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PROPAGATION OF *POUZOLZIA SANGUINEA* (Blume) Merr. THROUGH HARDWOOD STEM CUTTINGS UTILIZING LOCALLY SOURCED ROOTING MATERIALS

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ABSTRACT

Pouzolzia sanguinea is progressively harvested in Bhutan due to high demand from the traditional incense industry, raising concerns about pressure on natural populations and the need for efficient vegetative propagation methods. This study evaluated the effectiveness of four locally sourced rooting media: fine sand, tree bark, sawdust, and tree fern powder on the rooting performance of hardwood stem cuttings without the use of synthetic hormones. A total of 400 cuttings (100 per treatment) were established in a nursery experiment at Darla, Chhukha, Bhutan, and monitored over 60 days. Growth responses, including survival rate, shoot development, and root characteristics, were analyzed using ANOVA and Pearson correlation to determine treatment effects. Results showed that sand was the most effective medium, recording the highest survival rate (92%), followed by tree bark (77%), sawdust (65%), and tree fern (57%). Sand also produced superior shoot and root development, while tree bark recorded the longest root length, indicating medium-specific effects on root architecture. A positive but weak correlation was observed between root and shoot number ($r = 0.218$, $p < 0.01$), suggesting coordinated growth responses. Overall, sand is recommended as an efficient and low-cost substrate for propagation of *P. sanguinea*. We recommend sand media as primary rooting material for the vegetative propagation of *P. sanguinea* due to its superior performance in producing root and shoot development, resulting in a higher survival rate.

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Keywords

Rooting medium; root development; survival rate; shoot development; vegetative propagation; & *Pouzolzia. sanguinea*

INTRODUCTION

Pouzolzia sanguinea (Blume) Merr, a flowering shrub in the Urticaceae family, grows at altitudes between 1300-1900 masl [11, 33]. It is native to Southeast Asia, including India, China, Thailand, Vietnam, Nepal, and Bangladesh [19]. In Bhutan, the plant is recorded from 8 Districts (Sarpang, Punakha, Wangdue Phodrang, Mongar, Samdrup Jongkhar, Trongsa, Tashigang, and Zhemgang) [11]. *P. sanguinea* has been used in traditional medicine for the treatment of fever, cough, and sore throat, as the plant contains several bioactive compounds, including flavonoids, phenolic acids, and alkaloids [1, 7, 19]. Further, the tender leaves are used as vegetables and fodder, and the bark is used to make ropes and fishing nets [29]. According to [15], the root is used in soap making and as a pectin in traditional homemade recipes.

In Bhutan, the bark is commonly used as a key adhesive ingredient in traditional incense. In recent years, there has been an increasing number of incense-making industries, leading to increased demand for *P. sanguinea*, and 84 incense-making industries recorded in Bhutan are consuming 5.1 tons of *P. sanguinea* annually [8]. This increase is attributed to the growing significance of this species in economic, medicinal, and cultural values. Additionally, there are observations of increasing trends of illegal collection and export of these materials to neighbouring countries [31]. Consequently, there is a growing concern amongst traditional incense manufacturers, the pharmaceutical industry, and the Department of Forests and Park Services about over-exploitation and depletion of wild populations [9]. Despite *P. sanguinea* having economic, cultural, and medicinal values, studies on propagation of *P. sanguinea* are limited in Bhutan. Vegetative propagation is considered the most rapid and cost-effective multiplication of species that offers the opportunity to produce a reliable and adequate supply of superior planting stock locally by maintaining genetic stability [5, 12, 23]. It is widely practiced in horticulture for the rapid multiplication of desirable cultivars and for the perpetuation of sterile plants [2]. This method supports the sustainability of *P. sanguinea* resources by enabling rapid multiplication, preserving genetic traits, and reducing dependence on seeds. It also ensures conservation and community livelihood.

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Several studies on vegetative propagation were carried out using different rooting hormones [4, 5, 6, 10, 14, 18, 20, 30]. However, none of them were easy approaches that can be adopted easily by rural communities. Owing to the increased demand for the species and its probable threats in the wild [25], *P. sanguinea* propagation through stem-cutting, employs cost-effective materials for rooting. The findings of this study will enable the private enterprise to meet their demands and also reduce the pressure on wild populations.

