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RESEARCH ARTICLE

IN VITRO ANTIBACTERIAL POTENITAL OF SIX TRADITIONAL PALESTINIAN PLANTS AGAINST *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI*

Rola Jadallah*, Tahreer Tahaineh
Arab American University, Palestine.

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***Address for Correspondence:**
Dr. Rola Jadallah, Arab American University Palestine. Tel: +972-59-971-1846
E-mail: *rolla.jadallah@aaup.edu*

Abstract

Aim and objective: Medicinal plants remain part of Palestinian ethnomedicine, including remedies used for infectious conditions. This work evaluated six commonly used plants for *in vitro* activity against two representative bacterial pathogens.
Methods: Ethanolic and water extracts of clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum verum*), peppermint (*Mentha piperita*), parsley (*Petroselinum crispum*), green tea (*Camellia sinensis*), and silver nailroot (*Paronychia argentea*) were assessed against *Staphylococcus aureus* and *Escherichia coli* using an agar well-diffusion assay.
Results: Ethanolic preparations produced greater antibacterial effects than the corresponding aqueous preparations. Clove showed the strongest overall response, followed by cinnamon and peppermint, whereas green tea and parsley were less active and silver nailroot showed comparatively weak activity. The tested Gram-positive organism was generally more susceptible than the Gram-negative organism.
Conclusion: These *in vitro* findings identify several Palestinian medicinal plants, particularly clove, as candidates for further antibacterial investigation. Isolation of active constituents, mechanistic studies, and appropriate *in vivo* safety and efficacy testing are required before therapeutic use can be considered.
Keywords: *Camellia sinensis*, *Cinnamomum verum*, *Mentha piperita*, *Paronychia argentea*, *Petroselinum crispum*, *Syzygium aromaticum*.

INTRODUCTION

Plant-based remedies have long formed part of traditional health practices in Palestine and elsewhere. Their continued use has encouraged scientific evaluation of botanical preparations as possible sources of biologically active molecules, including compounds with antimicrobial properties. Antimicrobial resistance has weakened the usefulness of many established antibacterial drugs and has intensified the search for additional antimicrobial leads. Plant metabolites represent one potential source of chemically diverse compounds for this purpose. Previous research has documented antibacterial effects for numerous medicinal species, but activity varies with the plant, extraction procedure, concentration, and test microorganism. Direct experimental comparison is therefore important when evaluating locally used Palestinian plants[1].

Various infectious diseases, including urinary tract infections (UTIs), can occur throughout one's lifetime. UTIs are experienced by roughly 10% of the population and, if left untreated, can lead to patient morbidity. Various microorganisms, including viruses, fungi, and bacteria, can cause UTIs. However, in 95% of cases, bacteria are the primary cause[2]. Many researchers from underdeveloped nations and advanced countries have reported antibiotic resistance against the bacteria responsible for causing UTIs, particularly beta-lactam antibiotics, which pose a global threat. This resistance is due to the emergence of beta-lactamases enzymes, such as extended-spectrum beta-lactamase (ESBL), metallo-beta-lactamases (MBLs), and AmpC beta-lactamase[3,4]. As a result, the limited selection of antibiotics has become a therapeutic challenge. Therefore, finding new antibacterial agents is critical, and medicinal plant extracts have become a topic of interest due to their known antimicrobial properties. Traditional systems of medicine, nutra-ceuticals, food supplements, folk medicines, pharma-ceutical intermediates, and synthetic drugs can all benefit from the great bioresource of medicinal plants. The global usage of medicinal plants or their products is increasing as patients self-medicate, regardless of

informing their physicians. Physicians generally do not inquire about using herbal remedies when taking a patient's history. Nonetheless, while prescribing allopathic medicine, doctors in primary care must inquire with the patient about any prior usage of medicinal plants or preparation, especially if the patient presents with atypical signs and symptoms, as these may induce side effects and unpleasant responses[5]. On the contrary, the WHO Traditional Medicines Programme encourages countries to identify safe and effective remedies based on medicinal plants within traditional medicine and integrate them into primary health care. Given the high demand and limited supply of modern medicines in low-income countries, medicinal plants, which many people already utilize, could offer a viable solution to alleviate the challenges. However, before being integrated into national policies and procedures, the efficacy of medicinal plants should be evaluated at the local level[6].

