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Ethnobotanical Analysis of Antibacterial and Antifungal Activity in Five Medicinal Plants from Sonamukhi Block, Bankura District

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ABSTRACT
Five medicinal plants, *Ipomoea obscura, Tylophora indica, Abroma augustuma, Glinus oppositifolius*, and *Aristolochia indica*, were collected ethnobotanically from Sonamukhi Block in Bankura District, for analysis of antibacterial and antifungal activity. The antimicrobial characteristics were determined against *Aspergillus niger, Candida albicans, Escherichia. coli, Pseudomonas. aeruginosa, Staphylococcus. aureus*, and *Streptococcus. mutans*. Potato Dextrose Broth and Luria Bertani Broth were employed for fungal and bacterial cultures, respectively. Sample concentrations were produced in Dimethyl Sulfoxide (DMSO). The results demonstrated that *I. obscura* and *T. indica* had strong antibacterial and antifungal action against a variety of bacterial and fungal strains, with wider zones of inhibition reported at increasing dosages. These results emphasize the potential of medicinal herbs as natural antibacterial agents, highlighting the need for further study to better understand their medicinal uses, mechanisms of action, and ethnobotanical significance.
Keywords: Antibacterial, Antifungal, Ethnobotanical, Medicinal plants

INTRODUCTION

In the realm of ethnobotany, the antimicrobial activity of medicinal plants has generated a great deal of study and interest. Phytotherapeutic agents, also referred to as medical herbs or medicinal plants, are plants with pharmacological qualities that are employed in medicine. The therapeutic

benefits of these plants are attributed to a wide range of bioactive chemicals, such as phenolic compounds, terpenoids, flavonoids, and alkaloids (Huidrom *et al*., 2024).

The capacity of medicinal plants to impede the growth of microorganisms, such as bacteria, fungi, and viruses, is known as their antimicrobial activity. In traditional medicine systems, where plant-based treatments have been utilized for generations to cure infectious disorders, this action is essential (Sharmin, T. *et al.*, 2017). Medicinal plants may fight a variety of infectious disorders, and their antimicrobial activity has been thoroughly investigated (Kumari, M.S. and Ammani, K., 2020). Among those with known traditional medicinal uses were *Ipomoea obscura* (Convolvulaceae), *Tylophora indica* (Apocynaceae), *Abroma augustum* (Malvaceae), *Glinus oppositifolius* (Molluginaceae), *and Aristolochia indica* (Aristolochiaceae) (Bhadra, A. and BT, M., 2024). These species are especially noteworthy in this regard (P. Murmu., 2023). The ethnobotanical acquisition of these plants attests to their historical and cultural relevance in regional traditional medical treatments. Researchers have looked into the antimicrobial qualities of these plants against strains of *Pseudomonas aeruginosa*, *Escherichia coli* (MTCC 433), *Staphylococcus aureus* (MTCC 96), and *Streptococcus mutans* (MTCC 497), which are linked to bacterial infections, as well as *Aspergillus niger* and *Candida albicans*, which are found in fungal infections (Tshikalange, T.E et al.,2017).

In traditional medicine systems, where plant-based treatments have been utilized for generations to cure infectious disorders, this action is essential. Investigating the antimicrobial activity of medicinal plants provides important information about their potential as natural sources of antimicrobial agents, which may be used to tackle the rising danger of antibiotic resistance and create new therapies for infectious illnesses (Olasehinde, G.I. *et al.*, 2016).

Since it may result in the creation of novel therapeutic medicines, more investigation into the molecular components and processes underlying their antibacterial action is necessary (Machaba, T.C. *et al*., 2024). There is potential for resolving contemporary healthcare issues by utilizing knowledge of ethnobotanical techniques and the antibacterial qualities of medicinal plants, especially in light of the rise in antibiotic resistance (Pepeljnjak, S., 2020) (Thomas, E. *et al.*, 2007).

MATERIALS AND METHODS

Collection of plant material and preparation of powder

Plant samples were collected from several areas within Sonamukhi Block, Bankura district, West Bengal, pertaining to five medicinal plants: Ipomoea obscura, Tylophora indica, Abroma augustum, Glinus oppositifolius, and Aristolochia indica. Experts in taxonomy at FRLHT (Foundation for

Revitalisation of Local Health Traditions) and TDU (The University of Trans-Disciplinary Health Sciences and Technology) affirmed the ethnobotanical identification and analysis of these plants. Using a mortar and pestle, the gathered medicinal herbs were dried and crushed.