STUDY OBJECTIVES

This study aims to address how different locally sourced rooting materials affect the adventitious rooting capacity of *P. sanguinea*. The specific objectives are:

1. To determine the overall rooting percentage, root architecture development including root number and length, and survival rates of mature hardwood cuttings across different local substrates.

2. To characterize the physical and structural properties of fine sand, tree bark, sawdust, and tree fern powder to assess how they correlate with vegetative propagation success.

3. To establish an affordable, hormonally independent vegetative propagation protocol tailored for smallholder nurseries.

MATERIALS AND METHODS

Study site

The experiment was conducted in the nursery of Non-wood Forest Products and Agroforestry Technology Sub-centre, Darla, Chhukha Dzongkhag over a period of 60 days (9^{th} May to 9^{th} July, 2023). The study area is located at 26.864°N and 89.560°E at an elevation of 1700 masl (Figure 1). It is characterized by a Warm Broad-Leaved Forest with a humid climate. This area showcases a diverse and rich ecosystem, supporting a wide variety of plant and animal species.

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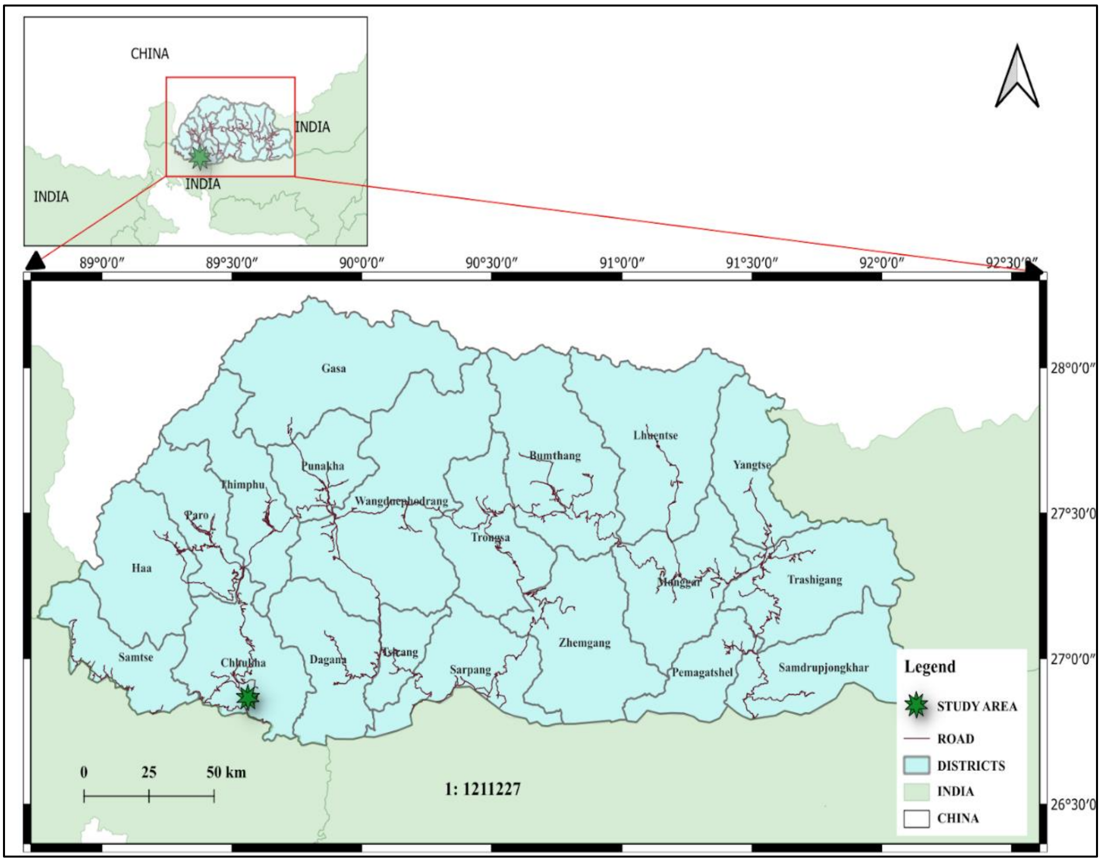

Figure 1. Study Area at Non-wood Forest Products and Agroforestry Technology Sub-Centre at Darla, Chhukha.

