# Cinnabarinic acid: Enhanced production from *Pycnoporus cinnabarinus*, characterization, structural and functional properties

# Sinnabarinik asit: *Pycnoporus cinnabarius*'tan artırılmış üretimi, karakterizasyonu, yapısal ve işlevsel özellikleri

Research Article

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**ABSTRACT**

The white-rot fungus, *Pycnoporus cinnabarinus*, produces the natural phenoxazinone pigment, cinnabarinic acid (CA), which is formed by laccase-catalyzed oxidation of the precursor 3-hydroxyanthranilic acid (3-HAA). This reaction is necessary for the production of antibacterial pigment compounds by the fungus. In this study, the optimum conditions were determined to produce pigment from *P. cinnabarinus* in batch cultures. Isolated pigment was characterized as cinnabarinic acid by spectroscopic techniques, FT-IR; 1H NMR and LC-MS. Temperature-dependent change of CA was also investigated for the first time by using these techniques. It was observed that CA was converted to 3-HAA with increasing temperature and therefore its antibacterial effect was decreased.

**Key Words**
*Pycnoporus cinnabarinus*, cinnabarinic acid, 3-hydroxyanthranilic acid, antimicrobial pigment.

**ÖZET**

Beyaz çürükçül mantar olan *Pycnoporus cinnabarinus*, sinnabarinik asit (CA) olarak adlandırılan ve öncülü olan 3-hidroksiantranilik asitin (3-HAA) lakkaz-katalizli oksidasyonu sonucu doğal fenoksizon pigmenti üretmektedir. Bu reaksiyon mantar tarafından antimikrobiyal pigmentin üretilmesinde önemlidir. Bu çalışmada, optimum koşullarda *P. cinnabarinus* tarafından pigment üretimini belirlenmiştir. İzole edilen pigment spektroskopik yöntemler, FT-IR, 1H NMR ve LC-MS kullanılarak sinnabarinik asit olarak karakterize edilmiştir. Bu teknikler kullanılarak ilk defa CA'in sıcaklığa bağlı değişimi bulunmuştur. Sıcaklığın arttırılmasıyla CA, 3-HAA'e dönüşmektedir ve bu nedenle pigmentin antimikrobiyal etkisi azalmaktadır.

**Anahtar Kelimeler**
*Pycnoporus cinnabarinus,* sinnabarinik asit, 3-hidroksiantranilik asit, antimikrobial pigment.

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## INTRODUCTION

Cinnabarinic acid (CA; 2-amino-3-oxo-3H-phenoxazine-1,9-dicarboxylic acid), a red phenoxazinone compound including numerous pigments and certain antibiotics, is responsible for the *Pycnoporus* species colour [1-8]. Several surveys of the production of antibacterial substances by fungi have been published recently from which it appears that many of the Ascomycetes, Basidiomycetes and Fungi Imperfecti show marked antibacterial activity [9, 10]. A fraction obtained from the culture fluids of *Pycnoporus* sanguineus fungus was shown to contain a compound with biological activity against strains of *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and members of the genus *Streptococcus*. The fraction was clearly more active on Gram-positive cocci than on Gram-negative bacilli [11-13,15,16]. The 20-day-old liquid culture filtrate of *Pycnoporus cinnabarinus* showed good antibacterial effects against the growth of the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* as well as Gram-positive Staphylococcus aureus. The culture filtrate was also used against mycelial growth and mycelial weight of three plant pathogenic fungi Botrytis cinerea, *Colletotrichum gloeosporioides* [Glomerella cingulata] and *Colletotrichum miyabeanus*, showing good inhibitory effect [9]. The pigment has no defined role in the physiology of producing strains but it has been reported to have antifungal, antibacterial, algicidal, antiprotozoal/ antimalarial activities, immunosuppressive and anticancer activities [3,5].

