Analysis of phytochemical constituents and bioactivity of infusion and decoction extracts from *Pycnoporus cinnabarinus* (Jacq) P. Karst: A comparative study

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Abstract

Pycnoporus cinnabarinus is a red-colored polypore fungus that has been used by the aboriginal people of Nepal to treat ear infections of child and also used in wounds. It has a wide variety of essential bioactive components that showed a promising pharmacological effect. Infusion and decoction are two efficient extraction processes that are employed to extract essential bioactive chemical constituents and subjected for the determination of antioxidant and anti-inflammatory properties of *P. cinnabarinus*. The result has shown that decoction has a significantly higher quantity of chemical constituents than infusion. Therapeutic activities (in-vitro antioxidant and anti-inflammatory) are also found to be higher in decoction than infusion. This study is focused on the comparative study of bioactive components and investigation of bioactive properties of two different extracts from *P. cinnabarinus* to show how two different water extracts prepared through different heat treatment influences the chemical composition and pharmacological activities.The study may also be helpful in the exploration of bioactive components and therapeutic prospects of the fungus so that it could be incorporated as a natural drug in herbal medicine against oxidative stress and inflammation-associated diseases.

Keywords: Antioxidant, anti-inflammatory, decoction, infusion, *Pycnoporus cinnabarinus*.

1. Introduction

As oxidative stress is a major problem in the human body, much attention is given to the research of the antioxidant properties of mushrooms to develop nutritional and medicinal provisions for mankind because of their significant capacity to prevent free radical-induced damage in the body. Free radicals are generated when all living cells are exposed to various challenges, and they are dangerous to every biomolecule, causing oxidative damage to DNA, proteins, and lipids [1, 2, 3]. They are also major reasons for many diseases such as cardiovascular diseases, aging, and cancer observed in the body [4, 5]. As endogenous antioxidant defense mechanisms cannot be strong enough to scavenge free radicals, natural antioxidants from mushrooms could be an efficient approach to defend against free radicals.

Inflammation is a defensive response developed in the body due to infection or injury. For defending host cells, macrophage produces mediators of inflammation such as nitric oxide (NO) and pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) [6]. Because of the overproduction of these mediators, chronic inflammation appears which is generally associated with many chronic diseases

such as Alzheimer's, cancer, and arthritis [7]. Compounds from natural sources inhibited these mediators which may be an effective approach to developing new anti-inflammatory agents.

Mushrooms have a long history of uses in different fields of medical science throughout the world and are a rich source of bioactive chemical constituents that are essential for the cure of different dangerous diseases [8, 9]. It has remarkable bioactive properties such as antioxidant, anti-inflammatory, and anticancer properties [10, 11]. Besides their promising medicinal aspects, they also have good nutritional benefits like high protein content, fibers, vitamins, minerals, natural antioxidants, etc. Almost 700 species of mushrooms belonging to higher basidiomycetes have been recognized to exhibit outstanding therapeutic activity [12, 13].

The biological effect of mushrooms is not only dependent on their chemical nature but also depends on the extraction procedures of metabolites that are used. Reports have found that infusion and decoction are very famous preparations in Ayurveda and Traditional Chinese Medicine to extract active constituents from botanicals [14]. In both these procedures external heat is applied in different ways. These two conventional methods are very easy to prepare, less time-consuming, less laborious, and beneficial to herbs.

Pycnoporus cinnabarinus is one of the three edible species of *Pycnoporus* belonging to the division Basidiomycota, order Polyporales, class Agariomycetes. It was described by P. Karst in 1881. It grows in a semicircular or amorphous-elongated formand is distinguished from other species of *Pycnoporus* by having thick context, angular pore on the lower surface of the fruiting body, and deep pore tube. It has been reported that people of Nepal and Hongkong have used *Pycnoporus cinnabarinus* as medicine [15]. In West Bengal, they grow on dead deciduous and conifer trees. The aim of this study is to investigate the bioactive constituents and pharmacological activities of *P. cinnabarinus* so that people are enriched with this scientific knowledge which could be helpful in its exploration in herbal medicine. This study also highlights how different heat treatments (infusion and decoction) during extraction affect the phytochemical compositions and therapeutic effects of the mushroom.

