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Plant Based Remedies against the Microbial Maladies with a Focus on Bacterial Wilt Caused by *Ralstonia solanacearum* in Ginger (*Zingiber officinale* Roscoe)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi & al. is one of the worst disease-causing pathogens to ginger (*Zingiber officinale* Roscoe) that incur severe crop loss and affect the economic security of the farmers. Management of the disease involves traditional and modern scientific techniques that necessitate or demand synthetic chemicals. In this connection, a study was conducted with certain endemic plants of the Western Ghats to unravel the ability of these plants to control bacterial wilt that affect the ginger crop. The plant species selected were *Hydnocarpus macrocarpus (*Bedd.) Warb., *Colubrina travancorica* Bedd., C*ynometra beddomei* Prain, *Prioria pinnata* (Roxb. ex DC.) Breteler and *Cynometra travancorica* Bedd. The plant samples

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were collected from different areas of Western Ghats particularly the Wayanad district of Kerala. Phytochemical investigations, both qualitative and quantitative means, revealed that all of them possess chemical compounds both potential therapeutic and antimicrobial properties. Studies to check the ability of these plants against *R. solanacearum* revealed that *Prioria pinnata* has the proper ability to inhibit bacterial growth. Further investigation showed that the alkaloid fractions of the plant extract showed the highest antimicrobial properties compared to that of phenols and terpenoid fractions. The GC-MS and FTIR studies indicate the presence of the good amount of chemical compounds in the tree species of *Prioria pinnata,* possessing antimicrobial and therapeutic potential. The study points out the conservation of plant wealth of theWestern Ghats that hides many biomolecules of clinical and industrial potential. The threat caused by climate change to plant wealth can be minimized by appropriate public and private partnership-oriented conservation programs.

Keywords: Bacterial wilt; Western Ghtas plant; Microbial Maladies; Ralstonia solanacearum; Ginger.

1. INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) considered as the confluence of spice and medicine. William Roscoe described this plant and gave the name *Zingiber* which was derived from Sanskrit. In India, the area under cultivation of Ginger is 63,000 ha, and the production is 3 tons/ha. India is the world's highest producer of Ginger with 1/3 of the production, being produced by China, Nigeria, Nepal, and Indonesia (Priyanka & Khanal, 2021). Madhya Pradesh has the maximum production with 31.18% of the share (2021–22). Kerala ranks 9th with 2924 MT during 2021–22, and Wayanad is the district with the most significant area under cultivation, 1232ha (2021-22). The soft rot in ginger caused by *Pythium aphanidermatum* (Edson) Fitzpn, that severely damages the yield and make the yield drop up to 50%. caused soft rot in ginger is caused by and it causes a crop loss of up to 50%. The Bacterial wilt Caused by *Ralstonia solanacearum* (Smith) Yabuuchi & al; and it is called 'Mahali disease' and makes a yield drop of nearly 40%, however, in conducive conditions, the yield reduces up to 100% (Mathew et al., 1979; Dohroo, 1991). Ginger farming become a nightmare (Yadav et al., 2023) in Kerala state because of microbial disease, and the decline in the area under cultivation of ginger usual trend that is prevailing in districts like Wayanad. While looking into the area under cultivation of Ginger in the district, during the year 2001-2002, Ginger was cultivated in 10706 ha (2012-13); however, in 2021-22 it considerably came down to 2924 ha (Agriculture Statistics 2022–23). This indicates that the percentage of reduction in the area under cultivation was nearly 73%. In this context, an eco-friendly approach that can best control the microbial disease in need of the hour can better save the lives and livelihood of the ginger farmers of the state and nation.

The present study focuses on plant-based remedies in the control of microbial diseases with a focus on bacterial wilt or Mahali disease caused by *Ralstonia solanacearum*. Five selected plants that are endemic to the Western Ghats were tested for their ability to control the bacterial wilt with an assumption that the phytochemicals present in the plants would have the right potential to check the growth of microorganisms that are pathogenic to crops. A detailed study could unravel the chemical constituents' quantity and mode of action in controlling the pathogen would be of greater use for farmers and ginger production.

