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Anti-inflammatory Action of Polypore Mushroom *Bondarzewia berkeleyi* in Western Ghats of Kanyakumari District, Tamil Nadu

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ABSTRACT: Mushrooms have been utilised for both food and medicinal from the beginning of human history. The ancient Greeks and Romans both held the firm idea that mushrooms, often called "Food of the Gods" in the former, would provide a tactical advantage to seasoned soldiers". Mushrooms have long been revered in Chinese culture as a "elixir of life" and a source of nutrition. The goal of the current research was to assess the anti-inflammatory activity of a particular medicinal fungus, *Bondarzewia berkeleyi.* Lipoxygenase and cyclooxygenase, Nitric oxide synthase, myeloperoxidase were all tested and reported. In Lipoxygenase and cyclooxygenase at the concentration of 100 µl/ml, the inhibitory activity of was 33.55% and 30.41% respectively. Myeloperoxidase at the concentration of 100 µl/ml provided the best inhibitory action, which is dose-dependent. The largest nitric oxide scavenging effect for nitric oxide synthase was shown by mushroom extract, which had a 41.53% efficiency in 50 µl /ml. Recent reports indicate that edible mushroom extracts have beneficial therapeutic and health-promoting effects, especially in diseases associated with inflammation. With certainty, edible mushrooms can be called "superfoods" and are recommended as a valuable component of the daily diet. The aims of this review are to report the different types of bioactive metabolites and their relevant producers, as well as the different mechanisms of action of mushroom compounds as potent anti-inflammatory agents.

Keywords: Mushroom, *Bondarzewia berkeleyi*, COX, LOX, MPO, anti-inflammatory.

INTRODUCTION

Polypore mushrooms have been employed for their therapeutic properties since ancient times. New medications are often discovered by looking to nature as a source of inspiration. In the natural world, an enormous variety of significant physiologically active substances have developed, influenced by evolution and spanning a great diversity of species across several kingdoms (Paintner *et al.,* 1998). In the fungus kingdom, therapeutic (edible) mushrooms have been utilised for treating infections and illness for a long time. Moreover, according to many studies (Blagodatski *et al.,* 2018; Lindequist *et al.,* 1990; Brandt & Piraino 2000), fungi have a great deal of promise as sources of antibacterial, antifungal, antiviral, anti-inflammatory, immunostimulant, and anticancer compounds. There is a long tradition of using mushrooms as food and medicine. Mushrooms have anti-inflammatory properties due to the necessary and optional amino acids they contain, which are known to affect prostaglandin metabolism. The inclusion of amino acids like leucine, isoleucine, tyrosine, and phenylalanine in *Pleurotus ostreatus* (oyster mushroom) has been partly credited with the anti-inflammatory qualities of the fungus (Jedinak *et al.,* 2011). Due to their high quantity of unsaturated fatty acids, mushrooms' fatty acids may help humans' natural anti-inflammatory activities (Ayaz *et al.,* 2011). Among the most significant classes of secondary metabolites, phenolic compounds are abundant in fungal fruiting bodies and have been shown to have anti-inflammatory and antioxidant activities (Ferreira *et al.,* 2009). Human metabolic activities produce free radicals, chiefly reactive oxygen and nitrogen species, an abundance of which may damage cell structure and trigger an inflammatory response. In instance, mushrooms contain active oxidants as well as vitamins including ascorbic acid, carotenoids, and tocopherols (Ferreira *et al.,* 2009). A major category of fungi-derived chemicals with anti-inflammatory properties is called terpenoids. Numerous publications on the identification of novel, active terpene compounds from various species, including non-edible mushroom species, have been made public. Nineteen studied mushroom species from Japan yielded unique terpene chemicals (Yaoita *et al.,* 2014). Nowadays, nonsteroidal anti-inflammatory drugs (NSAIDs) are usually the most commonly administered drugs to reduce inflammation in the body. Many studies, however, have shown that the long-term

administration of NSAIDs has the potential for significant side effects on the gastrointestinal tract (GIT). These include numerous harmful effects such as mucosal lesions, bleeding, peptic ulcers, and intestinal perforation (Smalley *et al*., 1995; Sinha *et al.,* 2013). Recently, therefore, much effort has been devoted to the discovery of alternative anti-inflammatory compounds of plant origin as potential natural and safe medicines without the harmful side effects of NSAIDs (Yuan *et al.,* 2006). While a variety of plants have traditionally been used in human medicine, mushrooms also have a long history as important components of folk medicine and have been widely used. Polypore mushroom terpenoids with potential anti-inflammatory, anti-proliferative, and anti-cancer effects were also identified in tree-dwelling mushrooms. The Polyporaceae family has one such species, *Poria cocos* (poroid mushrooms). In vitro studies employing RAW 264.7 macrophages show that extracts from this species have anti-inflammatory properties. The observed reduction in pro-inflammatory mediator production was due to the blocking of the NF-kB signalling pathway (Jeong *et al.,* 2015). The mushrooms' fungal terpenoids were tested for their anticancer effects on the U937 cell line. Its anti-proliferation and proapoptotic action, confirmed by testing extracts at various concentrations and times, has been connected to the release of cytochrome C to the cytosol, activation of caspases 3, 8, and 9, degradation of PARP, and loss of mitochondrial membrane potential. The collected data highlight *P. cocos's* potential as a leukaemia therapy (Choi, 2015).

