

EVALUATION OF ANTIBACTERIAL AND INSECT REPELLENT ACTIVITY OF CARBON NANOPARTICLES DERIVED FROM *Acorus calamus* RHIZOME

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ABSTRACT

The *Acorus Calamus rhizome* powder was incinerated and subjected to ultra-probe sonication to generate carbon nanoparticles. Their dynamic light scattering analysis verified the formation of carbon nanoparticles, and the tuned carbon nanoparticles were further characterised by UV-Visible absorption and photoluminescence spectroscopy. Both dry powder (D) and incinerated powder (I) were prepared and tested for antibacterial activity in aqueous medium, mother milk and insect-repellent activity using model plant species. The antibacterial activities against Gram-negative (*E. coli*) and Gram-positive bacteria (*S. aureus*) were evaluated through an MIC assay following the resazurin test. The MIC of the powdered rhizome incinerated in mother's milk was 0.62 mg/mL, indicating better antibacterial activity than the dry preparation under the tested conditions. The leaves of *Combretum indicum* and *Hibiscus rosa-sinensis* coated with the carbon nanoparticles from incinerated *A. calamus* powder exhibited comparatively less injury by insects. All these findings confirm that *A. Calamus*-derived carbon nanoparticles can be considered a material for further investigation as an eco-friendly source for antibacterial and insect-repellent applications. Results have been obtained, which serve as a basis for future investigations on their mechanism of action, safety, stability and suitability for practical applications. The research aligns with SDG 3 – good health and well-being, through its focus on exploiting the natural (botanical) resources to combat microbial issues.

Keywords: *Acorus calamus* Rhizome, Incineration, MIC, Antibacterial Activity, Insect Repellent Activity.

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INTRODUCTION

The increasing occurrence of bacterial infections and the growing concern regarding antimicrobial resistance (AMR) have created an urgent need for effective, safe, and sustainable antimicrobial materials. Conventional antimicrobial agents are potential contributors to the spread and emergence of resistant microorganisms when used inappropriately and excessively, which has great implications for infection control. Therefore, researchers have tried to find other antimicrobial agents from different sources in nature and to synthesise various materials having greater biological activity. Due to their small size, high specific surface area, high surface reactivity and the capability of tuning their physicochemical properties, nanomaterials have garnered much attention in recent years.¹ In addition, biomass-based and plant-based materials for developing functional nanomaterials have attracted considerable interest, thus adding to the push for sustainable development of nanomaterials.^{2,3} The optical, catalytic, surface and antimicrobial properties of these materials, along with their potential application in biomedical, environmental and sensing systems, fuel renewed interest in them. SWEET FLAG (*Acorus calamus* L.) is an aromatic semi-aquatic herb with a creeping rhizome. The plant has been used traditionally to treat various conditions, and it was reported to harbour multiple bioactive phytoconstituents with various biological properties.⁴ Cultivation of rhizome-based *A. calamus* as a renewable plant-derived raw material for functional material development will be supported by the discoveries described above about antimicrobial characteristics and biologically active plant constituents.^{5,6,7} Overall, the research on *A. calamus* rhizome exhibits its potential as a source of biologically active compounds and suggests future applications for functional material development derived from a natural resource. Nanotechnology has further enhanced the possible use of *A. calamus*. Some recent studies have proved that *A. calamus* can be integrated within various interfaces of nanoparticles.^{8,9,10} The intersection of *A. calamus* and nanotechnology, for instance

