Cinnabarinic Acid from *Trametes Coccinea* Fruiting Bodies Exhibits Antibacterial Activity Through Inhibiting the Biofilm Formation

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Research Article

Keywords: Antimicrobial, Bioactive metabolites, Biofilm formation, Cinnabarinic acid, Trametes coccinea

Posted Date: September 16th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-876241/v1

Version of Record: A version of this preprint was published at Archives of Microbiology on February 15th, 2022. See the published version at https://doi.org/10.1007/s00203-022-02782-4.

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 activity, anticancer activity, antidiabetic activity and antioxidant 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*. Fruiting 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 potent 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. Further, 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.

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 (Smânia 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 20th century. Besides the use of wild mushrooms as nutritive food stuffs 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 many medicinal properties. Several important metabolites have been reported from mushrooms having different bioactive properties. Some of them are clinically proven to exhibit significant bioactive properties. For instance,

ganodermin and rufuslactone have been found to show potent antifungal properties (Luo et al. 2005; Wang and Ng 2006). Other bioactive metabolites from higher fungi includes ganoderic acid T, ganoderic acid F (antimetastatic effect), ganoderic acid Me (Anti-metastatic effect, reduce tumor invasion), ganoderic acid DM (anti-proliferative effect), lucidenic acid N, lucialdehyde B, lucialdehyde C (anticancer activity), 11β-Hydroxy-3,7-dioxo-5α-lanosta8,24(E)-dien-26-oic acid (Cytotoxic effect), Cortinellin (antibacterial, antifungal), sesquiterpenoid and hydroquinones (antibacterial activity), *trans*-cinnamic acid, photocattheuric acid, caffeic acid, gallic acid, ascorbic acid and benzoic acid (antioxidant activity) (Herrmann 1962; Wu et al. 2001, 2012; Gao et al. 2002; Tang et al. 2006; Dembitsky and Maoka 2007; Chen et al. 2008; Zhong and Xiao 2009; Xu et al. 2010; De Silva et al. 2013; Kozarski et al. 2015).

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 brightred colour pigments, which is 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, cinnabarin, tramesanguine and few other phenoxazinone derivatives were characterized as bioactive metabolites of *T. cinabarina* 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 of *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 6,000 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_{600} 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 ºC 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 was 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 – 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 R_f 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 fraction were assessed against *Bacillus subtilis*, *B. cereus* and *Escherichia coli* as described earlier.

Further, the crude extracts and purified bioactive fraction were loaded onto a Cosmosil C18 column using the auto sampler 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, 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 200-400 nm. The identity of cinnabarinic acid and phenoxazine corresponding fractions were further confirmed by LC-ESI-MS using the same chromatographic parameters. Mass of each compound was analyzed based on positive mode of ionization.

Biofilm inhibition assay:

The inhibition of biofilm in *Bacillus subtilis* and *B. cereus* was assessed in presence of different concentrations of bioactive pigment. The bacterial cells were grown in liquid medium till OD_{600} 1.0 at 30 ºC. A 10 µl of culture was added to different wells of a sterile 96 wells microtiter plate. 170 µl of fresh liquid broth was added to each well containing bacterial culture. The TLC purified active fraction (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 ºC 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).

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 basiodiocarp 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 in to 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 potent 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 were 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 were 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 same species is 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 cinnabarina*), an evolutionary close species to *Trametes coccinea*, possess antibacterial properties against *Bacillus subtilis, Escherichia coli*, *Klebsiella pneumonae*, *Salmonella enteritidis*, *Staphylococcus aureus* and *Streptococcus* spp. The MIC of *P. cinnabarinus* extracts against these bacterial strains was recorded between 0.02 – 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). 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, herterologous production of cinnabarinic acid has been described in a metabolically engineered *Pseudomonas chlororaphis* GP72 (Yue et al. 2019).

