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INVESTIGATION OF THE ANTI-CANCER POTENCY OF NANOMEDICINE, GREEN SYNTHESIZED FROM *SPIRULINA PLATENSIS*, AGAINST BREAST CANCER

V. D. Mendhulkar * and L. Shetye

Department of Botany, Institute of Science, 15- Madame Cama Road, Mumbai - 400032, Maharashtra, India.

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Correspondence to Author:
V. D. Mendhulkar

Professor,
Department of Botany,
Institute of Science, 15- Madame Cama Road, Mumbai - 400032, Maharashtra, India.

E-mail: profmendhulkar@gmail.com

ABSTRACT: Breast cancer is the most common type of cancer found in women. Although considerable advances have been achieved in the diagnosis and treatment of breast cancer, medical research routinely thrives towards the development of newer technologies for the treatment of breast cancer. The current study aimed at the development of nanomedicine, in the form of silver nanoparticles from the aqueous extract of algae *Spirulina platensis*, for the treatment of breast cancer. The synthesis of silver nanoparticles was exhibited by UV-visible spectroscopy, and the nanoparticles were characterized by NTA, FTIR and TEM. The anti-tumor potential of this nanoparticle formulation was evaluated on *MCF7* breast cancer cell line and its cytotoxicity was compared with crude aqueous algal extract as well as the recommended breast cancer chemotherapy drugs. The results obtained were promising. NTA analysis indicated a mean particle size of 72 nm. FTIR spectrum showed the presence of alcohol, phenols, carboxyl, and aromatic groups in the nanoparticle formulation, which were responsible for the biological activity. The anti-cancer activity studies revealed that nanoparticle formulation exhibited an IC_{50} value of 102.9 µg/ml higher than the crude aqueous extract (544 µg/ml), but the potency of this nanoparticle formulation was lower than the commercially available chemotherapy drugs like Abraxane and Amebiciclib. This ascertained the anti-cancer potential of the synthesized silver nanoparticles as effective nanomedicines although further research has to be undertaken to improve the potency of this bio-drug.

INTRODUCTION: The synthesis of nanoparticles is one of the most valuable research achievements of humans because of their significant applications in the field of manufacturing, electronics, medicine, and basic sciences. Its medical applications in cancer diagnosis and therapy are highly noteworthy. The need for such advanced technology for cancer treatment is evident in the statistics indicating that cancer incidence, prevalence, and mortality remain at exceedingly high levels [1].

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The clinical success of nanoparticles in cancer treatment is due to their stability and extended blood circulation time, their ability to access the tumor site, bioavailability at the disease site, and safety profile [2]. The ability to differentiate between malignant and non-malignant cells and selectively eradicate malignant cells is central to cancer treatment by nanotechnology, which is achieved by passive and active targeting [3]. Passive targeting takes advantage of the enhanced permeability and retention (EPR) effect [4, 5] to increase the concentration of nanoparticles in the tumor, while active targeting involves selective molecular recognition of cell-surface antigens or proteins to localize nanoparticles to malignant cells [6, 7]. Once localized to the tumor, nanoparticles evoke a cytotoxic response by drug release [8], hyperthermia [9], and reactive oxygen species (ROS)-mediated killing [10].

In the past few years, breast cancer has been the leading cause of mortality amongst women worldwide. This type of cancer commonly develops in cells from the lining of milk ducts and the lobules that supply the ducts with milk. Age, family history of breast disease, genetic predisposition, reproductive and lifestyle factors, and hormonal exposure have been associated with an increased risk for the development of female breast cancer [11]. Genetic factors linked with the risk of developing breast cancer include *BRCA1* and *BRCA2* mutations and *TP53* gene mutation [12]. Screening techniques for the diagnosis of breast cancer include a biopsy of the lump, mammography, MRI scan, and prognostic indicators like the estrogen and progesterone receptor status or *HER2* protein expression [11]. Breast cancer treatment can be done through surgery, radiation therapy, chemotherapy, and hormonal therapy [11].