2. MATERIALS AND METHODS

2.1 Collection of the Plant Samples

The study conducted in the Wayanad district of Kerala and the plant samples collected from evergreen patches of the Western Ghats that spread over the district. Five plants selected based on the survey in which the plants were used by the farmers of the district to protect the ginger from the attack of pests and diseases. The selected plants identified after consulting the authentic specimens deposited at MH which are *Hydnocarpus macrocarpus (*Bedd.) Warb., *Colubrina travancorica* Bedd., *Cynometra beddomei* Prain., *Cynometra travancorica* Bedd., and *Prioria pinnata* (Roxb. ex DC.) Breteler (≡*Kingiodendron pinnatum* (DC.) Harms). The samples collected in a sterile polythene bag and taken into the laboratory. The samples surface sterilized under tap water several times to remove residual contaminants followed by distilled water (Ahmad et al*.,* 2009).

2.2 Microorganism Culture

Ralstonia solanacearum cultures collected from the Indian Institute of Spices Research (IISR), Calicut. The microbial culture collection number *Ralstonia solanacearum* IISR-GRS-SPR. The cultures were kept in nutrient agar plates and incubator at 28-32˚C. The cultures sub-cultured for the experiments in nutrient agar plates (Nnamdi et al*.,* 2011; Sreena et al., 2013).

2.3 Qualitative and Quantitative Determination of Phytochemicals

Preliminary phytochemical analysis carried out for the extract as per standard methods. The methanol extract of the sample was prepared by following the procedure mentioned by Parekh et al., (2006) in which10g of shade-dried powder was taken in 100ml methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker for 24 hours. After 24 hours, the methanol extract was centrifuged at 2000rpm for 10 minutes and the supernatant was collected and filtered by using Whatman no.1 filter paper. The solvent was evaporated to make the final volume which is one-fourth of the original volume and stored at 4˚C in an airtight bottle.

The extracts subjected to a qualitative chemical test for various phytoconstituents like carbohydrates (Sadasivam & Manickam, 1991); reducing sugars, phenols, tannins, pholobatannins, sterols and saponins (Raaman, 2006); flavonoids (Shamaki et al., 2012); alkaloids (Mayer' test & Wagner' test as described by Raaman 2006); glucosides (De et al*.,* 2006) and terpenoids (Shamaki et al., 2012). The quantitative determination of alkaloids and phenolic compounds was taken up by adopting the methods of Dattatreya et al. (2020).

2.4 Antimicrobial Assay

A disc diffusion assay (Jaishree & Kumar, 2017) performed to determine the antimicrobial activity of the selected five plant species. The test organism grown in SDA plates diluted in distilled water (1×10^8) and seeded into the petri plates. The methanol extract of the selected five plant samples at varying concentrations impregnated in Whatman no.1 filter paper in 6mm diameter. These discs were kept at 4˚C for one hour to effectively diffuse the extracts to the media and thereafter the plates were incubated at 37˚C for 24 hours. The inhibition zone (IZ) that formed around the disc after 24 hours measured. The Activity Index (AI) calculated by the formula

$$\text{Activity Index} = \frac{\text{Inhibition Zone of the Test}}{\text{Inhibition Zone of the Control}}$$

The experiments repeated three times to reduce the errors and values statistically assessed.

2.5 GC MS and FTIR

Gas Chromatography-Mass Spectroscopy (GC-MS)

The phytochemical analysis performed by using a combined 7890A gas chromatograph system (Agilent 19091-433HP, USA) and a mass spectrophotometer. The system equipped with an HP-5 MS fused silica column (30.0 m × 250 μm, film thickness 0.25 μm), and interfaced with a 5675C Inert MSD with a Triple-Axis detector. Helium gas used as the carrier gas, adjusted to a column velocity flow of 1.0 ml/min. Plant extract that dissolved in Dichloroethane taken for the GC-MS studies (Olivia et al., 2021).

