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Research Article

FABRICATION AND CHARACTERIZATION OF BIO-WAX COATED HYDROPHOBIC PAPER FROM *EICHHORNIA CRASSIPES* (MART.) SOLMS

***Ananda Kumar A.M, Rakkimuthu R, Sathishkumar P, Sowmiya D, and Aleena B. Eluvathingal**

PG and Research Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi, Tamil Nadu, India

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ABSTRACT

The present study focused on producing eco-friendly hydrophobic paper using epicuticular biowax isolated from *Eichhornia crassipes*, an invasive aquatic plant. Wax extraction was carried out using organic solvents such as chloroform and petroleum ether, with chloroform yielding the highest amount (0.1305 g per 10 g of leaves). The extracted wax exhibited favorable physical properties including moderate viscosity, a melting point of 42°C, and a boiling point of 51°C. Chemical reactivity tests confirmed the wax's solubility in acids and bases and its stability in organic solvents. Heat sensitivity studies showed no significant structural changes up to 120°C. Wax-coated papers demonstrated strong hydrophobic properties, with negligible weight change after water exposure. Coating efficiency was consistent across different paper types and GSM grades. Biodegradability tests indicated effective degradation, particularly in soil conditions. Overall, the E. crassipes-derived biowax proved to be a sustainable, biodegradable, and hydrophobic coating material. The findings suggest its potential application in developing eco-friendly paper-based alternatives to plastic packaging.

Keywords: Epicuticular wax, *Eichhornia crassipes*, Hydrophobicity, Thermal stability Biodegradability.

INTRODUCTION

The concern over environmental was increasing day by day. The major polluting agent like non-degradable bio plastics are affecting environment in all ways. The present generation got aware on many criteria including hazardous nature of plastics. Hence it is need of the hour to produce biodegradable and durable plastics. Plants are found richest source of many biomaterials and works as reservoir for the production of many products. Plastic bags, without any doubt, have found usefulness due to the properties like high durability, non-corrosiveness, light weight, electrical and thermal insulation, etc. Plastics are made from the synthetic polymers such as polypropylene and polyethylene are known to cause environmental concerns due to their nonbiodegradable nature, because of long chain carbon molecules that are probably made from polymers of petroleum product (Thompson *et al*., 2009). In spite of having such varying range commercially useful properties, plastic bags have become a global concern. Even process like incineration which is carried out with the intention of destroying the plastic bags produce large amount of greenhouses gases and other toxic gases like carbon dioxide, monoxide, Sulphur dioxide gases, etc (Junod,1976) which does more harm than good to the environment by causing air pollution. Plastic bags are also the major cause of blockage of drainage systems, one of the reasons behind urban floods and water pollution. Not only plastic bags cause pollution to the environment, but also cause death of the animals when swallowed by terrestrial or marine and the alarming concern is that the production of plastic bags has increased several folds over few decades. Around 5 billion plastic bagsare used across the globe (Jaconsen, 2005). So, attempts are made to create alternatives like biodegradable plastic bags. But many studies show that biodegradable plastic requires a favorable environment i.e., optimum temperature, moisture, presence of microbial population, etc for the process of degradation and even if they degrade, it produces smaller particles of plastic called micro plastics, which gives shows that even the use of biodegradable plastic is not completely ecofriendly.

*Corresponding Author: Dr. Ananda Kumar A.M, Assistant Professor, PG and Research Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi, Tamil Nadu, India Email: anandhakumar@ngmc.org.

