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Development and Evaluation of *Nigella sativa L*. Topical Cosmeceutical Formulations with Antiacne Activity

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ABSTRACT
The inflammation of the skin's sebaceous follicles is the cause of acne vulgaris. Certain bacterial species, such as *Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, are responsible for its initiation. Novel therapeutic treatments for acne vulgaris must be introduced due to the development of antibiotic resistance by these microorganisms and side effects from the present treatment regimens. In order to create new gel formulations from Nigella sativa L. seeds and assess their antibacterial efficacy against certain bacterial species that cause acne, this study was conducted. Using the agar well diffusion technique, the antibacterial activity of seed extracts was first tested against *S. aureus* and *P. acnes*. After that, topical gels were created using three distinct strengths of N. sativa seed ethyl acetate extract. The stability of these topical formulations was assessed over a 30-day period, and antibacterial activity experiments were conducted S. aureus and P. acnes growth was inhibited by all three formulations; however, the formulation containing 15% of the seed extract exhibited the most antibacterial action. Remarkably, this formulation's antibacterial efficacy against S. aureus outperformed that of the commercial synthetic product that served as the positive control. Furthermore, the antibacterial efficacy was maintained during the storage period, and no changes in color, odor, homogeneity, washability, consistency, or pH were seen. Topical gel formulations made from N. sativa ethyl acetate extract have strong antibacterial activity, indicating their potential as substitutes for current antiacne medications in the treatment of acne vulgaris.

INTRODUCTION

Regardless of gender, over 80% of teens and over 10% of adults suffer with acne vulgaris, a dermatological disorder. It is characterized by the development of seborrhea, inflammatory lesions, comedones, and nodules and arises from the persistent inflammation of a sebaceous follicle [1, 2]. Recent studies indicate that the bacterial species Propionibacterium acnes, Staphylococcus epidermidis, and Staphylococcus aureus may have a pathogenic role in acne vulgaris. Because P. acnes, an obligatory anaerobic microbe, may activate complement and convert sebaceous triglycerides into fatty acids, which subsequently chemotactically attract neutrophils, it is specifically responsible for the development of inflammatory acne. Conversely, superficial infections in the skin's sebaceous unit are often caused by aerobic Staphylococcus species [3]. Acne vulgaris is now treated with oral drugs such as retinoids and antibiotics of the tetracycline and macrolide families or topical use of benzoyl peroxide, retinoids, and antibiotics like erythromycin or clindamycin. However, combinational therapies are typically used for severe acne. Despite the fact that antibiotics can both target P. acnes and decrease the inflammation that is symptomatic of acne, the development of novel treatment drugs is necessary due to the acquisition of antibiotic resistance by P. acnes and other acne-causing bacterial species [4]. In this regard, natural substances that have been utilized in ancient medical systems, such as minerals, spices, and various plant parts, might be researched as powerful sources of new antiacne medicines.

In addition to their use as coloring agents and flavor enhancers, spices are valued for their various pharmacological qualities, including analgesic, antipyretic, anti-inflammatory, anti-microbial, and anticancer effects [5]. In particular, spices like cinnamon, saffron, mustard, and anise have been reported to have potential uses in pharmacology and cosmetics [6]. Because of its many uses in dermatology and other medical disorders, Nigella sativa L., sometimes known as black cumin, is a very significant spice [7]. For instance, N. sativa seeds and oil have been shown to possess a variety of bioactivities, including antibacterial [8], immunomodulatory [9], antioxidant [10], anti-inflammatory [11], and anticancer activity [12]. These bioactivities were mostly ascribed to the presence of thymoquinone [13]. For ages, several civilizations have utilized the seeds of N. sativa to treat a variety of dermatological

problems, including burns, wounds, acne vulgaris, and other inflammatory skin illnesses [7]. A clinical investigation that found 20% N. sativa oil extract in lotion formulation was more effective and less toxic than 5% benzoyl peroxide lotion, the standard therapy for mild to moderate stage acne vulgaris, validated these traditional assertions [14]. Additionally, N. sativa is a common dermatological cure in Sri Lankan folklore medicine and a key ingredient in a number of topical preparations used in traditional medicine to treat acne vulgaris [15], indicating potential antiacne properties.

