



## Debarring the Pathological Features Associated with *Curvularia lunata* and *Cladophialophora bantiana* using Cream Prepared from Essential Oil Extracted from *Monodora myristica*

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| Abstract  | Article History   |
|---|---|
| <p>The increasing reports of pathogenic infections caused by <i>Curvularia lunata</i> and <i>Cladophialophora bantiana</i>, particularly among farmers, pose a growing public health concern. Identifying a preventive solution that is natural, cost-effective, eco-friendly, and readily available is therefore of critical importance. This study evaluated the prophylactic potential of a topical cream formulated with essential oil extracted from <i>Monodora myristica</i> against infections caused by these fungi. Soil samples were randomly collected from various farmlands and analyzed using standard microbiological techniques to isolate <i>C. lunata</i> and <i>C. bantiana</i>. The pathological effects of these fungi and the preventive efficacy of the cream were assessed in albino Wistar rats, which were topically treated over a 15-day period. Results showed a significant (<math>P &lt; 0.05</math>) reduction in dermatological symptoms such as alopecia, scaling, erythema, macules, and ulceration. These effects became more pronounced at three-day intervals and were especially notable against <i>C. lunata</i>. The findings suggest that the <i>Monodora myristica</i> essential oil-based cream offers promising prophylactic potential against infections caused by <i>C. lunata</i> and <i>C. bantiana</i>, and may serve as an effective natural alternative in managing related dermatological conditions.</p> <p><b>Keywords:</b> <i>Curvularia lunata</i>, <i>Cladophialophora bantiana</i>, <i>Monodora myristica</i> essential oil, Topical antifungal cream, Dermatological prophylaxis</p> | <p>Received: 08 May 2025<br/>Accepted: 23 May 2025<br/>Published: 27 May 2025</p> <p>Scan QR code to view*</p>  <p>License: CC BY 4.0*</p>  <p>Open Access article.</p> |
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### Introduction

The increasing incidence of infections caused by *Curvularia lunata* and *Cladophialophora bantiana*, particularly among

individuals frequently exposed to soil such as farmers, has become a growing public health concern. These dematiaceous fungi are known to exhibit significant pathogenic potential in

humans, often leading to cutaneous and subcutaneous infections that can compromise the health and productivity of affected individuals. With the limitations of conventional antifungal treatments—such as cost, accessibility, side effects, and environmental impact—there is a pressing need to explore alternative, natural, and eco-friendly prophylactic options.

Plant-derived essential oils are complex mixtures which typically consist of a variety of low molecular weight compounds which can range in number up to 100 or more with a select few being the most abundant (Lopes *et al.*, 2016; Gupta *et al.*, 2017; Lopes *et al.*, 2017; Abdellatif *et al.*, 2021; Messaoudi *et al.*, 2021). The essential oils extracted from medicinal plants have been shown to exhibit antibacterial and antifungal activities both *in vitro* and *in vivo* (Gupta *et al.*, 2016). While most subcutaneous infections are not life-threatening and respond well to currently available topical treatment with over-the-counter (OTC) fungal agents, some subcutaneous infections can, however, be difficult to treat, require prolonged therapeutic regimens, and are increasingly resistant to conventional antifungal therapies. Previous estimates put the total cost for treatment of subcutaneous infections in the US at \$1.67 billion (Flores *et al.*, 2015). In addition to costs associated with treatment, many currently used antifungal agents have significant side effects thus underscoring the need for identification of therapeutic alternatives including those from natural products such as plant-based bioactive compounds, essential oils, and/or their components (Elaissi *et al.*, 2012).

The composition of essential oils can vary due to several factors including the extraction method used, the type and species of plant from which they are derived, the composition of the soil, and the exact stage of growth at the time of harvest. For this reason, careful chemical analyses must be performed using methods such as gas chromatography-mass spectrometry (GC-MS) to verify and standardize the composition of essential oils to ensure batch-to-batch consistency over time (Dias de Castro *et al.*, 2015).

Previous investigators have evaluated the use of essentials against dermatophytes focusing largely on melaleuca, thyme, eucalyptus, oregano, and lavender. Additional studies have been conducted that investigated the antifungal effects of specific components of these and other essentials including mono-, di-, and sesquiterpenes, phenolic terpenes, phenylpropanoids, hydrocarbons, and other cyclic compounds. Some investigations have led to the conclusion that the anti-dermatophytic activity resulted from the synergy between major and minor components rather than the result of the presence of a single compound (Dai *et al.*, 2016).