The Fourier Transform Infrared Spectrometer (FTIR) assessment one of the most essential tools for assessing the functional groups and bodies in the given chemical samples. It relies on the principle that the light absorbed would be related to the characteristic of the chemical compound present in the sample. Hence, the chemical compounds can be identified by interpreting the wavelength that was absorbed. In the present study used the methodology described by Ashokkumar and Ramaswamy (2014). The instrument used was Shimadzu, IRAffinity1, Japan, with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

3. RESULTS

3.1 Phytochemical Screening

The results of the phytochemical screening revealed that the selected plants from the Western Ghats possess different phytochemical components that may have therapeutic and antimicrobial properties. The outcomes of the qualitative experiments indicate that all the selected plants showed positive results for most of the tests. However, negative results shown in the case of sterols and saponins as given in Table 1. Phlobotannins showed positive results only in *Colubrina travancorica,* and the rest of them showed negative results.

Table 1. Qualitative determination of the phytochemicals present in the selected five endemic plant species from the Western Ghats of Kerala

Test	Plant species				
	Hydnocarpus macrocarpus	***Colubrina travancorica***	***Cynometra beddomei***	***Prioria pinnata***	***Cynometra travancorica***
Carbohydrates	+	+	+	+	+
Reducing sugar	+	+	+	+	+
Phenols	+	+	+	+	+
Tannins	+	+	+	+	+
Flavonoids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Phlobotannins	-	+	-	-	-
Glycosides	+	+	+	+	+
Sterols	-	-	-	-	-
Saponins	-	-	-	-	-
Terpenoids	+	+	+	+	+

+ Present; - Absent

3.2 Determination of Phenolic Compounds

All the selected plants possess varying amounts of phenolic compounds (Table 2). The *Colubrina travancorica* 0.80±0.00 exhibited the highest amounts of Tannic acid followed by *Prioria pinnata* with 0.57±0.01. The composition of flavonoids and phenols was found to be varying in all the selected plants.

3.3 Determination of Alkaloid Content

The result of the determination of Alkaloid content provided in Table 3. *Colubrina travancorica* possesses the highest amount of alkaloid content with 0.95±0.03mg/ml followed by *Hydnocarpus macrocarpus* with 0.92±0.02. However, all the plants show the presence of alkaloids in varying concentrations.

3.4 Antimicrobial Assay

Varying concentrations of the plant extract, such as 5%, 10%, and 15% taken for the antimicrobial activity assessment against the pathogen *R. solanacearum*, and the results enumerated in Table 4. However, as table indicates, none of the plants in any of the chosen concentrations showed any impact on the growth of *R. solanacearum*. From the Table 4, it is clear that only *Prioria pinnata* in all the selected concentrations of 20%, 40%, and 60% showed inhibitory effects against the selected organism, *R. solanacearum*. None of the other plant extracts exhibited any significant inhibition against the pathogen.

Table 2. Determination of the Total Phenolic Compounds present in the selected plant species

Samples (mg/ml)	***Hydnocarpus macrocarpus***	***Colubrina travancorica***	***Cynometra beddomei***	***Prioria pinnata***	***Cynometra travancorica***
Flavonoids	0.14±0.04	0.15±0.005	0.10±0.006	0.15±0.02	0.14±0.02
Tannic Acid	0.07±0.009	0.80±0.00	0.075±00	0.57±0.01	0.08±00
Phenols	0.13±0.04	0.141±0.01	0.12±0.05	0.15±0	0.15±0

n=3±standard deviation

Table 3. Determination of the total alkaloid composition in selected plant species.

Samples	***Hydnocarpus macrocarpus***	***Colubrina travancorica***	***Cynometra beddomei***	***Prioria pinnata***	***Cynometra travancorica***
Alkaloid Content (mg/ml)	0.92+0.02	0.95+0.03	0.89+0.02	0.90+0.01	0.87+0.04

n=3± standard deviation

3.5 Antimicrobial Activity Index

The antimicrobial activity index of phenolics, alkaloid, and terpenoid fractions of *P. pinnata* against *R. solanacearum* are presented in Table 5. The alkaloid fractions of the *P. pinnata* showed the highest inhibitory actions against *R. solanacearum.* All samples taken with 20% concentration in which the highest activity index was sexhibited by the alkaloid fraction followed by phenolics and terpenoids.