2. Materials and methods

2.1. Collection of Mushroom material

Mushroom material was collected from the Paschim Medinipur district of West Bengal, India. Identification was done using standard literature [16] and the voucher specimen (Accession no: CUH AM1028) was submitted at CUH Herbarium following the method of Pradhan et al., (2015) [17]. Then collected basidiocarp was dried at 40°C overnight.

2.2. Extraction procedure

After drying basidiocarps were powdered using a clean mixer grinder and passed through a sieve (BS-60) to get fine powder. Infusion and decoction extracts were prepared according to the standard protocol of Martins et al., (2015) [18].

Preparation of infusion extract- 0.1 g mushroom powder was taken in a test tube and then 20 ml of boiling water was added. After 5 minutes extract was filtered using Whatman filter paper.

Preparation of decoction extract- 0.1 g mushroom powder was taken in a test tube and 20 ml of distilled water was added. After heating in boiling water for 5 minutes, the extract was filtered with Whatman filter paper.

2.3. Determination of chemical constituents

2.3.1. Total phenol content

Determination of the total phenol content of two different extracts (infusion and decoction) was done by the method of Singleton and Rossi, (1965) [19]. 0.1 ml of the extract was mixed with 0.9 ml of distilled water and 1 ml of Folin Ciocalteu reagent. After incubation for 3 min,1 ml of 35% sodium carbonate was added. The volume of the reaction mixture was made up to 10 ml by adding distilled water and then the test tubes were incubated in the dark at room temperature. After 90 min of incubation, the absorbance was read at 725 nm. Gallic acid was used as standard. Total phenol content was expressed as µg of GAE (Gallic Acid Equivalent)/mg of extract.

2.3.2. Total flavonoid content

The determination of the total flavonoid content of two different extracts (infusion and decoction) was done by the method of Adebayo et al., (2012) [20]. 0.1 ml of extract was mixed with100 µl of 10% aluminum nitrate and 100 µl of 1 M sodium acetate.Then 4.1 ml of 80% methanol was added to the reaction mixture. After 40 minutes of incubation, absorbance was read at 415 nm. Different concentrations of quercetin were used to prepare a standard curve.The value was expressed as µg of QE (Quercetin Equivalent)/mg of extract.

2.3.3. β-carotene content

Determination of β-carotene contents of two different extracts (infusion and decoction) was done according to Nagata and Yamashita., (1992) [21]. 0.1 mL of extract was mixed with 4 ml of acetone and 6 ml of hexene. After vigorously shaking for 1 minute absorbance of the mixture was measured immediately at 453 nm, 505 nm, and 663 nm. Calculation of β-carotene content was done according to the following formula:

β-carotene (µg/mg)

$$= 0.216\times A_{663} - 0.304\times A_{505} + 0.452\times A_{453}$$

2.3.4. Lycopene content

Lycopene content was determined according to the standard protocol of Nagata and Yamashita, (1992) [21]. The procedure is like the determination of the β-carotene content mentioned above. Lycopene content was calculated by the following formula.

Lycopene (µg/ mg)

$$= 0.0458\times A_{663} + 0.373\times A_{505} - 0.0806\times A_{453}$$

2.3.5. Ascorbic acid content

The ascorbic acid content was determined according to a modified method of Rekha et al., (2012) [22]. 0.1 ml of extract was mixed with 9.9 ml of 0.6% oxalic acid and titrated with 2, 6 Dichlorophenol indophenol (DCPIP) dye. The amount of dye consumed was equivalent to the amount of ascorbic acid (V_1 mL). Similarly, titration of the sample extracts (W µg/mL) was done with the dye (V_2 mL). The amount of ascorbic acid was calculated using the formula:

ascorbic acid content

$$= [\{(10\ \mu g/V_1 mL) \times V_2\} \times W\ \mu g/mL] \times 100$$

2.4. Determination of the antioxidant activity

2.4.1. Total antioxidant capacity (TAC) assay

Determination of total antioxidant capacity (TAC) was carried out by phosphomolybdate method Prieto et al.,(1999) [23] with few modifications. Reaction mixtures containing 0.1 ml of extract and 0.2 ml of distilled water mixed with 3 ml of solution 1. After heating at 95°Ctemperature for 1.5 hours, absorbance was read at 695 nm. Solution 1 is prepared by mixing 0.6 M sulphuric acid, 28 mM sodium sulfate, and 4 mM ammonium molybdate. Different concentrations of ascorbic acid were used to prepare a standard curve and expressed as µg of AAE (Ascorbic Acid Equivalent)/ mg of extract.

