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Original Research Article

Antibacterial Activity of the Leaf Extracts of Terminalia Bellerica, Terminalia Chebula, Emblica Officinalis and Their Formulation Triphala

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ABSTRACT

In current era, natural products are measured to be the symbols of safety in comparison to the synthetic products that are regarded to be hazardous to human life and environment. Although herbs had been priced for their therapeutic importance, their phytochemical and pharmacological activities are conducted on different parts. With this, an attempt has been made to investigate the antibacterial activity of leaf extracts of Terminalia bellerica (TB), Terminalia chebula (TC) and Emblica officinalis (EO) and their formulation Triphala. The antimicrobial activity was evaluated using agar well diffusion method against the both gram (+) (Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Bacillus cereus) and gram (-) (Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella, Escherichia coli and Azotobacter) bacterial isolates using ethanol, methanol, acetone, chloroform and aqueous leaf extracts of TB, TC, EO and Triphala. It was observed that ethanolic extract exhibited good activity against the tested bacterial isolates, compared with methanol, acetone, chloroform and aqueous extract, respectively. From this study, it can be concluded that Triphala and its constituents reveal antibacterial activity against various human pathogenic bacteria.

Key words: Antibacterial, Triphala, Terminalia bellerica, Terminalia chebula, Emblica officinalis

INTRODUCTION

Infectious diseases are the leading cause of death worldwide, and the numbers of deaths are increasing day by day. Of all infectious disorders pneumonia, diarrhea, tuberculosis and malaria have been the leading causes of death. [1] According to recent literature 50,000 men, women and children are dying every day due to these chronic diseases. [2] Microbes that cause illness are also known as pathogens. The most common pathogens are bacteria, though a number of other microorganisms, including some kinds of virus, fungi and protozoa, also cause diseases. In the human host, a microorganism causes disease by either disrupting a vital body process or stimulating the immune system to mount a defensive reaction. [3] Resistant microorganisms are able to survive attack by antimicrobial drugs, so that standard treatments become ineffective and infections persist, increasing the risk of spread of these microorganism to others hosts. Multidrug resistance (MDR) creates serious challenges to the medicinal field and infections caused by MDR bacteria. [4]

Recent studies also indicate that synthetic drugs are emerging as drugs of abuse for any age of person. [5] Nowadays, people suffering from the side effects of antimicrobial resistance are trying to find alternative solution in natural products. Herbs are used to treat various infectious diseases worldwide. Interestingly, some herbs have antimicrobial activity against bacterial pathogens in addition to their flavouring effects. [6] From the earliest

times, herbal spices are added for improving taste which naturally and safely renews shelf life of food products. Bacterial pathogens are sensitive to extracts from many medicinal plants. [7] Therefore, the natural plants have been used as antimicrobial agents which provide a promising safe solution, cheap to produce, biodegradable and readily available. [8]

Triphala is an Indian tridoshic herbal formulation consisting of fruits of T. bellerica (TB), T. chebula (TC) and E. officinalis (EO) in 1:1:1 ratio. Triphala is used against constipation, act as restoratives for gastrointestinal tract and also detoxifies the whole body and improves digestion and assimilation. [9] Fruits of TB contain different tannins, flavonoids and other phenolic compounds. [10] that may responsible for various biological activities. As per literature Ayurvedic plant parts are good in cold, cough, chronic diarrhea, dysentery and helps to increases appetite. [11] TB leaf showed good in vitro antioxidant activity against different reactive oxygen species (ROS). [12]

TC is known as the “King of Medicine” in Tibet. [13] The plant is found to contain various phytochemicals such as phloroglucimol and pyrogallol, along with phenolic acids such as ferulic, pcoumaric, caffeic and vanillic acids. The powder of the dried fruits of TC is used for the various therapeutic purposes to promote longevity. EO is commonly known as Indian gooseberry in English and locally as Amla. EO fruit is used either alone or in combination with other plants to treat many common ailments such as cold, fever, peptic ulcer, dyspepsia and as digestive aid as well as serious diseases like cancer and cardiovascular disease. [14] It is one of the richest sources of vitamin C, responsible for antioxidant, anti-inflammatory and antimicrobial activities. Gallic acid and tannic acid are the major phytoconstituents of EO. [12,15] Thus Triphala and its constituents is emerging herbal warrior against infectious microorganisms. However, scientific exploration of leaf extracts of TB, TC and EO along with Triphala, growing in India, for antimicrobial properties is very little. The present study focused on evaluation of antibacterial activity of Triphala and leaf extracts of its constituents against both gram (+) and gram (-) bacteria.