Most of the cytotoxic drugs used in chemotherapy are synthetic in origin. Although they exhibit enhanced and explicit activity, their long-time presence in the human body is unsafe. With this perspective, the use of chemotherapeutics of biological origin is the need of the hour. The criteria for the selection of such bio-therapeutics are biocompatibility, biodegradability, safety, and ease of assembly in the structures with the desired characteristics. The use of extracts from medicinal plants exhibiting tumor inhibition activity has been extensively reported. Similarly, a free-living blue-green microalga, *Spirulina*, widely recognized for its nutritive and health benefits, is a potential source of bio-therapeutic metabolites with proven antioxidant and anti-tumor activity. In addition to this aspect, the industrial methods developed for the synthesis of metal nanoparticles involve the use of toxic solvents and have high energy consumption. Thus there is a need for developing a clean, non-toxic, and environmentally friendly technology for nanoparticle synthesis. An attractive possibility is employing microorganisms like bacteria [13], fungi [14,] and alga [15] for nanomaterial synthesis. Taken together, biomaterials and nano-technology can greatly improve the survival rates of cancer patients.

The present study is thus aimed at evaluating the anti-tumor activity of the crude extract and silver nanoparticles synthesized using *S. platensis* aqueous extract as a source of capping and reducing agent, on *MCF7* breast cancer cell line.

MATERIALS AND METHODS:

***Spirulina* Biomass Development and Preparation of Algal Extract:** Algae *Spirulina platensis* was used in the current research work as the source organism since it is being reported to possess anti-cancer metabolites. The algal species were obtained from the culture collection of the National Facility for Marine Cyanobacteria (NFMC), Bharathidasan University, Trichi. The organism was cultured in Zarrouk's media under the illumination of 1500 – 2000 lux and light period 16 h light / 8 h dark at 24 ± 2 ºC. After sufficient biomass was obtained, the cells were centrifuged; the cell pellet was dispensed in sterile distilled water and sonicated to obtain the algal extract.

Green Synthesis of Algae Mediated Silver Nanoparticles: Silver nanoparticles were synthesized by mixing 10 ml of an aqueous algal extract with 90 ml of 1 mM silver nitrate solution followed by incubation on a shaker at 37^{o}C up to 48 h [16]. The formation of silver nanoparticles was detected by visually monitoring the change in color of the solution and measuring absorbance from 200 nm to 800 nm using a UV-visible spectrophoto-meter (Shimadzu UV-1800) from 0 h to 48 h.

Characterization Studies of Silver Nanoparticles by NTA, TEM & FTIR: Nanoparticle tracking analysis was performed using Nanosight UK-LM20 to estimate the particle size distribution of the synthesized nanoparticles. NTA is a light-scattering technique that uses a laser light source to illuminate metal particles that appear scattered in the solution under Brownian motion. The sample preparation procedure for NTA involved brief sonication of the nanoparticle solution followed by injection of 10 µl of the sample using a sterile syringe.

Transmission Electron Microscope (PHILIPS CM-200) was used to assess the morphology of synthesized silver nanoparticles. Sample preparation for TEM analysis was performed by sonicating the nanoparticle solution for 10 min followed by evaporation of solvent from the colloidal solution on carbon-coated copper grids by exposure to infrared light. TEM was operated at an

accelerating voltage of 200 kV with a resolution of 0.22 nm.

The identification of functional groups of the biomolecules involved in nanoparticle synthesis and acting as surface capping and reducing agents was performed by Fourier Transform Infra-Red spectroscopy (3000 Hyperion Microscope with Vertex 80). The sample was prepared by mixing 2–3 drops of the colloidal solution of silver nanoparticles with KBr powder to produce a pellet for the study of nanoparticles by IR.

***In-vitro* Anti-Cancer Assessment of *Spirulina* Extract & Silver Nanoparticle on *MCF7* Breast Cancer Cell Line:** The algal extract and silver nanoparticles were tested on breast cancer cell line *MCF7* for evaluation of their anti-cancer activity. Approximately 1×10^4 cells were seeded in a 96-well plate and incubated at 37 ºC overnight under 5% CO_2 concentration. Once the cells attained confluence, they were treated with different concentrations of the algal extract and silver nanoparticles in the range of 2.5 µg/ml to 100 µg/ml and incubated overnight at 37 ºC under 5% CO_2 concentration.