CA is synthesized by laccase-catalyzed oxidation of the precursor 3-hydroxyanthranilic acid [3,6,8]. The white-rot fungus Pycnoporus cinnabarinus, a basidiomycete, is known to produce laccase but no LiP or MnP, which is more thermostable than others. Laccase (p-diphenol oxygen oxido-reductase EC 1.10.3.2), which is appertained to the blue copper enzyme family, catalyses the oxidation of phenolic compounds by molecular oxygen [7,12-16] and it is absolutely essential for lignin degradation by *P. cinnabarinus* [14, 16]. Laccase can not degrade non-phenolic lignin structures without a chemical redox mediator (2,2′-azino-bis-[3-ethyltiazoline-6-sulfonate] (ABTS) or 1-hydroxybenzotriazole (HBT)) because of their low redox-potentials [13-19,21]. To overcome the redox potential barrier, *P. cinnabarinus* produces a metabolite; 3-hydroxyanthranilate that can mediate the oxidation of nowphenolic substrates by laccase in other words, *P. cinnabarinus* produces 3-hydroxyanthranilic acid (3-HAA), a laccase mediator [14,20-22]. 3-HAA is a member of the o-aminophenol which is a metabolite of the kynurenine pathway and it is a precursor of the CA which gives the spesific orange-red color to the fruit body of the *P. cinnabarinus* [6,23,24] (Figure 1).

Also it is known that 3-HAA is important in the shikimic acid pathway. Anthranilic acid is an intermediate of the tryptophan synthesis and 3-HAA is occured by hydroxylation of anthranilic acid in the shikimic acid pathway [14]. This reaction is necessary for the production of antibacterial compounds by the fungus and concentrated culture fluid of the wood-rotting basidiomycete *Pycnoporus cinnabarinus* had biological activity against a variety of bacterial strains [3,6]. Although, 3-hydroxyanthranilic acid (3-HAA), has been proposed to play role in lignin degradation, Li et al., has been demonstrated that 3-HAA does not play an important role in the fungal degradation of lignin [14].

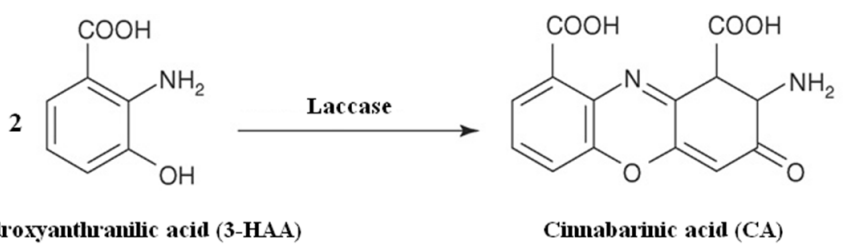


**Figure 1.** The oxidation of 3-hydroxyanthranilic acid to cinnabarinic acid by laccase.

**Table 1.** Enzyme Production Mediums (EPMs).

| **EPM I (pH: 6.6)** | |
|---|---|
| Maltose* | 2.5 g/L |
| Yeast extract | 1.0 g/L |
| Sodium tartrate | 2.3 g/L |
| Ammonium tartrate | 1.8 g/L |
| Copper sulphate pentahydrate ($CuSO_4.5H_2O$) | 0.01 g/L |
| **EPM II (pH: 5.5)** | |
| Maltose* | 20 g/L |
| Diammonium tartrate | 1.84 g/L |
| Disodium tartrate | 2.3 g/L |
| Potasiumdihidrogenephospate | 1.33 g/L |
| $CaCl_2.2H_2O$ | 0.1 g/L |
| $MgSO_4.7H_2O$ | 0.5 g/L |
| $FeSO_4.7H_2O$ | 0.07 g/L |
| $ZnSO_4.7H_2O$ | 0.046 g/L |
| $MnSO_4.7H_2O$ | 0.035 g/L |
| $CuSO_4.5H_2O$ | 7.0 mg/L |
| Yeast extract | 1.0 g/L |
| Vitamine solution* | 1.0 ml |
| **EPM III (pH: 4.5)** | |
| Glucose* | 3 g/L |
| Potasiumdihidrogenephospate | 1 g/L |
| $NaH_2PO_4$ | 0.26 g/L |
| $(NH_4)SO_4$ | 2.4 Mm |
| $MgSO_4.7H_2O$ | 0.5 g/L |
| $CuSO_4.7H_2O$ | 0.5 g/L |
| 2,2-dimethylsuccinic acid | 2.2 g/L |
| $CaCl_2.2H_2O$ | 74 mg/L |
| $ZnSO_4.2H_2O$ | 6 mg/L |
| $FeSO_4.7H_2O$ | 5 mg/L |
| $CoCl_2.2H_2O$ | 1 mg/L |
| Vitamine solution* | 500 ↔l |

*Glucose, maltose and vitamine solution were added into medium in an aseptic conditions.