MATERIALS AND METHODS

Plant materials

Plant materials collected from Herbal Garden of Narendra Dev (ND) University of Agriculture and Technology Kumarganj, Faizabad, Uttar Pradesh, were chopped, dried, powdered and stored in polythene bags at 4°C till further analysis. Identification of different plant samples was carried out and confirmed with the help of Dr. MN Srivastava, Senior Scientist, Botany Division, CSIR-Central Drug Research Institute, Lucknow, India and the voucher specimens were submitted in CDRI herbarium.

Chemicals and Reagent

All media, chemicals and reagents were purchased from HiMedia and SRL, India. All reagents and chemicals used were of analytical (AR) grade.

Extraction procedure for antioxidant assay

Twenty grams of dried and powdered plant samples of T. bellerica leaf (TBL), T. chebula leaf (TCL), Emblica officinalis leaf (EOL) and Triphala were extracted with 70% ethanol, methanol, acetone and chloroform solvent (in water) including aqueous system until decoloration. The extracted solvent was evaporated at 40°C in a vacuum rotary evaporator and lyophilized till dryness. The powdered form of plant extract was stored at -4°C and used for the antioxidant activity determination.

Bacterial culture

Clinical isolates of bacterial strains were obtained from the Department of Microbiology, Dr. Ram Manohar Lohia Avadh University, Faizabad, Uttar Pradesh, India. All the test strains were maintained at 4°C on nutrient agar (Hi-media). [16]

Antibacterial screening

Antibacterial activities of different extracts were evaluated by the agar well diffusion method [17] and Minimum inhibitory concentration (MIC). [18]

Media preparation and its sterilization

For agar well diffusion method [17] antimicrobial susceptibility was tested on solid (Agar-agar) media in petri plates. For bacterial assay nutrient agar (NA) (40 gm/l) was used for developing surface colony growth. The minimum inhibitory concentration (MIC) values were determined by serial micro dilution assay. The suspension culture, for bacterial cells growth was done by preparing 2% Lauria Broth (w/v). All the media prepared was then sterilized by autoclaving the media at (121°C) for 20 min.

Agar well diffusion method

Agar well-diffusion method was followed to determine the antimicrobial activity. [17] Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 8 hour old broth culture of respective bacteria. Wells (10 mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of each plant extract was prepared at a concentration of 1 mg/ml in different plant extracts viz. ethanol, methanol, acetone, chloroform and aqueous. About 100 µl of different concentrations of plant solvent extracts were added by sterile syringe into the wells and allowed to diffuse at room temperature for 2 hrs. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 h. The diameter of the inhibition zone (mm) was measured. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

Broth microdilution method

The broth microdilution method was carried out in a 96-well microtiter plate to determine the minimum inhibitory concentration (MIC). The different concentrations of compounds (200, 150, 100 and 50 µg/ml) were diluted in Mueller Hinton broth and the final volume was maintained at 100 µl. The final concentration of DMSO was less than 1%. 5 µl of an overnight grown bacterial culture was added to the test medium to bring the final inoculum size to 1×10^5 cfu/ml. [19] The agar plates were incubated at 37°C for 24h and the absorbance was read at 600 nm. The percent growth inhibition was calculated by comparison with a control using the formula indicated below. The lowest concentration of the compound that inhibits the complete growth of the bacterium was determined as the MIC.

$$\text{\% of growth inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Statistical analysis

Statistical analysis was done using prism software. Values from antibacterial activities were reported as mean ± standard deviation (SD) of three determinations.