Well diffusion method to check the Antimicrobial activity

With the use of the well diffusion method, the Antimicrobial activity of the following samples was determined: *Ipomoea obscura, Tylophora indica, Abroma augustum, Glinus oppositifolius,* and *Aristolochia indica*

Sample Preparation

The samples (*Aristolochia indica, Glinus oppositifolius, Abroma augustuma, Tylophora indica,* and *Ipomoea obscura*) each had 100 mg dissolved in 1 mL of dimethyl sulfoxide (DMSO). Pipetting 10µL (1 mg), 20µL (2 mg), 30 µL (3 mg), and 40 µL (4 mg) from 100 mg of sample resulted in different sample concentrations. DMSO was then added to get the total volume up to 50µL (Valgas, C, et al., 2007).

Bacterial Culture preparation

In four individual Erlenmeyer flasks, 30 mL of Luria Bertani (LB) broth (Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Distilled water 1000mL) was made by adding Tryptone 0.3g, Sodium chloride 0.3g, Yeast extract 0.18g, and Distilled water 30mL. The flasks were then autoclaved for 15 minutes at 121°C. Subsequently, 30 mL of sterilised LB broth flasks were inoculated with the *E. Coli* strain (MTCC 433), *P.aeruginosa* strain (two bacteria, MTCC 2453), *S. aureus* strain (MTCC 96), and *S. mutans* strain (MTCC 497), respectively, and incubated at 37˚ C for 24 hours.

In order to dissolve the pellets, cultivated strains were centrifuged at 6000 rpm for 10 minutes at a time, with the supernatant being disposed of. The pellets were then adjusted to an absorbance of 1.000 at 600 nm using a UV spectrophotometer (Genesys 10S UV-VIS Spectrophotometer) (Valgas, C, *et a*l., 2007).

Culture Media Preparation for Fungi

To make the fungal culture medium, 6g of potato was boiled in 30mL of distilled water, filtered, and then added 20g of dextrose (PDB: Potato-200g, Dextrose-20g, Distilled water-1000mL). The filtrate was mixed with 0.6g of dextrose, and 30mL of distilled water was added as the final volume. For fifteen minutes, the mixture was autoclaved at 121°C. Following this, flasks containing

Aspergillus niger culture, which was obtained from a natural source, and Candida albicans (MTCC 3958) were infected and allowed to incubate for 72 hours at 25°C (S. Magaldi, et al., 2004).

Positive Control Preparation

Tetracycline 10mg was dissolved in 1mL of DMSO. Different concentration of Tetracycline was prepared by pipetting 10µL (100µg), 20µL (200µg), 30µL (300µg), 40 µL (400µg) and make upto 50 µL by using DMSO.

Fluconazole 100mg was dissolved in 1mL of DMSO. Different aliquots were prepared for *A.niger* by pipetting 10µL (1mg), 20µL (2mg), 30µL (3mg), 40µL (4mg) and for *C.albicans* Fluconazole 10mg was dissolved in 1mL of DMSO. Different aliquots were prepared by pipetting 10µL (100µg), 20µL (200µg), 30µL (300µg), 40µL (400µg) and the final volume was made upto 50µL respectively by adding DMSO.

Media preparation

Potato Dextrose Agar media (PDA: Potato-200g, Dextrose-20g, Agar-20g, Distilled water-1000mL) 500mL was prepared in Erlenmeyer's flask by boiling100g of Potato in 200mL distilled water and filtered, final volume was made upto 500mL with distilled water and Dextrose 10g, Agar 10g was added respectively and autoclaved at 121°C for 15 mins. Luria Bertani (LB) agar media (Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Agar 20g, Distilled water 1000mL) 500mL was prepared in 2 Erlenmeyer flasks by adding Tryptone 5g, Sodium chloride 5g, Yeast extract 3g, Agar 10g, Distilled water 500mL respectively and autoclaved at 121°C for 15 mins.