2.4.2. DPPH radical scavenging assay

Determination of the antioxidant activity of two different extracts (infusion and decoction) was carried out by DPPH radical scavenging assay according to the method proposed by Khatua et al., (2017) [24]. 0.2 ml reaction mixture containing different concentrations of extracts was mixed with 0.004% DPPH solution. After 30 minutes of incubation in the dark, absorbance was read at 595 nm using a microplate reader (Bio-Rad iMarkTM Microplate Reader, USA). Ascorbic acid was used as a positive control. EC_{50} value was calculated which indicates the effective concentration of the extract at which 50% of the DPPH radical was scavenged. The degree of scavenging activity (%) was calculated by the following equation: Scavenging activity (%) = $A_{control}$ - $A_{sample}/A_{control}$ × 100 where, $A_{control}$ and A_{sample} denote the absorbance of the control and the sample at various concentrations of the extracts, respectively.

2.4.3. ABTS radical scavenging assay

Investigation of the antioxidant activity of two different extracts (infusion and decoction) was performed by ABTS radical scavenging assay by the following method of Khatua et al., (2017) [24] using a 96-well microtiter plate. ABTS solution was prepared by dissolving 1 mL of 7 mM ABTS in water and mixing it with 1 mL of 2.45 mM potassium persulfate, yielding a greenish tint in the solution. Before use, the stock solution was diluted to obtain an absorbance of 0.7 ± 0.02 at 750 nm. As a positive control trolox was used. Calculation of EC_{50} values was done from the calibration curve and ABTS radical scavenging activity (%) was measured using the previous equation.

2.4.4. Chelating assay for ferrous ion

The investigation of the chelating ability of ferrous ion (Fe^{2+}) of infusion and decoction extracts was measured by the following protocol of Khatua et al., (2017) [24]. Reaction mixtures (0.2 mL) containing 5 µL $FeCl_2$ (3 nM) and 10 µL ferrozine (0.12 nM) were mixed with different concentrations (0.1-0.5) mg/ml of each extract. After an incubation periodof 10 minutes, the absorbance was read at 595 nm in a microplate readeras mentioned in the previous assay, and EC_{50} values were calculated. EDTA was used as a positive control. Results were represented as a percentage of suppression of Fe^{2+} ferrozine complex formation.

2.4.5. Reducing power assay

The investigation of reducing power assay of the infusion and decoction extracts was performed according to the method proposed by Khatua et al., 2017 [24]. The reaction mixture (0.2 ml) containing 25 µL Na-phosphate buffer (0.2 M, pH 6.6) and freshly prepared 25 µL potassium ferricyanide solution (1%) were mixed with 10 µl sample solution of different concentrations (0.5 – 2.5) mg/ml in a 96 well microtiter plate. After incubation for 20 minutes at room temperature, 85 µl water, and 8.5 µl $FeCl_3$were added to each well and absorbance was read at 750 nm through a microplate reader as mentioned in the previous assay. Ascorbic acid was used as a positive control. EC_{50}value was calculated at the concentrations exhibiting a 0.5 absorbance value.

2.5. Anti-inflammatory activity

2.5.1. Protein denaturation assay

Investigation of anti-inflammatory activity was carried out by protein denaturation assay [25] with slight modifications. A reaction mixture containing 0.9 ml BSA, and 1.8 ml of phosphate-buffered saline (PBS, pH 6.4)was mixed with0.8 ml of varying concentrations (125 – 625 µg/ml) of extracts. As a positive control drug, diclofenac sodium was used. After incubation for 15 minutes at 37°C, the test solutions were heated at 95°C for 30 minutes. The reacted solutions were cooled at room temperature. Then absorbance was read at 660 nm. The experiment was performed in triplicates. The inhibition (%) of protein denaturation was calculated in the following way, Inhibition (%) of Protein Denaturation = $100 \times [A_{control} - A_{sample}/A_{control}]$ Where, $A_{control}$ = absorbance of the test control, A_{sample} = absorbance of the sample.