MATERIALS AND METHODS

Collections of specimens. The samples were taken early in the morning from the four locations in the Kanyakumari District. The samples were gently lifted up while being held near to the Rhizomorph with the stipe being held close to it, taking some soil with it. This prevents harming the mushroom's tissue. The samples were precisely labeled, air dried, and placed in transport polythene bags that were held loosely to ensure appropriate aeration of the samples.

Drying of Mushroom: The fruit bodies were properly cleaned using running water a minimum of three times and sterile distilled water once. The material for fruiting bodies was then sun-dried for a few days. The sun-dried mushrooms were crushed using a mortar and pestle to make fine powder.

Extraction of bioactive compounds from fruiting bodies of polypore mushroom. In the current study, a pestle and mortar was used to grind the mushroom material into a fine powder. Using a Micro Kjeldahl apparatus and 100 ml of each of the following solvents—ethyl acetate, chloroform, and aqueous—ten grams of mushroom powder were treated to Soxhelet extraction for ten hours. Filtered extracts was stored at 35°C for further analysis.

Anti- inflammatory assay. RAW 264.7 cells have been bought as from National Centre for Cell Sciences (NCCS) in Pune, India. Kept the cells alive in DMEM, or Dulbecco's modified Eagles media. The cell lines begun to grow in 25 cm in 2 culturing flask with antibiotic solution that included Streptomycin (100 g/ml), Penicillin (100 U/ml), Amphotericin B (2.5 g/ml), DMEM with 10% FBS, L-glutamine, and sodium bicarbonate. Cell lines have always been grown in an incubator to 5% CO_2 and a temperature of 37°C (NBS Eppendorf, Germany).

When the cells could be 60% complete, 1 g/mL of lipopolysaccharide was used to activated them up (LPS). RAW cells that had been activated by LPS were given sample at different concentrations of 6.25, 12.5, and 25 l/mL and left for 24 hours. The cell lysate was tested after incubation.

Cyclooxygenase (COX) activity. 5 mM/L of hemoglobin and 5 mM/Lof glutathione were mixed into 100 l of cell lysate along with Tris-HCL buffer (pH 8), and it was allowed to settle at 25°C about one minute. The process was terminated by adding 200 mL of 10% trichloroacetic acid in 1 N hydrochloric acid after 20 min of incubating at 37°C. To initiate the process, arachidonic acid (200 mM/L) was introduced. After centrifugation, 200 L of 1% thiobarbituric acid was added in to the tubes and then heated for 20 min. The tubes are cooled and then centrifuged for three minutes. The percentage of COX activity was calculated using the following method after measuring absorbance at 632 nm. The formula used to determine the enzyme's percentage of inhibition was:

% inhibition = (Absorbance of control-Absorbance of test)/Absorbance of control) × 100

Lipoxygenase (LOX) activity. Sodium linoleate (200 L), 50 L of cell lysate, and Tris-HCl buffer (pH 7.4) were all added to the reaction mixture that reached a final volume of 2 mL. Formation of 5-hydroxyeicosatetraenoic acid, which is indicative of the LOX activity, was seen as increased the absorbance at 234 nm (Agilent Cary 60).

Percentage of inhibition was calculated by using the formula:

% inhibition = (Absorbance of Control-Absorbance of Test)/Absorbance of Control) × 100

Myeloperoxidase (MPO) activity. Hexadecyltrimethylammonium bromide and 50 mM potassium phosphate buffer was used to homogenize the cell lysate (HTAB). The samples were centrifuged at 2000 rpm for 30 minutes at 4 °C, and the MPO activity of the supernatant was measured.50 mM phosphate buffer (pH 6) containing 1.67 mg/mL guaiacol and 0.0005% H_2O_2 was added to the sample to initiate the MPO activity. It was measured at the absorbance of 460 nm. Units of MPO activity were shown per milliliter of cell lysate. One minute of 1 μM peroxide degradation at 25°C was estimated to be one unit of MPO activity.

$$U = \frac{\Delta OD \times 4 \times Vt \times DF}{L \times £470 \times \Delta t \times Vs}$$

ΔOD = density change, Vt = total volume (mL) (1.1 mL), DF = 1, L=light path (1 cm), £470 = extinction coefficient for tetraguaiacol (26.6 $mM^{-1} \cdot cm^{-1}$), Δt = 5 minutes, Vs = 0.1.