Thus, the goal of the current study was to create topical cosmeceutical formulations that included N. sativa and then assess the antibacterial effectiveness of such formulations against specific bacteria that cause acne. Therefore, we think that the current study would support the traditional use of N. sativa as an antiacne agent while offering important insights for the commercial development of substitute antiacne drugs.

METHODOLOGY

Plant Material: The seeds of *N. sativa* were purchased in Natural products store-Vijayawada. The dried seeds (20 g) were initially soaked in hexane, ethyl acetate, and methanol (300–400 ml) separately for 24 hours in a shaker, and thereafter, the solvents were evaporated using a rotary evaporator (HS-2005V-N, South Korea). These crude extracts were subjected to anti-bacterial activity studies against *S. aureus* in order to select the best solvent for large-scale extraction.

Preliminary Screening of Crude Extracts Prepared from Seeds of N. sativa for Antibacterial Activity against S. aureus: The agar well diffusion technique was employed to assess the antibacterial efficacy against S. aureus, adhering to the protocol established by Soyza et al. [16] with minor changes.

Mueller Hinton Agar (MHA) plates were injected with a saline slurry of bacteria derived from isolated colonies of one-day-old pure cultures of S. aureus received from the Department of Microbiology at Andhra University. The turbidity of the bacterial suspension was calibrated to the McFarland 0.5 standard. Subsequently, wells (6 mm in diameter and 5 mm in depth) were created in the growth plates at equal intervals using a sterilized cork borer. Each well was filled with 50 µl of the respective test solutions (20mg/mL of hexane, dichloromethane, and ethyl acetate extracts dissolved in 2% DMSO) individually. Subsequently, the plates were incubated at 37°C overnight, and the inhibition zone surrounding each well was quantified. Co-amoxiclav served as the positive control, whilst 2% DMSO functioned as the negative control. The zones above 6 mm were regarded as inhibitions indicative of substantial antibacterial activity. The studies were conducted in duplicate, and the width of the inhibitory zone was reported as mean ± SD. The optimal solvent for large-scale extraction was determined based on the diameter of the inhibition zone. The minimum inhibitory concentration (MIC) of the selected extract was assessed using the broth microdilution method in 96-well microtitre plates, as outlined by Napagoda [17], with minor modifications; resazurin was employed for the visual identification of the lowest concentration of the test agent that inhibits bacterial growth. The MIC values were validated by subculturing the contents of the aforementioned microtitre plate wells onto agar plates, which also provided an indication of the MBC (minimum bactericidal concentration) values. The test was performed in triplicate.

Large-Scale Extraction of N. sativa. Based on the outcomes of the initial antibacterial evaluation against S. aureus, ethyl acetate was chosen as the preferred solvent for the extensive extraction of N. sativa seeds. Five hundred grams of dried seed materials were extracted with 2.5 liters of ethyl acetate for 24 hours in a shaker, following which the solvents were evaporated using a rotary evaporator (HS-2005V-N, South Korea). This extract was utilized to formulate the topical gel preparations.

Qualitative Screening for the Phytochemical Constituents in the Ethyl Acetate Extract of Seeds of N. sativa. The subsequent conventional qualitative screening procedures [18] were conducted to identify several classes of phytochemicals in the ethyl acetate extract of N. sativa seeds. All tests were performed with an appropriate positive control.

Test for Alkaloids.
Plant extract was dissolved in hydrochloric acid, and then few drops of Mayer's reagent were added and observed for the color change. Creamish precipitate indicates the presence of alkaloids.

Test for Phenolic Compounds*:*
A ferric chloride solution (3-4 drops) was introduced to the plant extract and monitored for the emergence of a bluish-black hue, indicating the presence of phenolic chemicals.

Test for Flavonoids:
A minimal amount of the extract was subjected to heating with 10 ml of ethyl acetate in boiling water for a duration of 3 minutes. The combination underwent filtration, and the filtrate was subsequently agitated with 1 ml of a 1% dilute ammonia solution. The layers were let to segregate and monitored for color alteration. The yellow hue in the ammonia layer signifies the existence of flavonoids.

Test for Saponins. Distilled water (6 mL) was added to the extract (2 ml) and shaken vigorously. Formation of persistent foam indicates the presence of saponins.