RESULT AND DISCUSSION

Different solvent extracts of TBL, TCL EOL and Triphala was tested for antimicrobial activity analysis by the disc diffusion method against both some gram (+) (Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus and gram (-) bacteria (Pseudomonas aeruginosa, Salmonella, Escherichia coli). In the present study ethanolic extracts of TBL, TCL EOL and Triphala showed good antibacterial activity at different concentration 100 and 200 µg/ml followed by methanol, acetone, chloroform and aqueous extracts (Table 1). Ethanolic leaf extracts showed strong inhibition to S. epidermidis, B. subtilis, K. pneumoniae, E. coli and Azotobacter, forming large zone of inhibition of 19, 19, 22, 18 19 mm by TBL; 26, 18, 21, 23, 29 mm by TCL and 25, 18, 22, 20, 22 mm by EOL at 200 µg/ml, respectively in comparison to standard antibiotics Gentamicin and Tetracycline. Ethanolic extracts of Triphala showed a broad spectrum of antibacterial activity against

both gram (+) and gram (-) bacteria than TBL, TCL and EOL with largest zone of inhibition of 21, 26, 21 26, 25, 33, 30, 26 and 29 mm at 200 µg/ml concentration (Table 1). This was supported by an earlier study on an alcoholic extract that exhibited greater activity than the aqueous and hexane extracts against bacteria, with no cellular toxicity. [20] Gupta et al. [21] reported that a Terminalia pallida fruit methanolic extract showed maximum activity against gram (-) bacteria, while that of T. bellerica showed the highest inhibition zones against P. aeruginosa and E. coli. [22] Two possibilities that may account for the higher antibacterial activity of alcoholic extracts are the nature of biological active components (alkaloids, flavonoids, essential oil, tarpenoids, tannins, etc.), which may be enhanced in the presence of ethanol; and the stronger extraction capacity of ethanol that may have yielded a greater number of active constituents responsible for antibacterial activity. [12,15] This is in agreement with a study by Taso and Deng [23] which showed that phenolic compounds are generally better extracted by using alcoholic solvents.

Table 1: Antibacterial activity of TBL, TCL, EOL and Triphala

Bacteria	S.	Extract Con. (µg/ml)	Name of plant					
			Tri	TBL	TCL	EOL	CN	TE
							30µg/disc	
Gram (+)								
S. aureus	E	100	19±0.20	18±0.54	17±0.73	16±0.52	22	23
		200	21±0.20	19±0.30	18±0.30	18±0.52		
	M	100	17±0.20	12±0.73	11±0.73	12±0.52		
		200	18±0.20	15±0.73	15±0.52	16±0.52		
	A	100	14±0.20	09±0.54	08±0.73	11±0.52		
		200	16±0.20	10±0.30	10±0.52	12±0.54		
	C	100	09±0.20	07±0.54	10±0.73	09±0.57		
		200	11±0.20	09±0.30	11±0.73	11±0.52		
	Aq	100	07±0.20	ND	ND	06±0.57		
		200	08±0.20	07±0.30	06±0.73	07±0.52		
	M	100	19±0.20	15±0.54	16±0.73	16±0.57		
		200	21±0.20	17±0.30	17±0.73	18±0.52		
	A	100	17±0.20	12±0.21	14±0.73	12±0.52		
		200	18±0.20	15±0.21	15±0.21	15±0.21		
	C	100	13±0.20	10±0.30	11±0.73	12±0.52		
		200	15±0.20	13±0.73	13±0.73	13±0.73		
	Aq	100	10±0.20	09±0.54	08±0.81	08±0.57		
		200	12±0.20	10±0.30	10±0.73	10±0.52		
B. subtilis	E	100	19±0.23	15±0.29	16±0.44	17±0.55	25	32
		200	21±0.15	19±0.24	18±0.39	18±0.50		
	M	100	15±0.20	13±0.30	14±0.73	12±0.52		
		200	18±0.20	15±0.30	15±0.30	14±0.30		
	A	100	12±0.20	11±0.54	11±0.73	09±0.57		
		200	14±0.20	12±0.30	13±0.73	12±0.52		
	C	100	11±0.20	09±0.54	09±0.73	09±0.57		
		200	12±0.20	10±0.30	10±0.73	10±0.52		
	Aq	100	07±0.20	ND	ND	ND		
		200	09±0.20	08±0.30	08±0.73	07±0.52		
B. cereus	E	100	23±0.00	11±0.51	15±0.55	14±0.66	30	30
		200	26±0.00	15±0.40	17±0.44	15±0.35		
	M	100	18±0.20	12±0.54	11±0.73	09±0.57		
		200	21±0.20	13±0.30	13±0.73	12±0.52		
	A	100	17±0.20	09±0.54	10±0.73	09±0.57		
		200	18±0.20	12±0.30	12±0.73	11±0.52		
	C	100	11±0.20	09±0.54	07±0.73	ND		
		200	13±0.20	10±0.30	10±0.73	09±0.57		
	Aq	100	09±0.20	ND	ND	ND		
		200	10±0.30	07±0.30	07±0.73	08±0.52		
Gram (-)								
P. aeruginosa	E	100	20±0.22	11±0.55	12±0.30	13±0.74	16	18
		200	25±0.00	15±0.55	14±0.30	15±0.55		
	M	100	20±0.20	12±0.30	11±0.73	12±0.52		
		200	22±0.20	14±0.30	12±0.73	14±0.30		
	A	100	17±0.20	10±0.54	11±0.73	09±0.57		
		200	18±0.20	12±0.30	10±0.73	12±0.52		
	C	100	12±0.20	09±0.54	07±0.73	ND		
		200	15±0.20	10±0.30	08±0.73	07±0.57		