Detection and identification of bioactive metabolite:

Cinnabarinic acid, a red pigmented derivative belonging to the group of phenoxazinone, is produced by a few *Tramates* (*Pycnoporus*) species as a byproduct of kynurenine pathway, due to the condensation of two molecules of 3-hydroxyanthranilic acid (Téllez-Téllez et al. 2016). 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 was 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*. 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 (Supplementary Table S1). Spectroscopic analysis suggested that this fraction has an absorption maxima of 427 nm in the visible region of the spectrum (Supplementary Fig. S2).

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. 4. 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 the TLC purified fraction (Fig. 5A). The identity of phenoxazine present in the samples as common metabolites (TLC R_f 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). 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). Similarly, bacterial pigment prodigiosin (an alkaloid) could inhibit biofilm production in *Pseudomonas aeruginosa* (Kimyon et al. 2016). There are several possibilities through which biofilm production is inhibited. Some antibiotics targets bacterial exopolysaccharide production mechanisms, thereby inhibiting the biofilm production. A few antibacterial compounds (for e.g., prodigiosin) targets bacterial DNA of the target cells and induce reactive oxygen species (ROS) production, which ultimately leads to the inhibition of bacterial biofilm production and cell death (Kimyon et al. 2016). Prodigiosin also cause damages to the plasma membrane of Gram-positive bacteria and show antimicrobial properties (Suryawanshi et al. 2017). It was reported earlier that cinnabarinic acid generated from the oxidation of 3-hydroxyanthranilic acid induced apoptosis in thymocytes of mouse through the production of ROS and DNA fragmentation (Hiramatsu et al. 2008). There is a strong possibility that cinnabarinic acid may use any of these mechanisms to exhibit antibacterial activity and inhibit bacterial biofilm formation.

Conclusion

We established the *in-vitro* antibacterial activity of the fruiting body extracts of *Trametes coccinea*, a wood rotting Basidiomycota native to the South-East Asia. The red pigment from *T. coccinea* fruiting body, which was identified as cinnabarinic acid, showed promising antimicrobial properties against Gram-positive *Bacillus subtilis*, *B. cereus* and Gram-negative *Escherichia coli*. Cinnabarinic acid showed remarkable potential for 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. Further

investigations will surely unveil the molecular mechanism of biofilm inhibition by identifying the potential targets of cinnabarinic acid in bacterial cells.

Declarations

Acknowledgment: 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.

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 influence over experimental design, findings and data interpretation.

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

Availability of data: All the necessary data generated through this study are either provided as electronic supplementary information with this manuscript, or those will be available upon conditional request.

Code availability: Not applicable.

Authors' contributions: RCB conceived the idea and acquired funding; MK and RCB designed the study; MK and DJH performed the bioactivities, 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, critically revised the manuscript and agreed to its content.

Ethics approval: Not needed.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

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Tables

Table 1: Antibacterial activity of the *T. coccinea* fruiting body extracts against the test bacterial strains. 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 Duncun multiple range test ($p \leq 0.05$) using SPSS 25.0.

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 ± 0.58^{c}	100
	Bacillus cereus S6	16.33 ± 0.67^{a}	125
	Escherichia coli K12	15.33 ± 0.33^{b}	100
APS2	*Bacillus subtilis* SCB-1	12.33 ± 0.33^{d}	100
	Bacillus cereus S6	14.67 ± 0.33^{bc}	125
	Escherichia coli K12	14.00 ± 0.58^{c}	100
F3	*Bacillus subtilis* SCB-1	13.67 ± 0.33^{c}	125
	Bacillus cereus S6	14.67 ± 0.33^{bc}	125
	Escherichia coli K12	14.33 ± 0.33^{c}	100