in nano-encapsulation approaches, highlights the emerging applications of the plant rhizome and the feasibility of using it as a source of biologically relevant nanoscale materials.^{11,12} Green-synthesised silver and zinc nanoparticles prepared using *A. calamus* rhizome extract have been investigated against *Sitophilus oryzae*, underscoring the potential of plant-mediated nanoparticles for insect-management applications.¹³⁻¹⁶ Overall, these studies illustrate the expanding interest in utilising *A. calamus* rhizome for the synthesis and investigation of functional nanoparticles with relevance across various fields. Besides extract-based and metallic nanoparticle systems, *A. calamus* has also been assessed to obtain carbon-rich materials. The production of functional carbon-based materials from *A. calamus* biomass would show promise in the following fields: Nathaniel et al. investigated the use of biochar produced from *A. calamus* consumed in nitrogen removal and wastewater treatment.¹⁷ Green and sustainable synthesis of nanoparticles has been a field of recent research, with studies pointing out the importance of renewable biological precursors for activated carbon production, such as *A. calamus*, to develop functional nanomaterials.¹⁸ Recent research has also underscored the increasing significance of carbon dots and related carbon-based nanomaterials, focusing on their physicochemical and biological properties and their possible applications in biomedical and sensing fields.^{19,20} Earlier, all such studies were mainly done on the extract of *A. calamus*, metallic nanoparticles, nanoencapsulated extract of *A. calamus*, biochar/activated carbon, depending upon the antimicrobial, insecticidal, insect repellent or nanoparticle/carbon activities, respectively.²¹⁻²³ In comparison, few studies have investigated carbon nanoparticles derived directly from *A. calamus* rhizome and employed for both antimicrobial and insect-repellent activities. So, further studies are needed to obtain multifunctional biological activity through carbon nanoparticles obtained from this plant. The present study fills this lacuna by preparing carbon nanoparticles from the powder of the rhizome of *A. calamus* through incineration and ultra-probe sonication and assessing the antibacterial and insect-repellent activity of carbon nanoparticles. The use of *A. calamus* rhizome is also related to its traditional use in certain parts of southern India. The rhizome used in traditional medicine has been burnt with an oil lamp, and its powder mixed with water or mother's milk has been used in the treatment of infantile colic. The present study investigates the antibacterial efficacy of mother's milk containing the rhizome material based on this traditional practice. Dry powder (D) and incinerated powder (I) were formulated and tested for biological activity to study the effect of processing on its biological activity. Antibacterial activity was tested against Gram-negative bacteria (*E. coli*) and Gram-positive bacteria *Staphylococcus aureus*; (*S. aureus*) using a Resazurin-based (MIC) method. Further, the ability of the carbon material obtained from *A. calamus* to repel insect was also studied in model plant species *Combretum indicum* and *Hibiscus rosa-sinensis* by observing the formation of pores on the leaves by insects. Further, the experiments were extended to use incinerated *Acorus calamus* powder as a source of carbon nanoparticles on the leaves of model plant varieties that showed considerable efficacy in blocking insect-induced poration. The whole synthesis and application process of the synthesised carbon nanoparticles from *Acorus calamus* is summarised in the scheme as presented in Fig.-1.

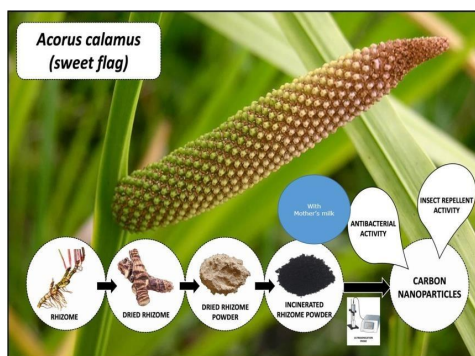


Fig.-1: Schematic Diagram of AC-carbon Nanoparticle Synthesis and Application

Significance of the Proposed Study

The present study also has relevance to (SDG 3), good health and well-being. The study on the potential of plant-derived products in the fight against bacteria is still significant in light of the growing issues of

bacterial resistance to antimicrobial treatments. The study examines a renewable biological resource, *A. calamus*-derived carbon nanoparticles, and their antibacterial activity against Gram-positive and Gram-negative bacteria and could possibly have health-related applications. But the results are preliminary and laboratory-based, and further research on toxicity, biocompatibility, stability, mechanism of action and safety is required before practical or biomedical applications can be considered.