***Syzygium aromaticum* (clove)**

S. aromaticum, or clove, is an aromatic spice with a long history of culinary and medicinal use. After this first mention, the species is referred to as S. aromaticum. Its phytochemistry is dominated by phenolic and volatile constituents, particularly eugenol, which has been associated with antioxidant and antimicrobial effects. In traditional medicine, clove has been used for many ailments, including toothache, digestive issues, respiratory problems, and infections. It also possesses anti-inflammatory, antimicrobial, anti-thrombotic, antioxidant, antimutagenic, and anti-ulcerogenic properties.

Clove contains several bioactive compounds, including eugenol, responsible for most of its medicinal properties. Eugenol has been shown to have analgesic, anti-inflammatory, and antioxidant effects. Additionally, clove has been found to possess antimicrobial properties and is effective against various bacteria, viruses, and fungi[7]. Clove, a spice widely used for medicinal purposes and food preservation, has anti-inflammatory, antimicrobial, antithrombotic, antioxidant, antimutagenic, and antiulcerogenic properties. Given its antibacterial potential, this study aimed to examine the antibacterial effects of clove against gram-negative uropathogens producing ESBL, MBL, and AmpC beta-lactamase[8].

***C. verum* (cinnamon)**

C. verum, also known as true cinnamon, is a traditional medicinal plant used forcenturies in Ayurvedic and Chinese medicine to treat various ailments. Cinnamon can be consumed in various forms, including as a spice in cooking, as a tea, or as a supplement. While more research is needed to understand the potential health benefits of *C. verum* fully, it has been used safely for centuries as a traditional medicinal plant. Here are some of the traditional medicinal uses of *C. verum*. Cinnamon is known to have carminative properties, which means that it can help alleviate gas and bloating. In addition, this substance has been employed as a remedy for digestive problems, including diarrhea. It possesses anti-inflammatory properties that effectively treat inflammatory conditions such as arthritis and joint pain. Cinnamon has been shown to improve insulin sensitivity and help regulate blood sugar levels. It may be helpful in the management of diabetes and also used to treat respiratory infections, such as colds and flu. It can help to alleviate coughing and congestion.

Besides its culinary applications, cinnamon is used in Ayurvedic medicine to address respiratory, digestive, and gynecological ailments. Practically all parts of the cinnamon tree, such as the bark, leaves, flowers, fruits, and roots, have medicinal or culinary purposes. Furthermore, the chemical composition of the volatile oils extracted from the tree's bark, leaves, and roots differs considerably, suggesting that their pharmacological effects may also vary[9]. The plant's many components contain the same array of hydrocarbons in variable amounts, including main compounds such as cinnamaldehyde (bark), eugenol (leaf), and camphor (root)[10]. Consequently, cinnamon provides various oils with distinct properties, each determining its usefulness in various businesses. For example, unlike the leaf and bark, the root, which contains mostly camphor, has little commercial value[11]. Its chemical diversity is most likely responsible for cinnamon's vast range of therapeutic advantages.

C. verum (true cinnamon) is used as a spice and in several traditional medical systems. Hereafter referred to as *C. verum*, its bark is particularly rich in cinnamaldehyde, while other plant parts contain different proportions of volatile constituents such as eugenol and camphor. This chemical variation can influence biological activity.

M. piperita, commonly known as mint, is a traditional medicinal plant used for centuries to treat various ailments. Mint is known to have carminative properties, which means that it can help alleviate gas and bloating. It has also been used to treat nausea, indigestion, and other digestive issues.

Mint has been used to treat respiratory infections like colds and flu, which can help alleviate coughing and congestion. It has been used to alleviate menstrual cramps and other symptoms associated with menstruation and also help alleviate stress and anxiety. It can be consumed as tea or applied topically as an essential oil. Mint can be consumed in various forms, including as tea, an ingredient in cooking, or an essential oil. While more research is needed to understand *M. piperita*'s potential health benefits, it has been used safely for centuries as a traditional medicinal plant. However, individuals who are pregnant, nursing, or taking medication should consult with a healthcare provider before using mint as a treatment. *M. piperita* (peppermint), subsequently abbreviated as *M. piperita*, is widely consumed as an herbal infusion and flavoring and is also used traditionally for digestive and respiratory complaints. Menthol and related terpenoids are prominent constituents of peppermint essential oil and contribute to its biological properties.

