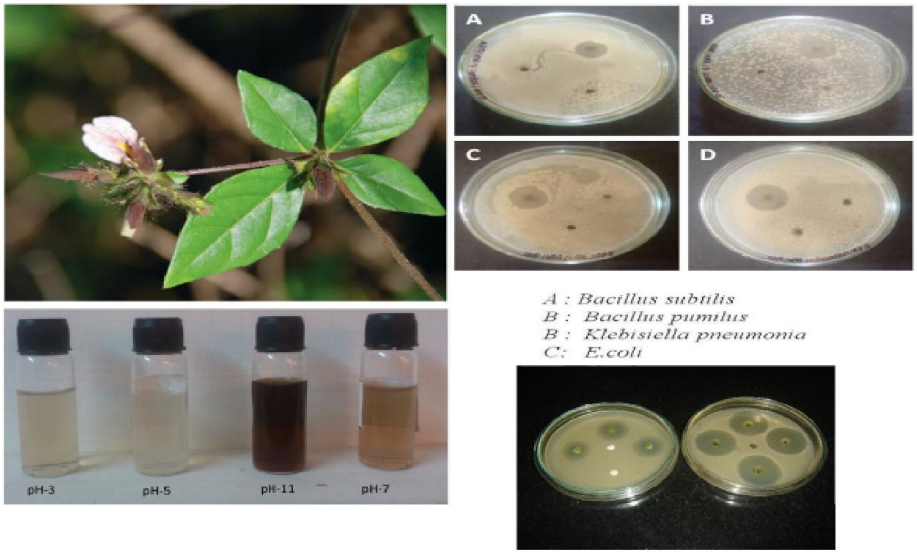

Fig. 6. blepharis leaf

Synthesis of silvernanoparticles from chemical and physical methods needs more time and temperature, pressure, energy etc., but the biosynthesis is cost effective and slow time consuming. They are eco friendly too. In the field of nanotechnology, the biosynthesis of metallic nanoparticles is an important step. Regarding antimicrobial activity the silver nanoparticles from the leaf extract shows the cell permeability and respiration activity. The characterization of silver nanoparticles was studied effectively used SEM, FTIR, UV-VIS, XRD and zeta potential. (Bar *et al*., 2009) Thus these silvernanoparticles may be used in future for further studies related with microbes and health purposes. However our studies are preliminary in blepharis maderaspatensis and synthesis of silver nanoparticles in its leaf extract but still work has to be carried out in future to know its mechanism to treat dreadful challenging diseases.

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Conflict of Interest

There is no conflict of interest.

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