Contributions of the Study

The main contributions of the present study are as follows:

Preparation of carbon nanoparticles from rhizome powder of *A. calamus* (incineration and ultra-probe sonication) was used as a plant-derived carbon nanoparticle preparation. Material characterisation: (DLS), UV-Visible absorption spectroscopy, and (PL) analysis of the prepared carbon nanoparticles were performed to examine the nanoscale and optical properties of these materials. Antibacterial testing: Antibacterial testing of the developed materials was conducted with Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus* using the resazurin-based MIC assay. Evaluation in mother's milk – The evaluation was done regarding the use of incinerated rhizome material in mother's milk as an antibacterial agent in relation to the traditional use of *A. calamus* preparations in infantile colic. Assessment of the insect-repellent potential: The insect-repellent efficacy of the formulated material for its ability to inhibit insect-induced pores in *C. indicum* and *H. rosa-sinensis* was checked. Multifunctional application potential: Since both the antimicrobial and insect-repellent properties were studied, the evidence showed the potential of *A. calamus*-derived carbon nanoparticles as a multifunctional plant-based material. Microbial control using a renewable material derived from plants relates to SDG 3: Good Health and Well-Being. The study is useful for SDG-3 as it examines an environmentally friendly substance derived from plants that may have antimicrobial properties and an alternative strategy to tackle the problem of microorganisms. Meanwhile, using a renewable plant source helps with the development of sustainable functional materials in general. The results served as a basis for further research into the mechanism of action of the antibacterial and insect-repellent activity, toxicity, safety, long-term stability, formulation, and potential uses in practical antimicrobial and plant-protection investigations.

EXPERIMENTAL

Materials and Methods

Acorus calamus rhizome was obtained from a local store. The solvents ethanol, acetic acid, and DMF were of analytical grade and obtained from SRL Chemicals Pvt. Ltd., India and used without any further purification. Double-distilled water was used throughout the study.

Preparation of Carbon Nanoparticles from *Acorus calamus* Rhizome

The process of preparing the carbon nanoparticles from *Acorus calamus* rhizome powder involved the following steps. Initially, the dry rhizome was carefully cut into small pieces and then ground in a mixer. Subsequently, to ensure a finely powdered consistency, it was further ground using a mortar and pestle. The next step in the experimentation process involved incineration in a muffle furnace at 250° C for 15 minutes, which was executed by first heating the dry rhizome within a lamp containing Gingely oil. Two distinct powders were obtained from this process, labelled as 'D' for the dry rhizome powder and 'I' for the incinerated counterpart. These powders were then transformed into solutions individually by dissolving 1 g in 10 mL of double-distilled water. After adding the water, the mixture was placed on a magnetic stirrer overnight to ensure thorough dispersion. The solution was then subjected to further refinement using an ultra-probe sonicator operating at 500 Hz. The resulting solutions, prepared through the above-mentioned techniques, were subsequently carried out for characterisation. The preparation of dry and incinerated *Acorus calamus* rhizome powder is illustrated in Fig.-2.

Characterization Techniques

The formation of carbon nanoparticles from the incinerated AC rhizome was confirmed through DLS measurements, which were conducted using a Horiba Scientific SZ 100 instrument at a temperature of 25 °C. This method allowed for the determination of the size distribution of the nanoparticles formed during the incineration process. The incinerated rhizome powder was subjected to FTIR using ThermoFischer

Scientific model to confirm the presence of β -asarone, an important component that is responsible for its medicinal properties.