Figures

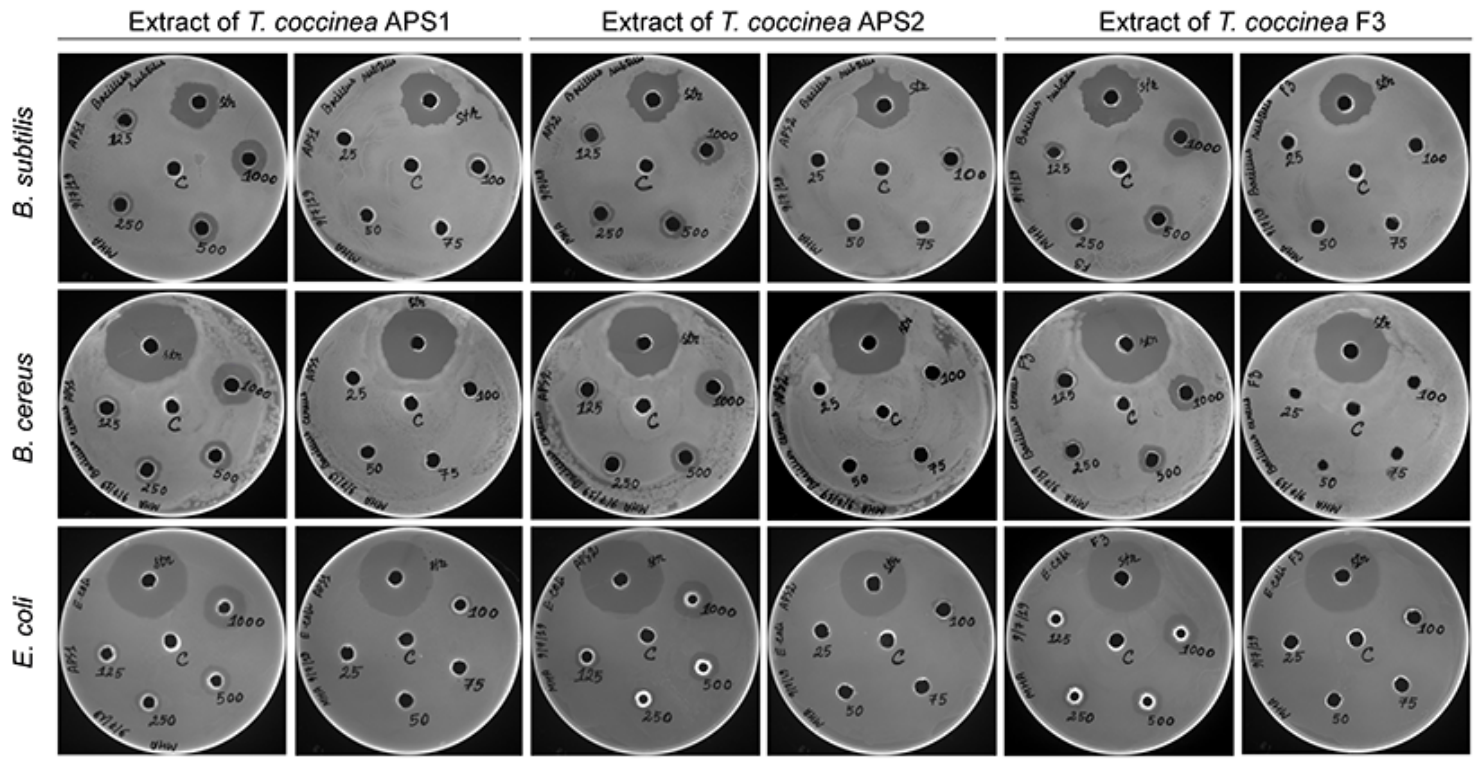

Figure 1

Photographs of plates showing zones of inhibition of the fruiting body extracts against the test bacterial strains. The numbers near each well signify the concentration (µg/ml) of extracts loaded into the respective wells. ‘Str’ denotes streptomycin (100 µg/ml) used as the positive control in each plate. ‘C’ signifies the negative control.