In addition to antibacterial properties, as first time, we demonstrated that cinnabarinic acid is also a good natural wool dyeing agent (unpublished data). This property is especially important for the manufacturing of eco-textiles with increasing trend. In this study, the optimum conditions were determined to produce antimicrobial pigment cinnabarinic acid from *Pycnoporus cinnabarinus* in lab-scale batch cultures. Isolated pigment was characterized as cinnabarinic acid by spectroscopic techniques, FT-IR; $^1$H NMR and LC-MS. Temperature-dependent change of CA was also investigated for the first time by using these techniques. Our next studies were focused on the production of CA in stirred tank reactor at optimized conditions for the large scale applications.

## MATERIALS AND METHODS

2,2′-azino-bis-[3-ethyltiazoline-6-sulfonate] (ABTS) and guaiacol were obtained from Sigma, ammonium tartrate, potato dextrose agar (PDA) and malt extract broth (MEB) were purchased from Fluka. 3-hydroxyanthranilic acid (3-HAA) was obtained from Aldrich. Silicagel 60 and sodium tartrate were provided from Merck. Other chemicals were provided from Fluka and Merck. Organic solvents were HPLC grade, while all other chemicals were analytical grade and obtained from Merck and Fluka.

### Organism and culture conditions

The *P. cinnabarinus* strains were isolated from Osmangazi University, Eskişehir and were maintained on 2% (w/v) malt extract agar plates at 24ºC and kept at 4ºC. The fungus was cultivated on potato dextrose agar at 30ºC for 6 days. After 6 days the spores were washed with 0.9% (w/v) NaCl and transfered into 250 mL Erlenmeyer flasks which contains 100 ml 2% malt exract (w/v) medium. These precultures were incubated for 3 days at 30ºC on a rotary shaker (125 rpm, Φ18 mm). For enzyme production, the pellets were taken into 250 ml Erlenmeyer flasks contains 100 ml Enzyme Production Mediums (EPMs) (Table 1). The cultures were incubated for 12 days at 30ºC on a rotary shaker (125 rpm, Φ18 mm). All cultivation steps were carried out in aseptic conditions.

### Enzyme assay for laccase

In order to detect ligninolytic enzymes produced from *P. cinnabarinus*, fungi were cultivated on PDA, containing indicator compound, guaiacol that enabled the detection of laccases as specific colour reactions. Guaiacol ($C_6H_4(OH)(OCH_3)$) was added to the media before autoclaving and cultivated at 30ºC. The spores were inoculated in aseptic conditions. Thus, laccase activity was visualized on plates containing 0.01% guaiacol since laccase catalyzes the oxidative polymerization of guaiacol to form reddish brown zones in the medium [19].

To remove the mycelium, the sample centrifuged 15 min at 5000 × g. Laccase activity was measured by the oxidation of 1 mM ABTS at 420 nm buffered with 100 mM Na-acetate buffer (pH 4.5). Unit of enzyme is given the transformation of 1μmol substrate in 1 min with 1 ml enzyme. ($\varepsilon_{420}$ = 36 000 $M^{-1}$ $cm^{-1}$). The experiments were performed in twice.

In vitro oxidation of 3-hydroxyanthranilic acid (3-HAA) and determination of cinnabarinic acid (CA)

For 3-HAA oxidation studies, either laccase semi-purified from Trametes trogii (0.5 mL) or commercially available Denilite laccase (20 μg) was added to a solution of 1 mM 3-HAA in 1 mL of 50 mM sodium tartrate buffer (pH 4.0) and incubated in 30ºC. For product analysis, reaction mixtures were incubated for 5 h at 30ºC. Oxidation of 3-HAA was monitored spectrophotometrically and scans were taken at intervals [6].

The supernatant of culture mediums were measured spectrophotometrically to determine CA. The sample was scanned from 200 nm to 700 nm to determine its maximal absorbance with a spectrophotometer. The measurements were carried out at 450 nm.