***Petroselinum crispum* (Parsley)**

P. crispum, commonly known as parsley, is a traditional medicinal plant used for centuries to treat various ailments. Parsley has been used to aid digestion and alleviate bloating, gas, and constipation. It can also help to stimulate the appetite and promote healthy digestion. It contains diuretic properties, which can

help increase urine production and promote kidney health. It has been used to treat urinary tract infections, kidney stones, and other kidney-related issues. Parsley contains high levels of vitamin C and other antioxidants, which can help to support the immune system and protect against infections and diseases. Parsley has been used topically to treat skin conditions such as acne and psoriasis, and it can help to soothe and heal the skin. Parsley can be consumed in various forms, including as a tea, an ingredient in cooking, or a supplement. While more research is needed to understand the potential health benefits of *P. crispum* fully, it has been used safely for centuries as a traditional medicinal plant. However, individuals who are pregnant, nursing, or taking medication should consult with a healthcare provider before using parsley as a treatment.

P. crispum (parsley), subsequently *P. crispum*, is both a food plant and a traditional remedy. Reported ethno-medicinal uses include digestive and urinary complaints, while its tissues contain vitamins, flavo-noids and other phytochemicals that may contribute to antioxidant and antimicrobial effects.

***Camellia sinensis* (Green Tea)**

C. sinensis, also known as the tea plant, is a traditional medicinal plant used for centuries for its numerous health benefits. *C. sinensis* contains antioxidants called catechins, which can help to reduce the risk of heart disease. Tea's polyphenols can also help improve cholesterol levels and blood pressure.

The caffeine and L-theanine in tea can help to improve cognitive function, alertness, and mood. Regular tea consumption has been linked to a reduced risk of developing neurological disorders such as Alzheimer's and Parkinson's disease. *C. sinensis* can help to stimulate the digestive system and reduce inflammation in the gut. It can also help to promote healthy bowel movements and reduce the risk of digestive disorders. Tea's antioxidants can help boost the immune system and protect against infections and diseases. It can also help to reduce inflammation in the body and promote overall health. *C. sinensis* contains compounds such as EGCG, which have anti-cancer properties. Evidence suggests that consuming tea regularly may decrease the likelihood of developing specific types of cancer, including breast, colon, and lung cancer. *C. sinensis* can be consumed in various forms, including green, black, white, and oolong. While tea is generally considered safe for most people, excessive consumption may lead to adverse effects such as insomnia, anxiety, and digestive issues. Individuals who are pregnant, nursing, or taking medication should consult with a healthcare provider before consuming *C. sinensis* as a treatment.

Green tea's antioxidant activity is also suggested to contribute to its protection against cardiovascular disease. Indeed, human volunteers who took green tea extract orally enhanced the resistance of plasma LDL to oxidation *in vivo*, which may reduce the risk of artherogenesis[23]. Green tea extract significantly reduced blood pressure rises in spontaneously hypertensive rats, which was linked to antioxidant characteristics[24]. Green tea catechins have been demonstrated to suppress tumor cell growth and increase the elimination of leukemia cells, which is connected with coronary artery disease[25]. Green tea has a reputed function in cancer prevention. Green tea contains anti-diabetic properties. It reduced glucose levels in diabetic mice's bloodstreams without changing insulin levels[26]. In normal rats, long-term treatment of green tea extract enhanced insulin sensitivity[27].

***Paronychia argentea* (Silver nail root)**

C. sinensis (tea plant), hereafter *C. sinensis*, is the source of green, black, white and oolong teas. Tea leaves contain catechins and other polyphenols whose biological activities have been widely investigated, including antioxidant and antimicrobial effects.