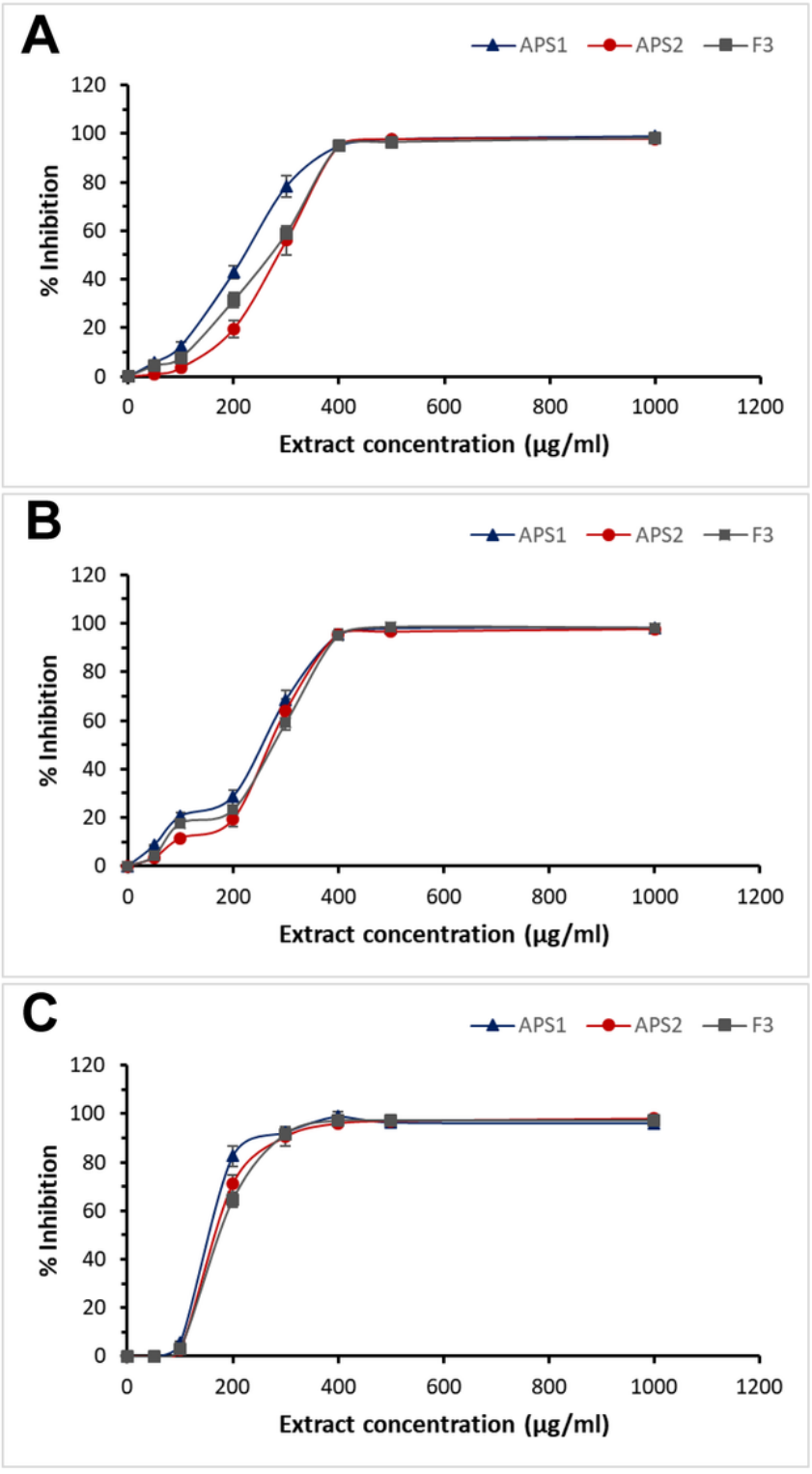

Figure 2

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.