Plating for antimicrobial activity against organisms.

Approximately 25mL of the media (PDA and LB agar) was poured into the sterilized petriplates and allowed it to solidify. 200µL prepared inoculum was poured respectively on a agar plates and spread thoroughly using a plate spreader. Five wells measuring 0.6cm was made in each plates using the borer and 50µL of Sample and Positive Control were loaded into the respective plate wells and 50µL of DMSO was loaded in the middle well as Negative Control. The bacterial plates incubated at 37°C for 24h and Fungal plates incubated at 25°C for 72h. Later, zone of inhibition was recorded in mm (millimeter) (S. Magaldi, *et al*., 2004) (Valgas, C, *et a*l., 2007).

RESULTS AND DISCUSSION

The antibacterial and antifungal zones caused by varying doses of plant extracts against a variety of diseases were determined. *Tylophora indica* showed moderate to good inhibitory effectiveness against *Candida albicans* (11mm in 1mg,12mm in 2mg, 13mm in 3mg, 15mm in 4mg), *Escherichia*

coli (9mm in 1mg, 10mm in 2mg, 13mm in 3mg, 14mm in 4mg) *Pseudomonas aeruginosa* (12mm in 1mg,13mm in 2mg, 14mm in 3mg, 15mm in 4mg), *Staphylococcus aureus* (12mm in 1mg, 14mm in 2mg, 15mm in 3mg, 17mm in 4mg) and *Streptococcus mutans* (11mm in 1mg, 12mm in 2mg, 12mm in 3mg, 15mm in 4mg). *T. indica*'s inhibitory effect was dose-dependent, with greater doses producing broader zones of inhibition. Notably, the highest dose of *T. indica* (4mg) (upto 15mm) demonstrated the greatest inhibition against all tested pathogens, demonstrating its potential as a useful antibacterial agent (Table 2). Similar readings were investigated by (Shahid Mohd., 2012) who reported that differed doses of the alcoholic extract of *Tylophora indica* had effective antibacterial action, considerably suppressing the development of microorganisms such as *Aspergillus niger* and *Pseudomonas aeruginosa*. At higher doses (4mg), the extract demonstrated mild to moderate efficacy against *Escherichia coli*. However, at greater doses, a substantial antibacterial impact was shown.

Abroma augustuma had minimal action against just *E. coli* (11mm in 2mg, 12mm in 3mg, 14mm in 4mg) and *P. aeruginosa* (11mm in 1mg, 12mm in 2mg, 12mm in 3mg, 14mm in 4mg), with no substantial suppression against the other pathogens examined. *A. augustuma's* inhibition was quite modest in comparison to the other plant extracts examined. Even at the maximum dose (14mm in 4mg), *A. augustuma* failed to have a significant inhibitory impact on most bacteria. This shows that *A. augustuma* may not be as effective against a variety of diseases as other plant extracts (Table 3). In the experiment, the observed inhibition zones were within the estimated range; however, according to (Kumar *et al*., 2023), the inhibition zones were much larger, ranging from 12 to 26 mm. The study indicated that susceptible and conventional bacteria, such as *Staphylococcus aureus* and *Escherichia coli*, had broader zones of inhibition than drug-resistant variants. The increased antibacterial efficacy found by Kumar et al. might be related to the large surface area of antimicrobial agents, which allows for better contact with microbes.

Glinus oppositifolius inhibited all studied pathogens, particularly *E. coli* (22mm in 4mg) and *S. mutans*. (23mm in 4mg). *G. oppositifolius'* inhibition was strong and dose-dependent, with greater zones of inhibition detected as the extract concentration increased. At the maximum dose (4mg), *G. oppositifolius* showed substantial inhibition against all infections (*C. albicans* -14mm, *E. coli* - 22mm, *P. aeruginosa*-15mm, *S..aureus*-20mm, *S.mutans*-23mm), showing broad-spectrum antibacterial action (Table 4). These findings indicate that *G. oppositifolius* may be a potential source of antibacterial chemicals. (Martin-Puzon J.J. *et al* 2015) reported comparable results. According to Martin et al. (2015), there have been very few investigations on the antibacterial capabilities of the medicinal plant *G. oppositifolius*. The antimicrobial susceptibility results for *G.*

oppositifolius reported in this paper suggest that leaf extracts can be an alternative source of antibacterial metabolites that can be developed into new antibacterial agents against Gram-negative bacteria, specifically *E. coli* and *P. aeruginosa*.