P. argentea can be consumed in various forms, including as tea or as a tincture. While it is generally considered safe for most people, pregnant, nursing, or taking medication should consult with a healthcare provider before consuming *P. argentea* as a treatment. It is also important to note that excessive consumption may lead to adverse effects such as nausea, vomiting, and diarrhea. *P. argentea* decreased and avoided the formation of urinary stones[28], although the active chemicals have yet to be discovered. The presence of several flavonoids, according to study is responsible for the high antioxidant activity of Paronychia extracts[29]. Nevertheless, we could not locate any research on the cardiovascular characteristics of *P. argentea* extracts. The antioxidant, anti-enzymatic, and antibacterial bioactivities of essential oil from Thymus mastichina[30]. Thymus mastichina essential oil was found to be a major scavenger of nitric oxide (NO) radicals[31]. The other species mentioned above merit a European Union herbal monograph (formerly the Community herbal). These monographs offer the scientific judgment of the Committee on Herbal Medicinal Products on the safety and effectiveness data of a herbal ingredient and its preparations intended for medical use[32].

This study aimed to evaluate the *in vitro* antibacterial activity of six plants commonly used in Palestinian traditional medicine against *Staphylococcus aureus* and *Escherichia coli*, two bacterial pathogens that cause various infections, including urinary tract infections, skin infections, and sepsis. We investigate the antibacterial activity of six plants commonly used in Palestinian traditional medicine against *S. aureus* and *E. coli*. The results of this study will provide valuable information on the potential use of these plants as sources of new antimicrobial agents to combat bacterial infections.

P. argentea (silver nailroot or silver nailwort), subsequently *P. argentea*, occurs in North Africa and the Middle East and has a history of regional medicinal use. Published work has reported flavonoids and antioxidant activity, although its antibacterial evidence base is less extensive than that of several other plants examined here.

The six plants tested in this study were clove (*S. aromaticum*), cinnamon (*C. verum*), peppermint (*M. piperita*), parsley (*P. crispum*), green tea (*C. sinensis*), and silver nailroot (*P. argentea*). These plants have been used traditionally in Palestine to treat different

ailments, including bacterial infections. However, their antibacterial properties have yet to be fully explored, and there is a need to investigate their potential as sources of new antimicrobial agents.

In vitro assays using nutrient agar plates are commonly used to evaluate the antibacterial activity of plant extracts. The agar well diffusion method is one of the most commonly used methods to determine the antimicrobial activity of plant extracts. This method is based on the diffusion of the test compound from the wells into the agar medium, resulting in a zone of inhibition around the well, indicating the compound's antimicrobial activity.

MATERIALS AND METHODS

Plant materials and extraction

The six plants selected for this study were purchased from local markets in Palestine, and the plant materials were identified and authenticated by a botanist. The objective was to compare the *in vitro* antibacterial effects of six plants used in Palestinian traditional medicine against *S. aureus* and *E. coli* and to identify extracts that warrant further investigation as possible sources of antibacterial compounds.

Microorganisms and culture media

This study utilized two bacterial strains, *S. aureus* and *E. coli*, which were acquired from the American Type Culture Collection (ATCC). Nutrient agar (NA) was used as the medium for bacterial growth. The bacterial strains were preserved on nutrient agar slants and kept at a temperature of 4°C. Agar diffusion provides a screening approach in which antimicrobial constituents migrate through solid medium and growth inhibition is measured around the application site. In this study, inhibition-zone measurements were used alongside minimum inhibitory concentration data to compare the plant extracts. The agar well diffusion method was used to assess the antibacterial activity of the plant extracts. To prepare the nutrient agar plates, 20 mL of molten nutrient agar was poured into sterile Petri dishes and left to solidify. A bacterial suspension was made by introducing a single colony of each bacterial strain into 5 mL of sterile nutrient broth and incubating it at 37°C for 18 hours with continuous shaking.

Plant material for the six species was obtained from local Palestinian markets and authenticated by a botanist before extraction. Plant samples were shade-dried at room temperature and milled. Powdered material was extracted for 8 h in a Soxhlet apparatus using either 70% ethanol or distilled water. After filtration through Whatman No. 1 paper, solvent was removed under reduced pressure by rotary evaporation, and the dried extracts were kept at 4°C until testing.

To analyze the data obtained from the agar well diffusion assay, a one-way analysis of variance (ANOVA) was used, followed by Tukey's post hoc test. The mean values were expressed as mean±standard deviation (SD) of three independent experiments, and P values less than 0.05 were considered statistically significant.