Test for Terpenoids:
Plant extract was taken to a test tube and chloroform was added, and then the test tube was held at 45^{o}. Thereafter, concentrated sulfuric acid was added using a dropper along the wall of the test tube and observed for color change. A reddish-brown coloration of the interface is an indication for the presence of terpenoids.

Table 1: Qualitative Screening for the Phytochemical Constituents in the Ethyl Acetate Extract

Phytochemical	Test Used	Reagent	Positive Indication
Alkaloids	Mayer's test	Mayer's reagent	Cream or pale yellow precipitate
Phenolics	Ferric chloride test	Ferric chloride solution	Bluish-black or dark green color
Flavonoids	Ammonia test	Ammonia solution (1%)	Yellow coloration
Saponins	Foam test	Distilled water	Stable persistent foam
Terpenoids	Salkowski test	Chloroform + conc. H_2SO_4	Reddish-brown interface

Preparation of Topical Gel Formulation:

Three formulations were prepared by incorporating the ethyl acetate extract of seeds of ***N. sativa*** at different concentrations, as indicated in Table 2. These concentrations were selected based on the amount of *N. sativa* used in traditional herbal preparations. In order to ensure that the incorporation of above concentrations of ethyl acetate extract for the gel formulations has an advantage of inhibiting the bacterial growth, the antibacterial activity of the extract at these concentrations was evaluated by the agar well diffusion method against ***S. aureus*** and ***P. acnes*** (under anaerobic conditions, against erythromycin as the positive control).

The antiacne gel base was formulated by using carbopol 940, glycerin, phenoxyethanol, EDTA, rosewater, cetyl alcohol, fuller's earth, polyethylene glycol, triethanolamine. Carbopol 940 was dissolved in rosewater until it gets completely soaked. Glycerin, phenoxyethanol, EDTA, rosewater, cetyl alcohol, fuller's earth, polyethylene glycol, and triethanolamine were added to the carbopol mixture while stirring in a vortex. Thereafter, the seed extract was incorporated into this mixture at varying percentages.

Table 2: Ingredients of gel formulations F1, F2, and F3 (in grams per 100 g of formulation)

Ingredient	F1 (g)	F2 (g)	F3 (g)
Carbopol 940	1.10	1.10	1.10
Phenoxyethanol	1.00	1.00	1.00
Glycerin	3.00	3.00	3.00
Polyethylene glycol (PEG)	0.05	0.05	0.05
Triethanolamine	Quantity sufficient	Quantity sufficient	Quantity sufficient
Fuller's earth	0.10	0.10	0.10
Cetyl alcohol	0.01	0.01	0.01
Ethylenediaminetetraacetic acid (EDTA)	0.10	0.10	0.10
Rosewater	Quantity sufficient	Quantity sufficient	Quantity sufficient
N. sativa extract	5.00	10.00	15.00

Antibacterial Activity Studies for the Gel Formulations. All gel formulations were solubilized in methanol to assess their antibacterial activity against S. aureus and P. acnes. The agar well diffusion technique was utilized to assess the antibacterial efficacy of the gel formulations against S. aureus as per the outlined procedure. A synthetic commercial antiacne gel served as the positive control, while the gel base and methanol acted as the negative controls. Subsequently, the minimum inhibitory concentration (MIC) of these formulations was ascertained using the broth microdilution technique in 96-well microtiter plates, adhering to the prescribed methodology. The test was performed in triplicate.

The agar well diffusion test was utilized under anaerobic circumstances to assess antibacterial activity against P. acnes. Wells of 6 mm in diameter and 5 mm in depth were created using a sterilized cork borer in blood agar plates infected with clinical isolates of P. acnes. The wells were filled with the test formulations, and the agar plates were incubated at 37°C for 48 hours in an anaerobic jar, following which the zones of inhibition were determined. A commercial anti-acne gel served as the positive control, and the gel base and methanol functioned as the negative controls. The experiment was performed three times.