Owing to its demonstrated antimicrobial and antioxidant properties, nutmeg essential oil is considered to be a promising biopreservative. Recently, the interest in essential oils (EOs) and their application in food preservation has been amplified because (1) it is desired to reduce the potential health risks and increasing negative consumer perception of synthetic preservatives; and (2) foodborne diseases are a growing public health problem worldwide, calling for more effective preservation strategies (Hyldgaard *et al.*, 2012; Djenane *et al.*, 2013).

Several researchers have studied the effects of essential oil extracted from different plant materials against different fungi isolated from different ecological sites. Studies but little or no published study has been conducted on the effect of organic cream or any form of cream prepared from essential extracted from

*Monodora myristica* (African Nutmeg) on filamentous fungi, mostly the dematiaceous fungi. This shows that there is a paucity of information on this aspect of mycological study. In this study, we investigate the prophylactic efficacy of a topical cream formulated from the essential oil of *Monodora myristica*, a plant known for its medicinal attributes, against infections caused by *Curvularia lunata* and *Cladophialophora bantiana*. Soil samples from various farmlands were analyzed to isolate the fungal strains, and the pathological impact as well as the protective potential of the formulated cream were assessed through topical application in albino Wistar rats over a 15-day period.

## Materials and Methods

### Isolation and Characterization of Test Isolate from Soil Sample

**Sample Collection:** This was carried out using the method described in the study published by Iheukwumere *et al.* (2021). The litter from the soil surfaces was carefully scrapped out using a sterile stainless spoon. The soil auger was derived to a plough depth of 15 cm in the farmland, and soil sample was drawn up to 10 samples from each sampling unit into a sterile tray. The samples were thoroughly mixed and foreign materials such as roots, stones, pebbles and gravels were carefully removed. The soil sample was then reduced to half by quartering the sample. Quartering was carried out by dividing the soil sample into four equal parts and the two opposite quarters were discarded and the remaining two quarters were mixed. The process was repeated for the rest of soil samples used for this study. The samples were carefully labeled and then kept in a disinfected cooler, to maintain the temperature and stability of the number of the isolates. The samples were transported to the laboratory for analysis.

**Isolation of the Fungal Isolate:** This was carried out using the method described in the study published by Iheukwumere *et al.* (2021). One gram of the soil sample was weighed into a 50 mL beaker (Pyrex) using analytical weighing balance (JJJ430BC), little normal saline (0.85% NaCl) was added; this was shake thoroughly and made up to 10 mL using the normal saline. The sample was aseptically plated on Sabouraud Dextrose Agar (SDA) prepared according to the manufacturer's direction, and supplemented with chloramphenicol antibiotics (0.05%). This was incubated at room temperature (30±2°C) for 7-10 days. The fungal isolates that were darkened on the reverse side were aseptically sub cultured on SDA containing chloramphenicol antibiotics (0.05%) and incubated at room temperature (30±2°C) for 7-10 days.

**Identification of Fungal Isolate:** The fungal isolate was identified to the genus/species level based on macroscopic and microscopic characteristics of the isolate obtained from pure cultures (Iheukwumere *et al.*, 2020).

**Macroscopy:** The colony was carefully examined for fungal characteristics. The rate of growth, color, shape, texture, consistency of the growth and other peculiar features of the colony were observed according to the method described in the work published by Iheukwumere *et al.* (2020).

**Slide culture technique:** This was carried out using the method described in the study published by Iheukwumere *et al.* (2020). A filter paper was cut and placed on the bottom of Petri-dish. Two slides were crossed over each other on top of the filter paper and the filter paper was moistened. The set-up was sterilized by

autoclaving at 121°C for 15 minutes. Approximately one centimeter square agar block was cut from already prepared Potato Dextrose Agar (PDA) and placed on the intersection of the two slides. The four edges of the agar block were inoculated with the test organisms. It was then covered with sterile cover slip and incubated at room temperature for 5 days. After 5 days of growth, the cover slip was removed and inverted over a slide containing a drop of lactophenol cotton blue (LCB). The agar block was removed and discarded. A drop of LCB was also placed on top of the adherent colony on the slide and covered with sterile cover slip. The edges of the cover slip were sealed with nail polish to prevent evaporation of the stain. The slides were examined under the microscope using x10 and x40 objective lenses.

### Prophylactic Potential of the Prepared Cream against Superficial Infection Caused by Test Isolate

**Preparations of Plant Materials:** The fresh fruits of *Monodora myristica* (African Nutmeg) were collected from Onitsha, Anambra State, Nigeria. The plant material was authenticated appropriately Dr B. Garuba, in Botany Department, Michael Okpara Federal University of Agriculture, Umudike. The plant material was washed and dried under shade at room temperature for 14 days. The dried plant material was ground to powder form using a sterile electric grinder (Iheukwumere *et al.*, 2016; Iheukwumere *et al.*, 2020; Iheukwumere *et al.*, 2025a; Iheukwumere *et al.*, 2025b).