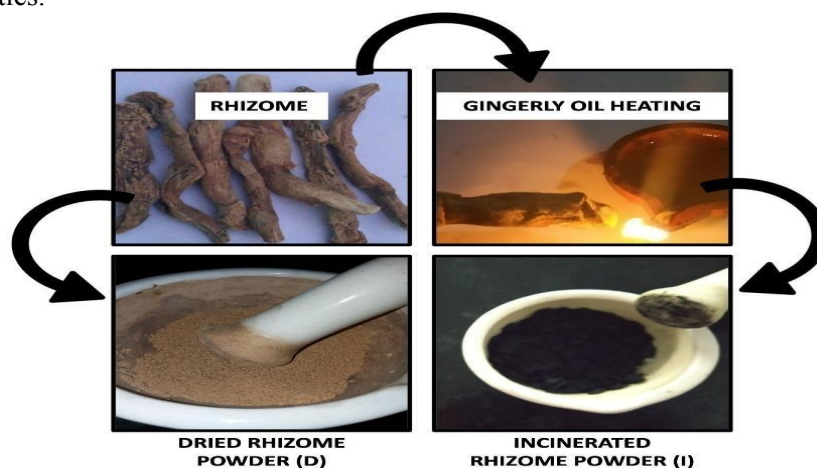


Fig.-2: Preparation of Dry and Incinerated Powder from *Acorus calamus* Rhizome

Subsequently, the carbon nanoparticles from incinerated AC rhizome powder were subjected to fluorescence property analysis, which involved measuring their absorption and emission spectra. The UV-Visible spectra were recorded using a model CARY 60 Agilent spectrophotometer, while the Photoluminescence signal was captured using the Flouromax_PLUS_CP011-7D0516 FMPLUS R928P instrument. Notably, both absorption and emission spectra were recorded in four different solvents, including (i) H₂O, (ii) DMF, (iii) DMF + EtOH, and (iv) DMF + EtOH + Acetic Acid.

Antibacterial activity: Determination of MIC

The MIC is a crucial parameter, representing the smallest amount of an antimicrobial substance, such as antibiotics, antifungals, or bacteriostatic agents, required to prevent observable growth of a microorganism following an overnight incubation period. To prepare the resazurin solution, 270 mg of the compound was dissolved in 40 mL of sterile distilled water, ensuring thorough dissolution and homogeneity using a vortex mixer. The MIC testing was conducted under aseptic conditions in a 96-well plate. Each well of the sterile plate was appropriately labelled. Initially, 100 μ L of the sample was pipetted into the first well, while 50 μ L of nutrient broth was added to all other wells, followed by serial dilution. Subsequently, 10 μ L of the resazurin indicator solution was introduced into each well, along with 10 μ L of the bacterial suspension. To prevent dehydration, each plate was loosely covered with cling film and then incubated at 37 °C for 18–24 hours. Visual inspection was employed to identify any colour change, particularly from purple to pink or colourless, which was recorded as a 'positive indication'. MIC was referred to as the minimum concentration at which there is no visible bacterial growth and which is signified by the lack of a colour change caused by the presence of live bacteria, usually through resazurin. In the current research, the MICs of both dry (D) and incinerated (I) rhizome powder were determined in aqueous medium as well as in the presence of mother's milk, focusing on their effectiveness against *E. coli* and *S. aureus*.

Insect Repellent Activity

Insect repellent activity was investigated through a process involving the use of carbon nanoparticles derived from AC rhizome powder. These nanoparticles were meticulously dispersed in water and subjected to a process of sonication to ensure their effective distribution. The resulting nanoparticle solution was then consistently sprayed and monitored for 25 days onto two distinct plant species, *Combretum indicum* (commonly known as Rangoon Creeper or Irangun Mali) and *Hibiscus rosa-sinensis*.

RESULTS AND DISCUSSION

The dynamic light scattering method confirmed the existence of carbon nanoparticles whose average diameter was 79.2nm, as shown in Fig.-3. The PDI of 0.556 suggests that there is great uniformity among

the particles in the water solvent. The PDI of 0.556 indicates a high level of particle uniformity in the water solvent.