Table 3: Antibacterial Activity Studies for the Gel Formulations

Parameter	Description
Method	Agar well diffusion
Medium	Mueller–Hinton Agar
Well size	6 mm diameter
Bacterial standard	0.5 McFarland (~1×10^8 CFU/mL)
Incubation	37°C for 18–24 h
Positive control	Erythromycin / commercial anti-acne gel
Negative control	Gel base / solvent
Outcome measure	Zone of inhibition (mm)

RESULTS AND DISCUSSION

Preliminary Screening of Crude Extracts for Antibacterial Activity: Among the three crude extracts tested, the highest activity against *S. aureus* was observed in the ethyl acetate extract with a zone of inhibition of 12 ± 0.0 mm in diameter. The zones of inhibition of 7 ± 0.0 mm in diameter were detected for both hexane and methanol extracts. The positive control, co-amoxiclav, exhibited a zone of inhibition of 31 ± 0.0 mm while any zone of inhibition was not observed for the negative control, 2% DMSO. Therefore, further investigations were conducted using the ethyl acetate extract. In addition, the MIC value of 31.25 *μg*/mL reflected the high antibacterial potency of this ethyl acetate extract which is quite comparable with that of the positive control co-amoxiclav. The ethyl acetate extract was incorporated into the gel base at three different concentrations, and all three concentrations were capable of inhibiting the growth of *S. aureus* and *P. acne*, as visualized by distinct zones of inhibitions in agar plates.

Table 4: Minimum Inhibitory Concentration (MIC) of Ethyl Acetate Extract against Staphylococcus aureus

Test Sample	*MIC (μg/mL)*
Ethyl acetate extract of Nigella sativa	*31.25*
Co-amoxiclav (Standard drug)	*7.8*

Qualitative Screening for the Phytochemical Constituents in the Ethyl Acetate Extract Prepared from Seeds of *N. sativa*. The qualitative phytochemical analysis revealed the presence of alkaloids, phenolics, and flavonoids in the above extract.

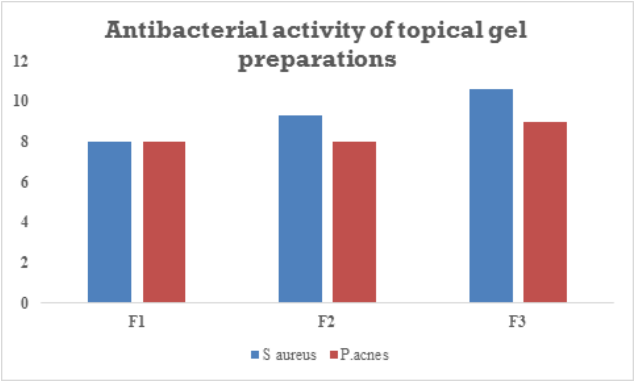

Fig 1: Antibacterial activity of topical gel preparations

Antibacterial Activity in the Gel Formulations.

As indicated in Table 5, the formulation F3 with 15% of the seed extract (Fig.1) displayed the highest activity against both

bacterial species. The negative controls, i.e., gel base and methanol, did not exhibit any inhibition while zones of inhibition with a diameter of 8.3±1.5 and 10.5±0.7 were observed for the synthetic commercial antiacne gel product (comprised of sulfur, isopropylmethylphenol, stearyl glycyrrhetinate, vitamin E, and vitamin B6 as active ingredients) against *S. aureus* and *P. acnes*, respectively.

Table 5. Antibacterial Activity of *Nigella sativa* Gel Formulations

Formulation	Extract Concentration	Zone of Inhibition (mm) *S. aureus*	Zone of Inhibition (mm) *P. acnes*
F1	5 % (w/w)	Moderate inhibition	Moderate inhibition
F2	10 % (w/w)	Higher than F1	Higher than F1
F3	15 % (w/w)	Highest inhibition	Highest inhibition
Commercial anti-acne gel	Standard product	8.3 ± 1.5	10.5 ± 0.7
Gel base	Negative control	No inhibition	No inhibition
Methanol	Solvent control	No inhibition	No inhibition

Table 6: Antibacterial activity of topical gel preparations

Microorganism	F1 (mm)	F2 (mm)	F3 (mm)	Positive	Negative
S. aureus	8.0 ± 0.0	9.3 ± 0.5	10.6 ± 0.5	control (mm)	control (mm)
P. acnes	8.0 ± 0.0	8.0 ± 0.0	9.0 ± 0.0	8.3 ± 1.5	0.0 ± 0.0