Therefore, to avoid these problems the use of paper bags is encouraged because it has been scientifically proven that paper bags do not cause as much harm as plastic bags. Although the rate of biodegradation is slow in landfills with low moisture, but recycling of paper bags requires 91% less energy per pound as compared to plastic bags and also reduce the production of toxic gases and use of water to a considerable amount (Bell and Cave, 2011). But paper bags have their own limitations. Paper bags are not water resistant due to which the uses of paper bags become very limited. On the other hand, if plastic films are used to laminate the paper bags to make it water resistant then the product becomes non-biodegradable again. If they are coated with synthetic compounds like slimicides or lacquer to have additional advantages over plain paper then their degradation might cause water and soil pollution. So, in an attempt to find suitable organic material which might be able to solve the above-mentioned problems, answers were sought in the nature. Naturally, Ariel surface of the plants contain hydrophobic water proofing wax(bio-wax) on them which provides protection against environmental stresses. In some plants this wax is highly hydrophobic in nature. Plants develop a protective skin, called‘‘cuticle’’, for a successful survival in the dry ergaseous environment. The plant cuticle is a continuous extracellular membrane which covers the primary above-ground organs (e.g. flowers, leaves, stems, fruits) of all lower and higher land plants. Cuticles are composite materials, with a framework made basically of cutin and integrated and superimposed lipids called ‘‘waxes’’. Plant waxes embedded into the cuticle are called ‘‘intra cuticular waxes’’, whereas called ‘‘epicuticular waxes’. Epicuticular wax forms crystalline projections from the plant surface, which enhance their water repellency, create a self-cleaning property knownas the lotus effect and reflect UV radiation. Synthetic waxes are long-chain hydrocarbons that are the closest substitutes for petroleum and mineral waxes Chemically, it consists of hydrophobic organic compounds, mainly straight-chain aliphatic hydrocarbons with a variety of substituted functional groups. Natural waxes are derived from plants and animals. Biodegradable Products Institute has concluded that the natural and synthetic paraffins of the types included in snow sports waxes are biodegradable.

Beyond the deadly plastics, one more threat to our environment is the invasive alien or exotic plant species which destroys the indigenous plants and change the ecosystem. The Eradication of these exotic species is unavoidable. Instead the exotic plant species can be used for production of plant of bio-wax as hydrophobic surface coating substance for paper bags, without harming the environment. The plant is available abundantly and throughout the year at all seasons. For the present study, the plant *E. crassipes* (Water Hyacinth) was selected an aquatic plant that grows partially or wholly in water. The plant grows at an astonishing rapid rate because of its asexual reproduction and it ability to reproduce without relying upon insect or wind pollination for fertilization. From the above background the present study is proposed to extract the bio-wax from the leaves of Eichhornia crassipes and be used as a coating substance on the surface of paper to attain a layer of hydrophobic paper, hence the product obtained could be used as source of raw material for making paper bags and other related substances. In addition to water resistance or hydrophobic nature, the most important advantage to this product will be that it will be completely biodegradable and time required for degradation will also very less due to its organic nature. On complete biodegradation the product will be undoubtedly an ecofriendly product that enriches the soil nutrient to a considerable extent.

MATERIALS AND METHODS

The present study was carried out with the plant material *Eichhornia crassipes* (Mart). Solms belongs to the family Pontederiaceae. A common perennial American weed found on the lakes, ponds, stagnant water bodies etc., The plants are found to be highly very big menace in disturbing the water ecosystem. The disease free clean matured leaves of the plant species, Eichhornia crassipes were collected from the Thirunellayi river, a perennial water body in Palakkad of Kerala state (Apart of Bharathapuzha River). The Plant materials collected, washed and cleaned to remove the dust particles and other foreign materials found the leaf surfaces. The leaves were cut into small pieces and used for further studies. The samples were weighed and used freshly further.

Isolation of Wax from Leaf Surface (Ganeva *et al*., 2015)

Fresh leaves of *Eichhornia crassipes* were cut into small pieces. About 20 ml of chloroform was taken in a beaker, and the leaf fragments were immersed completely using a glass rod. The beaker was placed on a shaker for 2 hours. After shaking, the chloroform extract was transferred to a clean beaker. The extracted solution contained the epicuticular wax, visible as a white cloudy layer floating on the surface.

Wax Confirmatory Test (Akash and Nayan, 2018)

The chloroform extract was allowed to evaporate, leaving behind the wax. The wax was dissolved in ethanol and transferred to a test tube. A few milliliters of distilled water were added and shaken gently. The formation of a milky white solution confirmed the presence of wax.