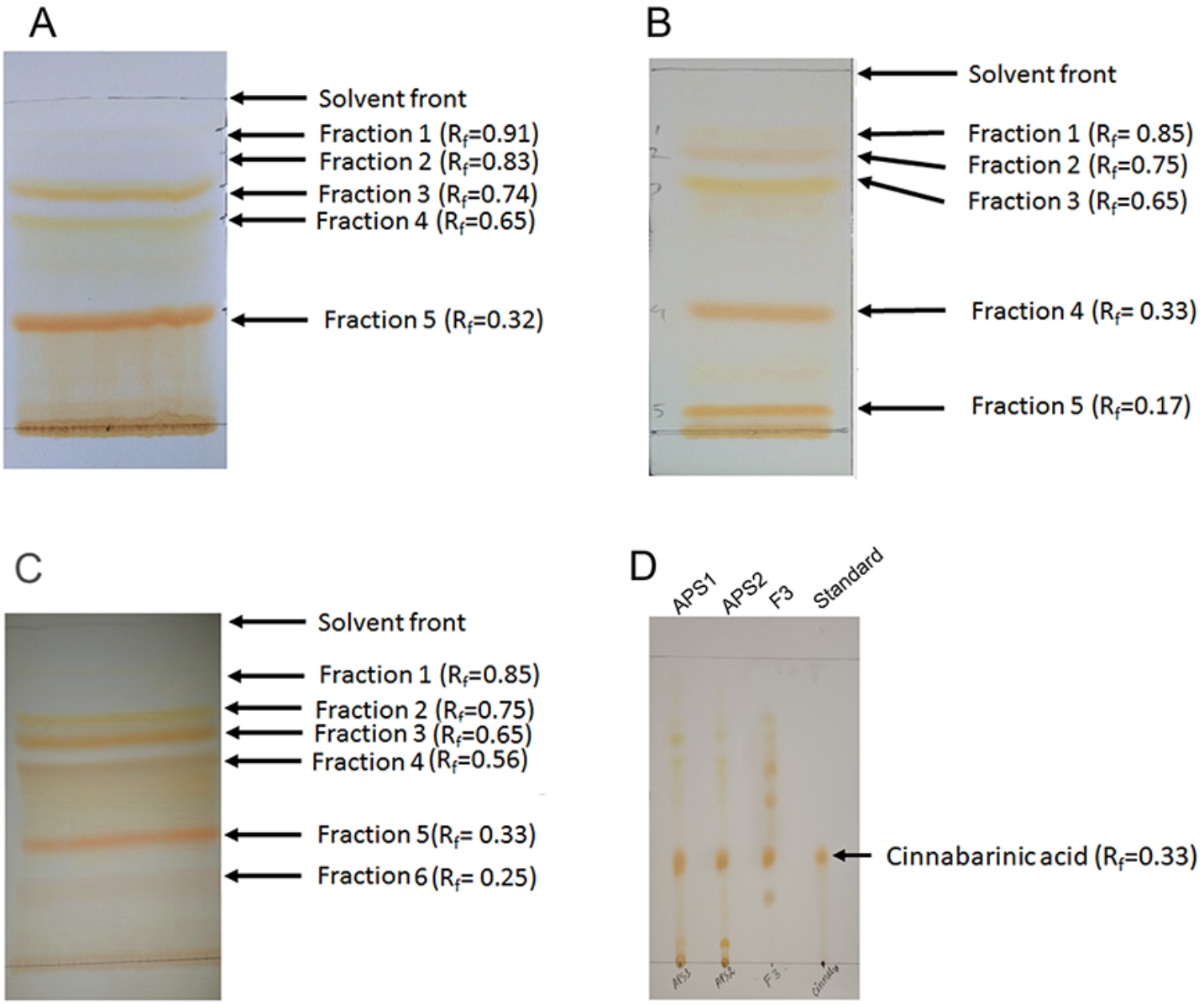

Figure 3

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 corresponding fraction by comparing with the standard.

