

In vitro screening of *Cichorium intybus*, a local medicinal plant for antimicrobial activities

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Abstract

The use of herbal medicines for research purposes is an elemental and essential step in achieving the results of excellent research. The investigation of a plant for medicinal purposes involves the extraction and discovery of the quality and quantity of the bioactive compounds. In this study, *Cichorium intybus* was examined to determine in vitro antimicrobial activity against the various bacterial strains, such as *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus atrophoeus*, *Salmonella typhi*, *Klebsella pneumoniae*, and *Escherichia coli* by applying the disc diffusion method. *Cichorium intybus* is used to cure many diseases. It was found that *Cichorium intybus* showed an inhibitory effect on the tested bacteria. The current study offers a scientific background for the significant utilization of plants under investigation for the discourse of several pathological diseases. Plant extracts of *Cichorium intybus* contain a considerable amount of antibacterial and antifungal components, which are commonly bioactive with variable quantities of inhibitory properties, linked to organisms and solvents. In different plant extracts; the ethanolic extract was supplementary active, followed by methanol, ethyl, hexane, and aqueous extracts.

Key words: Antimicrobial, *Cichorium intybus*, Disc Diffusion, Microbes.

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1. Introduction

Plants have been conventionally used to treat a wide range of human diseases [1]. The Therapeutic and pharmacological properties of medicinal plants have been attributed to a variety of chemical constituents related to their antioxidant properties [2]. Antioxidant substances are responsible for their protective possessions in the variability of deteriorating disorders [3]. Plant extracts are biologically potent natural moieties that work with different types of nutrients and fibres to produce a built-in uniformity of the defence system in conflict to diverse stress circumstances and diseases [4]. Phytochemicals are predominantly distributed into sets like secondary and primary ingredients on the base of their metabolic function in plants [5]. The primary components comprise the amino acid, chlorophyll, proteins, and common sugars whereas secondary moieties comprise saponins, alkaloids, terpenoids, phenolic contents and flavonoids [6]. Nowadays, plants derivative in different forms is used for curing various infectious diseases [7]. Herbal medications are plant-derived agents which possess therapeutic properties [8]. They may have raw or administered ingredients from more or one plant which is beneficial for human well-being [9]. Plants having medicinal belongings are necessary with reverence to the pharmaceutical productiveness and the novel drugs. *Cichorium intybus* belongs to the family Asteraceae (Fig 1). *Cichorium intybus* is cultured as an annual herb or may originate as a weed in *the Trifolium field* [7]. It is found in Europe, New Zealand Pakistan and India [10]. In Pakistan, it is found in areas of Hazara, Swat, Kurram, Gilgit and Kashmir [11]. Phytochemical findings showed that the plant derivative contains vitamins i.e., ascorbic acid, thiamine, retinol, riboflavin, niacin carotenoids, inulin i.e. cichorin, esculetin, esculin, lactucin and lactucopicrin [12]. The dried roots of *Cichorium intybus* are used as coffee substitutes and additives [13]. The significance of the plant extracted from microorganisms has been investigated by a number of investigators in different parts of the world [13].



Fig 1. Image of *Cichorium intybus*

2. Material and methods

Extracts of *Cichorium intybus* medicinal plant namely ethanol, methanol, ethyl acetate, hexane, and aqueous were explored for antimicrobial activities. The plant extracts were adjusted to one milligram/6 microliter in DMSO (dimethyl sulfoxide) solvent. The solution of stock was prepared; every 6 microliters of the solution contains one milligram of the extract.

2.1. Disc Diffusion Method

In this work, the disc diffusion technique has been used for antimicrobial accountability. To explore the presence of antibacterial components in the plant extracts, bacterial culture was adapted to 0.5 turbidity McFarland principles. Petri dishes were dried for 15 minutes before being used for antibiotic testing. The disc that was immersed with the extracts of *Cichorium intybus* was employed on the Mueller-Hinton agar. Each test plate consists of four discs. One for standard action as a positive control disk (commercial antibiotic), one for negative control and two for treated disks, samples under investigation. The standard antibiotic discs were Coltrimazole Ciprofloxacin, Azithromycin for the above-mentioned bacteria. The plates were then incubated for 18 to 24 hours at 37°C, depending on the types of bacteria used in the trial samples. Following incubation, the plates were examined for the inhibition zone. The inhibit zone was then calculated with callipers and then recorded. The tests were repeated 4 times to verify the reliability of the activity.

