International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Special Issue 4, 2024

Original Article

ANTIINFLAMMATION AND ANTIFUNGAL EFFECTS OF SILVER NANOPARTICLES GREENLY SYNTHESIZED USING *PHYLLANTHUS EMBLICA* L. EXTRACT AS A REDUCING AGENT AGAINST DERMATOPHYTOSIS

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Received: 27 Apr 2024, Revised and Accepted: 10 Jul 2024

ABSTRACT

Objective: This research aims to determine whether the extract of *Phyllanthus emblica* can synthesize silver nanoparticles as an alternative bioreductive synthesis that is safer and free of hazardous waste. Silver nanoparticles were tested for their anti-inflammatory and antifungal activity on *Trichophyton mentagrophytes* and *Microsporum canis*.

Methods: This study makes ethanol extract of *Phyllanthus emblica* fruit, synthesizes silver nanoparticles utilizing green synthesis, and then characterizes and tests for anti-inflammatory and antifungal activities.

Results: This research shows that *Phyllanthus emblica* ethanol extract can be used to produce silver nanoparticles with characterization using a UV-Vis spectrophotometer at 430 nm, and FTIR shows specific wave numbers at 3739, 1703, 1347, 1054 and 875 cm-1. Particle Size Analyzer (PSA) and Scanning Electron Microscope (SEM) analysis of silver nanoparticles showed a colloid size of 25.93 nm and dry silver nanoparticle powder of 112.68 nm with spherical particles and nanometer scale size. In anti-inflammatory activity with a weak IC_{50} and optimal antifungal activity was seen at concentrations of *Trichophyton mentagrophytes* (125 mg/ml) and *Microsporum canis* (150 mg/ml).

Conclusion: The ethanol extract of *Phyllanthus emblica* fruit produced silver nanoparticles with anti-inflammatory and antifungal properties.

Keywords: Silver nanoparticles, Green synthesis, *Phyllanthus emblica*, Anti-inflammatory, Antifungal

DOI: https://dx.doi.org/10.22159/ijap.2024.v16s4.06 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Phyllanthus emblica, also known as Indian gooseberry, is frequently employed as a constituent in traditional medicinal practices. This botanical specimen has been used to manage various medical conditions, including cancer, diabetes, hepatic disorders, cardiovascular ailments, and hematological deficiencies. The observed biological activity is hypothesized to be attributed to bioactive chemicals derived from secondary metabolites inside it, particularly molecules belonging to the phenolic and flavonoid categories [1]. The presence of these compounds can be used in synthesizing various kinds of nanoparticles, such as Au, Ag, Pd, Pt, ZnO, and TiO_2. Compounds such as terpenoids and flavonoids can act as stabilizing and reducing agents in the nanoparticle synthesis process [2].

The production of silver nanoparticles by eco-friendly techniques is gaining popularity in chemistry and materials technology. The advantages of the green synthesis method are that it is environmentally friendly, low cost, and does not require too much energy. Apart from the method, the main precursor and reductant used in synthesizing silver nanoparticles determine the success of forming Ag ions. So far, the silver nitrate salt $Ag(NO_3)$ $_4H_2O$ is the precursor. The reducing agent used is $NaBH_4$ or hydrazine. These two substances are very dangerous if the resulting solution is wasted in the environment. A green synthesis approach or natural extracts is used to reduce the level of danger of reducing agents used to synthesize silver nanoparticles [3]. Silver nanoparticles can be a production alternative because this method can minimize the use of hazardous materials from the waste produced. Bioactive compounds contained in plants, such as antioxidant compounds, and certain secondary metabolite compounds, such as terpenoid and flavonoid compounds, are thought to play a role in the metal ion reduction process [3].

The cause of dermatophytosis is the dermatophyte fungus group, including the *Microsporum canis* fungus and the *Trichophyton mentagrophytes* fungus. The diseases that can be caused include tinea capitis (92.8%) and tinea corponis (65.4%). This fungus will produce the protease enzymes Sub^3 and Mep^3 in the infection process. Another disease caused by ringworm. This dermatophytosis fungus likes most body parts that contain lots of keratin, such as skin, hair, and nails [4].