**Extraction of the essential oil:** A 2000 mL Soxhlet extractor that has three main sections: a percolator (boiler and reflux) which circulates the solvent, a thimble (usually made of thick filter paper) which retains the solid to be extracted, and a siphon mechanism, which periodically empties the thimble was used for the process. Twenty grams (100 g) of the plant material to be extracted was placed inside the thimble. The thimble was then loaded into the main chamber of the Soxhlet extractor. Then 1000 mL of n-hexane was placed in a 1000 mL distillation flask. The flask was placed on the heating mantle (2000 mL, 220 V, 500 W). The Soxhlet extractor was placed at the top of the flask. A reflux condenser was placed at the top of the extractor. When the n-hexane was heated to reflux, the solvent vapour travelled up a distillation arm, and flooded into the chamber housing the thimble of solid. The condenser ensured that any solvent vapour cooled, and dripped back down into the chamber housing the solid material. The chamber containing the solid material slowly filled with a warm solvent. When the Soxhlet chamber was almost full, the chamber was emptied by the siphon. The solvent then returned to the distillation flask. The thimble ensured that the rapid motion of the solvent did not transport any solid material to the still pot. This cycle was allowed to repeat many times for 12 h. After extraction, the solvent is removed, typically by means of a rotary evaporator to collect the essential oil.

**Preparation of organic cream:** The shea butter (one full 500 mL beaker) and Almond oil (50 mL) were mixed in a small stainless pot (2 litres capacity). They were stirred thoroughly and heated using cooking gas until all the ingredients were completely melted. This was followed by the addition of essential oil (100 mL) into the mixture and these were stirred and mixed properly. Then the mixture was enriched with vitamins (A, D and E), colourant and fragrance. The cream was then dispensed (20 mL) into a container and allowed to solidify (Iheukwumere *et al.*, 2021).

**Test Isolate:** The test isolate was prepared using 0.5 MacFarland matching standard prepared by mixing 0.05 mL of 1 % BaCl<sub>2</sub>.2H<sub>2</sub>O and 9.95 mL of 1 % conc. H<sub>2</sub>SO<sub>4</sub>. The test isolate was prepared using normal saline (0.85 % NaCl). The culture plates were flooded with the normal saline and these were thoroughly macerated and filtered using Whatman no 1 filter paper. The filtrate was further diluted to match with turbidity of the prepared 0.5 MacFarland standard (1.5 × 10<sup>8</sup> cells/mL).

**Experiment Animal:** The laboratory animal use in this study was albino wistar rats purchased from animal house at university of Nigeria, Nsukka (UNN). The rats were transported to the animal house at Department of Biochemistry, Faculty of bioscience, Nnamdi Azikiwe University (NAU) Awka. The rats were randomly examined for their suitability for the study. Those that were not suitable for the test was excluded in the study.

**Animal study:** This was carried using the method described in the study published by Iheukwumere *et al.* (2021). A total of 12 albino wistar rats were used for this study. The body of each rat was disinfected with 70% ethanol. Three locations were selected and the hairs in each location were thoroughly removed, and these were labeled A, B and C. Location A was topically administered 0.5 mL test isolate, B was applied cream and later applied 0.5 mL test isolate whereas C was left without applying anything as control. The procedure was repeated for the remaining rats. The pathological parameters of the test isolates were monitored and healing potential of the cream was also monitored in every three days for 15 days.

### Statistical Analysis

The data obtained in this study were presented in tables. Chi square (χ<sup>2</sup>) was used to determine the significance of the sample sources 95% confidence level. Pairwise comparison was carried out using student “t” test (Iheukwumere *et al.* 2021; Iheukwumere *et al.*, 2025c).

### Results

The characteristic features of the fungal isolate are shown in Table 1 and Plate 1. The study revealed the macroscopic characteristics of the isolate such as the texture of the colony, shape of the colony, colour of the colony, reverse side of the colony and growth rate of the colony. The microscopic characteristics such as the nature of the hyphae, nature of conidiophore, nature of conidia, their shapes, texture, and colours were also revealed in Table 1 and Plate 1 and 2.

The sample of organic cream prepared using essential oil extracted from *Monodora myristica* (African Nutmeg) was shown in Plate 3. The prepared cream had good consistency, soft, dark colour, satisfactory fragrance and moderate oily. One of the major advantages of this cream was that the cream exhibited similar quality of emulgel by possessing the gel and penetrating qualities.