Table 1 to be continued…

	Aq	100	09±0.20	ND	ND	ND		
		200	11±0.20	08±0.30	08±0.73	09±0.52		
K .pneumoniae	E	100	28±0.20	19±1.00	18±1.00	19±1.00	33	33
		200	33±0.00	22±1.00	21±1.00	22±1.00		
	M	100	25±0.20	16±0.54	16±0.73	16±0.57		
		200	28±0.20	19±0.30	18±0.73	19±0.52		
	A	100	19±0.20	13±0.54	11±0.73	14±0.57		
		200	23±0.20	15±0.30	15±0.73	16±0.52		
	C	100	17±0.20	11±0.54	10±0.73	09±0.57		
		200	18±0.20	13±0.30	12±0.73	12±0.52		
	Aq	100	13±0.20	08±0.73	09±0.73	07±0.63		
		200	16±0.20	10±0.73	10±0.73	10±0.52		
Salmonella	E	100	28±0.00	12±0.05	13±0.35	13±0.33	30	28
		200	30±0.00	15±0.04	15±0.33	15±0.24		
	M	100	22±0.20	11±0.54	11±0.73	11±0.57		
		200	25±0.20	14±0.30	14±0.73	14±0.52		
	A	100	16±0.20	09±0.54	08±0.73	09±0.57		
		200	20±0.20	12±0.30	11±0.73	12±0.52		
	C	100	12±0.20	ND	ND	06±0.57		
		200	15±0.20	09±0.30	10±0.73	08±0.52		
	Aq	100	09±0.20	ND	ND	ND		
		200	11±0.20	ND	08±0.73	07±0.52		
E. coli	E	100	26±0.33	16±0.24	21±0.28	18±0.28	27	20
		200	30±0.25	18±0.20	23±0.25	20±0.27		
	M	100	23±0.20	14±0.54	16±0.73	15±0.57		
		200	26±0.20	16±0.30	18±0.73	18±0.52		
	A	100	18±0.20	12±0.54	13±0.73	12±0.57		
		200	21±0.20	14±0.30	15±0.73	15±0.52		
	C	100	17±0.20	09±0.54	10±0.73	09±0.57		
		200	18±0.20	11±0.30	12±0.73	12±0.52		
	Aq	100	10±0.20	09±0.54	08±0.73	07±0.57		
		200	12±0.20	10±0.30	10±0.73	09±0.52		
Azotobacter	E	100	22±0.03	16±0.33	25±0.36	19±0.33	16	22
		200	29±0.02	19±0.25	29±0.33	22±0.29		
	M	100	21±0.20	13±0.54	19±0.73	16±0.57		
		200	25±0.20	16±0.30	24±0.73	19±0.52		
	A	100	17±0.20	11±0.54	13±0.73	11±0.57		
		200	18±0.20	13±0.30	17±0.73	13±0.52		
	C	100	12±0.20	09±0.54	11±0.73	07±0.57		
		200	15±0.20	10±0.30	13±0.73	12±0.52		
	Aq	100	09±0.20	ND	06±0.73	06±0.57		
		200	11±0.20	06±0.30	09±0.73	09±0.52		