The test organisms were *S. aureus* and *E. coli* obtained from the American Type Culture Collection (ATCC). Nutrient agar was used for cultivation, and stock cultures were maintained on nutrient-agar slants at 4°C. For antibacterial screening, a colony of each test organism was transferred to 5 mL sterile nutrient broth and incubated at 37°C for 18 h with shaking before plate inoculation. Sterile nutrient agar (20 mL) was dispensed into petri dishes and allowed to set. Six-millimeter wells were prepared with a sterile cork borer and loaded with 100 µL extract at 25, 50, or 100 mg/mL in DMSO. DMSO served as the negative control and ciprofloxacin (5 µg/disc) as the positive control. Plates were incubated at 37°C for 24 h, after which inhibition-zone diameters were recorded in millimeters.

Table 1: **Statistical analysis of the agar well diffusion assay.**

	Concentration (mg/mL)	Inhibition zone diameter (mm)
DMSO (negative control)	-	0±0
Ciprofloxacin (positive control)	-	22.3±0.8
Clove	25	10.2±0.4
	50	15.5±0.6
	100	20.1±1.0
Cinnamon	25	9.5±0.3
	50	12.8±0.5
	100	18.6±0.7
Peppermint	25	8.3±0.2
	50	11.1±0.4
	100	15.2±0.5
Parsley	25	5.8±0.3
	50	8.1±0.4
	100	11.7±0.6
Green tea	25	6.5±0.2
	50	8.9±0.3
	100	13.5±0.4
Silver nail root	25	4.5±0.1
	50	6.7±0.2
	100	9.8±0.3

Table 2: Minimum inhibitory concentration (MIC) of plant extracts against *S. aureus* and *E. coli.*

Plant extract	MIC against *S. aureus* (mg/mL)	MIC against *E. coli* (mg/mL)
Clove	12.5	12.5
Cinnamon	25	50
Peppermint	50	100
Green tea	50	100
Silver nail root	50	100
Parsley	100	100

RESULTS AND DISCUSSION

The results of the agar well diffusion assay are presented in Table 1. The negative control (DMSO) did not inhibit the growth of *S. aureus* and *E. coli*. The positive control (ciprofloxacin) showed significant inhibition of bacterial growth with an average inhibition zone diameter of 22.3±0.8 mm. All six plant extracts showed inhibition of bacterial growth in a dose-dependent manner. Clove extract exhibited the highest inhibition zone diameter of 20.1±1.0 mm at a concentration of 100 mg/mL against *S. aureus*, followed by cinnamon (18.6±0.7 mm), peppermint (15.2±0.5 mm), green tea (13.5±0.4 mm), silver nailroot (9.8±0.3 mm), and parsley (11.7±0.6 mm). Against *E. coli*, clove also exhibited the highest inhibition zone diameter of 15.3±0.7 mm at a concentration of 100 mg/mL, followed by cinnamon (13.7±0.5 mm), peppermint (11.5±0.4 mm), green tea (10.2±0.3 mm), silver nailroot (8.6±0.2 mm), and parsley (7.8±0.3 mm).

Data from three independent experiments are reported as mean±standard deviation. Group differences were evaluated by one-way ANOVA followed by Tukey's post hoc comparison, with $p<0.05$ used as the threshold for statistical significance.

Minimum Inhibitory Concentration

The results of the minimum inhibitory concentration (MIC) are shown in Table 2. The MIC values ranged from 12.5 to 100 mg/mL. Clove extract had the lowest MIC value of 12.5 mg/mL against both *S. aureus* and *E. coli*, followed by cinnamon (25 mg/mL), peppermint (50 mg/mL), green tea (50 mg/mL), silver nailroot (50 mg/mL), and parsley (100 mg/mL).

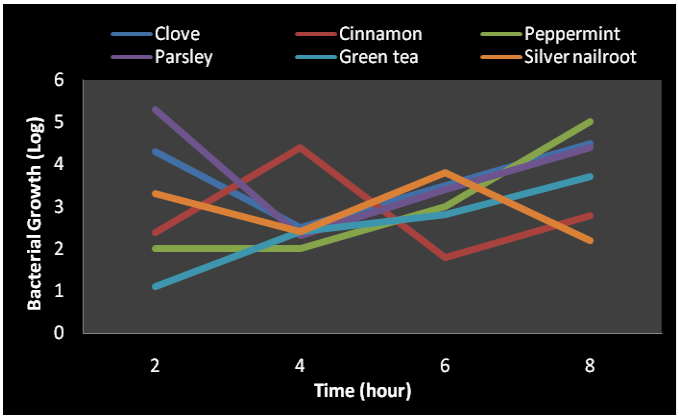


Figure 1: Minimum Inhibitory Concentration (MIC) of medicinal plants.