The clinical features exhibited by *Curvularia lunata* and *Cladophialophora bantiana* such as alopecia, discoloration, scaly, swelling, erythema, patches, macule, erosion, formation of vesicle and ulceration are shown in plates 4 and 5. The study revealed that alopecia, discoloration, patches, swelling and erythema were mostly seen as the clinical manifestation of the organism. The study also revealed that the essential cream that was used as prophylaxis significantly (P < 0.05) reduced the clinical

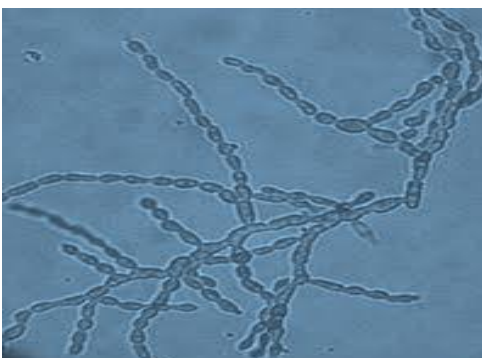
manifestation of the isolate to minimal or 0% level as shown in plates 4 and 5.

**Table 1:** Macroscopic and microscopic characteristic of the fungal isolate

| Macroscopic Characteristic   | Microscopic Characteristic   | Possible Isolate                 |
|--|--|----------------------------------|
| The colony was pinkish gray Initially and turned olive brown When fully matured. The reverse Side was brown to black. The growth rate was rapid. | The hyphae were brown and septate. The conidiophore was brown, simple, branched and bent where the conidia originated forming sympodial geniculate growth. It had proconidia that were straight, pyriform, brown, multiseptate with dark basal protuberant hila. | <i>Curvularia lunata</i>         |
| The colony was woolly, olivaceous brown in front and dark on the reverse side. The growth rate was moderate to rapid.                            | The hyphae were septate. It produced unicellular, long chains of conidia that had lemon shaped. The conidia were brown without attachment scar.  | <i>Cladophialophora bantiana</i> |



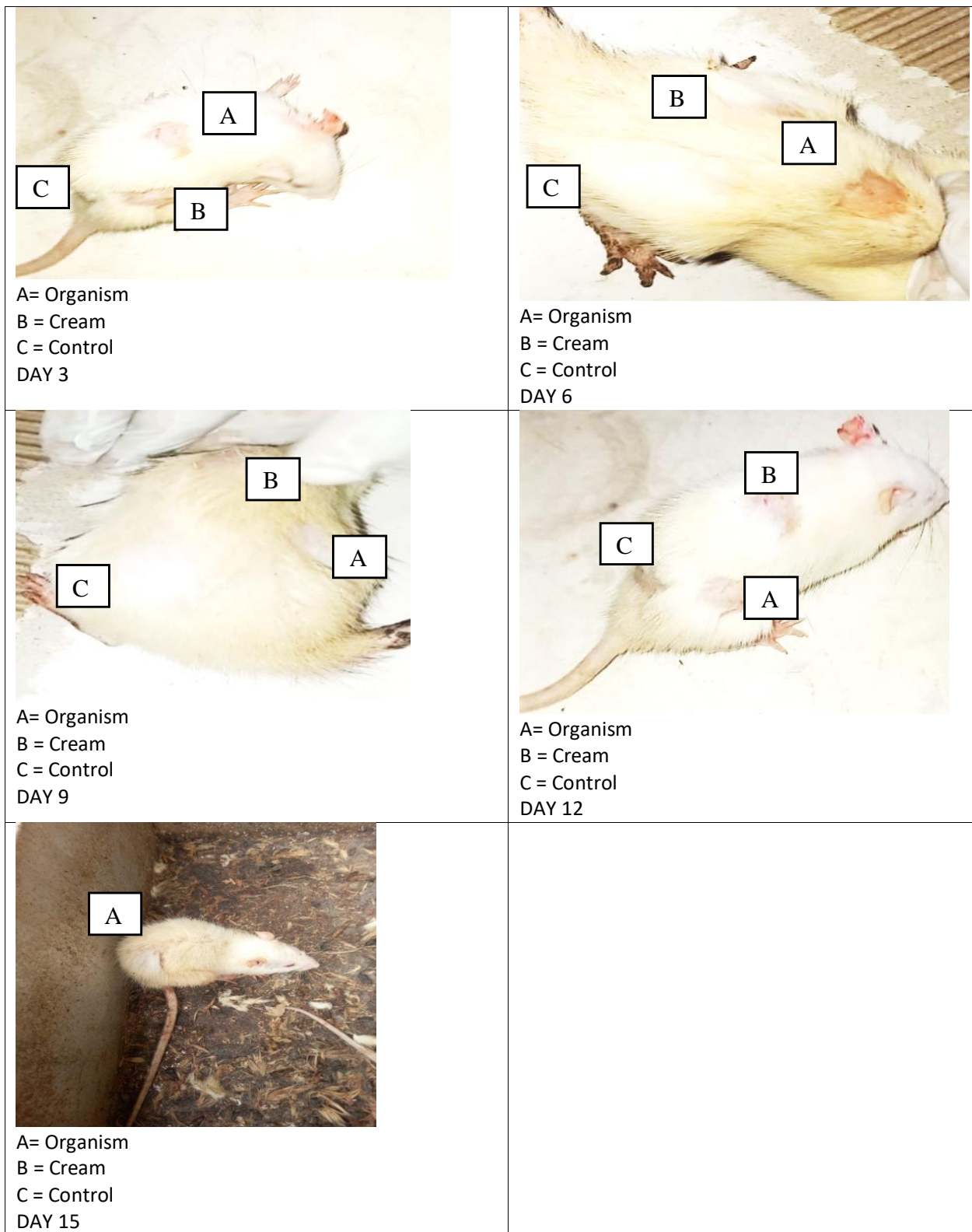
**Plate 1:** Macroscopic and microscopic feature of *Curvularia lunata*