2.2. Applying Test

Plates under observation comprising standardized microbial inoculums were reserved in a post-absorption biological safety cabinet. Whatman No.1, sterilized filter paper discs drenched with plant extracts in 1, 2 and 3 mg discs in concentrations of 6, 12 and 18 microlitre were applied on the platters. Microbial (Fungal and bacterial) cultures were reserved at 37 °C for 18 hours. Antibiotics (Clotrimazole Ciprofloxacin, Azithromycin,) were used at a dose of 6 microliters/disc as a positive control in contradiction of fungi, Gram-negative and Gram-positive bacteria, expressed in Graph 1 and Fig 2.

3. Result and Discussion

3.1. Use of Standard Antibiotics against different Bacteria and Fungi

The Ciprofloxacin (as standard) antibiotic was utilized beside three Gram-negative bacteria namely *Escherichia coli*, *Klebsiella pneumonia* and *Salmonella typhi* and Azithromycin against three Gram-positive

bacterial strains namely *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus atropoeus* while Coltrimazole was utilized beside fungal strains namely *Candida albicans*, *Aspergillus sp.* and *Rhizopus sp.* In 50 mg per 6 microliter concentration. Azithromycin confirmed (50mm, 48mm and 65mm zone of inhibition) against *Bacillus subtilis*, *Staphylococcus aureus* and *Bacillus atropheous*. Ciprofloxacin showed (40mm, 35mm and 45mm against *Salmonella typhi*, *Escherichia coli* and *Klebsella pneumonia*, respectively. While Coltrimazole showed 25mm, 65mm and 60mm against *Candida albicans*, *Aspergillus* and *Rhizopus* respectively.

3.2. Antimicrobial Activity of *Cichorium intybus*

Different solvents such as aqueous, ethanol, methanol, hexane, and ethyl acetate were utilized for the antimicrobial activity of *Cichorium intybus* plant.

3.3. Ethanolic Extract of *Cichorium intybus*

The findings of the antimicrobial activities were calculated as a function of the diameter of the zone of inhibition in mm. The antimicrobial assay of the Ethanolic extract of *Cichorium intybus* has expressed in Graph 2 and Fig 3. Ethanolic extract of *Cichorium intybus* expressed the extreme inhibitory potential contrarily to the Gram-positive bacterium, *B. subtilis* (22 ± 0.1 mm zone of inhibition), against the Gram-negative bacterium, *K. Pneumonia* (22.5 ± 0.5 mm zone of inhibition) and fungus, *C. Albicans* (19 ± 0.1 mm zone of inhibition) while the reference results were 50 ± 0.0 mm, 40 ± 0.0 mm and 25 ± 0.0 mm, respectively.

3.4. Methanolic Extract of *Cichorium intybus*

The antimicrobial activity of the Methanolic extract of *Cichorium intybus* is expressed in Graph 3. Methanolic extract of *Cichorium intybus* explored the best inhibitory ability beside the Gram-positive bacterium, *B. Subtilis* (15.5 ± 0.5 mm zone of inhibition), against the Gram-negative bacterium, *E. coli* (17 ± 0.1 mm zone of inhibition) and fungus, *C. Albicans* (14 ± 0.5 mm zone of inhibition). While the reference findings were 50 ± 0.0 mm, 35 ± 0.0 mm and 25 ± 0.0 mm, respectively.

3.5. Ethyl Acetate Extract of *Cichorium intybus*

The antimicrobial activity of the ethyl acetate extract of *Cichorium intybus* is shown in Graph 4. Ethyl acetate extract of *Cichorium intybus* showed the maximum inhibitory activity against the Gram-positive bacterium, *S. aureus* (13 ± 0.1 mm zone of inhibition), against the Gram-negative bacterium, *E. coli* (18 ± 0.1 mm zone of inhibition) and fungus, *C. albicans* (18 ± 0.1 mm zone of inhibition). While the control results were 48 ± 0.0 mm, 35 ± 0.0 mm and 25 ± 0.0 mm, respectively.