Table 4. Antimicrobial activity of selected five plant species against *Ralstonia solanaceaurm*

Sl. No	Plant extract	Concentration (%)	Inhibition (mm)
1.	*Hydnocarpus macrocarpus*	5	No zone
		10	No zone
		15	No zone
		20	No zone
		40	3
		60	10
2.	*Prioria pinnata*	5	No zone
		10	No zone
		15	No zone
		20	12
		40	17
		60	20
3.	*Colubrina travancorica*	5	No zone
		10	No zone
		15	No zone
		20	No zone
		40	No zone
		60	No zone
4.	*Cynometra travancorica*	5	No zone
		10	No zone
		15	No zone
		20	No zone
		40	No zone
		60	7
5.	*Cynometra beddomei*	5	No zone
		10	No zone
		15	No zone
		20	6
		40	7
		60	9

Table 5. Antimicrobial activity index of phenolics, alkaloid and terpenoid fractions of *Prioria pinnata* against *Ralstonia solanacearum*

Sl. No.	Extracts	Concentration (%)	Diameter of zone of inhibition (mm)	Activity index
1.	Alkaloids	20	5	0.15
			6	0.18
			5	0.15
2.	Phenolic Compounds	20	4	0.125
			3	0.09
			4	0.125
3.	Terpenoids	20	3	0.09
			2	0.06
			2	0.06

The Minimum Inhibitory Concentration (MIC) of alkaloid extracts against *R. solanacearum* provided in Table 6 indicates that the increasing concentration of the alkaloid fraction of the plant *P. pinnata* is having a positive impact on the growth of the selected pathogen *Ralstonia solanacearum*. The highest concentration taken 30% and that showed an inhibition of 6mm in the growth of the pathogen followed by 25% with 4mm and 20% with 3.5mm.

Table 6. Minimum inhibitory concentration (MIC) of alkaloid extracts against *Ralstonia solanacearum*

Extract	Concentration in (%)	Diameter of zone of inhibition (mm)
Alkaloids	5	No zone
	10	2
	15	3
	20	3.5
	25	4
	30	6