2.6. Statistical analysis

All experimental data was expressed as mean ± standard deviation (SD) and statistical analysis was done using Microsoft® Office Excel (Microsoft®, USA). Results were compared by ANOVA to determine variances among samples and values of $p<0.05$ was considered as statistically significant.

3. Results

After extraction yeild (%) of each extract (Infusion and Decoction) was recorded. Yeild (%) of infusion and decoction were 14 and 16.3.

3.1. Chemical constituents

Various pharmacologically active constituents were estimated in infusion and decoction extracts of *Pycnoporus cinnabarinus*. The composition of chemical constituents is given in Table 1. Infusion and decoction extract of *P. cinnabarinus* have notable amounts of total phenol, total flavonoid, β-carotene, lycopene, and ascorbic acid. Total phenol, total flavonoid and ascorbic acid are the most important mycoconstituents which were present in *P. cinnabarinus* in the quantities of 14.77, 3.57, 17.58 µg/mg in infusion and while decoction had 23.97, 5.73 and 28.63 µg/mg. Some other chemical constituents like β-carotene and lycopene were also estimated from *P. cinnabarinus* which were present in the quantity of 0.02591, 0.01086 and 0.03947, 0.01941 µg/mg in infusion and decoction extracts, respectively.

3.2. Antioxidant activity

To determine the antioxidant activity of *P. cinnabarinus*, five different assays were employed such as total antioxidant capacity (TAC) assay, DPPH radical scavenging assay, ABTS radical scavenging assay, chelating assay of ferrous ion, and reducing power assay. In all these assays two different extracts (infusion and decoction) of *P. cinnabarinus* showed promising antioxidant activity. In TAC assay, the colour reacted solution turned in to green due to the reduction of molybdenum (Mo) into phosphate/Mo complex in the presence of antioxidant compounds in the extracts. Total antioxidant activity of infusion extract was 15.6 µg/mg AAE while decoction had 28.5 µg/mg AAE (Table - 2). In the DPPH assay, DPPH radical produced in the reaction mixture is scavenged by antioxidant molecules present in extracts resulting in different shades of colour. Scavenging activity depends on the concentration of extracts. At concentrations of 225 and 300 µg/ml, infusion extract of *P. cinnabarinus* showed 52.5 % and 63.2% scavenging activity (Fig - 1A), where as decoction extract showed 60% and 72.1% scavenging activity at 225 and 300 µg/ml concentration. And decoction (EC_{50} = 165.5 µg/ml) has higher antioxidant activity than infusion (EC_{50} = 214.7 µg/ml). EC_{50} value indicates the concentration of extract at which 50% scavenging activity is achieved. Similar result was

found in the ABTS assay like DPPH assy. At 120 and 160 µg/ml concentrations, the decoction extract of *P. cinnabarinus* showed 65.6% and 75.5% scavenging activity (Fig - 1B),while infusion showed 50.5% and 65.1% scavenging activity at 120 and 160 µg/ml. Decoction (EC_{50} = 77.9 µg/ml) has higher antioxidant activity than infusion (EC_{50} = 118 µg/ml).

In chelating assay, violet coloured reaction mixture formed by a reaction of ferrozine with ferrous ion gradually decreases due to the presence of chelating agent in the extracts. Chelating activity (%) of infusion extract were 36.2% and 51.8% at 450 and 600 µg/ml of concentration, and decoction showed 53.7% and 61.8 % (Fig - 1C). Decoction (EC_{50} = 466 µg/ml) has higher metal chelating ability than infusion (EC_{50} = 601 µg/ml). In reducing power assay, the colour of the reacted solution turned in to deep green due to the reduction of Fe^{3+}/ferricyanide complex to Fe^{2+} form and colour intensity of the reacted solution is increased in a concentration-dependent manner. Both the extracts (infusion and decoction) showed a moderate result. Absorbance of infusion extract of *P. cinnabarinus*were0.335 and 0.50 OD at 1 and 1.5 mg/ml concentration and decoction showed 0.519 and 0.676 OD (Fig - 1D). Decoction (EC_{50} = 989.7 µg/ml) had higher antioxidant activity than infusion (EC_{50} = 1560 µg/ml).

