Cinnabarinic acid from *Trametes coccinea* fruiting bodies exhibits antibacterial activity through inhibiting the biofilm formation

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Received: 3 September 2021 / Revised: 18 January 2022 / Accepted: 25 January 2022 / Published online: 15 February 2022 © The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract
Wild mushrooms are rich sources of natural compounds with potent bioactive properties. Several important metabolites have been reported from mushrooms, which possess clinically important bioactive properties like antibacterial, anticancer, antidiabetic, and neuroprotective activity. In this study, we have evaluated the antimicrobial activity of *Trametes coccinea* fruiting body extracts against different bacterial isolates, viz., *Bacillus subtilis*, *Bacillus cereus*, and *Escherichia coli*. Fruit-bearing bodies of three *T. coccinea* samples, of which two were collected from Santipur, Arunachal Pradesh and one collected from Jorhat, Assam, were used for extraction using methanol. The extracts showed significant antimicrobial activity against all the test bacteria. Minimum Inhibitory Concentration (MIC) of the extracts against *Bacillus subtilis*, *Bacillus cereus*, and *Escherichia coli* was recorded as 400 µg/ml, 400 µg/ml, and 300 µg/ml, respectively. Furthermore, the bioactive compounds of the extract were separated and detected using Thin Layer Chromatography (TLC). Presence of cinnabarinic acid (CBA)—a potent antimicrobial compound—was detected in TLC, which was further confirmed through High Performance Liquid Chromatography (HPLC) and Electrospray Ionization-Mass Spectrometry (ESI-MS). Cinnabarinic acid was able to inhibit the formation of biofilms in *Bacillus subtilis* and *B. cereus*, suggesting that the compound can be beneficial in the management of biofilm-based antimicrobial resistance.

Keywords Antimicrobial · Bioactive metabolites · Biofilm formation · Cinnabarinic acid · *Trametes coccinea*

Introduction
Antibiotics have gained place among the most important medical interventions needed for the treatment of complex clinical causalities. Till the discovery of penicillin in 1929, several antibiotics have come into the scenario for combating infectious diseases (Smaïnia et al. 1995). However, many of the antibiotics have failed to serve in persistent manner because of resistance mechanisms employed by several pathogenic bacteria. These mechanisms include enzymatic degradation or alteration of antibiotic molecules, mutational changes of the antibiotic target sites, decreased permeability of antibiotics inside the cells, efflux of antibiotic molecules from the cells, formation of protective layer such as biofilm, and many others (Munita and Arias 2016). Biofilm formation is one of the most effective and wide spread mechanisms used by several Gram-positive and Gram-negative bacteria to show resistance against common antibiotics. This mode of defense mechanism is associated with the chronic infections with their inherent resistance to antibiotic chemotherapy (Stewart and William Costerton 2001; Høiby et al. 2010). Therefore, investigations of new antimicrobials that can work for the inhibition of biofilm formations is becoming a needful strategy to combat antibiotic resistance in clinical cases.

Natural products from different natural sources such as microbes, plants, and animals have played a significant role in doubling the human life span during the twentieth century. Besides the use of wild mushrooms as nutritious foodstuffs since ancient time (Sharma and Gautam 2015), they have been a prime source of attraction due to their potential...
for biosynthesis of various secondary metabolites in their fruiting bodies. Many of these metabolites serve as bioactive components with different bioactive properties, some of which are clinically proven (Rai et al. 2005; Venturella et al. 2021). For instance, ganodermin and rufuslactone have been found to show potent antifungal properties (Luo et al. 2005; Wang and Ng 2006).