**Plate 2:** Macroscopic and microscopic feature of *Cladophialophora bantiana*



**Plate 3:** Organic cream prepared with essential oil extracted from *Monodora myristica*



**Plate 4:** Effect of the cream prepared from African Nutmeg essential oil on the infection caused by the *Curvularia lunata*



**Plate 5:** Effect of the cream prepared from African Nutmeg essential oil on the infection caused by the *Cladophialophora bantiana*

### Discussion

The characteristic features of *Cladophialophora bantiana* and *Phomopsis azadirachtae* observed in the present study shared similar characteristic features of dematiaceous fungi isolated and characterized by other researchers (Revankar, 2007; Revankar

and Sutton, 2010; Yew *et al.*, 2014; Ozgok and IIShan, 2020). The pathological features associated with the implicated fungi agrees with the findings of Revankar and Sutton (2010), Derber *et al.* (2010), Brandt *et al.* (2013) and Yew *et al.* (2014). Different species of dematiaceous fungi were isolated and characterized

from different ecological soil samples by many other researchers (Nagano *et al.*, 2008; Sudhadham *et al.*, 2010; Bensch *et al.*, 2012; Giraldo *et al.*, 2014; Asl *et al.*, 2017). The pathologic potential of this fungus is its ability to produce skin lesions as a result of its extracellular enzymes. Nosanchuk *et al.* (2015) reported production of melanin significantly enhanced the virulence of the dematiaceous fungi and also aided them to survive in diverse hostile environments. The pronounced activity of the essential cream supported the fact that the active; plant extract contained some bioactive substances that showed both healing and cicatrical activity on the implicated lesions. The pathological features observed from some dematiaceous fungi isolated and characterized in the present study corroborated with the findings of many researchers (Zeng *et al.*, 2007; Rossetto *et al.*, 2010; Queiroz-Telles *et al.*, 2009; Welsh *et al.*, 2007; Hoffmann *et al.*, 2011). Hoffmann *et al.* (2011) recorded the ability of dematiaceous fungi to produce lesions in laboratory animals. Zeng *et al.* (2007) observed that dematiaceous fungus was responsible for subcutaneous infection of the lower limbs. Silva *et al.* (2005) also documented the subcutaneous infection known as phaeohyphomycosis on the scrotum caused by dematiaceous fungus, which was characterized by dark patches on the scrotum. Kouame *et al.* (2015) investigated the antifungal activity of essential oils extracted from *Monodora myristica* and recorded optimum inhibition against plant pathogens. Enabulele *et al.* (2014) recorded an optimum antimicrobial and nutritional potential of *Monodora myristica* essential oils. Osuagwu *et al.* (2015) screened essential oils extracted from *Monodora myristica* and recorded optimum antimicrobial activity. Uwakwe *et al.* (2008) documented the potential of *Monodora myristica* to guard the body against infectious diseases.

## Conclusion

The study has shown that *Cladophialophora bantiana* and *Phomopsis azadirachtae* isolated from soil samples showed significant dermatological lesions in albino Wistar rats, and these were effectively prevented among the rats topically applied essential cream produced from *Monodora myristica*.

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**Availability of Data and Materials:** All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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