The study area is characterized by a mean temperature of 9.63 °C - 21.11 °C and a mean precipitation of 4.16 mm - 508.6 mm (Figure 2). The mean relative humidity ranged from 66% in January to 90% in July (32).

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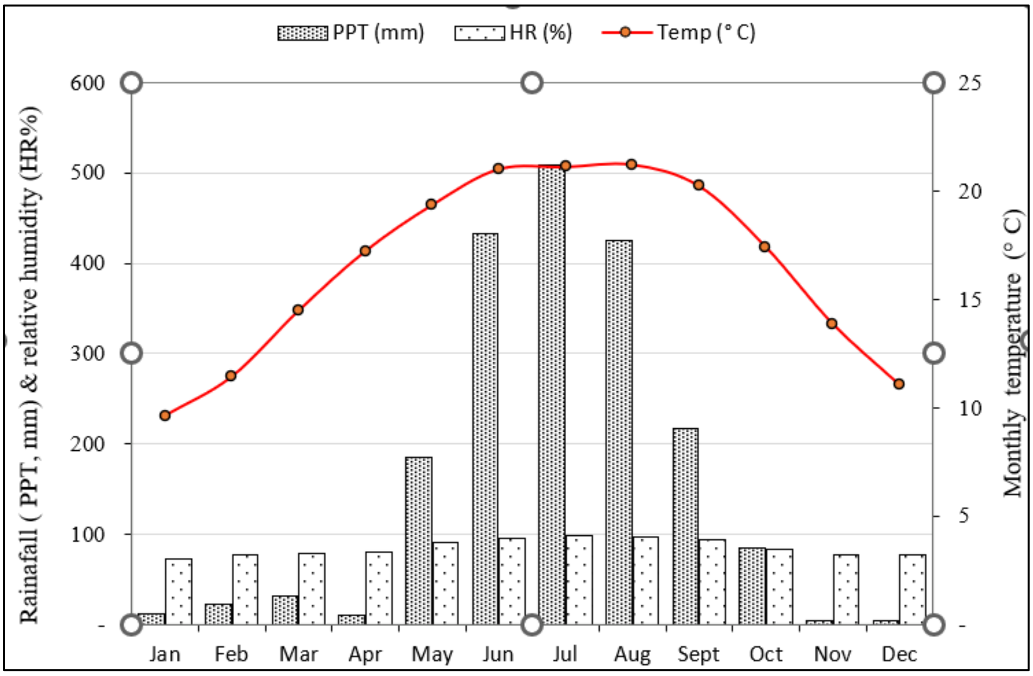

Figure 2. Mean monthly rainfall (PPT), temperature and relative humidity of the study area.

Preparation of media

The different propagation media were used: fine sand (S), hardwood tree-bark (TB), tree-fern (TF) = *Alsophila spinulosa* (Wall. ex Hook.) R.M Tyron, and sawdust (SD). The TB and TF were pulverized into fine pieces using a manual mortar and pestle. The four media were prepared in separate sunken beds (2 m x 1 m x 0.3m), edged by a cement wall. The beds were irrigated to maintain moisture before placing cuttings into the propagation beds. The beds were covered with transparent plastic (3 m x 2 m x 1 m) to retain soil moisture and maintain temperature for optimal growth (Figure 3, A).

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Figure 3. (A). Preparation of sunken beds sealed with plastic (B) and propagation of cuttings in the bed.

Collection of plant materials

The stems were collected in late spring to promote better growth and ensure they develop into seedlings for planting in the summer, when rainfall and temperatures are favourable. Following the method of [16], young (1-2 years) & healthy stem cuttings were collected from mother plants with desirable traits from the adjacent natural forests. Stems were cleanly cut with a sharp knife below the leaf node, measuring 0.1-2.90 cm in diameter and 16-20 cm in length, with 3-5 nodes retained. The outer bark from the cuttings were removed at the lower end (2.5 cm) to expose the cambium for inducing root formation (Figure 4).