The skin condition caused by dermatophytosis causes the body to carry out a natural immune reaction to fight this fungal attack, namely inflammation. Inflammation is a reaction to tissue damage and infection. During inflammation, a vascular response occurs when fluid, blood components, white blood cells, leukocytes, and chemical mediators gather around the tissue damage or infection site. Inflammation is a protective response by the body to eliminate harmful substances at the injury site and promote tissue healing [5].

Based on the description above, researchers express their interest in synthesizing silver nanoparticles using an ethanol extract from *P. emblica,* which was then characterized and tested for anti-inflammatory activity using the denaturation inhibition method using UV-Vis spectrophotometry and antifungal against the fungi *Trichophyton mentagrophytes* and *Microsporum canis*.

MATERIALS AND METHODS

The study utilized glasses from Iwaki and Pyrex brands. High-grade supplies and reagents from Merck and Sigma-Aldrich were used in the study. *Trichophyton mentagrophytes* and *Microsporum canis* fungi were acquired from the USU pharmacy faculty laboratory, while Fruit samples of *Phyllanthus emblica* were collected from Badung Regency, Bali Province.

Preparation of extract

The fresh *Phyllanthus emblica* fruit was washed thoroughly and then drained. The fruit was deseeded, sliced into little pieces, and dried in

a drying cupboard at 40 – 50 °C. The desiccated state of *Phyllanthus emblica* fruit was characterized by its brittleness. Subsequently, the desiccated simplicia was pulverized via a blender and preserved within a hermetically sealed plastic receptacle. The levels water were determined using the Azeotropy method (toluene distillation).

Dry simplicia powder (1 part) was mixed with a solvent (10 parts) in a glass container. For the first 6 h, the mixture was stirred occasionally. After that, it was left to sit for 18 h. Filtration was used to isolate the macerate. The filtering process was repeated at least twice, and then the water was evaporated [6].

Synthesis of silver nanoparticles

The production of silver nanoparticles using $AgNO_3$ as a precursor was noted, while the ethanol extract derived from *Phyllanthus emblica* fruit served as a bioreduction and stabilizing agent. This process resulted in the transformation of the solution into a dark brown hue, signifying the successful development of silver nanoparticles [7].

Silver nanoparticles were synthesized by gradually introducing *Phyllanthus emblica* ethanol extract at 0.125%, 0.25%, and 0.5% into a 1.0 mmol $AgNO_3$ solution, which was constantly stirred for 20 min at temperatures between 50-60 °C. Subsequently, the solution was placed in a light-restricted environment and maintained at ambient temperature for 12 h. A comparative investigation was conducted to verify the production of silver nanoparticles from the phytochemical compounds in the ethanol extract of *Phyllanthus emblica* fruit. This was accomplished by utilizing an $AgNO_3$ solution and distilled water. The presence of a dark brown color indicated the reduction of silver ions [8].

The silver nanoparticles were separated from the solution using centrifugation at 10,000 revolutions per minute for 10 min. The nanoparticles were then washed with distilled water until all contaminants were no longer visible. Finally, the silver nanoparticle powder was dried in an oven at a temperature of 100 °C until it reached a state of dryness [8].

Characterization of silver nanoparticles

UV-Vis spectrophotometry is used to identify absorbance peaks within a specific range to analyze the creation of silver nanoparticles. Based on the characteristics of silver, an absorbance peak appears in the wavelength range of 400-450 nm [9].

Functional Group Analysis employs Fourier-Transform Infrared Spectroscopy (FTIR) to identify the functional groups involved in the reduction process and to assess the stability and efficiency of the produced silver nanoparticles. Two mg of sample were mixed with 100 mg KBr, made into pellets, and then subjected to IR with a wave number range of 4000-500 cm-1 [9].

Analyzing particle size via a Particle Size Analyzer (PSA). The silver nanoparticle sample, consisting of 10 drops of colloidal silver nanoparticles and 10 mg of silver nanoparticle powder, should be carefully introduced into the designated location within the particle size analyzer. Subsequently, the tool should be securely closed, and the measurement results can be observed on a computer monitor directly linked to PSA [10].

Analysis of Particle Surface Morphology Using Scanning Electron Microscope (SEM). The film sample was cut to 1 x 1 cm and then broken by dipping it in liquid nitrogen. The membrane was attached to the specimen holder, cleaned, and coated using Cu in a vacuum chamber. Once the sample is ready to be inserted into the specimen chamber, the equipment conditions are set and run. The magnification and focus are adjusted to obtain an optimum image [10].