*Trametes coccinea* (also known as *Pycnoporus coccineus*), is one of the white-rot fungus (Basidiomycetes) belonging to the family Polyporaceae and the class Agaricomycetes of fungi, which most commonly grows in the outer layer of decaying woods and also found on the surface of deciduous trees (Zhang et al. 2020). This mushroom produces bright red colour pigments, which are deposited in the fruiting bodies. Few other species of *Trametes* including *T. sanguinea, T. cinnabarina,* and *T. punicea* also produce this type of pigments (Eggert 1997) (Téllez-Téllez et al. 2016). Few studies have characterized the pigments from *T. cinnabarina* (synonym. *P. cinnabarinus*) for their bioactive properties including the antibacterial activity. Cinnabarinic acid, cinnabin, tramesanguine, and few other phenoxazinone derivatives were characterized as bioactive metabolites of *T. cinnabarina* and *T. sanguinea* (Sullivan and Henry 1971; Eggert et al. 1996; Téllez-Téllez et al. 2016). However, scarce information is available for bioactive properties of metabolites isolated from *T. coccinea*. In this study, the pigmented fruiting body extracts of three *T. coccinea* samples were used to determine the antibacterial activity against *Bacillus subtilis, B. cereus,* and *Escherichia coli*. The active metabolites were also characterized using analytical chromatographic techniques.

## Materials and methods

### Microorganisms and culture condition

The bacterial isolates used in this study, viz., *Bacillus subtilis* SCB-1 (Accession no MF893336.1), *Bacillus cereus* S6 (Accession no MF187565.1), and *Escherichia coli* K-12, were obtained from the Microbial Technology Laboratory, Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat, Assam. All the test bacterial strains were regularly sub-cultured on Nutrient Agar (NA) medium (Himedia, India) for further use and maintenance.

### Source material

Fruiting bodies of three mushroom samples belonging to *Trametes coccinea* were used in this study. Fresh fruiting bodies of two samples viz. *Trametes coccinea* APS1 (GenBank Accession Number MK851556.1) and *T. coccinea* APS2 (GenBank Accession Number MK851557.1) were collected from Santipur, Arunachal Pradesh. Another sample *T. coccinea* F3 (GenBank Accession Number MK168589.1) was collected from Jorhat, Assam. Samples were identified based on their morphology and molecular data of ribosomal RNA internal transcribed spacer (ITS) region (Dullah et al. 2021; Kakoti et al. 2021).

### Preparation of extracts

For extraction of pigments from fruiting bodies, the samples were cut into small pieces and dried in a hot air oven at 45 °C for 6 h. Dry samples were powdered using an electric grinder and sieved through a 0.5 mm sieving net. A 1 g of fruiting body powder was extracted with 20 ml of methanol and supernatant was collected through centrifugation at 6000 rpm for 15 min. The residue was re-extracted twice with another 20 ml of methanol each time. The supernatant thus obtained were filtered through a 0.22 µm syringe filter (GE Healthcare, USA) and dried under vacuum. The concentrated extract was re-dissolved in dimethyl sulphoxide (DMSO) or acetonitrile at specific concentrations as per the further requirements.

### Evaluation of antibacterial activity

The DMSO-solubilized methanol extracts of the samples were prepared at different concentrations, viz., 1000, 500, 250, 125, 100, 75, 50, and 25 µg/ml for the assessment of antibacterial activity. Well-diffusion method was employed to study the inhibitory activity against 3 bacterial strains mentioned earlier. The bacterial strains were pre-grown in Nutrient Broth (NB) medium till OD of 1.0 and spread on to 90 mm Petri plates containing Mueller Hinton Agar (MHA). Wells were made (5 mm diameter) using a sterile well borer. Extracts at aforesaid concentrations were loaded into the respective wells of the plates. DMSO was used as negative control. Streptomycin (100 µg/ml) was considered as positive reference and tested against each bacterial strain in the similar way as the samples. The plates were incubated at 30 °C for 16 h. The presence of zone of inhibition around the wells indicated the positive antibacterial activity. The minimum concentrations at which the extracts showed detectable zone of inhibition were considered as minimum zone-forming concentration. The experiment was performed with three biological replicates for each bacterial strain.

### Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations were determined using the microdilution method in Luria Bertani (LB) broth. A 0.5 McFarland scale inoculum of each bacterial strain was prepared from 24 h old suspension culture. Mushroom
extracts at different concentrations were prepared and tested against the three bacterial isolates. The 5 ml culture broth consisted of 4.4 ml sterile LB broth, 0.1 ml standardized bacterial suspension culture, and 0.5 ml extract (with final concentrations in the range between 50 and 1000 µg/ml). The initial absorbance was noted at 600 nm and the culture tubes were incubated at 37 °C for 16 h with shaking followed by recording the final absorbance at the same spectrophotometric condition. MIC was determined as the last dilution of extract at and above which >90% inhibition of bacterial proliferation was recorded.

Metabolite production in solid culture medium

The in-vitro mycelial cultures of the mushroom samples were generated by inoculating on to potato dextrose agar (PDA) plates, which were then maintained at 28 °C for 7 days. Production of the red pigment was optimized in PDA medium supplemented with different concentrations of (0.005%, 0.01%, and 0.015%) guaiacol. Extraction of the pigment from the in-vitro culture plate of mushrooms was carried out by taking out the freshly grown mycelia from PDA plates in a conical flask containing 20 ml of methanol, followed by continuous shaking for 4 h, which was then sonicated at maximum intensity for 5 min. The pellet was separated through centrifugation and re-extracted twice with another 20 ml of methanol each time. The supernatant was processed to obtain the crude extract as described earlier.

Detection and identification of metabolites

The crude extract of each mushroom sample was further separated in a silica gel thin-layer chromatography (TLC) plate using benzene:ethyl acetate:glacial acetic acid (12:6:2) as mobile phase. Presence of cinnabarinic acid and phenoxazine, two important metabolites of *T. coccinea*, were detected in TLC using commercial standards (Sigma, USA). The *Rf* values were calculated from the TLC chromatogram. The fractions containing cinnabarinic acid and phenoxazine were extracted from the TLC plate and re-dissolved in acetonitrile for further analysis. The absorption spectrum of the purified fraction was generated using an Evolution 202 UV–visible spectrophotometer (Thermo Scientific, USA). Bioactivity of the TLC fractions was assessed at a concentration of ~200 µg/ml against *Bacillus subtilis, B. cereus*, and *Escherichia coli* as described earlier.

Furthermore, the crude extracts and purified bioactive fraction were loaded onto a Cosmosil C18 column using the autosampler of Hitachi Chromaster HPLC system. The samples were eluted with the mobile phase containing 2.5% acetic acid in water (A) and acetonitrile (B) in a gradient mode. The gradient profile was as follows: 20% B at 0–5 min, 50% B at 10 min, and 80% B at 15 min, continued with 80% B till 23 min, 20% B at 25 min, and continued till 30 min. The cinnabarinic acid and phenoxazine were used as standards, and the peaks were detected using a diode array detector in a scan mode from 200 to 400 nm. Concentrations of these two compounds in the crude extracts of APS1, APS2, and F3 were determined based on the regression curve prepared using different concentrations of the reference standards. The identity of cinnabarinic acid and phenoxazine corresponding fractions was further confirmed by LC–ESI–MS (Waters Corporation, USA) using the same chromatographic parameters. Mass of the selected compounds was analyzed based on positive mode of ionization in a Xevo Triple Quadrupole Mass Spectrometer (Waters Corporation, USA).

Biofilm inhibition assay

The inhibition of biofilm in *Bacillus subtilis* and *B. cereus* was assessed in the presence of different concentrations of bioactive pigment. The bacterial cells were grown in liquid medium till OD$_{600}$ 1.0 at 30 °C. A 10 µl of culture was added to different wells of a sterile 96-well microtiter plate. 170 µl of fresh liquid broth was added to each well containing bacterial culture. The TLC-purified active fractions (20 µl) prepared in DMSO were added at different concentrations. Instead of extracts, DMSO was used in the control wells. The plates were incubated at 30 °C for 24 h. The plates were stained with 250 µl of 0.1% crystal violet and biofilm production was estimated as described previously (O’Toole 2011).