Aristolochia indica exhibited strong inhibitory action against all tested pathogens. *A. indic*a's inhibition was dosage dependent, with greater concentrations resulting in broader zones of inhibition. Notably, *A. indica* had substantial inhibitory effects even at lower dosages, indicating its efficacy as an antibiotic. At the highest quantity (4mg), *A. indica* demonstrated the most significant inhibition against all pathogens tested (*C.albicans*-21mm,*E.coli*- 17mm ,*P.aeruginosa*-18mm, *S.aureus*- 23mm ,*S.mutans*- 24mm showing its potential for therapeutic usage a wide spectrum of diseases (Table 5). These results are more positive than those of (I T Gbadamosi et al 2011), *Aristolochia indica* which demonstrated the greatest antibacterial activity of 19 mm against clinically isolated multidrug-resistant *E.coli*. In the study we conducted, the ethanol extract of A. *indica* demonstrated 18 mm against multidrug resistant *E.coli*. The ethanol extract of *Aristolochia indica* has antifungal activity against 9 out of 10 *Candida albicans* isolates, with zones of inhibition ranging from 14.00 mm to 50.00 mm. It was most effective against isolation (19.00 mm to 50.00 mm) and less effective against isolate 14.00 mm to 15.00 mm (Jirovetz, L. *et al.* 2000).

The inhibitory activity of *I. obscura* extract was tested against a variety of diseases at different doses. The zone of inhibition for *C. albicans* varied from 9 mm to 17 mm as the dose rose from 1 mg to 4 mg. Similarly, *E. coli* showed inhibition zones ranging from 10 mm to 15 mm, whereas *P. aeruginosa* showed inhibition from 11 mm to 15 mm. S. aureus showed zones ranging from 12 mm to 17 mm, whereas S. mutans showed inhibition ranging from 11 mm to 15 mm when the concentration was gradually raised (as shown in Table 1). Similar results by (Akinniyi G et al 2022) where Various *Ipomoea* extracts were tested for antifungal efficacy against two fungus strains. (*Candida albicans*, *Aspergillus niger*) strains utilising cup. The plate technique and zone of inhibition were estimated for each strain All three extracts demonstrated moderate antifungal activity against two fungal strains, with diameter of The zone of inhibition was measured if the diameter was smaller than 12. thus viewed as having no antifungal action. (Akinniyi G *et al* 2022). Furthermore, the antifungal and antibacterial activity of positive controls, Fluconazole against fungus and Tetracycline against bacteria was calculated. Fluconazole inhibited *A. niger* and *C. albicans*, but tetracycline inhibited *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. mutans*. These findings verify the assay's reliability and demonstrate the efficiency of typical antimicrobial drugs against the pathogens examined.

Plant extracts, particularly *G. oppositifolius* and *A. indica*, had promising inhibitory activities against a variety of infections, indicating their potential as natural antibacterial agents. Further research is needed to isolate and characterise the active chemicals responsible for the reported antibacterial activity, as well as to assess their safety and effectiveness for therapeutic use.

Aristolochia indica and *Glinus oppositifolius* were shown through this research to have strong antibacterial action, especially at higher dosages, with inhibition zones of up to 24 mm against pathogens such as *S. mutans* and *E. coli*. Strong, influenced by dose effects were continuously displayed by these plants, indicating their potential as broad-spectrum antibacterial agents. *Abroma augustuma* was just little affecting *E. Coli* and P. aeruginosa, whereas Tylophora indica was demonstrating considerable, dependent on concentration action. These differences in efficacy were consistent with other studies, showing that the phytochemical makeup of plant extracts and the pathogens under investigation might have a significant impact on their antibacterial activity (Diliarosta, S. *et al.*,2022).