Table 7. Preliminary Antibacterial Screening of *Nigella sativa* Seed Extracts Against *Staphylococcus aureus*

Extract / Control	Solvent Used	Zone of Inhibition (mm)
Hexane extract	Non-polar solvent	7 ± 0.0
Methanol extract	Polar solvent	7 ± 0.0
Ethyl acetate extract	Moderately polar solvent	**12 ± 0.0**
Co-amoxiclav (Positive control)	Standard antibiotic	31 ± 0.0
2% DMSO (Negative control)	Solvent control	No inhibition

Table 8. Minimum Inhibitory Concentration (MIC) of Gel Formulations against *Staphylococcus aureus*

Sample	MIC (µg/mL)
F1 formulation	250
F2 formulation	62.5
F3 formulation	62.5
Commercial anti-acne gel	125

The MIC values against *S. aureus* were observed as 250, 62.5, and 62.5 μg/mL for F1, F2, and F3, respectively. Interestingly, the MIC value of the synthetic commercial antiacne gel was observed as 125 μg/mL, indicating a potent antibacterial activity in formulations F2 and F3 against *S. aureus* in comparison with this synthetic product. Although the same MIC values were observed for F2 and F3 against *S. aureus*, the diameter of the zone of inhibition was slightly higher in F3 in comparison with F2 as observed in the agar well diffusion assay.

Stability of the Physical Parameters and the Antibacterial Activity: All three gel formulations demonstrated a good stability, without any change in the status of the initial physical parameters over an experimental period of 30 days (Table 9,10). Interestingly, the findings proved that the antimicrobial property of these gel formulations against S. aureus has retained during the storage period as evidenced by the presence of zones of inhibition of 10.5 ± 0.7, 11.5 ± 0.7, and 12.0 ± 0.0 mm in diameter for formulations F1, F2, and F3, respectively.

Table 9. Stability Study of *Nigella sativa* Gel Formulations (After 30 Days)

Formulation	Zone of Inhibition (mm) Against *S. aureus* After 30 Days
F1	10.5 ± 0.7
F2	11.5 ± 0.7
F3	12.0 ± 0.0

Table 10. Physical Stability of Gel Formulations After 30 Days Storage

Parameter	F1	F2	F3
Color	No change	No change	No change
Odor	Pleasant	Pleasant	Pleasant
Homogeneity	Uniform	Uniform	Uniform
Consistency	Smooth	Smooth	Smooth
Washability	Good	Good	Good
Phase separation	None	None	None
pH	Within skin-compatible range	Within skin-compatible range	Within skin-compatible range

DISCUSSION

The microbial flora isolated from acne patients include *P. acnes*, *S. epidermidis*, *S. aureus*, *Klebsiella pneumonia*, and *Streptococcus* whose pathogenic mechanisms and the genes associated with virulence factors are believed to play a significant role in the development of acne [19]. Although antibiotics have been included in the therapeutic regimes of acne, the number of reports on antimicrobial resistance by acne-causing bacterial species is escalating over the recent years. For example, in Europe and in the United States, antimicrobial-resistant *P. acnes* strains have been isolated frequently while a study conducted in Japan revealed a relationship between the use of antimicrobial agents and the emergence of antimicrobial resistance against *P. acnes*. Furthermore, the analysis of correlation between the antimicrobial resistance of *P. acnes* and *S. epidermidis* had revealed that more than 80% of the patients who carried clindamycin-resistant *P. acnes* also carried clindamycin-resistant *S. epidermidis* [20]. Apart from that, the adverse effects associated with benzoyl peroxide, retinoids, isotretinoids, azelaic acid, and salicylic acid and other widely used antiacne agents could not be neglected [21]. This necessitates the development of novel therapeutic agents with high efficacy and low side effect profiles. In this approach, a number of plant extracts and phytochemicals thereof have been reported with antibacterial activity against acne-causing bacterial species. For example, the extracts of *Punica granatum*, *Morus alba*, and *Angelica anomala* have exhibited MIC in the range of 4–50 μg/mL against *P. acnes*, along with a potent antibacterial activity against *S. epidermidis*. Similarly, MICs of 0.005–0.6 μL/mL have been reported in essential oils of *Citrus obovoides*, *Citrus natsudaidai*, *Cryptomeria japonica*, and *Cymbopogon nardus*, while phytochemicals such as pulsa- quinone, hydropulsaquinone, rhodomyrtone, and rhinacan- thin-C were found to possess MIC in the range of 0.5–12.5 μg/ ml against *P. acnes* [22]. These observations suggest that medicinal plants could be potential sources of novel phar- maceuticals for treating

acne. Hence, the present investigation was undertaken to develop topical formulations which are effective against acne-causing bacterial species using seed ex- tracts of *N. sativa*, a plant that has been reputed in Sri Lankan folklore medicine as a remedy for acne, eruptions of the skin, and related skin diseases [15].