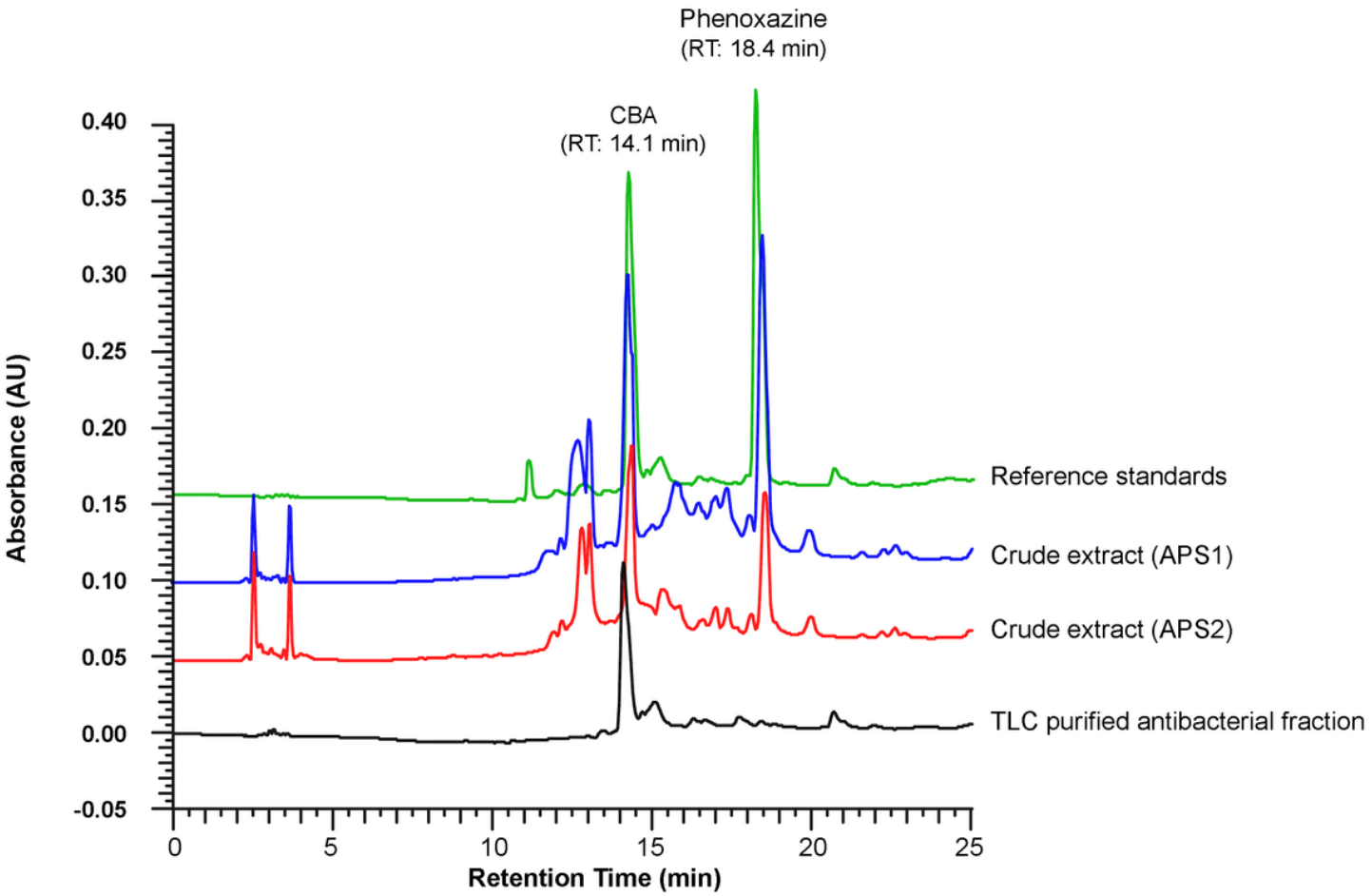

Figure 4

HPLC detection of cinnabarinic acid (CBA) in the crude extracts of T. coccinea and TLC purified bioactive fraction.