**Optimization the production conditions in batch cultures**

Effect of production medium components on enhanced production of CA by *P.cinnabarinus* was studied in shake flasks at initial (0.5; 1.0; 2.5; 5.0; 7.5 and 10 g/L) maltose and ammonium tartarate concentrations (0.46; 0.96; 1.84; 3.68 and 5 g/L). To determine the effect of carbon sources on CA production, 2.5 g/L glucose, maltose, mannose, cellulose, xylose, fructose and galactose were added into culture mediums. To detect the effect of temperature onto pigment and enzyme production the fungus was inoculated at 24ºC, 26ºC, 28ºC and 30ºC, respectively. During the pigment cultivation, fungus was incubated at 30ºC on a rotary shaker (125 rpm, Φ18 mm). Both pigment and enzyme productions were carried out at 450 nm (for CA) and 420 nm (for laccase) by using spectrophotometric methods.

**Antibacterial activity of cinnabarinic acid**

The antibacterial activity of culture filtrate was determined by AATCC 147-1998 test method. The culture fluid was incubated at different temperatures (25-80ºC) during 2 hours to determine the effect of temperature onto the structure of cinnabarinic acid. The sterile paper discs were soaked with 1 mL of the culture fluids which were incubated at different temperatures and placed on a bacterial (Staphylococcus aureus) seeded plate (90 x 15 mm) of nutrient agar. The plates were incubated at 37ºC for 24-48 h and the inhibition zones around the application points were then measured. An average inhibition zones were calculated for 2 replicates.

**Isolation of cinnabarinic acid**

Sample from the culture medium was removed and centrifuged, and the supernatant was extracted with ethyl acetate (EtOAc). The EtOAc extract was used to detect the presence of CA with spectrophotometry, spectroscopy and chromatography.

**Identification of cinnabarinic acid and 3-hydroxyanthranilic acid**

Analysis of 3-HAA and CA by TLC was performed on Kieselgel 60 $F_{254}$ (DC-Alufolien, Merck, Darmstadt, Germany) (20 cm x 20 cm) using butanol/acetic acid/water (4:1:1; v/v) as the eluant system. For identification of compounds in culture mediums, $R_f$ values were compared with those of the pure compounds.

All spectrophotometric measurements were performed on Perkin Elmer Lambda 35 UV/Vis Spectrometer.

FT-IR analysis was performed on Perkin Elmer Spectrum BX FTIR System to detect the differences between the structures of CA at different temperatures from 25ºC to 80ºC.

For the NMR measurements, the samples were dissolved in dimethylsulphoxide (DMSO). The $^1$H NMR spectra for synthesized and produced CA were obtained at 30ºC on Varian instruments (NMR-AS400) (Varian Associates Inc., Palo Alto, CA, USA).

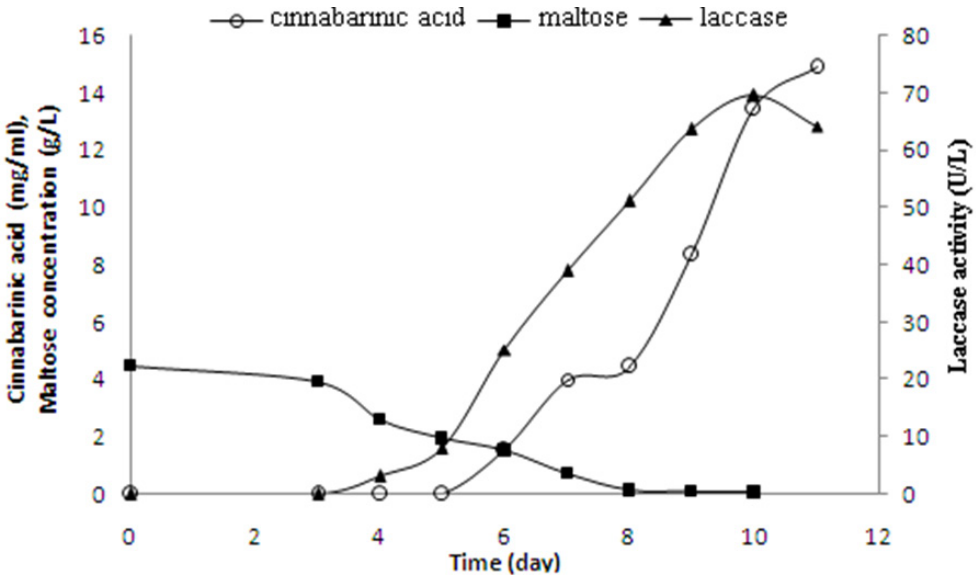


**Figure 2.** Time course of a laboratory cultivation of P. cinnabarinus in shaking cultures: Laccase and cinnabarinic acid production.