The plant extracts were demonstrating promising but less strong efficacy when compared to the positive controls, tetracycline and fluconazole, underscoring the need for more research. The findings highlighted these plants' potential as natural sources of antimicrobial compounds, especially in light of the growing prevalence of antibiotic resistance. To properly assess their medicinal potential, the study also emphasised how crucial it is to isolate and characterise the chemical molecules causing these effects (Bektaş, E. *et al.*,2018). While pointing up the need for more study to guarantee these plants' safety and performance in clinical applications, the results also supported the traditional usage of these plants in treating ailments.

Antimicrobial Activity of Plant Samples

Table 1. Antibacterial & Antifungal of *Ipomoea obscura* against Pathogens

Organisms	**Zone of inhibition (in mm) of *I. obscura* (in mg) against *Pathogens***			
	1mg	**2mg**	**3mg**	**4mg**
Plates	**1**	**1**	**1**	**1**
C.albicans	11	12	13	15
E.coli	9	10	13	14

P. aeruginosa	12	13	14	15
S.aureus	12	14	15	17
S.mutans	11	12	12	15

Table 2. Antimicrobial activity of *Tylophora indica* against Pathogens

Organisms	**Zone of inhibition (in mm) of *T. indica* (in mg) against *Pathogens***			
	1mg	**2mg**	**3mg**	**4mg**
Plates	**1**	**1**	**1**	**1**
C.albicans	11	12	14	14
E.coli	10	12	14	14
P. aeruginosa	11	12	13	15
S.aureus	11	12	13	14
S.mutans	11	12	13	15

Table 3. Antimicrobial activity of *Abroma augustuma* against Pathogens

(-): no antimicrobial activity

Organisms	**Zone of inhibition (in mm) of *A. augustuma* (in mg) against *Pathogens***			
	1mg	**2mg**	**3mg**	**4mg**
Plates	**1**	**1**	**1**	**1**
E.coli	-	11	12	14
P. aeruginosa	11	12	12	14

Table 4. Antimicrobial activity of *Glinus oppositifolius* against Pathogens

Organisms	**Zone of inhibition (in mm) of *G. oppositifolius* (in mg) against *Pathogens***			
	1mg	**2mg**	**3mg**	**4mg**
Plates	**1**	**1**	**1**	**1**
C.albicans	11	13	13	14
E.coli	15	15	19	22
P. aeruginosa	12	13	14	15
S.aureus	14	15	17	20
S.mutans	13	14	19	23

Table 5. Antimicrobial activity of *Aristolochia indica* against Pathogens

Organisms	**Zone of inhibition (in mm) of *A. indica* (in mg) against *Pathogens***			
	1mg	**2mg**	**3mg**	**4mg**
Plates	**1**	**1**	**1**	**1**
C.albicans	13	15	18	21
E.coli	12	14	15	17
P. aeruginosa	13	14	16	18
S.aureus	13	14	17	23
S.mutans	13	15	18	24

Positive Control

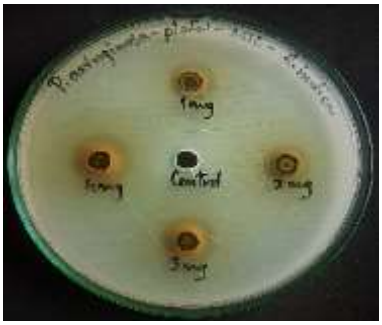
Table 6. Antimicrobial activity of Fluconazole against Fungi and Tetracycline against Bacteria

Organism	**Zone of inhibition (in mm) of Positive control (in mg)**			
	1mg	**2mg**	**3mg**	**4mg**
A.niger	15	20	22	24

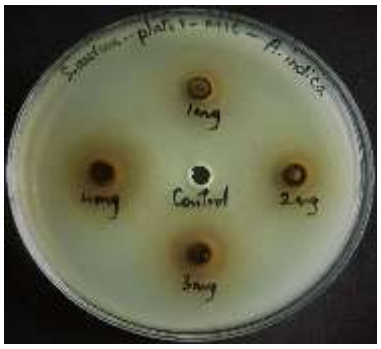
Organism	Zone of inhibition (in mm) of Positive control (in µg)			
	100µg	**200µg**	**300µg**	**400µg**
C.albicans	23	25	26	28
E.coli	30	32	34	36
P.aeruginosa	25	26	27	29
S.aureus	20	22	24	26
S.mutans	22	24	26	28