The quantitative determination of anti-tumor activity was performed by MTT assay. 10µl of 5mg/ml MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) reagent was added to each well and incubated for 4 h for color development which was measured spectrophotometrically at 595 nm. The anti-cancer activity of the algal extract and silver nanoparticles was expressed as IC_{50}. IC_{50} stands for half-maximal inhibitory concentration and is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. Lower IC_{50} indicates that the biological activity is inhibited by a lesser concentration of a compound, which implies the compound is more potent.

RESULTS: In the current study, silver nanoparticles were synthesized using *Spirulina* aqueous extract. The synthesized nanoparticles were characterized by NTA, TEM & FTIR, and their anti-tumor activity was evaluated on *MCF7* cell line using MTT assay.

Analysis of Nanoparticle Synthesis by UV-Visible Spectrophotometry: The reduction of $AgNO_3$ particles by *S. platensis* aqueous extract leading to the synthesis of silver nanoparticles occurred in the presence of visible light. This was evident from the change in color of the solution from light green to dark brown after 48 h of incubation **Fig. 1**. The UV-VIS spectrum of synthesized silver nanoparticles was also determined, and it showed an absorbance peak at 420 nm **Fig. 2**. The spectrum also indicated that the rate of nanoparticles synthesis enhanced with time and stabilized at 48 h. Further, incubation did not show any significant increase in nanoparticle synthesis.

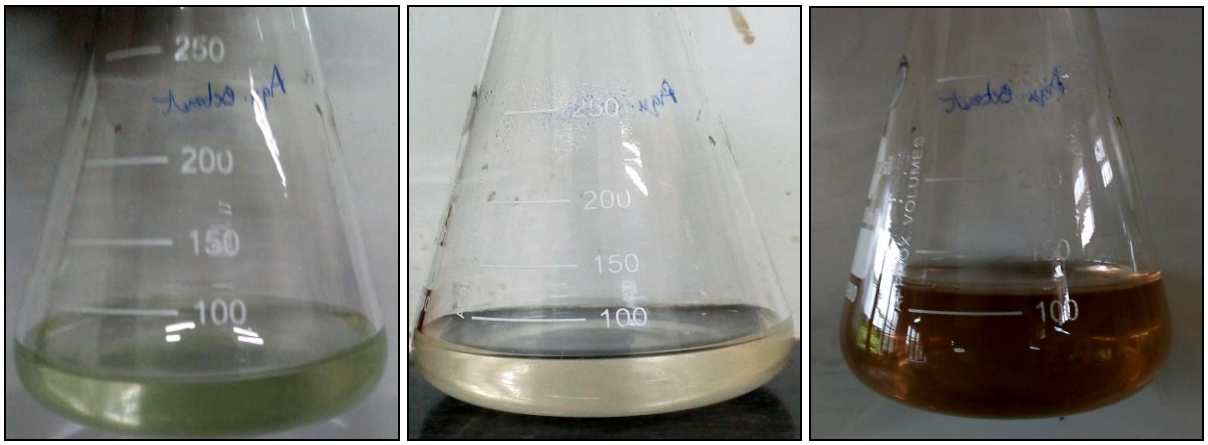


FIG. 1: COLOUR CHANGE INDICATING SYNTHESIS OF NANOPARTICLE

Characterisation of Nanoparticles by NTA, TEM and FTIR: NTA analysis provided information about the size, distribution, and concentration of nanoparticles. The mean particle size of silver nanoparticles synthesized from the algal extract was estimated to be 72 nm, and particle concentration was found to be 6.45×10^8 particles/ ml **Fig. 3**.