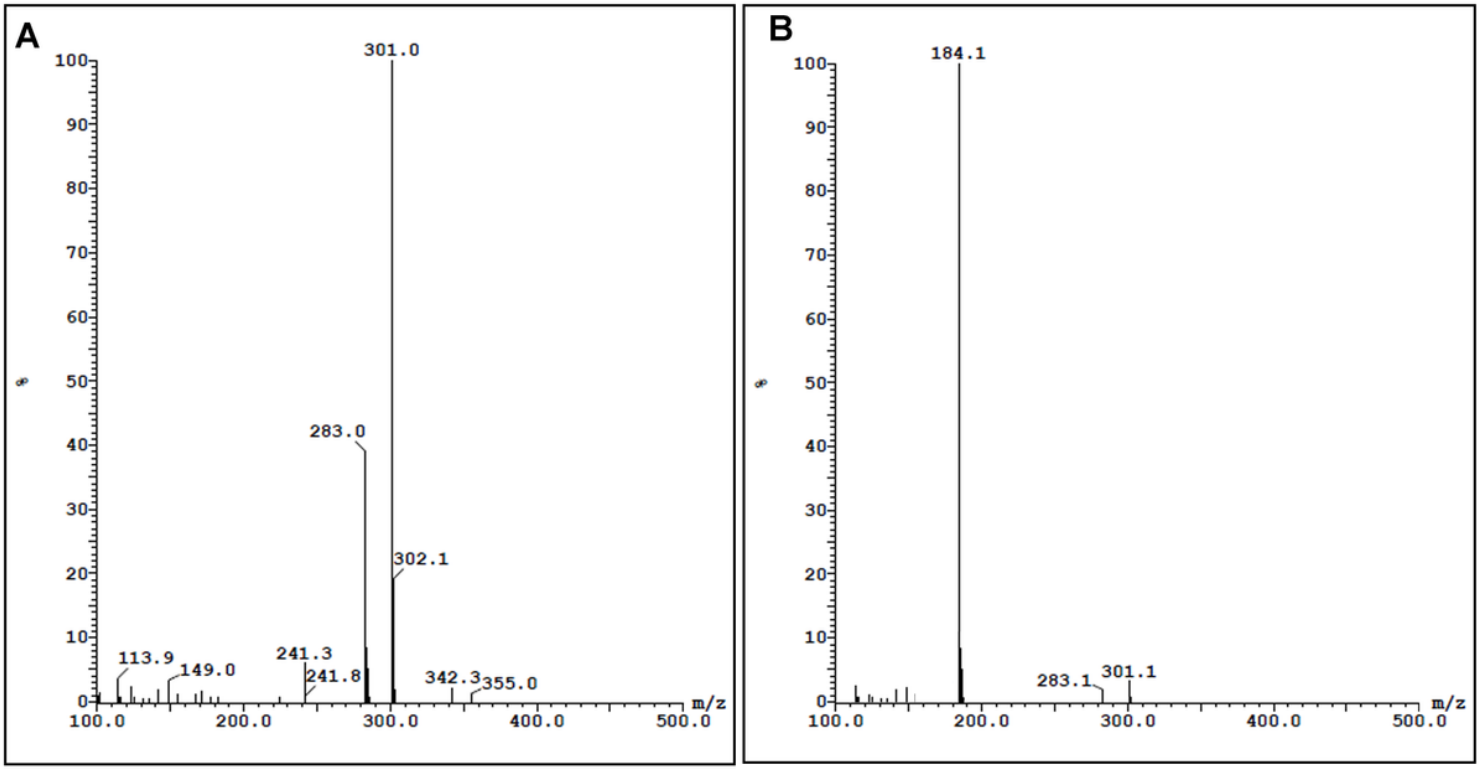

Figure 5

Identification of cinnabarinic acid and phenoxazine purified from the fruiting body extract of T. coccinea using mass spectrometry. A. MS spectra of cinnabarinic acid; B. MS spectra of phenoxazine.