Detection of damages in the plasma membrane:

The effect of cinnabarinic acid on the bacterial plasma membrane was assessed in *Bacillus subtilis* cells using BacLight™ bacterial viability kit (Life Technologies, USA). Bacterial cells pre-grown in LB broth up to 1.0 OD$_{600}$ were treated with 500 µg/ml of cinnabarinic acid purified from the sample APS1. The treated and untreated (no cinnabarinic acid) cells were incubated at 30 °C with continuous shaking, and the cells were observed under microscope at specific intervals (1 h, 2 h, 4 h, and 6 h) after staining with SYTO9 and propidium iodide as described earlier (Hazarika et al. 2021).

Effect of cinnabarinic acid on nucleic acid content

DNA isolation was performed from the cinnabarinic acid treated and untreated cells after 1 h, 2 h, 4 h, and 6 h of incubation using HiPurA bacterial genomic DNA isolation kit (Himedia, India) as per the manufacturer’s instructions. The isolated DNA samples were detected on a 1% agarose gel (Darshan and Manonmani 2016).
Results and discussion

Antibacterial activity of T. coccinea extracts

*Trametes coccinea*, synonym *Pycnoporus coccineus*, is a saprophytic white rod fungus belonging to the family Polyporaceae. This species and the related members of this genera are characterized by the presence of an annual sessile or effused-reflexed basidiocarp which is often pigmented (Lomascolo et al. 2011). Four species of this genus namely *Pycnoporus cinnabarinus*, *P. sanguineus*, *P. coccineus*, and *P. puniceus* were found to produce a typical red pigment which is deposited in the fruiting bodies, and due to this, the fruiting body turns into orange in colour. Except this characteristic feature, the rest of all morphological characters of the genus are similar to the genus *Trametes*, and therefore recent taxonomy have included these four species in to the genus *Trametes* (Welti et al. 2012).

In this study, the red pigment was isolated as crude extract using methanol. Here, the antibacterial activity of *Trametes coccinea* fruiting body extract was assessed against two Gram-positive bacteria, viz., *Bacillus subtilis* and *Bacillus cereus*, and one Gram-negative bacteria, viz., *Escherichia coli*. The extract showed significant inhibitory activity against the tested bacterial strains. The extracts of three mushroom samples were able to form visible zones of inhibition, as detected in the Mueller Hinton Agar plates. The photographs of plates showing zones of inhibition against the bacterial strains are shown in Fig. 1. However, the diameter of the zones of inhibition of the crude extracts at a concentration of 1000 µg/ml was smaller than that of pure streptomycin (100 µg/ml), the positive control used in this study. The minimum zone-forming concentrations of the three extracts against *Bacillus subtilis*, *B. cereus*, and *E. coli* are presented in Table 1. It was also observed that the extracts were effective at lower concentrations against Gram-negative bacteria (*E. coli*) than the Gram-positive bacterial strains (*B. subtilis* and *B. cereus*). Although, the three tested mushroom samples were collected from different places, but a very negligible amount of differences was recorded in the minimum zone-forming concentrations among all the three mushroom extracts. From this, it could be concluded that the active compound(s) present in the extracts of different samples of *Trametes coccinea* is the same and present in similar concentrations.