Antifungal activity assay

Three sterile petri dishes were each inoculated with 0.1 ml of fungal inoculum. Following inoculation, 15 ml of Potato Dextrose Agar (PDA) was added to each petri dish. The dishes were gently shaken on a table surface to achieve homogeneity between the media and fungal suspension. Subsequently, the petri dishes were left undisturbed until the media solidified. Antifungal activity was tested using the agar diffusion method with a paper backer, namely by dripping the paper backer with the test solution, positive control, and negative control, 50 µl** each, on solid media inoculated with the fungus. Then, they were incubated at 25 °C for 48 h. The positive control was $AgNO_3$, and the negative control was DMSO [3].

Anti-inflammatory activity assay

In the anti-inflammatory test, a silver nanoparticle test solution was used at 169.87 µg/ml, and a positive control solution with concentrations of 4.000, 2.000, 1.000, and 500 µg/ml. Anti-inflammatory activity was measured by taking 50 µl of each solution concentration (test solution and positive control solution), then adding 0.2% BSA solution until the volume reached 5 ml. This mixture produced a concentration of 1.6987 µg/ml of the silver nanoparticle test solution and 10, 20, and 40 µg/ml of the diclofenac sodium concentration solution. The solutions were then incubated at 25 °C for 30 min, heated for 5 min at 72 °C, and then left for 25 min at 23 °C. After cooling, the solutions were vortexed and measured at 660 nm [11].

RESULTS AND DISCUSSION

Preparation of extract

In this study, 8.1 kg of *Phyllanthus emblica* fruit pulp was subjected to a drying process within a drying cabinet. The objective was to obtain a weight of 1.2 kg for the resulting *Phyllanthus emblica* fruit simplicia, taking into account a shrinkage percentage of 85.5%. The simplicia powder was then macerated to obtain 580 g of thick extract. The results of examining the water content of Phylanthus emblica fruit simplicia were 7.76%. This level meets the general requirements for simplicia water content below 10%.

Synthesis of silver nanoparticles

The synthesize silver nanoparticles using natural ingredients like *Phyllanthus emblica* fruit ethanol extract, which contains secondary metabolites of flavonoid compounds (quercetin). The hydroxyl group in the flavonoid compound acts as a reducing agent to reduce the $AgNO_3$ precursor compound, namely the Ag^+ion, into a nanoparticle compound, Ag. Table 1 displays the yield of silver nanoparticles generated by manipulating the concentration and volume of *Phyllanthus emblica* fruit ethanol extract, considering variations in orientation.

Table 1: Results of the synthesis of silver nanoparticles

Variations in 1 mmol AgNO3 100 ml ethanol extract		Yield weight (g)	Percent yield (%)
Concentration (%)	Volume (ml)		
0.125	5	0.0010	0.10
0.25	5	0.0021	0.21
0.5	5	0.0054	0.54
0.5	10	0.0057	0.57

The *Phyllanthus emblica* fruit ethanol extract chosen showed differences in concentration and volume as observed in the orientation study. Because it produces silver nanoparticles with a yield weight of 0.54%, 0.5% concentration variation and 5 ml volume were chosen. In comparison, a volume of 10 ml resulted in a weight yield of only 0.57%. Despite the larger volume of extract added (twice as much), the observed outcome difference was relatively insignificant. Apart from that, the 0.5% 5 ml variation also showed a smaller particle size after

being measured using the PSA tool, namely 112.68 nm, while the 0.5% 10 ml variation showed a result of 202.98 nm.

The synthesis involved using 30 liters of 1 millimolar AgNO3 and 0.5% of 1.5 liters of *Phyllanthus emblica* fruit ethanol extract as the reductant to produce 1.5984 gs of silver nanoparticle powder, resulting in a yield of 49.33%. Fig. 1 displays the produced silver nanoparticle powder.

Fig. 1: Synthesized silver nanoparticle powder

Characterization of silver nanoparticles

Characterization UV-Vis spectra for determine the Surface Plasmon Resonance (SPR) of the synthesized silver nanoparticles. The spectrum of silver nanoparticles exhibits a peak wavelength ranging from 400 to 450 nm [9, 12]**.** The findings from the analysis of silver nanoparticles indicate a peak wavelength of 430 nm, as depicted in fig. 2. The observed wavelength aligns with the sSPR value, signifying the presence of silver nanoparticles within the 400-450 nm region.