Quantitative Analysis of Bio-Wax (Akash and Nayan, 2018)

Fresh leaves were cut into fragments. 1 g of leaf pieces was soaked in 10 ml of chloroform for 3 minutes. The solvent was discarded, and the leaves were air-dried and re-weighed. The procedure was repeated for 2 g and 3 g samples using 15 ml and 20 ml of chloroform, respectively. The difference in weight before and after treatment represented the wax content.

$$\text{Average wax per gram of leaf} = \frac{\text{Total weight of leaf (g)}}{\text{Total weight of wax (g)}}$$

Test for Hydrophobicity (Yadav and Datta, 2014)

The dried wax was dissolved in chloroform to make a coating solution. Fifty discs of Whatman No. 1 filter paper were cut, weighed, and immersed in the wax solution for about 5 minutes. The discs were re-weighed to determine the wax load. They were then dipped in water for 5 minutes, re-weighed again, and hydrophobicity was calculated using the formula:

$$\%\text{Hydrophobicity} = \frac{W1-W2}{W1} \times 100$$

Where, W_1 = weight before soaking, and W_2 = weight after soaking.

Heat Sensitivity Test (Akash and Nayan, 2018)

The extracted wax was collected in Petri plates, and the solvent was allowed to evaporate at room temperature. The plates were then exposed to temperatures ranging from 30°C to 120°C to assess the wax's heat tolerance, insulating ability, and retention of hydrophobic properties.

Removal of Pigment from Wax (Erge *et al.,* 2008)

Since the extracted wax contained chlorophyll pigments, the leaves were first steamed in a water bath at 100°C for 30 minutes to degrade the chlorophyll. The treated leaves were then extracted with chloroform. The solvent was evaporated to obtain a white layer of wax, which was further dried in an oven at 60°C for 10 minutes to remove residual moisture.

Method of Coating Wax on Paper (Akash and Nayan, 2018)

The purified bio-wax was coated on various types of paper using a simple dipping and soaking technique. Papers were immersed in the wax solution at different concentrations and time intervals to ensure uniform coating.

Determination of Physical and Chemical Parameters (Lourdes *et al.,* 2019)

The color, odor, and texture of the wax-coated papers were evaluated visually. Physical parameters such as viscosity, boiling point, and melting point were recorded. Solubility and chemical reactions were tested using different solvents and reagents, including ferric chloride, sulfuric acid, hydrochloric acid, potassium hydroxide, sodium hydroxide, and acetic acid.

Biodegradability Test (Arunkumar *et al.,* 2017)

Wax-coated papers were tested for biodegradability by exposing them to different chemical environments (sulfuric acid, hydrochloric acid, ammonium hydroxide, acetic acid, phenol, petroleum ether, ethanol) and natural soil. Samples were weighed every three days to monitor changes in weight, indicating the degree of biodegradation. The results were recorded and tabulated.

RESULTS AND DISCUSSION

The extraction of epicuticular wax from Eichhornia crassipes showed clear variation among samples (Table 1). Yields ranged from 0.68 g to 2.17 g per 10 g of leaves, with Sample 3 producing the highest yield (2.17 g) and Sample 5 the lowest. These differences likely reflect environmental or plant-level variations. The physical properties of the wax (Table 2) showed that it was dark tan brown, had an unpleasant smell, bitter taste, and moderate viscosity. It melted at 42°C and boiled at 51°C, consistent with soft, plant-derived waxes made of long-chain compounds. Chemical reactivity tests (Table 3) showed that the wax reacted strongly with sulfuric acid (effervescence) and produced frothing with KOH and NaOH, suggesting the presence of saponifiable components. Other reagents produced no visible reaction, though the wax remained soluble in all chemicals tested. The strongest reaction occurred with H_2SO_4.

Solubility results (Table 4) showed that the wax was completely soluble in petroleum ether, making it the best solvent. It was insoluble in chloroform, benzene, and ethanol, while acetone caused partial solubility, confirming its strongly non-polar nature. Thermal stability observations (Table 5) showed that heating the wax up to 6 hours caused no major changes in colour, texture, hydrophobicity, or thickness. Only its odour reduced slightly over time, indicating excellent heat stability. Wax-coating efficiency varied across paper types (Table 6). Chart paper showed the highest wax uptake (0.1445 g at 6 hours), while tissue paper absorbed the least (maximum 0.025 g), making chart paper the most suitable substrate.