To detect the amount of natural CA and 3-HAA in culture medium, the LC–MS analyses were performed. The EtOAc extracts were injected onto an Acquity UPLC BEH C18 (1.7 μm 2.1 x 100mm) column and detected with TQ LCMS/MS detector. Mobile phase was arranged as eluant A (water with 0.1% trifluoroacetic acid) and eluant B (acetonitrile:water (90:10) with 0.1% trifluoroacetic acid). The flow rate was adjusted as 0.3 mL/min. LC-MS analysis were performed to determine the differences between the amounts of CA at different temperatures from 25ºC to 80ºC.

## RESULTS

*P. cinnabarinus* was grown at 26ºC on a rotary shaking incubator with maltose (2.5 g/L) as a carbon source. Over the time course of cultivation, the culture color remained without change for three days but after that the yellow culture fluid turned to dark orange/red. Samples were centrifuged (15 min, 5 000 x g) prior to spectrophotometric measurements. The supernatant was scanned from 200 nm to 700 nm to determine its maximal absorbance. The maximal absorbance revealed in 450 nm for CA from *P. cinnabarinus* culture fluid

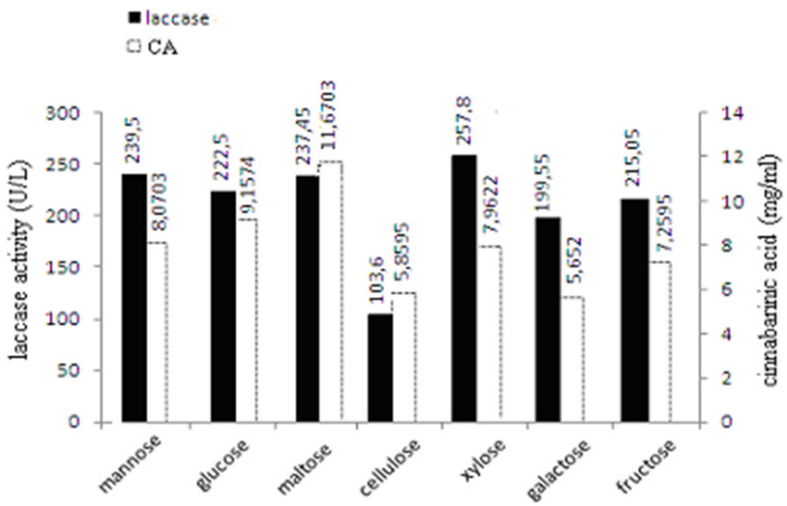


**Figure 3.** Laccase and cinnabarinic acid production by P. cinnabarinus in different carbon sources.

was appropriate with the absorbance spectrum of CA chemically synthesized in our laboratory as well as with spectra reported previously for CA [6].

The optimum conditions were investigated to produce CA from *P. cinnabarinus* both in the batch shaking cultures. According to this cause, the optimum conditions were determined in the erlenmeyer flasks. As a result of optimization, the suitable carbon source, nitrogen concentration and incubation temperature were determined as 2.5 g/L maltose, 5 g/L ammonium tartrate, 26ºC, respectively. All the cultivations were carried out at culture mediums pH. To show the important of the enzyme activity for pigment production, enzyme activity and pigment production were measured with spectrophotometer. During the cultivation, concentration of carbon source (maltose) was monitored. *P. cinnabarinus* was used the entire carbon source during the pigment production. CA production and laccase activity were correlated with each other (Figure 2).

To determine the enzyme production medium (EPM), three different mediums which include different sources were investigated. For enzyme production, the pellets from malt extract broth preculture were taken into 250 mL Erlenmeyer flasks that contained 100 mL EPMs. To detect the maximum laccase activity and CA production, samples were taken from culture fluid and measured spectrophotometrically. In EPM II and EPM III the maximum laccase activities were measured as 2.325 U/L in the 6$^{th}$ day and 0.915 U/L in the 7$^{th}$ day of cultivation. However in EPM I the maximum laccase activity was measured as 350.7 U/L in the 11$^{th}$ day of cultivation. Moreover these results, the color was changed bright yellow to dark orange/red only in EPM I. Hence, the optimum enzyme production medium was decided on EPM I.