The FTIR characterization of silver nanoparticles (fig. 3) reveals OH stretching phenol groups observed at a peak wavenumber of 3739 cm^{-1}. Additionally, the analysis indicates the presence of C=O groups originating from aldehydes and ketones, which exhibit a peak wavenumber of 1703 cm^{-1}. Furthermore, N=O groups are detected at wavenumber 1347 cm^{-1}. The ester's C-O group is 1054 cm^{-1}, while the alkene's C-H group peaks at 875 cm^{-1} [9, 12]**.**

The PSA quantifies the mean particle size of the silver nanoparticles generated. Silver nanoparticles in colloidal form have an particle size of 25.93 nm, whereas in dry powder form, they have an particle size of 112.68 nm.

The findings from the characterization process utilizing a SEM are depicted in fig. 4. The visual representation illustrates that the silver nanoparticles exhibit a spherical morphology and a significantly diminutive particle size measuring less than 20 µm. Nevertheless, there exists a conglomeration of several little particles [13]. The findings from the characterization process utilizing a Scanning Electron Microscope are depicted in fig. 4.

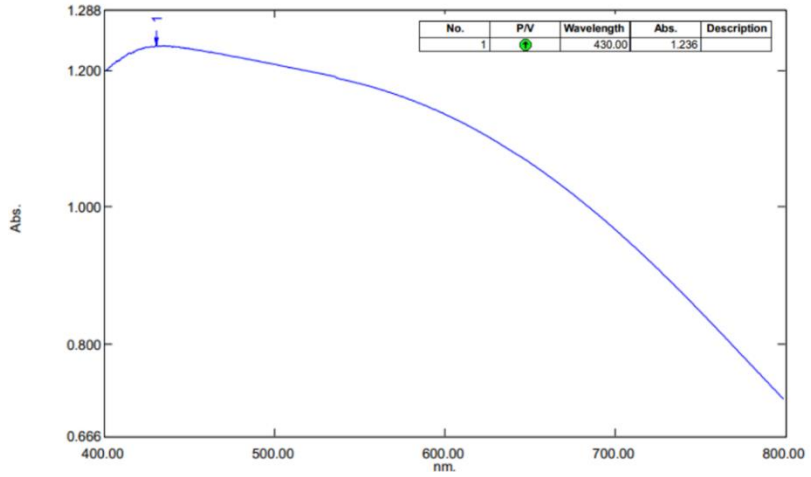

Fig. 2: Spectrum of silver nanoparticle

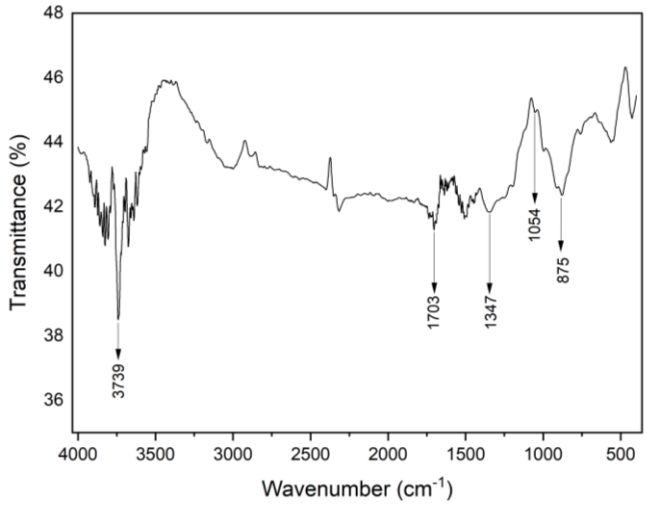

Fig. 3: FTIR spectrum of silver nanoparticles

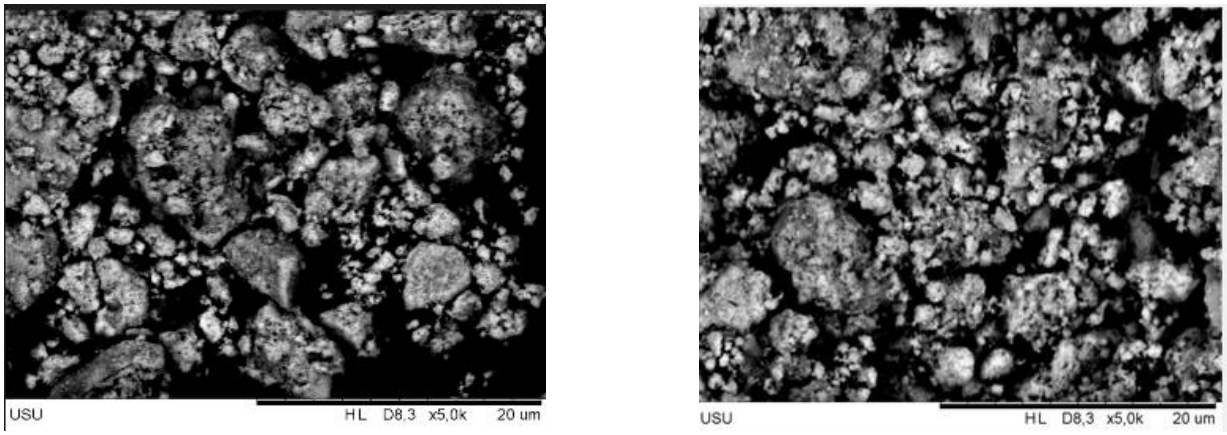

Fig. 4: Results of scanning electron microscope of silver nanoparticles

Anti-inflammatory activity

The finding shows that the anti-inflammatory activity at 5, 10, and 20 µg/ml is 19.70%, 22.10%, and 22.56% respectively. The positive control diclofenac sodium produced the largest was at 40 µg/ml with inhibition of 23.31%. The percent inhibition produced by the positive control diclofenac sodium was greater the greater the concentration. Anti-inflammatory activity with a protein denaturation inhibition percentage value greater than 20% means it is said to have very strong anti-inflammatory ability. Meanwhile, the anti-inflammatory activity produced by the silver nanoparticle test solution shows a percent inhibition of 16.80%, so the anti-inflammatory activity of silver nanoparticles is weak [12].