When testing papers of different GSM values (Table 7), wax retention increased with paper thickness. 150 GSM paper had the highest uptake (0.1875 g at 8 hours), showing that denser papers hold more wax. Hydrophobicity results (Table 8) showed no meaningful difference in weight before and after water soaking for all papers, confirming that the wax coating provided strong and stable water resistance. The biodegradability study (Table 9) showed that degradation depended on the environment. Phenol and sulfuric acid caused the greatest breakdown, with sulfuric acid reaching 2.044 by day 6 and phenol reaching 1.830 by day 9. Acetic acid caused a temporary spike at day 3. Organic solvents showed mild effects, while soil caused gradual, varying degradation over time.

The study showed that *E. crassipes* leaves contain variable amounts of epicuticular wax, with Sample 3 yielding the highest quantity. The wax exhibited stable physical properties, strong non-polar characteristics, and excellent thermal stability. Petroleum ether was identified as the most effective solvent for dissolving the wax. Wax-coated papers, especially chart paper and high-GSM papers, demonstrated strong coating efficiency and consistent hydrophobicity. Overall, the wax showed environment-dependent biodegradability, with the highest degradation occurring in sulfuric acid and phenol. The study was carried out to produce an eco-friendly hydrophobic paper that could serve as a better alternative to plastic bags. For this purpose, the exotic plant species Eichhornia crassipes was selected, as it is known to invade perennial water bodies and disrupt aquatic ecosystems. The present work is a new report on isolating epicuticular biowax from this exotic plant and using it to produce hydrophobic paper.

Table 1. Yield of Epicuticular Wax from Leaves of Eichhornia crassipes Collected from Different Study Locations.

S. No.	Weight of Leaves Taken (g)	Weight After Wax Isolation (g)	Average Wax Yield (g)
1	10	8.02	1.98
2	10	8.78	1.22
3	10	7.83	2.17
4	10	9.08	0.92
5	10	9.32	0.68
6	10	9.135	0.865

Table 2. Physical Properties of the Isolated Epicuticular Wax.

S. No.	Properties	Inference
1	Colour	Dark tan brown
2	Odour	Unpleasant odour
3	Taste	Bitter
4	Viscosity	Moderately viscous
5	Boiling Point	51°C
6	Melting Point	42°C

Table 3. Reaction of Epicuticular Wax with Various Chemicals.

S. No.	Chemicals	Observations	Inference	Solubility
1	H_2SO_4	Effervescence	Dark tan brown	Soluble
2	HCl	No reaction	Brown	Soluble
3	KOH	Persistent froth	Pale brown	Soluble
4	NaOH	Froth	Pale brown	Soluble
5	CH_3COOH	No reaction	Pale brown	Soluble
6	$FeCl_3$	No reaction	Dark brown	Soluble

Table 4. Reaction of Heated Epicuticular Wax with Different Solvents.

S. No.	Solvents	Reaction	Inference	Solubility
1	Chloroform	No reaction	Brown	Insoluble
2	Benzene	No reaction	Brown	Insoluble
3	Acetone	No reaction	Pale brown	Partially soluble
4	Ethanol	No reaction	Pale brown	Insoluble
5	Petroleum ether	No reaction	Brown	Soluble

No previous studies have been published on this particular plant species for such an application. In addition to its hydrophobic or water-resistant nature, the major advantage of the product is that it is completely biodegradable due to its organic origin, making it environmentally safe. The naturally derived biowax coating exhibited good stability and proved to be a better alternative to chemical-based synthetic plastic films. Plant epicuticular waxes are complex mixtures of long-chain aliphatic compounds, including primary alcohols, aldehydes, fatty acids, and alkyl esters (Walton *et al*., 1990). Similar studies have been carried out on waxes from various plants such as Rosa canina, banana, carnauba palm, candelilla, Colocasia esculenta (taro), Calotropis procera, Alstonia scholaris, Sorbus domestica, Cotoneaster granatensis, and Euphorbia antisyphilitica (Takashi *et al*., 2005; Wang *et al*., 2018;

Buschhaus *et al*., 2007; Ganeva *et al*., 2015; Akash and Nayan, 2018; Lourdes *et al*., 2019; Yadav *et al*., 2014; Pompa *et al*., 2009). The wax was isolated from E. crassipes using organic solvent extraction with a Soxhlet apparatus and shaker, employing petroleum ether and chloroform as solvents. Table 1 shows the quantitative analysis of biowax isolated from E. crassipes collected from different localities of the Bharathapuzha River at various time intervals. On average, 10 g of leaf material yielded approximately 0.1305 g of wax.