Figure 4. Stem cuttings with a 2.5 cm band of outer bark removed at the basal end.

Planting of cuttings

In each bed, 100 cuttings were planted at a 70–80-degree angle to the ground, with a distance of 10-12 cm between each cutting (Figure 3, B). The roofs of the beds were covered with a green nylon net to avoid direct sunlight. Cuttings were irrigated and monitored weekly to avoid drying of the growing medium.

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Data collection

After 60 days of the experimental period, cuttings were carefully removed from the rooting medium following the methods of [3, 17, 23]. The impact of different rooting mediums on cuttings were examined based on the following criteria: (1) survival rate, indicated by the retention of greenness and absence of rot; (2) shoot development, measured by the attainment of a minimum shoot length of 1 cm; and (3) root development, assessed by the presence of root attaining a minimum length of 1 cm [3].

Data analysis

The data analysis was conducted using the Microsoft Excel and IBM SPSS Statistics software. The survival rate in each medium was determined through the count of shoot and root development. The descriptive analysis was conducted to estimate the mean values of each growth (survival rate, shoot and root development). Effects of different media on growth of cuttings were determined by analysis of variance (ANOVA). Pearson correlation analysis was conducted to determine the association between stem diameter and survival rate/development (root & shoot).

RESULTS AND DISCUSSION

Survival percentage

The survival rate was assessed by counting the number of cuttings in each medium that remained green and showed no signs of rot. The highest survival rate of 92% was observed in medium S, while TB, SD, and TF exhibited survival rates of 77%, 65%, and 57%, respectively (Figure 5). This variation is attributed to the higher water absorption capacity of tree bark, tree fern, and sawdust, which likely influenced moisture availability for successful growth. The type of medium significantly affected the survival rate of stem cuttings, $F = 12.30$, $p < .001$.

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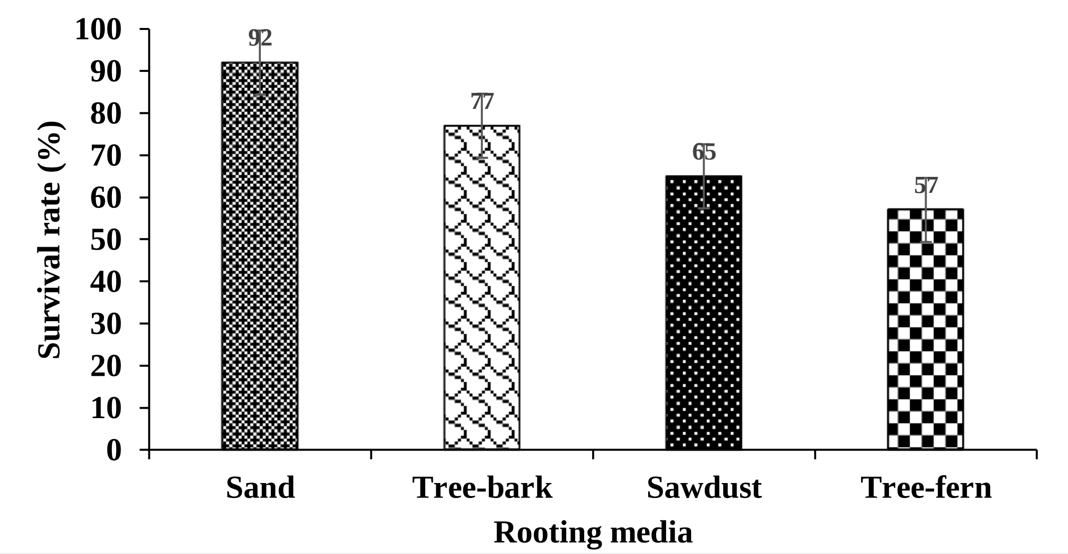

Figure 5. Survival rate of *P. sanguinea* in various rooting media after experimental period, including standard error bars.