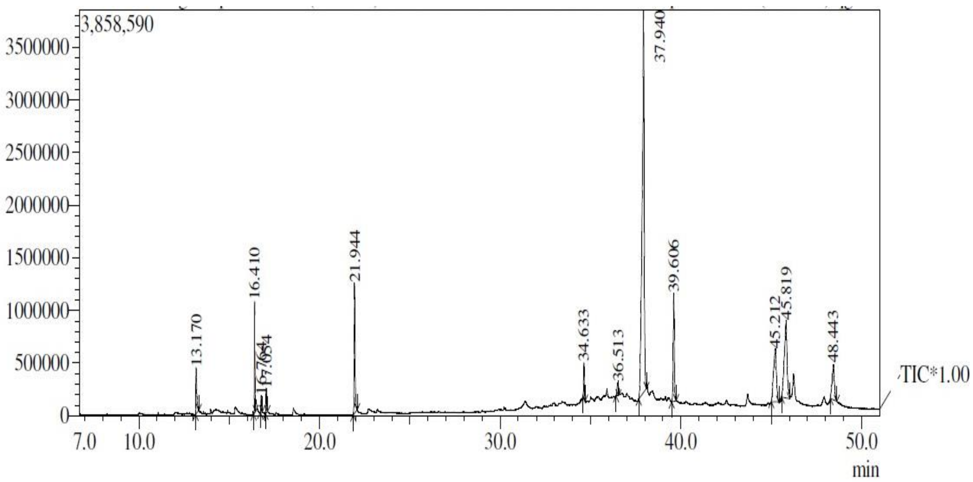

Fig. 1. GCMS Spectrum of the methanol extract of *Prioria pinnata*

Table 7. Major Compounds Present in *Prioria pinnata*

Peak	R. Time	Area	Area%	Height	Height%	Name	Base m/z
1	13.170	1499705	2.48	438333	4.46	3,'5'-Dimethoxyacetophenone	180.10
2	16.410	2421680	4.00	1057913	10.77	Neophytadiene	68.05
3	16.764	396647	0.65	159747	1.63	3,7,11,15- Tetramethyl - 2- Hexadecen-1-ol	82.05
4	17.054	597666	0.99	228600	2.33	Phytol, Acetate	71.05
5	21.944	4454073	7.35	1235878	12.58	Isophytol, Acetate	71.05
6	34.633	919434	1.52	342050	3.48	Sqalene	69.05
7	36.513	660935	1.09	126938	1.29	Beta- Sitosterol	55.05
8	37.940	28790903	47.54	3633482	36.98	Tetrakis (2,3- Ditert-Butylphenyl)-4,4'Biphenylene Diphosphonate	57.10
9	39.606	4349973	7.18	1022509	10.41	Vitamin E	165.10
10	45.212	5504834	9.09	501934	5.11	2-Tert-Butyl-4,6-Bis(3,5-Ditert-Butyl-4-HydroxyBenzyl) Phenol	57.10

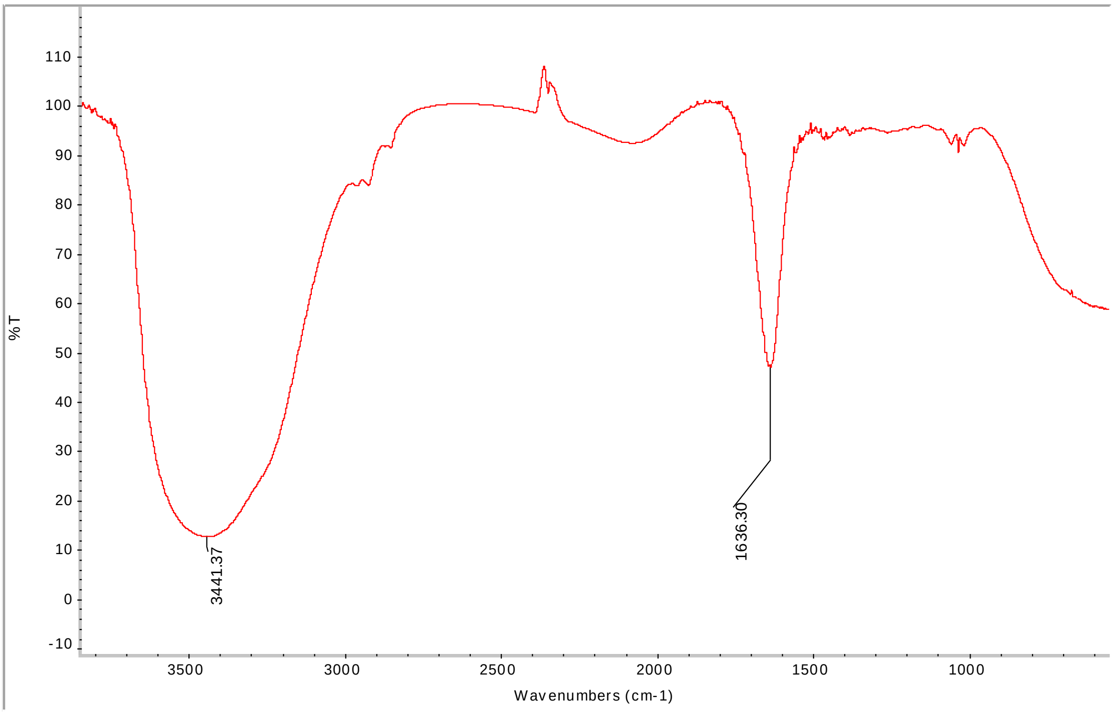

Fig. 2. FTIR- Spectrum of an alkaloid fraction of *Prioria pinnata*

Table 8. FTIR spectral peak values and functional groups obtained for the leaf extract in Methanol solvent

Extract Prepared In	Peal Values	Bonds	Functional Groups
Methanol (ME)	3441.37	O-H Stretch, free hydroxy	Alcohols and Phenols
	1636.30	-C=C- stretch	Alkenes

Table 7 shows that the components that have a high retention time were 2-Tert-Butyl-4,6-Bis(3,5-Ditert-Butyl-4-HydroxyBenzyl) Phenol with a value of 45.21% followed by Vitamin E with a retention time of 39.6%. Other compounds such as Tetrakis (2,3- Ditert- Butylphenyl)-4,4'Biphenylene Diphosphonate and Beta-Sitosterol also showed good retention values. Fig. 1 is the GCMS spectrum of the methanolic extract of the *P. pinnata*.