To remove the mycelium, the sample from culture fluid was centrifuged 15 min at 5000×g and the supernatant of culture medium was measured with spectrophotometry to determine CA. The sample was scanned from 200 nm to 700 nm to determine its maximal asbsorbance with a spectrophotometer. The maximal absorbance of CA was determined at 450 nm.

To determine the effect of carbon sources to laccase activity and CA production, maltose, glucose, mannose, cellulose, xylose, galactose and fructose (2.5 g/L) were added into EPM I in aseptic conditions. Laccase activity and CA production were measured spectrophotometrically. The maximum laccase activities and CA concentrations were given at Figure 3.

As a result of carbon sources optimization, the maximum CA concentration was determined as maltose. Temp and Eggert, 1999, has been declareted that, when glucose is the carbon source, the white rot fungus *P. cinnabarinus* produces a characteristic red pigment, cinnabarinic acid, which is formed by laccase-catalyzed oxidation of the precursor 3-hydroxyanthranilic acid. When

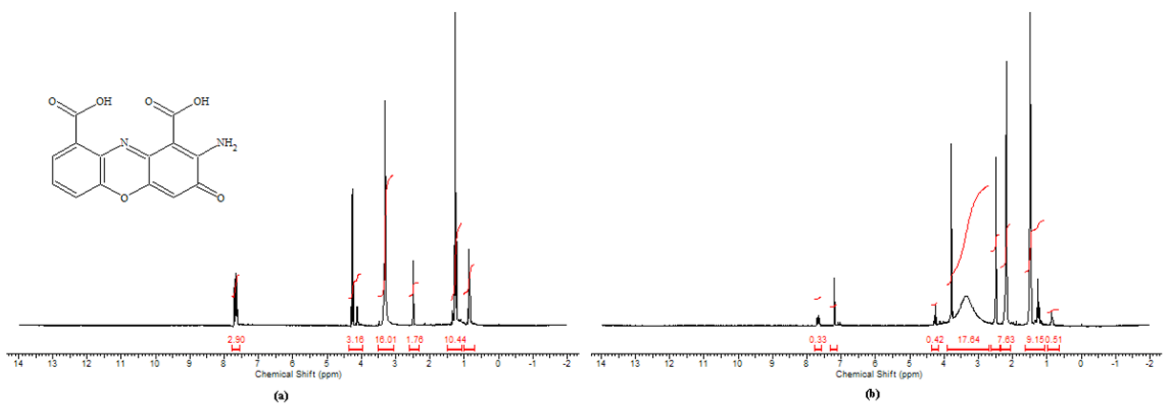


**Figure 4.** NMR spectrum of (a) cinnabarinic acid synthesized by enzymatic reaction and (b) cinnabarinic acid from culture fluid of *P. cinnabarinus*.

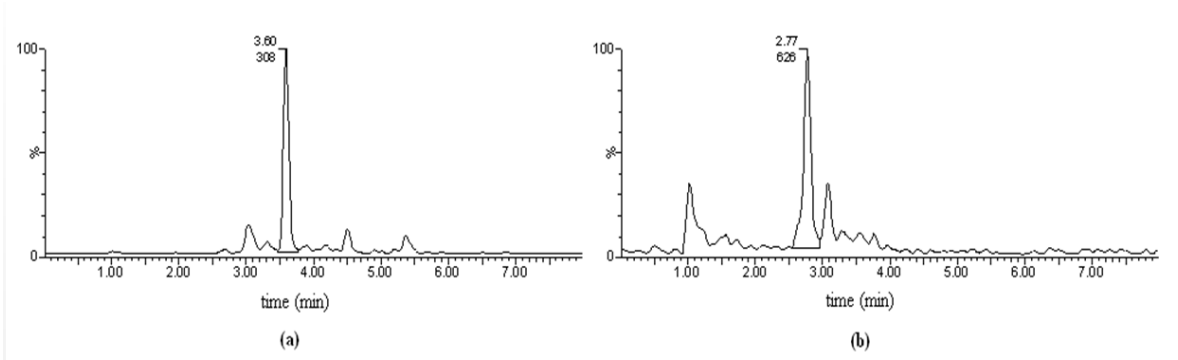


**Figure 5.** The LC-MS spectrum of (a) cinnabarinic acid and (b) 3-hydroxyanthranilic acid from the culture medium of P. cinnabarinus at 25 ºC.