Antifungal activity of extracts and silver nanoparticles

The antifungal activity test show that the ethanol extracts of *Phyllanthus emblica* fruit can inhibit the growth of the fungi *Trichophyton mentagrophytes* and *Microsporum canis* in table 3.

The antifungal activity test was carried out at a concentration of 500, 400, 300, 200, 150, 100, and 50 mg/ml using the disc paper diffusion method using PDA (Potato Dextrose Agar) media. Antifungal activity was demonstrated by forming an inhibitory zone around the paper disc, and its diameter was measured using a caliper.

Table 2: Results of percent anti-inflammatory inhibition

Concentration (µg/ml)	Percent inhibition (%)
Blank	-
Positive Control 5	19.70
Positive Control 10	22.10
Positive Control 20	22.56
Positive Control 40	23.31
AgNP Test Solution	16.80

Note: positive control = diclofenac sodium

The antifungal activity test results demonstrate that the created silver nanoparticles can hinder the growth of the fungi *Trichophyton mentagrophytes* and *Microsporum canis*, as presented in table 4.

Table 3: Results of antifungal activity of ethanol extract

Extract concentration (mg/ml)	Diameter of inhibition area±SD (mm)	
	Trichophyton mentagrophytes	***Microsporum canis***
500	18.10±0.20	17.90±0.10
400	15.93±0.25	15.83±0.20
300	15.03±0.57	14.67±0.15
200	11.67±1.52	12.63±0.11
150	9.06±0.20	9.67±0.15
100	8.20±0.10	8.50±0.10
50	7.06±0.15	8.00±0.17

N=3

Table 4: Results of antifungal activity test of silver nanoparticles

AgNP concentration (mg/ml)	Diameter of inhibition area±SD (mm)	
	Trichophyton mentagrophytes	***Microsporum canis***
150	13.63±0.60	12.93±0.20
125	11.4±0.75	10.03±0.20
100	9.63±0.25	8.30±0.26
75	8.96±1.07	7.60±0.30
50	8.30±0.43	7.50±0.20
25	7.20±0.40	6.76±0.30
Positive control (AgNO3 1 mmol)	18.10±0.3	17.53±0.25
Negative control (DMSO)	0	0