3.3. Anti-inflammatory activity

In protein denaturation assay, the external application of heat induces denaturation of protein that turned the colour of reacted solution into white turbid solution. In the presence of anti-inflammatory compounds, the extracts inhibited protein denaturation in a concentration-dependent manner. Inhibitory (%) of protein denaturation of infusion extract were 33.4 % and 46.1% at 125 and 250 µg/ml concentration, while decoction showed 43.5% and 60% (Fig - 1E). Decoction (EC_{50} = 161.2 µg/ml) had higher anti-inflammatory activity than infusion (EC_{50} = 283.5 µg/ml).

Table 1:. Chemical composition of infusion and decoction extracts obtained from *P. cinnabarinus*.

Chemical constituents (µg/mg of extracts)		
Name of the chemicals	Infusion	Decoction
Total phenol	14.77±0.9[a]	23.97±1.1[b]
Total flavonoid	3.57±0.2[a]	5.73±0.3[b]
β-carotene	0.02591±0.002[a]	0.03947±0.005[b]
Lycopene	0.01086±0.003[a]	0.01941±0.002[b]
Ascorbic acid	17.58±0.8[a]	28.63±1.4[b]

Results are presented in mean ± SD (n=3). Different letters in the column show statistically significant differences ($p<0.05$) according to ANOVA.

Table 2: Antioxidant activity of infusion and decoction extracts obtained from *P. cinnabarinus*.

Antioxidant assays	Infusion	Decoction extract	Standard
Total antioxidant capacity (µg/mg of extracts)	15.6±1.1[a]	28.5±1.8[b]	-
DPPH radical scavenging assay (EC_{50} = µg/ml of extract)	214.7±10.25[a]	165.5±3.53[b]	9.2± 1[c]
ABTS radical scavenging assay (EC_{50} = µg/ml of extract)	118±3[a]	77.9±1.4[b]	6 ± 0.5[c]
Chelating assay of	601±8.5[a]	466±5[b]	15.6 ± 1.8[c]

ferrous ion (EC_{50} = µg/ml of extract)			
Reducing power assay (EC_{50} = µg/ml of extract)	1560±28.3[a]	989.7±15.5[b]	18.8±1.2[c]

Results are presented in mean ± SD (n=3). Different letters in the column show statistically significant differences ($p<0.05$) according to ANOVA.

Table 3: Anti-inflammatory activity of infusion and decoction extracts obtained from *P. cinnabarinus*.

Anti-inflammatory assay	Infusion	Decoction	Standard
Protein denaturation assay (EC_{50}=µg extract /ml)	283.5±6.3[a]	161.2±4.2[b]	27± 1[c]

Results are presented in mean ± SD (n=3). Different letters in the column show statistically significant differences ($p<0.05$) according to ANOVA.