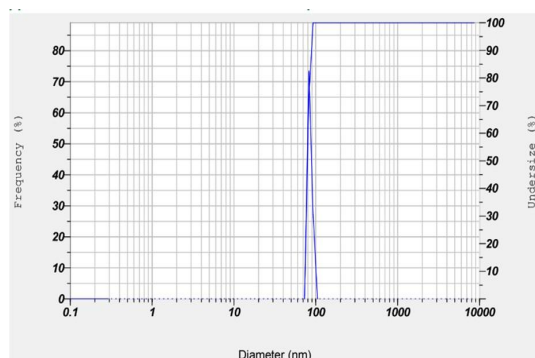


Fig.-3: DLS Histogram of Carbon Nanoparticles for Particle Size Measurement

The FTIR spectrum of the burned *A. calamus* rhizome powder is presented in Fig.-4. From the IR spectrum, there was evidence of the existence of the C-H stretch of the methyl group (2939 cm^{-1}), aromatic ring C-C stretch (1559 and 1377 cm^{-1}), and C-O-C stretch (1107 cm^{-1}).

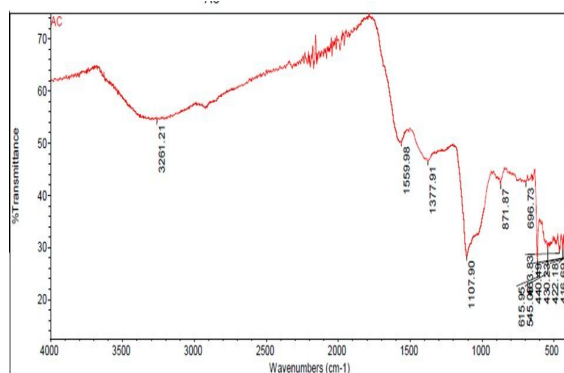


Fig.-4: IR Spectrum of Incinerated AC Rhizome Powder

The UV-Visible absorption and photoluminescence spectra of the *A. calamus*-derived carbon nanoparticles are presented in Fig.-5 in various solvents: (a) Water, (b) DMF, (c) DMF + Ethanol, and (d) DMF + Acetic acid. The UV-visible absorption and Photoluminescence data for these solvents are as follows: (a) In Water – absorption peak at 204 nm, emission peaks at 452 and 443 nm. (b) In DMF – absorption peak at 800 nm, emission peaks at 449, 453, and 455 nm. (c) In DMF + Ethanol – absorption peak at 799 nm, emission peaks at 442, 454, and 449 nm. (d) In DMF + Acetic acid – absorption peak at 205 nm, emission peaks at 443 and 445 nm. The outcome shows the effect of the solvent on fluorescence behaviour. In polar protic solvents, such as H_2O , the emission spectrum shifts to longer wavelengths, whereas in polar aprotic solvents like DMF and DMF + EtOH, it shifts towards shorter wavelengths. A similar shift towards shorter wavelengths is observed when DMF is mixed with the less polar acetic acid.

Antibacterial Activity

The lowest concentration of *Acorus calamus* rhizome powder that reduced the growth of bacteria was obtained using the MIC. The detailed growth-inhibition observations at the concentrations studied are given in Table-1, and a comparison of the corresponding MIC values is provided in Fig.-6. The rhizome powders were both Dry (D) and Incinerated (I); both powders have the same MIC (10 mg/mL) and the same bacteria, that is, *S. aureus* and *E. coli*. When the powders were applied in mother's milk, a significant increase in antibacterial effect was seen. The dry powder had an MIC of 1.25 mg/mL, which was further reduced to 0.62 mg/mL for the incinerated powder. Therefore, the incinerated preparation was the most effective in the tested antibacterial activity. The antibacterial activity of the incinerated preparation in mother's milk is increased due to the lower MIC value compared with the dry sample under the tested conditions.