Each value is mean ± SD of three replications (n = 3). S.: solvent; E: ethanol; M: methanol; A: acetone; C: chloroform; Aq: aqueous, CN: Gentamicin; TE: tetracycline; ND: not determined

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is defined as the highest dilution or least concentration of the extracts that inhibit growth of organisms. Determination of the MIC is important in diagnostic laboratories because it helps in confirming resistance of micro-organism to an antimicrobial agent and it monitors the activity of new antimicrobial agents. The ethanolic extract of Triphala, TBL, TCL and EOL was further subjected to the broth microdilution method to determine the MIC (Table 2). The maximum activity was observed against S. epidermidis, B. subtilis, K. pneumonia, E. coli and Azotobacter at a concentration of 200 µg/ml. This result is in agreement with the report of Phadke and Kulkarni [24] and Rani and Khullar [25] studied in T. chebula and T. arjuna leaves, respectively.

In this study, 90% growth inhibition was found against S. epidermidis and nearly 80% growth inhibition was observed against E. coli at a concentration of 200 µg/ml (Figure 1). This present study showed antibacterial activity at a low concentration, whereas Ahmad et al. [20] reported similar activity at a concentration of 200 mg/ml. About 70% growth inhibition of B. subtilis was found at a concentration of 200 µg/ml. T. arjuna was found to have antibacterial activity against B. subtilis and S. aureus (Perumalsamy and Ignacimuthu, 2000). Sato et al. [26] reported that a fruit ethanol extract

of T. chebula exhibited antibacterial activity against S. aureus and the compounds responsible for this activity were gallic acid and its ethyl ester. The clinical pathogen E. coli showed a MIC value of 62.5 µg/ml, [27] which is six times higher than the present study. Terpenoides from T. avicennioides showed antibacterial activity against S. aureus, E. coli and P. aeruginosa. [28] Nearly 90% growth inhibition was also observed in K. pneumoniae at a concentration of 200 µg/ml. This is in agreement with the report of Suguna et al. [29]

Table 2: Minimum inhibitory concentration (µg/ml) of ethanolic extracts of Triphala, TBL, TCL and EOL