The MIC of the three extracts were also evaluated in the liquid culture against the test bacteria, which revealed that the extracts could inhibit more than 90% of bacterial growth at a concentration of 400 µg/ml against *B. subtilis* and *B. cereus*. Similarly, the growth of *E. coli* was also inhibited by more than 90% at a concentration of 300 µg/ml of extracts (Fig. 2). It was earlier reported that the red pigments from *Pycnoporus cinnabarinus* (synonym *Trametes...
cinnabarinus), an evolutionary close species to Trametes coccinea, possess antibacterial properties against Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Salmonella enteritidis, Staphylococcus aureus, and Streptococcus spp. The MIC of P. cinnabarinus extracts against these bacterial strains was recorded between 0.02 and 0.7 mg/ml (Eggert 1997). The pigments of Pycnoporus cinnabarinus has been reported to contain a major bioactive compound called as cinnabarinic acid, along with few analogues of this compound (Eggert et al. 1995; Eggert 1997). Cinnabarinic acid is a metabolite of the kynurenine pathway, which derives from two molecules of 3-hydroxyanthranilic acid through a condensation reaction (Fazio et al. 2012; Téllez-Téllez et al. 2016). Laccase-mediated production of the red pigments by mycelial cultures of Pycnoporus cinnabarinus has been established earlier (Eggert et al. 1995). Laccase catalyzes the oxidative dimerization of 3-hydroxyanthranilic acid in a 6-electron oxidation reaction (Eggert 1997). Our findings also suggested the production of similar pigments in the mycelial cultures of T. coccinea (Supplementary Figure S1).

It was further confirmed in our study that supplementation of guaiacol (an inducer for laccase production) enhances the pigment production in mycelial cultures, suggesting the involvement of laccase in the synthesis process. This enables an advantageous biosynthesis of cinnabarinic acid over chemical synthesis where hazardous or expensive catalysts are used thereby making the synthesis process nonrenewable and environmentally unfriendly (Jabri and Overman 2013). Recently, heterologous production of cinnabarinic acid has been described in a metabolically engineered Pseudomonas chlororaphis GP72 (Yue et al. 2019).

### Table 1

| Sample name | Test bacterial strain      | Zone diameter (± SE) at 1000 µg/ml (mm) | Minimum zone-forming concentration (µg/ml) |
|-------------|---------------------------|----------------------------------------|-------------------------------------------|
| APS1        | *Bacillus subtilis* SCB-1 | 14.00 ± 0.58c                          | 100                                       |
|             | *Bacillus cereus* S6      | 16.33 ± 0.67a                          |                                            |
|             | *Escherichia coli* K-12   | 15.33 ± 0.33b                          |                                            |
| APS2        | *Bacillus subtilis* SCB-1 | 12.33 ± 0.33d                          | 100                                       |
|             | *Bacillus cereus* S6      | 14.67 ± 0.33bc                         |                                            |
|             | *Escherichia coli* K-12   | 14.00 ± 0.58c                          |                                            |
| F3          | *Bacillus subtilis* SCB-1 | 13.67 ± 0.33c                          | 125                                       |
|             | *Bacillus cereus* S6      | 14.67 ± 0.33bc                         | 125                                       |
|             | *Escherichia coli* K-12   | 14.33 ± 0.33c                          | 100                                       |

Zone diameters were represented as mean ± standard error (SE) of three independent replicates. The letters in superscript denote the levels of significance determined by one-way ANOVA with Duncan’s multiple range test ($p \leq 0.05$) using SPSS 25.0.

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**Fig. 2** Minimum inhibitory concentrations of the fruiting body extracts against A *Bacillus subtilis*, B *Bacillus cereus*, and C *Escherichia coli*. The MIC was considered to be the concentration that inhibited >90% of the bacterial proliferation.
Detection and identification of bioactive metabolites