Table 3 shows that *Phyllanthus emblica* Fruit Ethanol Extract offers resistance against the fungus *Trichophyton mentagrophytes* and *Microsporum canis*. Table 4 shows that Silver Nanoparticles (AgNP) exhibit resistance against the fungus *Trichophyton mentagrophytes*

and *Microsporum canis*. Comparing the impact of *Phyllanthus emblica* fruit ethanol extract and AgNP on *Trichophyton mentagrophytes* fungus at concentrations of 150, 100, and 50 mg/ml, it was noted that the inhibition zone diameter was greater with the AgNP sample. The inhibitory zones' diameters for AgNP were 13.63±0.60 mm, 9.63±0.25 mm, and 8.30±0.43 mm, whereas for the ethanol extract of *Phyllanthus emblica*, they measured 9.06±0.20 mm, 8.20±0.10 mm, and 7.06±0.15 mm. The AgNP sample shows a strong inhibitory diameter zone (10-20 mm) at a dose of 150 mg/ml, while the ethanol extract displays a medium inhibitory diameter zone (5-10 mm) at three different concentrations [14].

In the fungus *Microsporum canis*, with the same concentration of *Phyllanthus emblica* fruit ethanol extract and AgNP, namely at concentrations of 150, 100, and 50 mg/ml, it shows that the diameter of the inhibition zone produced by the AgNP sample is greater at 150 mg/ml with 12.93±0.20 mm, where the ethanol extract sample of *Phyllanthus emblica* fruit produced a zone of inhibitory diameter of 9.67±0.15 mm. Meanwhile, at 100 mg/ml and 50 mg/ml, the zone of inhibition diameter produced by samples of *Phyllanthus emblica* fruit ethanol extract was larger, namely 8.50±0.10 mm and 8.00±0.17 mm, respectively. The AgNP samples produced zones of inhibitory diameters of 8.30±0.26 mm and 7.50±0.20 mm, respectively. The AgNP sample concentration of 150 mg/ml has the inhibitory diameter zone in the strong category (10-20 mm), and in the ethanol extract of *Phyllanthus emblica* sample, the inhibitory diameter zone at the three concentrations is in the medium category (5-10 mm). The effective concentration of silver nanoparticles to inhibit the fungi *Trichophyton mentagrophytes* and *Microsporum canis* is at 150 and 125 mg/ml because the zone of inhibition diameter produced is in a strong category (10-20 mm) [14].

Silver nanoparticles can release silver ions continuously; these ions will stick to the cell walls and cytoplasmic membranes of microbes, thereby increasing the permeability of the cytoplasmic membranes and causing microbial cell disruption. After the entry of silver ions, the ATP (Adenosine Triphosphate) production process will be disrupted, affecting the DNA replication process, cell reproduction, and even killing the microbe. AgNPs can also act as antimicrobials because they can penetrate through cell walls, which then change the structure of the cell membrane [15].

CONCLUSION

Silver nanoparticles can be produced by utilizing a bioreductant derived from the ethanol extract of *Phyllanthus emblica* fruit. The synthesized silver nanoparticles have appropriate characteristics and potential anti-inflammatory activity with an inhibition percentage of 16.80%. In addition, antifungal activity against the fungi *Trichophyton mentagrophytes* and *Microsporum canis* with effective concentrations of 125 mg/ml (diameter of the inhibition zone respectively 11.4±0.75 and 10.03±0.20) and 150 mg/ml (diameter inhibition zone 13.63±0.60 and 12.93±0.20 respectively).

ACKNOWLEDGMENT

We would like to thank Universitas Sumatera Utara for funding this research project.

FUNDING

This research did not receive a specific grant from any funding agencies.

AUTHORS CONTRIBUTIONS

Concept – M., S.; Design – M., H. S.; Supervision – M., S., H. S.; Resources – M., H. S.; Materials – M., H. S.; Data Collection and/or Processing – H. S., V. F.; Analysis and/or Interpretation – M., S., H. S., V. F.; Literature Search – H. S., V. F.; Writing – M., H. S., V. F.; Critical Reviews – S.

CONFLICT OF INTERESTS

Declared none

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