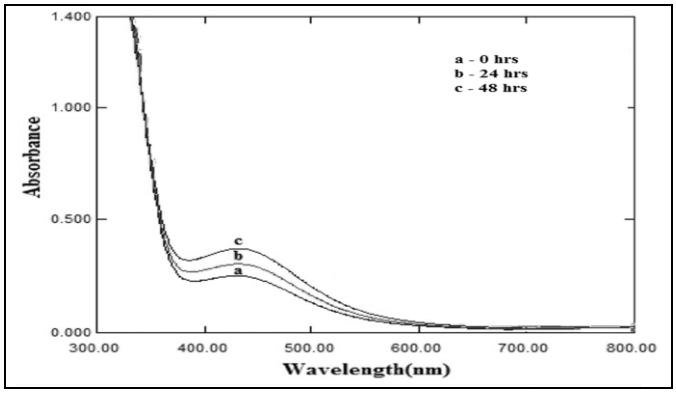


FIG. 2: UV- VISIBLE SPECTRUM OF SILVER NANOPARTICLES AT VARYING TIME

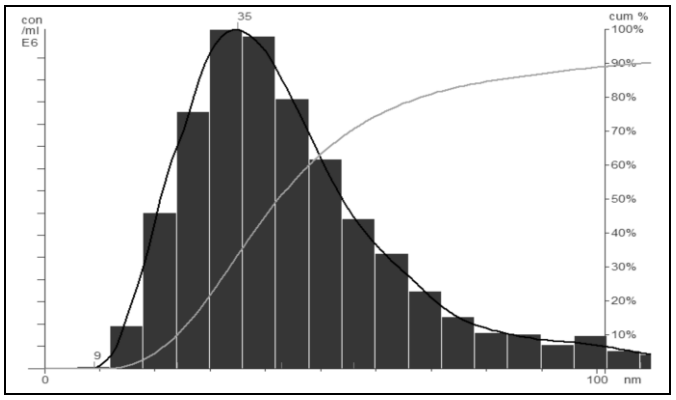


FIG. 3: NTA GRAPH SHOWING PARTICLE SIZE DISTRIBUTION

TEM examination demonstrated the presence of spherical shaped silver nanoparticles. The size of the nanoparticles ranged from 15 nm to 50 nm. These silver nanoparticles were aggregated, forming clumps **Fig. 4**.

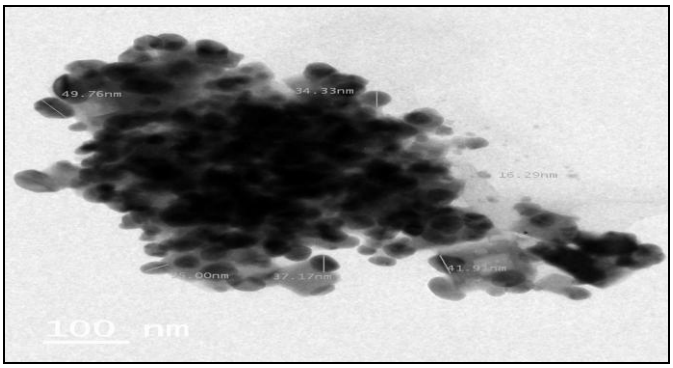


FIG. 4: IMAGES OF NANOPARTICLES VISUALISED BY TEM

FTIR analysis revealed the chemical nature of possible biomolecules acting as surface coating agents of the silver nanoparticles. The FTIR spectrum of the synthesized nanoparticles showed several absorption peaks corresponding to different biomolecules **Fig. 5**. The prominent peaks were detected at 3425 cm^{-1}, 2924 cm^{-1}, 1667 cm^{-1}, 1544 cm^{-1}, 1238 cm^{-1}, 1384 cm^{-1} and 1024 cm^{-1} corresponding to functional groups like O-H stretching of alcohols and phenols, N-H groups of tetrapyrroles, C-O of carboxylic acids, and N-O stretching. The peak at 697.13 cm^{-1} is an indication of the presence of an aromatic functional group.