The current results align with studies on *Garcinia lucida* Vesque in Cameroon, where sand was identified as the best medium for propagating *G. lucida* without rooting hormones, while sawdust proved to be the least effective [28]. Contrarily, a study conducted in Africa on the propagation of *Ximenia caffra* Sond, the highest survival and shoot development occurred in sawdust, followed by sand and other mixed media [21]. This discrepancy is due to the use of growth hormones and decomposed sawdust in their study, which were not used in our research.

An ANOVA conducted to determine the effect of stem diameter on growth performance indicated that stem diameter of the cuttings across the various growth media were not significantly different (F = .199, p = .656). However, [22, 27] reported that the diameter classes and growth media examined in their study significantly affected the survival rate, number of shoots, and root development.

Shoot development

The morphological development of shoots and roots observed in stem cuttings after 60 days of planting is presented in Figure 6. According to [13], sprouting is the basis for measuring the success of vegetative propagation. Following this approach, the present study evaluated propagation success by recording the number of shoots produced per cutting.

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Figure 6: Shoot and root development in stem cuttings after 60 days of growth.

The average number of shoots ranged from 2 to 3, and the mean shoot length ranged from 4.69 cm to 13.95 cm. The longest shoot length measured in medium S was 38 cm, followed by TB (29 cm), TF (16 cm), and SD (13 cm). Among different rooting media, S and TB (3 cm) showed better growth in shoot number, followed by TF (2 cm) and SD (2 cm). The maximum number of shoots was observed in S and TB medium (8 each), followed by TF (7) and SD (4) (Table 1). However, the study conducted by [17] on the propagation of cassava found that sawdust was the most effective medium for root and shoot development.

The Pearson correlation analysis conducted to assess the relationship between stem diameter and shoot number revealed an insignificant relationship (r = -.008, p = .898, n = 100), in contrast to a study conducted by [22]. This could be attributed to the limited duration of the experiment and propagation conditions such as edaphic factors, climatic factors, and age of the cuttings.

Table 1. Stem diameter, mean (±) and standard deviation on shoot and root development of *P. sanguinea* stem cuttings.

		Shoot development		Root development	
Rooting media	**Stem diameter [cm]**	**Shoot number**	**Shoot length [cm]**	**Root number**	**Root length [cm]**
Sand (S)	0.8-2.30	3 ± 1.76	13.95±8.49	7 ± 3.82	10.90±7.44
Tree-bark (TB)	0.1-2.40	3 ± 1.82	7.17 ± 6.54	4 ± 3.68	8.72± 7.95
Sawdust (SD)	0.8-2.20	2 ± 1.17	4.69 ± 4.66	5 ± 3.77	8.99 ± 7.26

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Tree-fern (TF)	0.4-2.90	2 ± 1.46	9.08±10	6 ± 4.46	14.04±9.61

Note: n=100 cuttings for rooting media (N=400)

Root development

The rooting media influenced both the number and length of roots produced by the stem cuttings (Table 1). Among the four media evaluated, cuttings established in sand produced the highest mean number of roots per cutting (7 ± 3.82), followed by those grown in tree-fern medium (6 ± 4.46). Cuttings planted in sawdust developed an intermediate number of roots (5 ± 3.77), whereas the lowest root production was recorded in tree-bark medium, with a mean of 4 ± 3.68 roots per cutting. Root length, however, exhibited a different trend. The longest roots were observed in the tree-fern medium, reaching a mean length of 14.04 ± 9.61 cm, followed by sand (10.90 ± 7.44 cm). Root lengths in sawdust (8.99 ± 7.26 cm) and tree bark (8.72 ± 7.95 cm) were comparatively shorter.

In accordance with the findings of [24, 26], it was reported that sand, when used as a single medium, yielded limited success and hindered growth. This was primarily attributed to the high porosity of sand, which made moisture retention difficult, as noted by [3]. However, in this study, sand media exhibited the highest survival rate and accelerated development of roots and shoots. This is likely attributed to the regular irrigation and the use of a plastic sheet to retain moisture. Further, the experimental plots were established in a naturally humid environment.