3.6. Hexane Extract of *Cichorium intybus*

The antimicrobial activity of the hexane extract of *Cichorium intybus* is expressed in Graph 5. Hexane extract of *Cichorium intybus* expressed the maximum inhibitory activity against the Gram-positive bacterium, *B. subtilis* (17 ± 0.3 mm zone of inhibition), against the Gram-negative bacterium, *E. coli* (17 ± 0.1 mm zone of inhibition) and fungus, *C. albicans* (19 ± 0.1 mm zone of inhibition). While the control results were 50 ± 0.0 mm, 35 ± 0.0 mm and 25 ± 0.0 mm.

3.7. Aqueous Extract of *Cichorium intybus*

The antimicrobial activity of the aqueous extract of *Cichorium intybus* is shown in Graph 6. Aqueous extract of *Cichorium intybus* showed the maximum inhibitory activity against the Gram-positive bacterium, *S. aureus* (11 ± 0.1 mm zone of inhibition), against the Gram-negative bacterium, *E. coli* (14 ± 0.0 mm zone of inhibition) and fungus, *C. albicans* (9.5 ± 0.1 mm zone of inhibition). While the control results were 48 ± 0.0 mm, 35 ± 0.0 mm and 25 ± 0.0 mm, respectively.

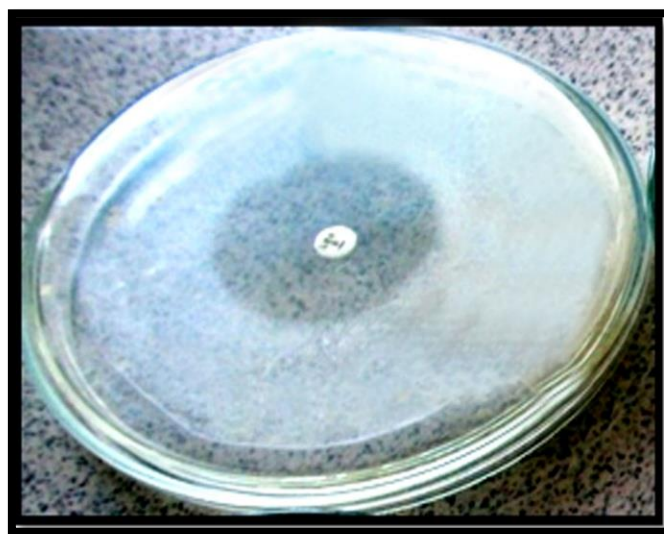


Fig 2. Use of standard drug (ciprofloxacin) against *K. pneumoniae*

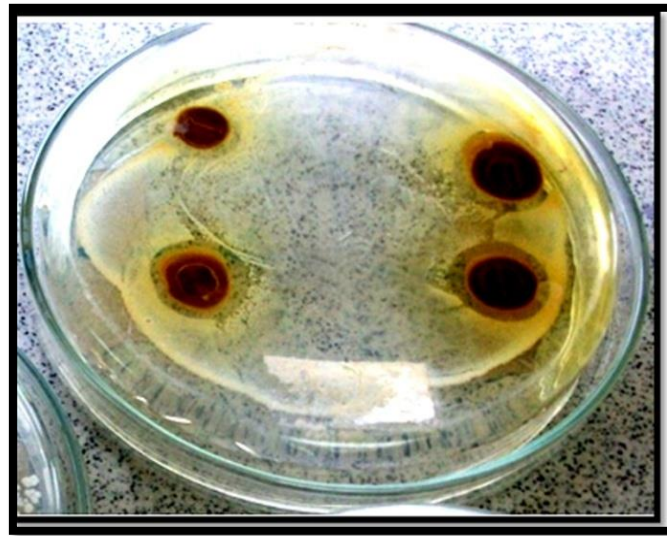
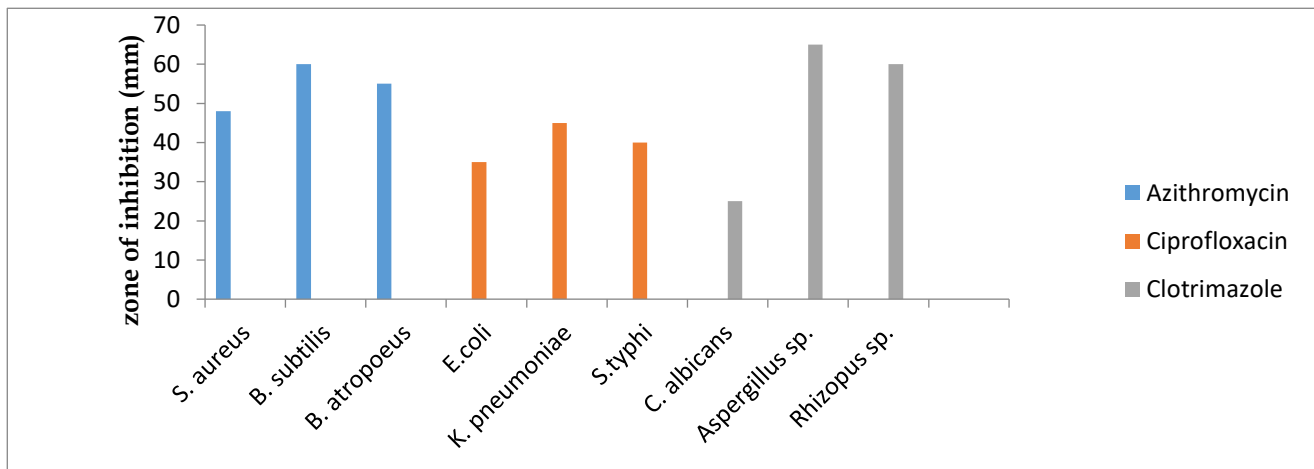
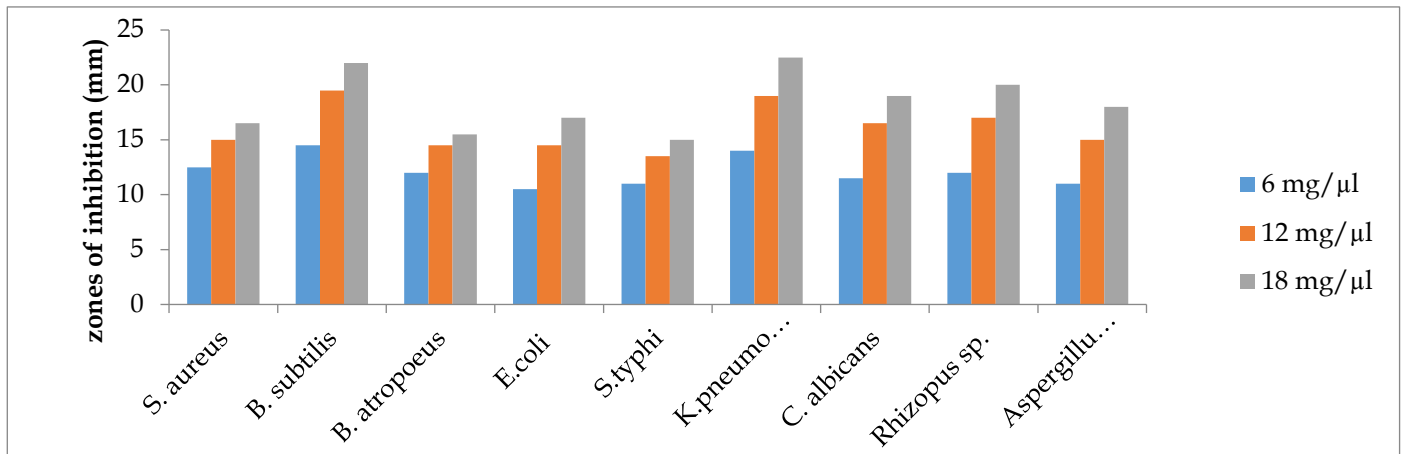