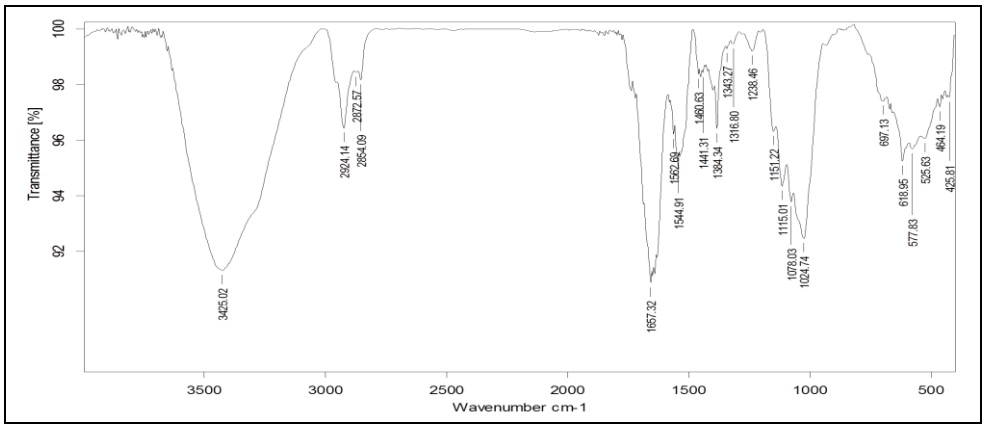
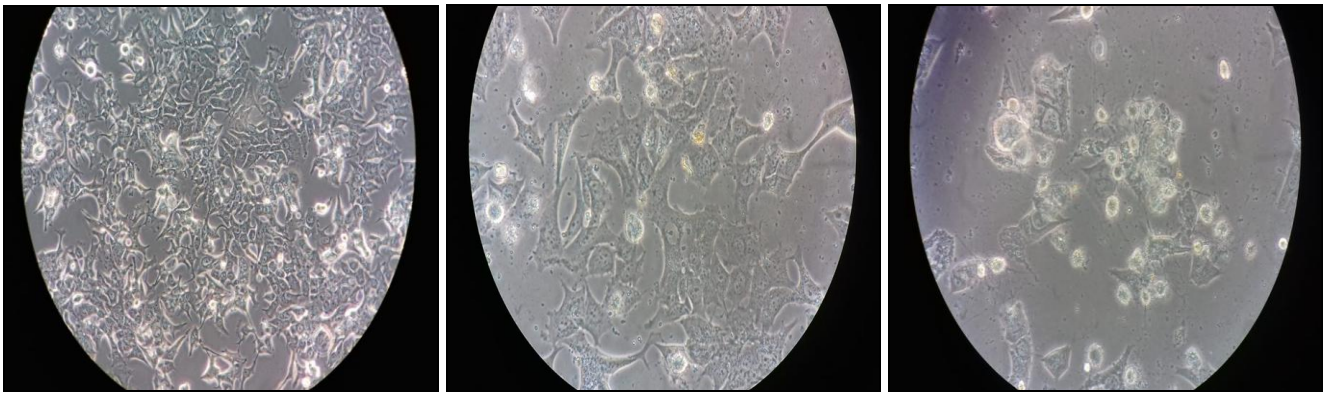


FIG. 5: FTIR SPECTRUM FOR EXTRACT

Assessment of Anti-Cancer Activity of Nanoparticles against *MCF7* Cell Line: *MCF7* was used as the model breast cancer cell line to assess the cytotoxicity of the silver nanoparticles against breast cancer. The anti-proliferative activity of nanoparticles against *MCF7* cell line was tested using MTT assay and results were expressed in terms of IC_{50} values. **Fig. 6** demonstrates the effect of the crude algal extract and silver nanoparticles on *MCF7* cells. The untreated cells exhibit a high level of confluence while the viability of these cells decreases after treatment which was indicated by a decline in cellular confluence. The IC_{50} value of the crude extract was found to be 544 µg/ml ± 5 while that of silver nanoparticles was 102.9 ± 10 µg/ml.



Untreated cells **Cells treated with algal extract** **Cells treated with silver nanoparticle**

FIG. 6: EFFECT OF ALGAL EXTRACT AND SILVER NTPs ON MCF-7 CELLS

DISCUSSION: The results demonstrated that silver nanoparticles can be synthesized from *S. platensis* aqueous extract via a green route. The synthesis of silver nanoparticles was detected by visual analysis and UV-Visible spectrophotometry. The silver nanoparticles appeared yellowish-brown to dark brown in color in aqueous solution due to the excitation of surface plasmon vibrations [17]. The UV-VIS spectrum showed a broadened absorption peak which implies that the particles were polydispersed [17]. The morphological characteristics of nanoparticles were studied by NTA and TEM analysis. It was found that the synthesized silver nanoparticles had size in the range of 15 - 50 nm with a spherical shape. This outcome complies with the study conducted by Sharma *et al.,* (2015) who reported that silver nanoparticles synthesized using aqueous extract of *S. platensis* are spherical in shape and exhibited particle size in the range of 30 -50 nm [18].