Effect of root development and shoot development

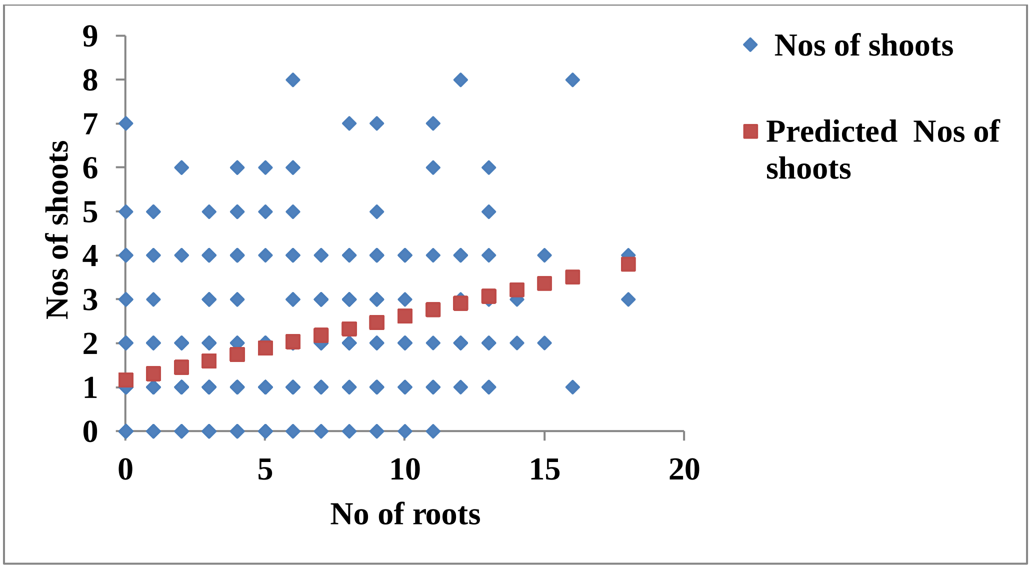

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Figure 7. Relationship between number of root and shoot development.

A Pearson correlation was conducted to assess the relationship between root number and shoot number. The analysis revealed a low relationship but a significant positive correlation between roots and shoots number (r =.218, $p<.01$). This indicates that as the number of roots increases, the number of shoots also tends to increase slightly (Figure 7). This could be due to the supply of nutrients and the initiation of shoot growth, supported by roots' absorption of nutrients and water. According to the study by [28], the number of roots developed in *G. lucida* species is influenced by the number of leaves or shoots present on the cutting during propagation, a finding which is consistent with the current study.

CONCLUSION

This study provides evidence that *P. sanguinea* can be successfully propagated through hardwood stem cuttings using locally available and low-cost rooting media. A sand medium consistently supported higher survival and more uniform root and shoot development compared to tree bark, sawdust, and tree fern powder. While tree bark showed comparatively longer individual roots, overall establishment success was strongest in sand, suggesting that physical properties such as aeration and drainage play a more decisive role, complemented by adequate moisture retention.

These findings contribute practical value to vegetative propagation research by demonstrating that effective rooting of *P. sanguinea* does not necessarily require synthetic hormones or specialized substrates. For nursery managers and local practitioners, this simplifies propagation protocols and reduces input costs, which is particularly relevant in community-based production systems and small-scale nursery settings. The results also provide a baseline reference for scaling up propagation efforts to support sustained raw material supply for the incense industry, potentially reducing harvesting pressure on wild populations.

At the same time, the study is limited by its relatively short duration, which may not fully reflect field performance after transplantation. Seasonal variability, longer-term survival, and nutrient dynamics were not assessed and would require further investigation. Future work integrating mixed media, longer monitoring periods, and physiological assessments would strengthen understanding of rooting mechanisms and improve operational guidelines.

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Overall, the study supports sand as a reliable and accessible medium for propagation of *P. sanguinea*, while also highlighting that rooting responses are influenced by multiple interacting factors that merit deeper, longer-term evaluation.

Competing interests

No competing interests from authors

Authors Contributions

All the authors have taken part in the research and have also contributed to the development of this manuscript through approval to publish the current paper.

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