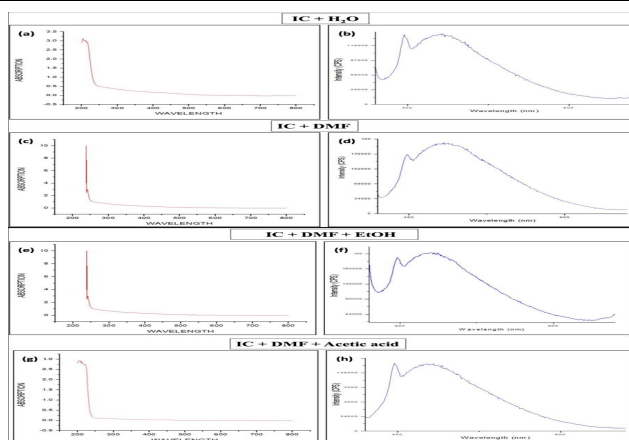


Fig.-5: UV-Visible Spectrum (left) and Emission Spectrum (right) of Carbon Nanoparticles Derived from Incinerated AC Powder with different Solvents

Both the preparation condition and the test medium may affect the antibacterial response, as evidenced by this difference. The data indicate the preliminary potential of the material prepared by using these incinerated *Acorus calamus* as an antibacterial preparation, but need to be followed by additional studies to determine its mechanism of action, reproducibility and safety. The quite low MIC value of the incinerated preparation in mother's milk suggests an increased antibacterial activity under the tested condition when compared with the dry one. The difference indicates that the antibacterial response might be affected by the two factors (preparation condition and test media). The results offer preliminary proof for the use of the incinerated *Acorus calamus* in the preparation of an antibacterial agent.

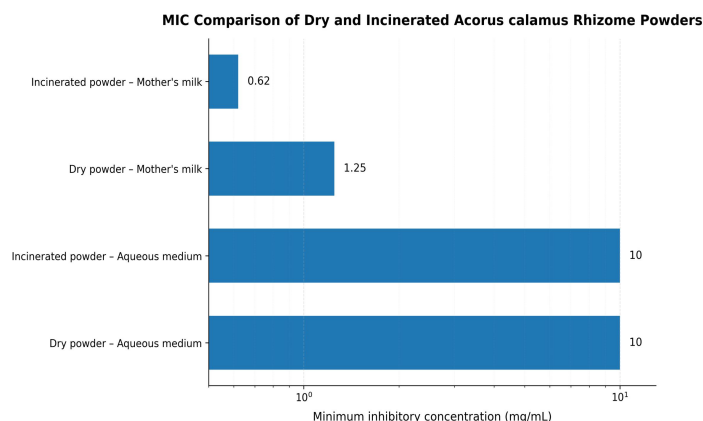


Fig.-6: Visual Determination of the MLD of Dry and Incinerated *Acorus calamus* rhizome Powders against *E. coli* and *S. aureus* in Aqueous Medium and Mother's Milk

Table-1: Comparison of MIC Values of Dry (D) and Incinerated (I) AC Rhizome Powder against *S. aureus* and *E. coli* in Aqueous Medium and Mother's Milk

S. No.	Sample	Growth inhibition										Culture
		10 mg/mL	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.62 mg/mL	0.31 mg/mL	0.15 mg/mL	0.07 mg/mL	std 10 µg	DMSO	
		<i>S.aureus</i>										
1	D (aq)	-	+	+	+	+	+	+	+	-	+	+
2	I (aq)	-	+	+	+	+	+	+	+	-	+	+
		<i>E.coli</i>										
3	D (aq)	-	+	+	+	+	+	+	+	-	+	+
4	I (aq)	-	+	+	+	+	+	+	+	-	+	+
5	D (M)	-	-	-	-	+	+	+	+	-	+	+
6	I (M)	-	-	-	-	-	+	+	+	-	+	+

Insect Repellent Activity

The insect repellent potential of the incinerated material of *Acorus calamus* rhizome was tested in model plant species *Combretum indicum* and *Hibiscus rosa-sinensis*. During the observation period, the treated leaves showed comparatively less damage due to insect infestation than the untreated leaves, as shown in Fig.-7. The lower amount of visible feeding damage indicates that the *A. calamus*-derived material might have a protective effect against the feeding damage by insects.²⁴ The present observation, however, presents a preliminary indication, and additional quantitative investigations are needed with controlled insect populations, dose-dependent treatment, feeding deterrence testing, and extended observations over a longer time period to substantiate its insect-repelling effect.