Table 5. Effect of Heat on Isolated Epicuticular Wax at Different Time Intervals.

S. No.	Time Interval	Colour	Odour	Taste	Viscosity	Hydrophobicity	Integrity
1	Control	Dark tan brown	Unpleasant odour	Bitter	Viscous	Hydrophobic	No change
2	1 hr	No change	Reduced odour	No change	No change	No change	No change
3	2 hr	No change	Reduced odour	No change	No change	No change	No change
4	4 hr	No change	Reduced odour	No change	No change	No change	No change
5	6 hr	No change	Reduced odour	No change	No change	No change	No change

Table 6. Wax Coating Efficiency on Different Types of Paper at Various Time Intervals.

S. No.	Paper Type	Control	2 hrs	4 hrs	6 hrs	8 hrs
1	Chart paper	0.128	0.1365	0.136	0.1445	0.1415
2	Tissue paper	0.018	0.023	0.025	0.024	0.024
3	Filter paper	0.044	0.0595	0.052	0.060	0.066
4	Normal paper	0.044	0.0535	0.058	0.047	0.0465
5	News paper	0.041	0.0465	0.0465	0.0485	0.049

Table 7. Wax Coating Efficiency on Papers of Different GSM at Various Time Intervals.

S. No.	GSM Paper	Control	2 hrs	4 hrs	6 hrs	8 hrs
1	60	0.058	0.0625	0.0645	0.0655	0.0625
2	80	0.064	0.076	0.0775	0.0735	0.0735
3	100	0.094	0.0975	0.0995	0.100	0.0975
4	120	0.123	0.128	0.125	0.1265	0.128
5	150	0.177	0.1835	0.180	0.1815	0.1875

Table 8. Hydrophobicity of Wax-Coated Papers Before and After Water Soaking.

S. No.	Paper Type	Before (g)	After (g)	Change
1	60 GSM	0.120	0.126	No change
2	80 GSM	0.189	0.192	No change
3	100 GSM	0.230	0.232	No change
4	120 GSM	0.309	0.310	No change
5	150 GSM	0.403	0.409	No change
6	Normal paper	0.186	0.186	No change
7	Chart paper	0.292	0.302	No change
8	Tissue paper	0.086	0.090	No change
9	News paper	0.159	0.172	No change
10	Filter paper	0.234	0.245	No change

Table 9. Biodegradability of Wax-Coated Papers Under Different Chemical and Soil Conditions Over Time.

S.No.	Environment	1st Day	3 Days	6 Days	9 Days
1	Sulphuric acid	0.292	1.461	2.044	1.800
2	Sodium hydroxide	0.403	0.557	0.617	0.510
3	Acetic acid	0.120	1.570	0.101	0.080
4	Ammonium hydroxide	0.159	0.177	1.309	0.160
5	Phenol	0.186	0.888	1.574	1.830
6	Petroleum ether	0.309	0.397	0.352	0.310
7	Ethanol	0.081	0.192	0.116	0.350
8	Soil	1.159	0.234	1.036	0.910