Cinnabarinic acid, a red pigmented derivative belonging to the group of phenoxazinone, is produced by a few *Trametes* (*Pycnoporus*) species. To confirm the presence of cinnabarinic acid and/or its analogues, the crude extracts of the mushroom fruiting bodies were separated in a TLC plate containing silica gel as stationary matrix. It was observed that the crude extract of sample APS1 was separated to 5 different fractions with $R_f$ values of 0.91, 0.83, 0.74, 0.65, and 0.32 (Fig. 3A). Likewise, APS2 and F3 showed 5 fractions (0.85, 0.75, 0.65, 0.33, and 0.17) and 6 fractions (0.85, 0.75, 0.65, 0.56, 0.33, and 0.25), respectively (Fig. 3B and C). The fractions with $R_f$ value 0.56 and 0.25 were exclusively detected in the sample F3, whereas fractions with $R_f$ value 0.17 were exclusive to sample APS2. While comparing with the standards of cinnabarinic acid (Sigma, USA) and phenoxazine (Sigma, USA), both the fractions were detected in sample APS1, APS2, and F3, suggesting that the two compounds were common active metabolites of the *T. coccinea*. It is noteworthy that the phenoxazine corresponding fraction was pale blue in colour, and therefore, the corresponding band was not clearly visible in the TLC plates. As cinnabarinic acid has been reported from other related species of *T. coccinea* that possess antibacterial activity (Eggert 1997), we assumed that this compound is responsible for the inhibitory activity against the Gram-positive and Gram-negative test bacterial strains. To confirm this, we tested the antibacterial activity of the major fractions in each sample after TLC separation. It was found that the cinnabarinic acid corresponding fraction contained significant antibacterial activity against the test bacterial strains, while the other fractions did not show significant antibacterial activity (Supplementary Fig. S2, Table S1).

To confirm the identity of cinnabarinic acid, we also considered the HPLC and ESI–MS data as evidence. The HPLC analysis revealed the presence of cinnabarinic acid in the crude as well as TLC-purified fraction, with a retention time of 14.1 min. The HPLC peaks of standards cinnabarinic acid (Sigma, USA) and phenoxazine (Sigma, USA), *T. coccinea* crude extracts from sample APS1 and APS2, along with TLC-purified bioactive fraction ($R_f$ value 0.33) are shown in Fig. 4A. Based on the regression curves prepared using different concentration of the reference standards, the concentrations of cinnabarinic acid and phenoxazine in the fruiting bodies were determined. Cinnabarinic acid was recorded between 15.4–19.6 mg/g of dry weight, while phenoxazine was found to be in the range of 8.1–13.4 mg/g dry weight (Fig. 4B). The molecular mass of cinnabarinic acid from *T. coccinea* was detected using ESI–MS which suggested the presence of cinnabarinic acid (m/z: 301.0 [M + H]$^+$) in

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Fig. 3 Thin-layer chromatographic separation of the fruiting body extracts of *T. coccinea*. A Different fractions present in the crude extracts of *T. coccinea* APS1; B different fractions present in the crude extracts of *T. coccinea* APS2; C different fractions present in the crude extracts of *T. coccinea* F3; D identification of cinnabarinic acid and phenoxazine corresponding fractions by comparing with the standards.
the TLC-purified fraction (Fig. 5A). The identity of phenoxazine present in the samples as common metabolites (TLC Rf value 0.83/0.85) was also confirmed by ESI–MS (m/z: 184.1 [M + H]+)(Fig. 5B); however, this fraction did not possess antibacterial activity.

Inhibition of biofilm production in Bacillus subtilis and B. cereus

Biofilm production is an important mechanism of antimicrobial resistance in many pathogenic bacteria. Biofilm production in bacteria is associated with several economic and environmental implications as well as medical complications. Recent strategies to overcome these problems focus on the use of antimicrobial agents that could target bacterial biofilm formation (Singh et al. 2017). Here, we performed the biofilm production assay to study the effect of cinnabarinic acid produced by T. coccinea on development of bacterial biofilms in B. subtilis and B. cereus. It was observed that a concentration of 500 µg/ml, cinnabarinic acid from APS1, APS2, and F3 could inhibit 95%, 87.7%, and 86.9% of biofilm formation, respective in B. subtilis compared to the control condition. Similarly, the same concentration was also found to be effective to inhibit (91.5–94.7%) biofilm formation in B. cereus (Fig. 6). As per our knowledge, no information is available regarding the bacterial biofilm inhibitory activity of phenoxazinone derivatives. Previously, a few natural compounds, including bacterial and fungal metabolites, have been reported to inhibit bacterial and fungal biofilms. For example, farnesol—a sesquiterpene group of quorum sensing molecule from Candida species—was reported to inhibit biofilm formation in Staphylococcus epidermidis, and pathogenic strains of Candida albicans (Ramage et al. 2002). In a separate study, it was that farnesol treatment could reverse the resistance of methicillin-resistant Staphylococcus aureus against methicillin by inhibition of biofilm production (Gomes et al. 2009).