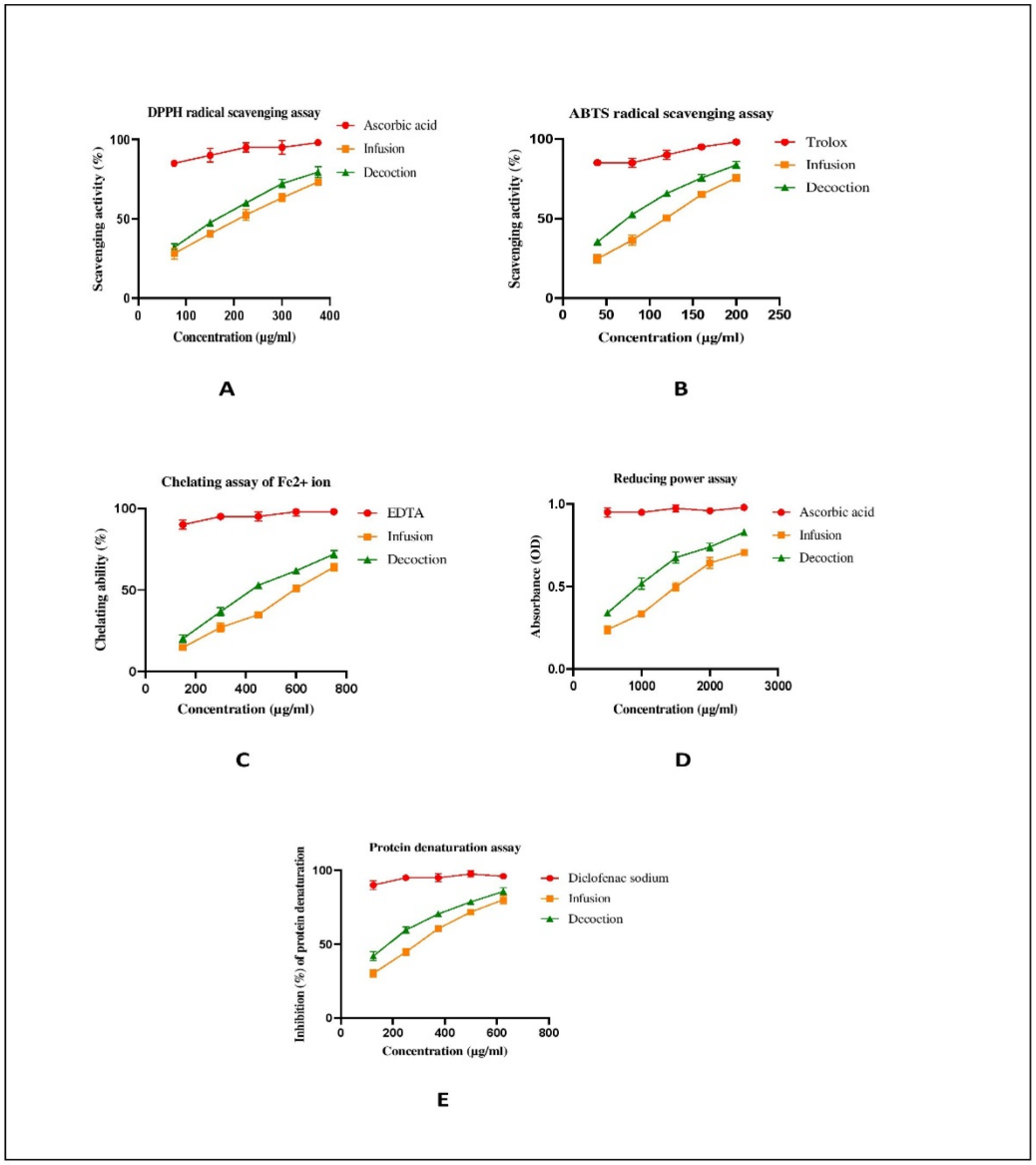

Figure 1: Antioxidant and anti-inflammatory activity of infusion and decoction extract from *P. cinnabarinus*. A - DPPH radical scavenging assay, B - ABTS radical scavenging assay, C - Chelating assay of Fe^{2+} ion, D - Reducing power assay, E - Protein denaturation assay.

4. Discussion

Infusion and decoction are two water extracts prepared through different heat treatments from the fruiting bodies of *P. cinnabarinus* and were subjected to assess essential phytochemicals and therapeutic activities. Total phenol, total flavonoid, and ascorbic acid contents were estimated, and therapeutic activities (antioxidant and anti-inflammatory) were determined. In addition, other phytochemicals like β-carotene and lycopene were also determined. Phytochemical investigation of *P. cinnabarinus* showed that two different extracts (infusion and decoction) have a significant quantity of essential chemical constituents. Decoction extract has a greater quantity of chemical constituents than infusion. The result is quiet different in *Lepista sordida* studied by Ghosh et al., 2018 [26] where infusion (Total phenol= 6.35 µg/mg, total flavonoid= 0.22 µg/mg) had higher amount of essential phytochemicals than decoction (Total phenol= 3.725 µg/mg, total flavonoid= 0.132 µg/mg). Phytochemical contents of both the extract of *P. cinnabarinus* was greater than *Lepista sordida* but lesser than *Auricularia delicata* studied by Ghosh et al., 2018 and Ghosh et al., 2021 [26, 27]**.** It is assumed that the external application of heat differently affects the chemical compositions in infusion and decoction extracts of *P. cinnabarinus*. As more heat is generated during the preparation of decoction extract (heated in boiling water for 5 mins), it releases a greater quantity of chemical constituents than infusion (added boiling water and kept it for 5 mins).