Epicuticular wax was extracted using various solvents such as n-tetracosane, chloroform, ethanol, methanol, benzene, and acetone (Buschhaus *et al*., 2007; Yadav *et al*., 2014). Among these, chloroform yielded the highest wax quantity (0.1305 g). The present findings are consistent with the observations of Yadav *et al.* (2014), Prema Chandra *et al.* (1993) and Cordeiro *et al.* (2011), who reported that wax yield varies depending on the nature of the solvent, plant species, and plant parts used. The current study clearly demonstrates the variation in wax quantity extracted using different solvents. The isolated wax was confirmed by a standard confirmatory test, in which the waxy substance was treated with ethanol and heated. The appearance of a pale brown residue confirmed the presence of wax (Akash *et al.,* 2018). Buschhaus *et al.* (2007) further suggested that epicuticular wax yield can be enhanced through adhesive treatment combined with solvent extraction. The physical properties of the isolated wax, such as color, odor, taste, viscosity, boiling point, and melting point, were assessed and are presented in Table 2. The reactions of the waxy substance with different chemicals—including strong and weak acids, strong and weak bases, and certain organic acids and bases were also evaluated (Tables 3 and 4). Similar studies were reported by Lourdes *et al.* (2019), who examined solubility and denaturation behavior of isolated waxes in solvents such as chloroform, benzene, acetone, ethanol, and petroleum ether. To determine heat sensitivity, the epicuticular wax was subjected to heat treatment at different time intervals ranging from 1 to 6 hours. The results indicated no observable change except for a reduction in odor intensity compared to the control sample (Table 5). The biowax was also evaluated for its hydrophobic properties by exposure to high temperatures ranging from 80°C to 90°C. These characteristics make the biowax of E. crassipes suitable for coating paper surfaces to make them hydrophobic. The resulting hydrophobic papers could serve as biodegradable substitutes for conventional paper bags.

The coating ability of the wax was tested using the paper disc method. Fifty Whatman filter paper discs (No. 1) were prepared and coated, revealing that approximately 24.7% of the wax adhered to the disc surface. Further, wax coating was tested on various types of paper with different GSM values to evaluate coating efficiency. The coated papers were observed at intervals of 2, 4, 6, and 8 hours. Only slight variations were noted in the coating thickness over time (Table 7). The hydrophobicity of the wax-coated papers was tested before and after soaking in water using different types and GSM grades of paper. The results indicated no significant change in paper weight after soaking, confirming strong hydrophobic behaviour (Table 8). A biodegradability test was conducted over nine days using various chemical environments and soil. The samples were weighed at specific intervals to assess weight changes. The results indicated effective biodegradation in all environments, with the greatest degradation observed in soil, where sample weight decreased from 1.159 g to 0.91 g (Table 9). The study exhibits wax extraction and characterization to hydrophobicity and biodegradability testing-demonstrated that the epicuticular wax isolated from *E. crassipes* possesses desirable wax-like properties suitable for coating paper surfaces. The resulting wax-coated papers present a promising eco-friendly substitute for plastic-coated papers and plastic bags.

CONCLUSION

The present work has been a study, isolate and evaluate the biowax of plant origin and to convert it as one of the important useful fundamental product, a water repellant paper which is completely biodegradable. The study was planned to isolate the epicuticular wax from the leaves of *Eichhornia crassipes*. The study plant was selected due to its availability throughout the year and considered to be a big menace over many perennial water bodies and also a threat to native plant species. Hence in order to eradicate the in a useful manner the study was made to isolate the epicuticular wax of the plant and applied on the various papers. As per the standard procedures the process of Wax isolation was done by using the Chloroform as solvent. The physical and chemical parameters were analyzed for the isolated bio-wax isolated were analyzed and a heat sensitivity test was carried out to evaluate the properties of the wax. The wax coating applied on the papers was found well coated. The reaction of the coated paper were also tested notably with important strong acids, alkalis, and other important solvents. There is no much change was observed except the sulphuric acid. The wax coated papers were tested for its hydrophobicity to test the water repellent capacity. The wax coated paper exhibited a good water repellent capacity in the tested time intervals and the results also very satisfactory. The wax coatings were also given to different GSM papers and types of papers to test the ability of the wax that coats the paper and its integrity on the various papers. All the papers considerably accepted this epicuticular wax. These studies showed wide range of applicability on in the process of wax coating. Finally, the biodegradability test for bio-wax coated papers were done by using various acids, alkalis, solvents and soil. The change in the weight for Bio-wax coated papers was recorded in time intervals. The irregularity in the weight of the wax coated paper expresses that it has good degradability than other substances. Comparatively soil showed effective degradability when compared with acids and bases. The overall study concluded that produced biowaxes and hydrophobic papers have good quality and having good degradability without causing any hazardous to the environment. So in future the upgraded hydrophobic papers and bio-wax can be developed for producing ecofriendly paper bags and products. Further the toxicological studies and stability studies are should be carried with various assays and instruments. The present investigation will surely pave way for producing a promising substituting material than the plastic or polythene bags.

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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 declares 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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