Effect of cinnabarinic acid on cellular targets of Bacillus subtilis

To identify the potential targets of CBA in bacterial cells, the effects of CBA on the cellular membrane and nucleic acid content were assessed. Staining of the cells with SYTO9 and propidium iodide revealed that damages in the plasma membrane of bacterial cells were recorded after 4 h and 6 h of treatment with CBA. Propidium iodide could enter the bacterial cells after prolonged treatment, while during the initial periods (1 h and 2 h after treatment), the cells remained unaffected (Fig. 7A). This finding suggested that the damages in the plasma membrane were not the direct effect of CBA treatment; rather, it was a secondary effect due to cell death triggered by CBA treatment.
Furthermore, analysis of the DNA integrity revealed that CBA affected the DNA integrity on the agarose gel, which increased with the time of CBA treatment (Fig. 7B). This suggested a potent role of CBA on DNA damage, which may trigger programmed cell death in bacterial cells (Darshan and Manonmani 2016).

**Conclusion**

We established the *in-vitro* antibacterial activity of the fruiting body extracts of *Trametes coccinea*, a wood-rotting Basidiomycetes native to the South-East Asia. The red pigment from *T. coccinea* fruiting body, which was
identified as cinnabarinic acid, showed promising antimi-
crobial properties against Gram-positive *Bacillus subtilis*,
*B. cereus* and Gram-negative *Escherichia coli*. Cinnabar-
inic acid showed significant inhibition of bacterial biofilm
formation. As per our knowledge, this is for the first time
the inhibition of biofilm formation by cinnabarinic acid
has been demonstrated. Preliminary investigations on the
potential targets of cinnabarinic acid suggested its effects
on nucleic acids, which ultimately leads to bacterial cell
death. Further investigations will surely unveil the molec-
ular mechanism of biofilm inhibition by identifying the
potential targets of cinnabarinic acid in bacterial cells.

Acknowledgements The authors hereby acknowledge Science and
Engineering Research Board (SERB), Department of Science and
Technology, New Delhi for providing fund for the present study under
the project sanction no. EEQ/2016/000631. The authors are also
thankful to the Department of Agricultural Biotechnology and DBT
North-East Centre for Agricultural Biotechnology, Assam Agricultural
University, Jorhat, Assam for providing laboratory space and facilities
for conducting the present study.

Authors’ contributions RCB conceived the idea and acquired funding;
MK and RCB designed the study; MK and DJH performed the bioactiv-
ities, and prepared the figures; MK, SD, and DJH performed analytical
characterization and data analysis; MK prepared the draft manuscript,
MB and RCB provided technical supervision during experimental data
generation, analysis, and validation. All authors read and approved the
final manuscript.

Funding This study was funded by Science and Engineering Research
Board (SERB), Department of Science and Technology, New Delhi,
India, vide project sanction no. EEQ/2016/000631 without any influ-
ence over experimental design, findings, and data interpretation.

Availability of data and materials All the necessary data generated
through this study are either provided as electronic supplementary
information with this manuscript, or those will be available upon con-
ditional request.
Ethics approval Not needed.

Conflicts of interest The authors declare that they have no conflict of interest.

Ethics approval Not needed.

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