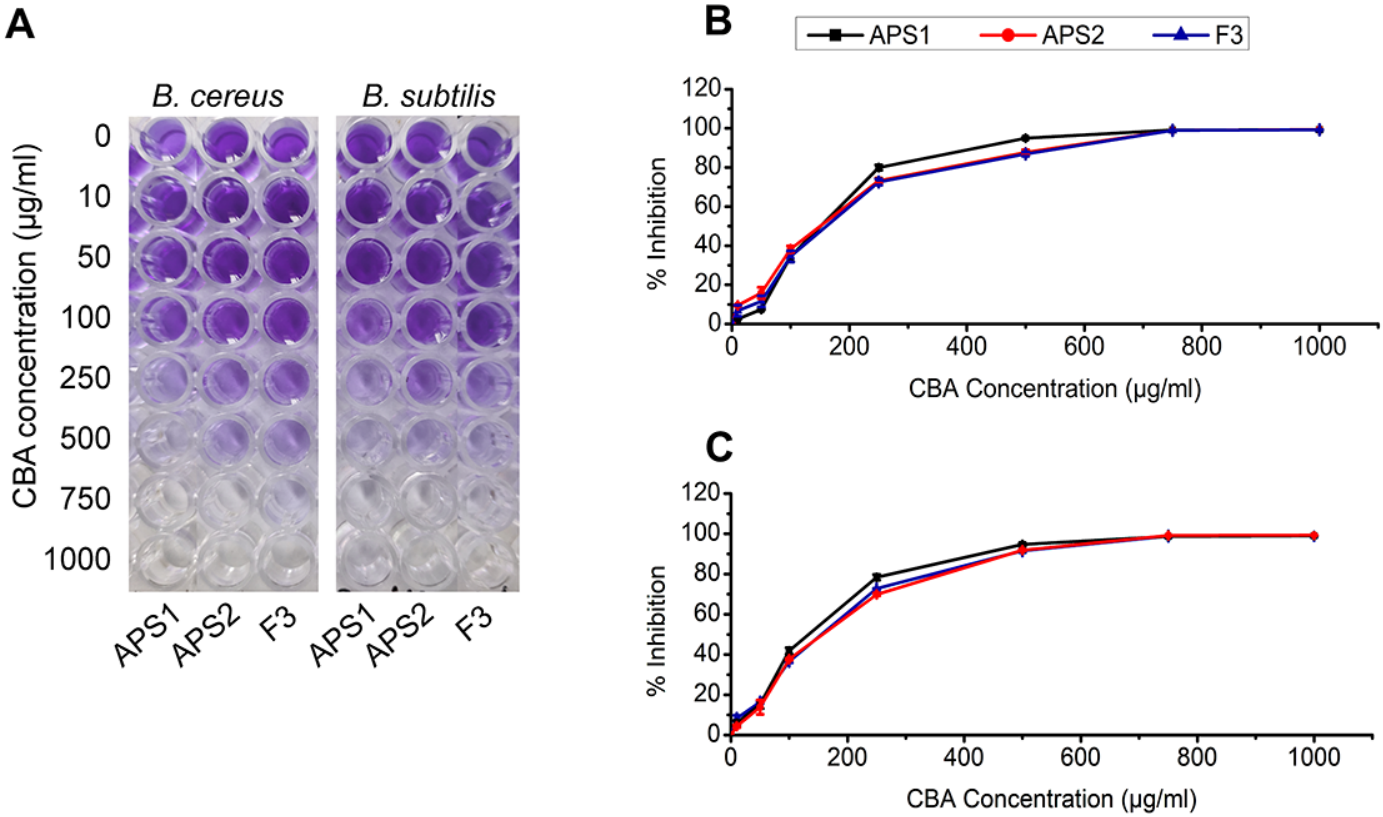

Figure 6

Biofilm inhibitory potential of T. coccinea extracts against biofilm formation in B. cereus and B. subtilis. A. Photographs showing crystal violet staining of biofilms in microtitre wells after treatment with different concentrations of cinnabarinic acid; B. & C. Graphical representation of biofilm inhibitory potential of cinnabarinic acid against B. cereus and B. subtilis, respectively.

Supplementary Files

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