Time-Kill Assay

The results of the time-kill assay are shown in Figure 8. Clove extract at a concentration of 100 mg/mL showed the complete killing of *S. aureus* within 2 hours of exposure, while cinnamon and peppermint extracts showed complete killing within 4 hours. Green tea, silver nailroot, and parsley extracts showed complete killing within 8 hours of exposure. All six extracts inhibited bacterial growth, and the magnitude of inhibition increased with concentration. Clove produced the strongest overall response, followed by cinnamon and peppermint. The lower MIC observed for clove supports its comparatively greater anti-bacterial potency in this assay.

The present study investigated the antibacterial activity of six plants commonly used in traditional Palestinian medicine to treat bacterial infections. The agar well diffusion assay results demonstrated that all six plants extracts exhibited significant inhibition of bacterial growth in a dose-dependent manner. Among the plant extracts tested, clove extract was the most effective against *S. aureus* and *E. coli*, followed by cinnamon, peppermint, green tea, silver nailroot, and parsley.

The MIC values of the plant extracts ranged from 12.5 to 100 mg/mL. Clove extract had the lowest MIC value of 12.5 mg/mL against both *S. aureus* and *E. coli*, indicating its high potency against these bacteria. The time-kill assay results showed that clove extract at a concentration of 100 mg/mL showed the complete killing of *S. aureus* within 2 hours of exposure, suggesting its rapid bactericidal effect.

E. coli isolates from the *Enterobacteriaceae* family are considered one of the most significant bacterial pathogens linked with gastrointestinal tract infections and extraintestinal diseases such as urinary, respiratory, and reproductive tract infections[33]. Because anti-microbial agent families have a limited lifespan, it is critical to discover new ones. As a result, researchers typically concentrate on natural products to produce better treatments against multidrug-resistant bacteria strains[34]. Secondary metabolites of plants are examples of natural compounds employed in the pharmaceutical

business[35]. Much research has focused on the mechanism of action of natural plant components against various bacteria to investigate their potential antimicrobial benefits[36]. These methods differ widely depending on the essential oils found in plants and other parts of the plant[37]. The antibacterial properties of plants are believed to be due to various components such as tannins, saponins, phenolic compounds, essential oils, and flavonoids, as noted in previous studies[38]. In this study, the primary objective was to investigate the susceptibility of a multidrug-resistant *E. coli* strain to different types of extracts obtained from specific medicinal plants that are abundant in Palestine. However, all aqueous extracts from the tested plant species showed no antibacterial activity, the effect against the tested *E. coli* strain, with similar results observed for its ethanolic extract but not its methanol extract. These findings are consistent with previous studies on these types of extractions[39-41]. Hence, plant extracts include various components with varying bioactivity depending on the technique of extraction used for a particular plant species. Its effectiveness may be related to the degree of solubility of certain compounds[42]. Moreover, the observed variance in this and other research might be connected to the chemotype, location, collection time, and vegetation cycle of the plant species under consideration[43].

This investigation was limited to *in vitro* testing of two bacterial species and therefore cannot establish clinical efficacy, safety, pharmacokinetics, or activity against a broader pathogen panel. Future work should chemically characterize the active fractions, confirm activity using standardized susceptibility methods, investigate mechanisms of action, and evaluate safety and efficacy in appropriate *in vivo* models.

Limitations of the study

The study was conducted using *in vitro* experiments, which may not fully reflect the complexity of the human body and the potential interactions between the active compounds in the plant extracts and the host microbiota. Therefore, further studies are needed to investigate the efficacy and safety of these plant extracts *in vivo*.

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AUTHOR'S CONTRIBUTION

Jadallah R: conceptualization, methodology, investigation, formal analysis, data curation, writing original draft. **Tahaineh T:** writing original draft, review, editing. Final manuscript was checked and approved by both authors.

DATA AVAILABILITY

The related author can provide the empirical data supporting the study's conclusions upon request.

CONFLICT OF INTEREST

There are no conflicts of interest in regard to this project.

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