Bacteria	Extract Con. (µg/ml)	Tri	TBL	TCL	EOL	CN	TE
S. aureus	200	19±0.5	19±0.1	21±0.1	19±0.1	16±0.1	20±0.1
	150	22±0.5	22±0.2	28±0.2	23±0.0	19±0.2	29±0.2
	100	29±0.5	29±0.2	34±0.3	31±0.0	25±0.5	35±0.5
	50	34±0.1	34±0.1	39±0.1	35±0.1	30±0.1	43±0.1
S. epidermidis	200	09±0.3	16±0.3	14±0.3	15±0.3	14±0.1	17±0.1
	150	12±0.5	28±0.2	25±0.4	23±0.0	20±0.2	24±0.2
	100	15±0.1	33±0.1	30±0.1	30±0.1	27±0.5	29±0.5
	50	30±0.5	38±0.1	35±0.1	36±0.0	32±0.1	34±0.1
B. subtilis	200	11±0.1	18±0.1	19±0.1	16±0.1	15±0.1	17±0.1
	150	18±0.5	28±0.0	33±0.0	31±0.0	28±0.2	28±0.2
	100	25±0.1	35±0.1	39±0.1	38±0.1	35±0.5	36±0.5
	50	33±0.1	43±0.1	46±0.1	49±0.1	42±0.1	45±0.1
B. cereus	200	36±0.1	54±0.1	73±0.1	76±0.1	28±0.1	38±0.1
	150	49±0.1	61±0.1	89±0.1	82±0.1	45±0.2	47±0.2
	100	78±0.5	109±0.2	108±0.1	98±0.0	57±0.5	67±0.5
	50	94±0.5	124±0.2	128±0.2	119±0.0	76±0.1	86±0.1
P. aeruginosa	200	31±0.1	37±0.1	39±0.1	31±0.1	34±0.1	34±0.1
	150	35±0.1	45±0.1	56±0.1	48±0.1	50±0.2	48±0.2
	100	52±0.5	63±0.5	71±0.0	67±0.0	63±0.5	65±0.5
	50	74±0.1	85±0.1	88±0.1	89±0.1	82±0.1	82±0.1
K. pneumoniae	200	11±0.5	17±0.2	15±0.0	16±0.0	11±0.1	15±0.1
	150	16±0.1	29±0.1	31±0.1	26±0.1	18±0.2	22±0.2
	100	19±0.1	38±0.1	36±0.1	32±0.1	26±0.5	29±0.5
	50	24±0.1	49±0.1	40±0.1	40±0.1	34±0.1	37±0.1
Salmonella	200	31±0.1	36±0.1	39±0.1	41±0.1	26±0.1	30±0.1
	150	42±0.1	62±0.1	59±0.1	57±0.1	37±0.2	39±0.2
	100	68±0.5	88±0.0	84±0.0	88±0.0	48±0.5	52±0.5
	50	92±0.5	107±0.0	113±0.0	112±0.0	62±0.1	68±0.1
E. coli	200	08±0.1	09±0.1	10±0.1	11±0.1	10±0.1	11±0.1
	150	17±0.1	19±0.1	20±0.1	19±0.1	18±0.2	20±0.2
	100	23±0.5	29±0.1	28±0.0	24±0.0	25±0.5	28±0.5
	50	29±0.5	36±0.2	39±0.0	37±0.0	34±0.1	38±0.1
Azotobacter	200	10±0.2	13±0.2	12±0.0	14±0.0	11±0.1	11±0.1
	150	19±0.2	21±0.2	27±0.2	20±0.2	18±0.2	19±0.2
	100	28±0.1	29±0.1	25±0.1	26±0.1	27±0.5	27±0.5
	50	34±0.2	34±0.2	32±0.2	36±0.2	32±0.1	35±0.1

Each value is mean ± SD of three replications (n = 3). S.: solvent; E: ethanol; M: methanol; A: acetone; C: chloroform; Aq: aqueous, CN: Gentamicin; TE: tetracycline; ND: not determined

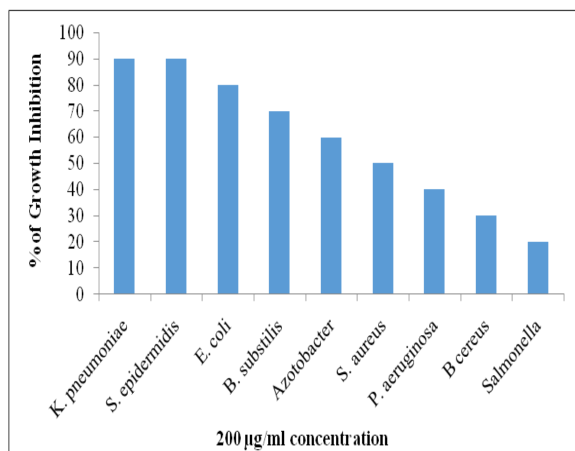

Figure 1: MIC against pathogenic microorganisms

CONCLUSION

Now a day, due to extensive use of antibiotics and vast majority of synthetic drugs, many multidrug resistant strains are developing especially in hospital environment. To overcome drug resistance and to avoid side effects associated with the commonly available antibiotics, there is need of an alternative treatment method to cure such infections by use of traditional medicinal herbs like TB, TC and EO. Triphala and leaf extracts of its constituents are clinically safe, economically cheap and

easily available in market. Present study proved that ethanolic extracts of Triphala and leaf extracts of its constituents are potent antibacterial agents against various human pathogenic bacteria. This study provide a lead for further in vitro and in vivo studies to understand the mechanism of antimicrobial action of Triphala and its constituents which may help in developing better therapeutic agents and healthy products.

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Conflict of interest

The authors declare that they have no conflict of interest.

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