(a)

(b)



(c)



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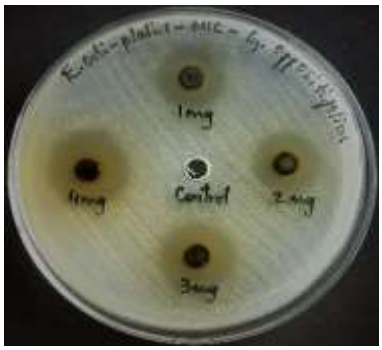

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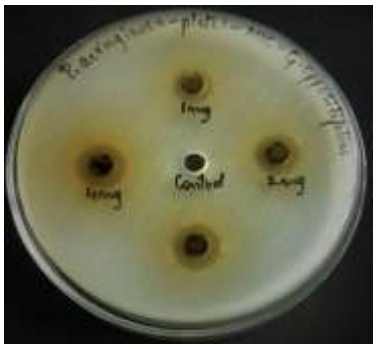

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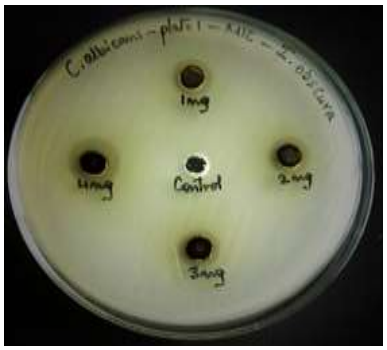

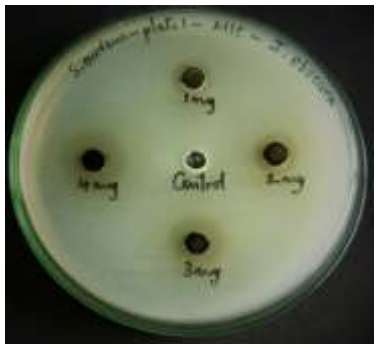

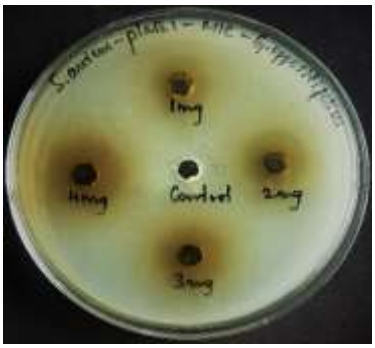

(g) (h) (i)

Fig (a-i). The antimicrobial findings shown on plates labelled A through I were examined for zones of inhibition against various pathogens treated with varying amounts of plant extracts and positive controls.

CONCLUSION

Eventually, the study's findings highlight the potential of plant extracts as promising antibacterial agents against a wide range of diseases. The investigation revealed the various degrees of inhibitory activity displayed by diverse plant extracts, with *Glinus oppositifolius* and *Aristolochia indica* appearing as especially powerful inhibitors against a wide variety of examined diseases. These findings emphasize the necessity of looking for new antibacterial chemicals in natural sources, particularly in light of rising antibiotic resistance. More study is needed to understand the mechanisms of action of these plant extracts, isolate and characterise their active components, and evaluate their safety and effectiveness for future therapeutic uses. Overall, this work provides useful insights into the search for alternate antibacterial treatments to tackle infectious illnesses.

The findings of this study have substantial significance for the field of ethnobotanical traditional medicine. The study highlights the powerful antibacterial activities of several plant extracts, including *Glinus oppositifolius* and *Aristolochia indica*, emphasizing the value of traditional medical knowledge in discovering natural sources of therapeutic compounds. These findings corroborate the practical usage of these herbs in traditional medicine systems by giving scientific evidence for their usefulness in treating microbial infections. Furthermore, by understanding the mechanisms of action and identifying active components in these plant extracts, future research inspired by these findings may lead to the creation of novel antimicrobial medications derived from traditional medicinal plants.

This merging of traditional knowledge with current scientific approaches not only verifies indigenous practices but also allows for the discovery of innovative medications based on ethnobotanical traditions, bridging the gap between traditional medicine and modern healthcare procedures.

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Conflict of Interest

The author(s) declare that there is no conflict of interest.

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