The antioxidant activity of *P. cinnabarinus* is investigated by five different assays: total antioxidant capacity assay (TAC), DPPH assay, ABTS assay, chelating assay of ferrous ion, and reducing power assay. In all assays, significant antioxidant activity is found in two different extracts (infusion and decoction) of *P. cinnabarinus*. In the TAC assay, higher antioxidant activity is found in decoction extract (28.5 µg/mg AAE) than in infusion (15.6 µg/mg AAE) (Table - 2). In the DPPH assay, decoction extract (82 %) has higher scavenging activity than infusion (73.2%) at a concentration of 375 µg/ml (Figure – 1A). And decoction has higher antioxidant activity than infusion with EC_{50} values of 165.5 and 214.7 µg/ml, respectively (Table -2). Scavenging activity of decoction (83.1%) is found to be higher than infusion (75.5%) in ABTS assay at a concentration of 200 µg/ml (Figure – 1B). Decoction has higher antioxidant activity than infusion with EC_{50} values of 77.9 and 118 µg/ml (Table - 2), respectively. Decoction (71.3%) has higher metal chelating activity than infusion (63.5%) in the chelating assay of ferrous ions at a concentration of 750 µg/ml (Figure – 1C). Decoction has higher antioxidant activity than infusion with EC_{50} values of 466 and 601.4 µg/ml, respectively (Table - 2). In reducing power assay, the absorbance of decoction (0.829 OD) is found to be higher than infusion (0.706 OD) at a concentration of 2500 µg/ml (Fig – 1D). Decoction showed higher antioxidant activity than infusion with EC_{50} values of 989.7 and 1560 µg/ml, respectively (Table - 2). Both the extracts of *P. cinnabarinus* showed significant antioxidant activity in the chelating and reducing power assay which was higher than other fungal species like *Lepista sordida* (EC_{50}= 410 and 610 µg/ml for decoction and infusion in chelating assay, EC_{50}= 3440 and 3010 µg/ml for decoction and infusion in reducing power assay) [26]. In DPPH and ABTS assay the extracts showed lower antioxidant activity than *Auricularia delicata* (EC_{50}= 159.9 and 135.1 µg/ml for infusion and decoction in DPPH assay, EC_{50}= 45 and 35.1 µg/ml for infusion and decoction in ABTS assay) [27]. Several reports are found on the antioxidant activity of *P. cinnabarinus* [28, 29]. But our present study is a comparative analysis of the chemical composition and therapeutic activities (antioxidant and anti-inflammatory) of infusion and decoction extracts from *P. cinnabarinus*. In all assays, antioxidant potential of decoction extract is higher as it contains higher amount of bioactive chemical constituents like total phenol, total flavonoid, and ascorbic acid. It is assumed that the generation of high heat in the decoction extract releases a higher quantity of bioactive components that directly affect the antioxidant profile of *P. cinnabarinus*.

In-vitro anti-inflammatory activity of *P. cinnabarinus* is investigated by protein denaturation assay. In these assays, decoction (86.5%) has higher inhibition of protein denaturation than infusion (81%) at a concentration of 625 µg/ml (Figure – 1E). The result showed that decoction has a greater anti-inflammatory effect than infusion with EC_{50} values of 161.2 and 283.5 µg/ml respectively (Table - 3). The result is similar with another researcher Ghosh et al, 2022 [30]. Anti-inflammatory activity

depends on the total flavonoid content of the mushroom. It exerts its role by inhibiting reactive oxygen species and cyclooxygenase enzymes. It also induces inhibition of prostaglandin synthesis [25]. Earlier reports says that ethyl acetate, water, dichloromethane, and methanol extracts of *P. cinnabarinus* have anti-inflammatory effects against the 5-lipoxygenase enzyme [31]. But in this section, a comparative analysis of the anti-inflammatory activity of infusion and decoction extracts of *P. cinnabarinus* is studied. It is supposed that generation of high heat in the decoction extract of *P. cinnabarinus* influenced the release of high flavonoid that directly affected the anti-inflammatory activity.

5. Conclusion

In this study, two important extraction procedures (infusion and decoction) are used to get bioactive compounds from the fruiting bodies of *P. cinnabarinus* and their therapeutic activities (antioxidant and anti-inflammatory) are investigated. A noticeable quantity of bioactive substances is found to be present in both the extracts of *P. cinnabarinus* and have remarkable bioactive properties that made it an appealing natural drug to combat oxidative stress and inflammation-associated diseases. This study concluded that infusion and decoction are two efficient extraction processes to extract maximum bioactive compounds that can be prepared by different heat treatments. It is also concluded that decoction is a more beneficial preparation than infusion.

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