This result is significant since the morphology of nanoparticles greatly influences their bio-distribution and cellular internalization in the host cell; both factors are critical for the biological activity of nanoparticles [19]. It is reported that spherical nanoparticles in the size range of 10 nm - 100 nm showed enhanced permeability and retention (EPR) effect and are rapidly and uniformly internalized in the tumors [20]. The morphological characteristics of nanoparticles synthesized in the current study comply with the reported scientific work, which implies that the nanoparticles are ideal for the delivery of drugs into the target cells. Further, FTIR analysis showed the presence of different functional groups like alcohols, phenols, carboxylic acid, and algal-specific aromatic compounds *i.e.,* tetrapyrroles.

The detection of these chemical groups implies that metabolites from the algal cells have got associated with silver nanoparticles as capping and stabilizing agents. These characterization studies presented encouraging results to illustrate the biological activity of silver nanoparticles under *in-vitro* conditions.

The current investigation revealed positive anti-proliferative activity of the synthesized nanoparticles against *MCF7* cell line. This biological activity is attributed to the presence of tetrapyrrolic metabolites, namely phycocyanobilin (PCB) and chlorophyll synthesized by *S. platensis* and acting as capping agents on the silver nanoparticles. PCB inhibits the enzyme cytochrome C oxidase in the mitochondria, which triggers the cells to undergo premature apoptosis, while chlorophyll exhibits potent reactive oxygen scavenging effects [21]. Earlier, researchers have demonstrated the anticancer activity of dried *S. platensis* powder water extract on human lung cancer A549 cell line [22].

Similarly, the effectiveness of water extracts of *S. platensis* as anticancer agents against colon carcinoma cells *HCT116* and liver cancer cell lines *HEPG2* has been reported [23]. Violaxanthin, a metabolite extracted from microalgae *Dunaliella tertiolecta* has been demonstrated to inhibit the proliferation of *MCF7* cell line [24]. Thus, it is evident that the anti-cancer activity of the synthesized silver nanoparticles is due to the presence of metabolites present in *S. platensis* extract which are capping the silver nanoparticles. The growth inhibition of *MCF7* cells was validated by calculating the IC_{50} value of the silver nanoparticles that were found to be 102.9 µg/ml.

Researchers have reported the IC_{50} value of synthetic drugs like Abraxane and Amebiciclib, used for the treatment of breast cancer, to be 4.18 µg/ml and 5.57 µg/ml [25, 26]. Thus, it is apparent that the potency of the synthesized nanomedicine is less than the available drugs. However, in the current study, the crude aqueous algal extract was used, and its activity cannot be directly compared with pure synthetic compounds in terms of bioactivity. Thus, further research directed towards extraction followed by recovery of pure active metabolites from the crude algal extract needs to be undertaken to deduce the commercial viability of the nanodrug.

Although drugs like Abraxane and Amebiciclib are available in the market for the treatment of breast cancer, researchers are always searching for new compounds with better efficacy. Plants have been the first choice of source organisms for such studies, but microalgae have recently attracted attention due to the various reports indicating their medicinal applications. In this context, the current study was significant as it showcased the development of a biopharmaceutical agent for cancer therapy by applying the principles of nanotechnology.

CONCLUSION: Nanotechnology is playing an increasingly important role in cancer treatment. The small size of nanoparticles permits their interaction with the diseased cells and improved delivery of therapeutic drugs facilitating active as well as passive therapy of cancer.

The present research work dealt with the synthesis of silver nanoparticles from *S. platensis* aqueous extract and evaluation of their anti-tumor activity against breast cancer. The results showed that the silver nanoparticles synthesized from *S. platensis* are more potent anti-cancer agents than the crude algal extract but less potent than the commercially available drugs for the treatment of breast cancer.

We believe that the presence of algal-specific metabolites in *S. platensis* has contributed towards inhibition of cell proliferation of *MCF7* breast cancer cells. Optimum extraction and purification of these metabolites followed by their conjugation with silver nanoparticles need to be undertaken to develop an effective nanomedicine for the treatment of breast cancer.

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CONFLICTS OF INTEREST: The authors hereby declare that they have no conflict of interest.

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