*P. cinnabarinus* was grown on media containing cellobiose or cellulose as the carbon source, the amount of cinnabarinic acid that accumulated was reduced or, in the case of cellulose, no cinnabarinic acid accumulated [8]. However, in this study, it was observed that cinnabarinic acid accumulation by *P. cinnabarinus* has been carried out in a medium containing cellulose even if just a quite low level.

As a result of optimization of CA production, the optimum carbon source was determined as maltose and the optimum maltose concentration was detected as 2.5 g/L. At 2.5 g/L maltose concentration, the maximum laccase activity and CA production was determined as 239.5 U/L on $11^{th}$ day and 4.1358 mg/mL on $9^{th}$ day of cultivation, respectively. Optimum nitrogen concentration was observed as 5 g/L ammonium tartrate (228.1 U/L for laccase activity and 1.65 mg/mL for CA production).

## Characterization of cinnabarinic acid

CA and 3-HAA were determined with TLC using butanol:acetic acid:water (4:1:1, v/v) as an eluant system. $R_f$ values for CA (0.775±0.05) and 3-HAA (0.550±0.01) were identical with authentic pigments that were synthesized at laboratory.

$^1$H-NMR spectroscopic analysis for isolated compound was also performed CA from culture medium and enzymatically synthesized CA were dissolved in dimethylsulphoxide (DMSO) and its protons were determined at 46.05 ppm for natural CA and 51.25 ppm for synthetic CA (Figure 4).

The EtOAc extract was taken to analyze by LC-MS, the amount of CA and 3-HAA in the maximum cultivation day of *P. cinnabarinus*. The concentration of CA and 3-HAA were determined as 7.209 mg/L and 3.73 mg/L, respectively at 25ºC (Figure 5).

## Antimicrobial properties of CA depending on temperature

Cinnabarinic acid has been reported to have antibacterial activity against strains of Staphylococcus aureus. To investigate the usage of CA in different industrial applications, the effect of antibacterial activity of CA at different temperatures were determined.

The results obtained from the paper disc method AATCC 147-1998, S. aureus was inhibited by the culture fluids at three temperatures (25ºC, 30ºC and 40ºC). Inhibition zones were counted 4.5, 4 and 3 mm, respectively and no inhibition zone were observed at high temperatures (50ºC-80ºC) (Table 2).

**Table 2**. Antibacterial activity of culture fluids at different temperatures.

| Temperature (ºC) | Inhibition zone(mm)* |
|---|---|
| 25 | 4.5 |
| 30 | 4.0 |
| 40 | 3.0 |
| 50 | - |
| 60 | - |
| 70 | - |
| 80 | - |

*Inhibition zone was measured by diameter of total inhibition zone minus diameter of paper disc after 48 h of incubation at 37ºC.