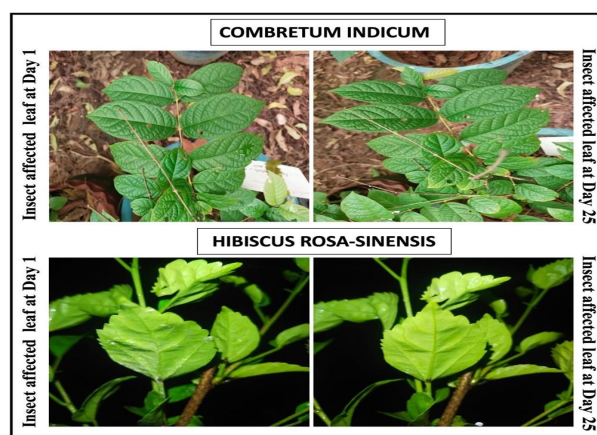


Fig.-7: Photographic Comparison of Control and Treated Leaves of *Combretum indicum* and *Hibiscus rosa-sinensis* on Day 1 and Day 25

Key Findings

The major outcomes of this research proved that the incinerated material of *Acorus calamus* exhibited better antibacterial properties than the dried preparation when tested under the experimental conditions. As demonstrated, the lowest MIC was recorded for the incinerated preparation in mother's milk, which showed good antibacterial properties. The treated *Combretum indicum* and *Hibiscus rosa-sinensis* leaves also exhibited less insect damage as compared to the untreated leaves. All these data support the possibility of the plant-based material developed to be explored in antimicrobial and plant-protection uses.

Contribution of the Developed Technology to SDG-3

The technology developed using CAC for the manufacture of carbon nanoparticles can be useful for SDG 3, which is related to the promotion of good health and well-being. The antibacterial evaluation revealed that the dry and incinerated *A. calamus* rhizome preparations demonstrated antibacterial activity against *S. aureus* and *E. coli* using mother's milk as a medium, leading to reduced MIC values, especially for the incinerated preparation. The results suggest the developed plant-based material can form a foundation for a sustainable antimicrobial strategy. The use of a naturally derived material also contributes to the overall goal of finding alternative sources for antimicrobial research. The findings of the current study, however, should not be interpreted as clinical suitability and are considered to be zebrafish laboratory-level evidence. For biomedical or infant-related applications, further investigations are needed to assess the cytotoxicity, biocompatibility, dose optimisation, stability, antibacterial action mechanism and long-term safety. Therefore, the present technology is contributing to the research aspect of SDG-3 through investigating plant-derived material which may be used to carry out its antimicrobial property.

CONCLUSION

The present study aimed to synthesise and assess carbon nanoparticles derived from *Acorus calamus* rhizome by incineration and ultra-probe sonication technique. The prepared formulations exhibited antimicrobial activity against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. MIC of dry and incinerated preparations in aqueous medium was 10 mg/mL, while in the mother's milk it was 1.25 mg/mL and 0.62 mg/mL, respectively. The lowest MIC value recorded for the incinerated

preparation shows that its antibacterial effect under the tested conditions improved. Besides, the leaves of the treated *Combretum indicum* and *Hibiscus rosa-sinensis* plants also showed relatively less visual injury caused by insects compared with untreated control plants, suggesting a preliminary level of insect repellency. The results prove that the carbon nanoparticles prepared from *A. calamus* could be used for antibacterial activity and plant protection and might be developed as a plant source material. Further, the study also addresses SDG 3: studying an alternative plant-derived material with potential antimicrobial properties. However, the current findings are based on preliminary laboratory-level observations and have not been determined to be suitable for clinical use, medication, or use in infants. Additional investigations are needed to assess for toxicity, cytotoxicity, biocompatibility, stability, dose optimisation, mechanism of antibacterial activity, and reproducibility. Also, there is a need for insect-repellent experiments under control conditions and with a better-defined insect population and quantitative measurements of feeding damage. The investigations will contribute to assessing the safety, effectiveness and suitability of the material developed for specific future usage.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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