Table 8 highlights the presence of O-H stretch, free hydroxy bonds that may indicates the alcohols and phenolic compounds present in the sample and -C=C- stretch bonds that are indicative of Alkenes in the samples. Fig. 2 shows the peaks of the representing functional groups present in the sample.

4. DISCUSSION

Ralstonia solanacearum is one of the dreadful pathogens that affect crops around the world and is considered one of the significant threats to ginger cultivation (Nair, 2013). *R. solanacearum* naturally inhabits soil, plants, and animals and has wide host ranges. Controlling of *R. solanacearum* is a difficult task since it has multiple host ranges, prolong survival in the soil, a vast mechanism for spreading, and the ability to associate with weeds in the cropland and grow endophytically (Sarkar & Chaudhari, 2016). Attempts to control the pathogen include cultural, biological, chemical or physical means (Cordoso et al., 2006). The present study revealed that among the five selected plant species, only *P. pinnata* showed inhibitory effects, starting with a concentration of 20%. While looking at the chemical components that inhibit the growth it can be noticed that the alkaloid fractions of the leaf extract showed maximum inhibitory effects compared to the phenols and terpenoids. Values of the alkaloid fraction's minimum inhibitory concentrations (MIC) of the alkaloid fractions further revealed that a concentration of above 5% only showed inhibitory effects. However, the treatment with alkaloid fractions with a concentration of more than 10% showed positive results with 2mm and a further increase in inhibition was observed ith an escalation in concentrations. Earlier research attempts with *P. pinnata* also showed similar results as reported by earlier research attempts (Sheik & Chandrashekar, 2014; Baheti et al., 2020). The dreadful pathogen can be controlled with leaf extracts of *P. pinnata* and a focused attempt to reveal the chemical constituents present in the alkaloid fractions is essential.

Antimicrobial compounds derived from plants have received wider attention since they used against drug-resistant microorganisms and other resistant microbes. Alkaloids are naturally found in plant kingdoms and have proven to be anticancer, antiviral, anti-inflammatory, and antimicrobial properties (Liu et al., 2020). Many drugs have developed out of alkaloid, and one of the best examples to cite is morphine- the narcotic analgesic (Rodrigues et al., 2020). The present study also corroborates that the alkaloids from the plants have antimicrobial properties and could best inhibit the most dreadful pathogen *R. solanacearum* that affects ginger cultivation. An alkaloid fraction of 10% can rightly check the growth of tested microorganisms and reveal that an increase in the testing concentration could control further microbial growth. The importance of the plant wealth in the Western Ghats is one of the areas of concern and it reported that out of the 4000 species of flowering plants known so far, 1500 species belong to the endemic category. These plant species to be studied in detail to unravel the chemical compounds present in them, which may lead to the development of novel biomolecules that have therapeutic values and disease-prevention abilities both in plant and animal kingdoms (Cardoso et al., 2006; Dattatray et al., 2020; Parekh et al., 2006).

5. CONCLUSION

Bacterial wilt caused by *Ralstonia solanacearum* is one of the dreadful diseases that cause significant crop loss to the farmers of the Kerala state, especially the Wayanad district. Farmers are manage to control this disease with cultural, physical, chemical, and biological means. Endemic plant species from the Western Ghats were tested in controlling the evil causing bacteria *R. solanacearum.* Out of the five plant species, the *Prioria pinnata* found to be control the growth of the tested microorganism *R. Solanacearum*. Further investigation revealed that among the alkaloid, phenolics, and terpenoid fractions of plant extract, an alkaloid portion could rightly control the microorganism. Detailed studies using GC-MS and FTIR further revealed that the plant *P. pinnata* possesses a series of chemical compounds with antimicrobial properties and therapeutic values. The FTIR study showed that the plant possesses functional groups that belong to alkaloids, phenols and alkenes. The study throws open light into the importance of chemical compounds that may hidden in the plant kingdom of Western Ghats, one of the unique mega biodiverse hot spots of the world.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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