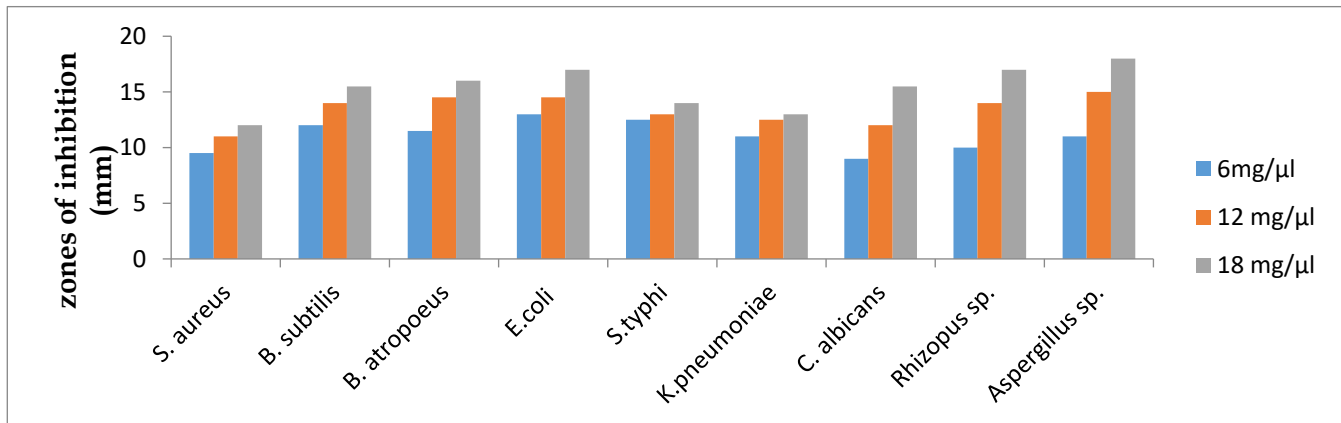
Fig 3. Ethanolic extract of *Cichorium intybus* against *K. pneumonia*



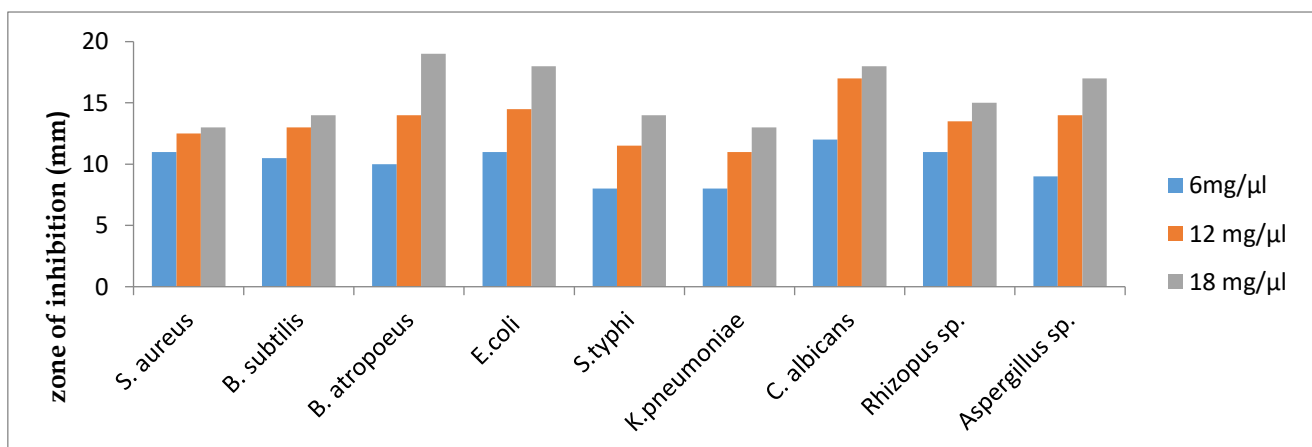
Graph 1. Antimicrobial activities of standard antibiotics against different bacteria and fungi