Although several publications exist about the antibacterial efficacy of crude extracts from N. sativa against acne-inducing bacterial species [23], the impact of topical gel formulations including N. sativa on these microorganisms remains undocumented. A recent work assessed the antibacterial efficacy of methanolic extract from N. sativa seeds against P. acnes; however, the gel formulation derived from this extract was not subjected to the pertinent antimicrobial evaluations [24]. Consequently, the current investigation offers novel insights into the potential antiacne effects of topical gel formulations including N. sativa extracts. Furthermore, it exhibits other advanced characteristics that were absent in the gel formulation described by Bhalani and Shah [24]. This incorporates cetyl alcohol, an emollient and moisturizer, to mitigate the natural irritant properties of N. sativa, along with fuller's earth for the absorption of excess sebum on the face skin. Our formulas are devoid of parabens, a prevalent chemical used in the cosmetics sector, which has often been debated over its carcinogenic potential. Additionally, phenoxyethanol was used as the preservative with EDTA to prevent rancidity, hence improving the product's cosmetic appeal and the gel's water washability. In these innovative compositions, rosewater was utilized as the carrier.

It was assumed that rosewater might aid in preserving pH levels while alleviating erythema, dermatitis, and eczema, due to its anti-inflammatory properties. Moreover, the pH values of the formulations developed in this study vary from 5 to 6, aligning with the ideal skin pH, which is recognized as 5.5. All three formulations generated in this investigation shown commendable stability for several physical characteristics while preserving their antibacterial efficacy, a characteristic not assessed in the prior work by Bhalani and Shah [24]. Among the three formulations, the antibacterial activity was prominent in the formulation containing 15% extract, exhibiting more efficacy against S. aureus compared to the commercial synthetic product used as the positive control. The comparison of MIC values of the gel formulations and the positive control against P. acnes could have enhanced the study's outcome; however, the constraints of the current laboratory facilities and the lack of validated and reliable experimental protocols have impeded the determination of MIC for P. acnes. This was the primary limitation of the current investigation. Nonetheless, the current study revealed the importance of these innovative gel formulations created using seed extracts of N. sativa as therapeutic and palliative solutions for acne vulgaris.

The present study successfully formulated and evaluated a polyherbal refreshing drink using medicinal plants known for their antioxidant and health-promoting properties. The prepared formulations satisfactory organoleptic characteristics, acceptable physicochemical parameters, and significant antioxidant activity. Phytochemical screening confirmed the presence of bioactive compounds responsible for the therapeutic potential of the beverage. Antioxidant studies revealed strong radical scavenging activity, while microbiological evaluation confirmed the safety of the formulations. Among the tested formulations, F2 was identified as the optimized formulation due to its balanced sensory properties, adequate antioxidant activity, and acceptable physicochemical characteristics. The developed herbal refreshing drink can serve as a natural functional beverage and a healthier alternative to synthetic soft drinks. Further studies involving shelf-life determination, large-scale production, and clinical evaluation are recommended to establish its commercial viability.

CONCLUSION

The topical gel formulations derived from N. sativa seeds were effectively developed and assessed for several parameters, including antibacterial activity. The findings demonstrate that the developed formulations exhibit significant antibacterial efficacy against acne-inducing bacteria, with the formulation containing 15% extract showing the most pronounced action. The in vitro bactericidal efficacy of this formulation exceeded that of the synthetic commercial anti-acne formulation, and the incidence of hypersensitive responses among patients using the improved formulation was negligible. This work demonstrated the potential for producing commercial products utilizing N. sativa for the treatment of acne vulgaris, while substantiating its application as an antiacne cure.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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