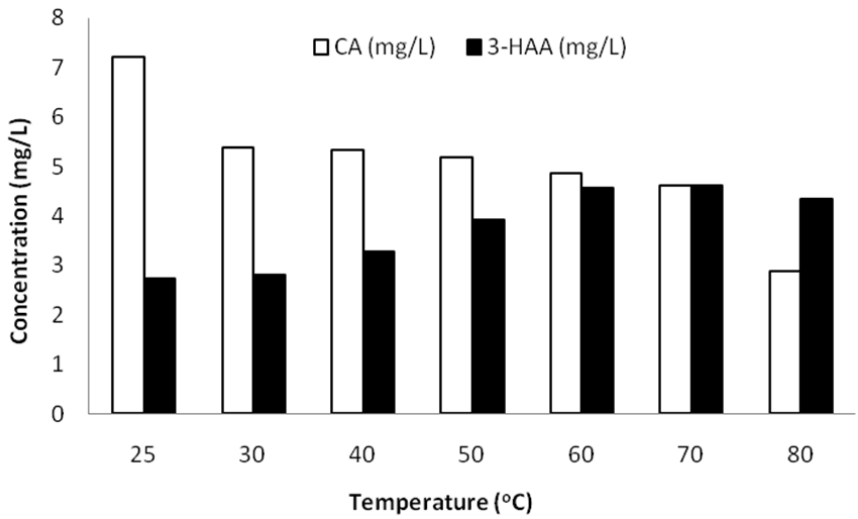


**Figure 6.** Alternation of cinnabarinic acid and 3-hydroxyanthranic acid concentrations between 25-80ºC.

To detect the effect of temperature on CA structure, culture medium was incubated during 2 hours at different temperatures (from 25ºC to 80ºC). Samples were analyzed by FT-IR and LC-MS. It was shown that, the structure of CA was destructured due to break with –COOH groups upon 40 ºC. The microbial activity of pigment was sinking during the temperature increasing (Figure 6).

The concentration of CA and 3-HAA were determined as 2.87 mg/L and 4.35 mg/L, respectively at 80 ºC by LC-MS. Depending on the increment of temperature, the amount of CA was decreased despite the amount of 3-HAA was increased. With respect to these results, the amount of 3-HAA from the culture fluid that contains the maximum CA concentration, was half the amount of CA. This results proved the oxidation of 2 molecules of 3-HAA to 1 molecule of CA.

## DISCUSSION

This paper reports the optimum conditions for cinnabarinic acid production from white-rot fungi, P. cinnabarinus. In batch cultures, enzyme production medium (EPM), carbon source, carbon and nitrogen concentrations and temperature were optimized. In the optimum EPM, culture fluid was turned bright yellow to orange/red. Increasing in laccase activity and pigment production were correlated in the culture medium. The supernatant of culture medium was scanned from 200 nm to 700 nm to determine its maximum absorbance with spectrophotometer. The maximal absorbance detected in 450 nm for CA from *P. cinnabarinus* culture fluid and same as chemically synthesized in laboratory.

With respect to optimum conditions for CA production, 2.5 g/L maltose was detected the optimum carbon source. Except cellulose, all monosaccarides were used as carbon sources by *P. cinnabarinus*. However, when polysaccaride was used as carbon source for *P. cinnabarinus*, laccase activity and CA production were not detected as well as the other monosaccarides.

Nitrogen concentration is important for pigment production. To point out this, different concentration of ammonium tartrate were added into culture conditions, CA production and laccase activity were measured spectrophotometrically. The culture fluid that contained 5 g/L ammonium tartrate had the maximum CA production and laccase activity.

Temperature is effective upon enzyme and pigment production. According to this phenomenon, different temperatures were studied to explain the effect on CA production. At 24ºC, laccase activity and CA production

were determined lower than other temperatures and the change of color in culture fluid was not determined. Despite the low laccase activity, the maximum CA production was measured at 26ºC. With reference to these results, the optimum temperature was detected as 26ºC for CA production.

After the production of CA in optimum conditions, CA and 3-HAA were identified by spectrophotometry, spectroscopy and chromatography. As a result of TLC, $R_f$ values for CA and 3-HAA were identical with authentic pigments that were synthesized at laboratory. After designate the CA and 3-HAA in the culture medium, spectroscopic and spectrophotometric measuremens were performed. According to the $^1$H-NMR results, natural CA protons were determined nearly the same with synthetic CA. This was established that the structure of orange pigment was nearly same with synthetic CA. The result of LC-MS analyses were clarified the oxidation of 3-HAA to CA during the cultivation of *P. cinnabarinus*. With reference to LC-MS results, 3-HAA concentration was half the amount of CA in the culture medium. To reveal the effect of temperature on CA structure, the culture fluid was incubated during 2 hours at different temperatures, and the antibacterial activity of CA was determined. According to the antibacterial test method, upon the 40ºC, the antibacterial effect of CA could not occured. To prove this phenomenon, the orange/red pigment was analyzed by LC-MS and FT-IR. The –COOH groups of CA were broken down above 40ºC. Consequently, the effect of temperature onto CA was observed that, as long as the temperature was increased, depending upon the reaction of CA from 3-HAA, CA concentration was decreased despite 3-HAA concentration was increased.

This report shows the optimum conditions for CA production in batch cultures by *P. cinnabarinus*. Identification CA structure and the antibacterial activity of CA at different temperatures were determined. These conditions can be remarkable for CA production in industrial scale. On the occasion of antibacterial property of CA, this pigment can be used for different industrial fields such as textile.

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