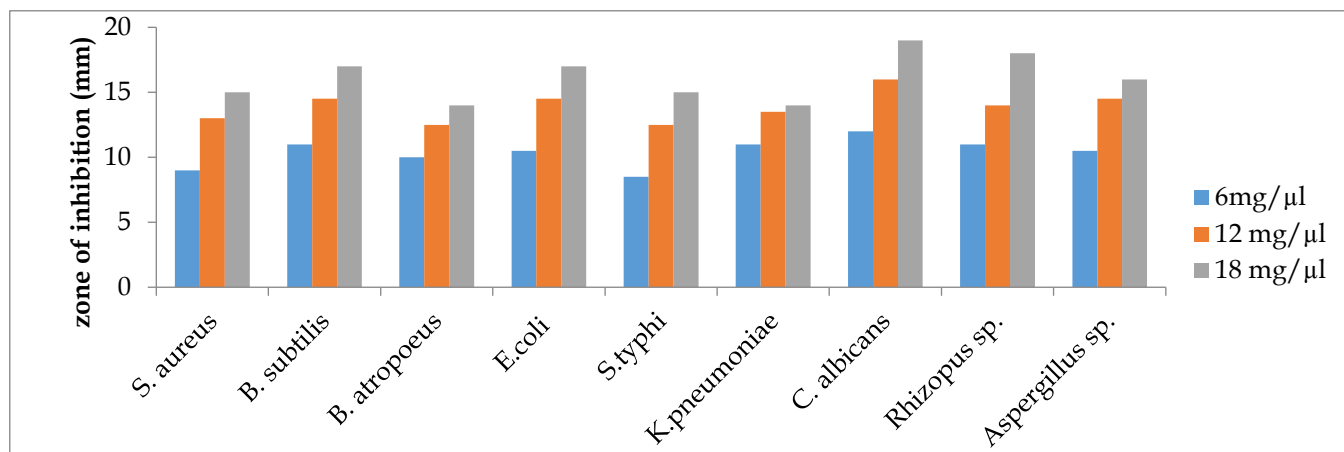
Graph 2. Antimicrobial activity of the ethanolic extract of *Cichorium intybus*



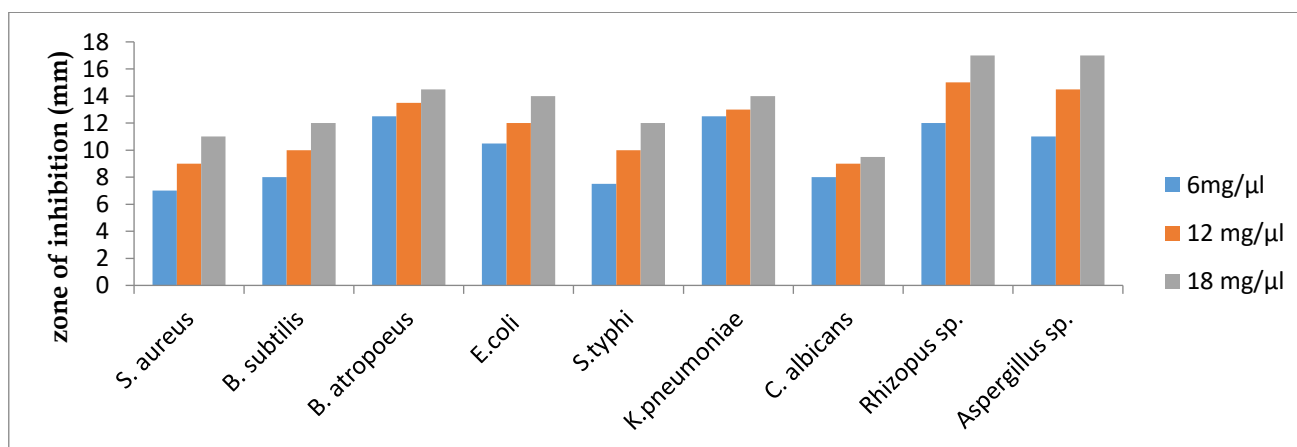
Graph 3. Antimicrobial activity of the methanolic extract of *Cichorium intybus*



Graph 4. Antimicrobial activity of the ethyl acetate extract of *Cichorium intybus*



Graph 5. Antimicrobial activity of the hexane extract of *Cichorium intybus*



Graph 6. Antimicrobial activity of the aqueous extract of *Cichorium intybus*

4. Conclusion

In this study, the antimicrobial and antifungal activities of *Cichorium intybus* were examined using the disc diffusion method. Based on the attained result we concluded. *Cichorium intybus* extract showed excellent antimicrobial potency. Efficiency order for plant extracts, *Cichorium intybus* solvent, ethanol > methanol > ethyl acetate > hexane > aqueous extract. The order of inhibition for different collections of organisms was fungi > Gram-negative bacteria > Gram-positive bacteria.

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Conflict of Interest:

The authors declare that they have no potential competing interests.

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