Inducible Nitric Oxide Synthase. 2 ml of HEPES buffer was used to homogenize the cell lysate. The

testing system contains 0.1 ml of L-Arginine with a 2 µmol/L concentration, 0.1 ml of manganese chloride with a 4 µmol/L concentration, 0.1 ml of tetrahydropterin with a 4 µmol/L concentration, 0.1 ml of oxygenated hemoglobin with a 10 µmol/L concentration, and 0.1 ml of cell lysate. The following equation was used to calculate the enzyme activity after increasing the absorbance at 401 nm. The formula used to determine the enzyme's percentage of inhibition was:

% inhibition = (Absorbance of control-Absorbance of test)/Absorbance of control) × 100

Estimation of Cellular Nitrite Levels. The combination of 0.5 mL of cell lysate and 0.1 mL of 3% sulphosalicylic acid was vortexed vigorously for 30 minutes. The materials were then centrifuged over 15 minutes at 5,000 rpm. The assessment of nitrite levels was done using the protein-free supernatant. 300 µL of Tris-HCl buffer was added after 30 µL of 10% NaOH to 200 µL of the supernatant, and everything was well mixed. After adding 530 L of Griess reagent (consisting of 2% phosphoric acid, 0.1% N-1-naphthyl ethylene diamine dihydrochloride and 1% sulphanilamide) and allowing the mixture to incubate in the dark about 10-15 minutes, its absorbance was determined at 540 nm using a Griess reagent blank as a reference. The reference solution was sodium nitrite solution. The standard curves developed were used to determine the quantity of nitrite contained in the samples.

RESULT

Lipoxygenase (LOX) activity. The LOX was measured by increasing the absorbance at 234 nm, which shows the formation of hydroperoxylinoleic acid. When there was more crude extract, the effect was strongest. In 100 µ l/ml of extract, the lipoxygenase was inhibited by 30.41 %, which was much higher than in the other two concentrations of crude extracts (Table 1).

Table 1: The percentage of Lipoxygenase (LOX) activity determined by OD at 234 nm.

Volume (µl/ml)	OD at 234nm	Percentage inhibition
Sample code- BMP		
LPS	0.2972	0.00
25	0.236	20.59
50	0.2216	25.43
100	0.2068	30.41

Myeloperoxidase (MPO) activity. Additional earlier research has shown that various anti-inflammatory medications may reduce MPO activity, and this suppression may be responsible for their anti-inflammatory impact. Table 2 shows how polypore mushroom crude extracts affect MPO's peroxidative activity. The amount of MPO activity was decreased by each extract in a concentration-dependent manner. The 100µl/ml ethyl acetate extracts exhibited the best myeloperoxidase activity inhibition under these circumstances.

Table 2: The percentage of Myeloperoxidase (MPO) activity determined by OD at 460nm.

Volume (µl/ml)	OD at 460nm	Enzyme Activity (U/ml)
Sample code- BMP		
LPS	0.0181	0.0059
25	0.015	0.0049
50	0.0074	0.0024
100	0.0058	0.0019

Inducible Nitric Oxide Synthase. Comparing the incubation bacterial cells to the LPS control group, the generation of Nitric Oxide was significantly reduced in a dose-dependent manner at different doses (25, 50, and 100 µl/ml) by the incubation bacterial cells. As seen in Table 3, When protein extract containing 100µ l/ml of LPS was added, the inhibition of Nitric Oxide considerably increased to levels of 41.53%. Mushroom extract seemed to have the largest impact on scavenging Nitric Oxide, with a 41.53% reduction, followed by extracts containing 50µ l/ml and 25µl/ml at 27.07% and 16.30%, respectively.

Table 3: The percentage of inhibition by mushroom for Inducible Nitric Oxide Synthase at 401 nm.

Volume (µl/ml)	OD at 401 nm	Percentage of Inhibition (%)
Sample code- BMP		
LPS	0.0325	0.00
25	0.0272	16.30
50	0.0237	27.07
100	0.019	41.53

Estimation of Cellular Nitrite Levels. The ethyl acetate extracts of the crude protein from polypore mushrooms were tested using a nitrite radical scavenging assay at concentrations ranging from 25 to 100µl/ml. As demonstrated in Table 4, the percentage of extracts that scavenged free radicals was measured against extract concentration. In order to scavenge for the nitrite radical, the mushroom crude extract engaged in oxygen-based competition. As the extracts' concentration increased, thus increased their antioxidant activity. There was an increase in nitrite concentrations when the extract concentration was increased (Fig. 1).

Table 4: Estimation of Cellular Nitrite Levels at 540nm.

Volume (µl/ml)	OD at 540nm	Concentration of Nitrite (µg)
Sample code- BMP		
LPS	0.0243	0.00
25	0.0189	22.63
50	0.0135	45.13
100	0.011	53.36

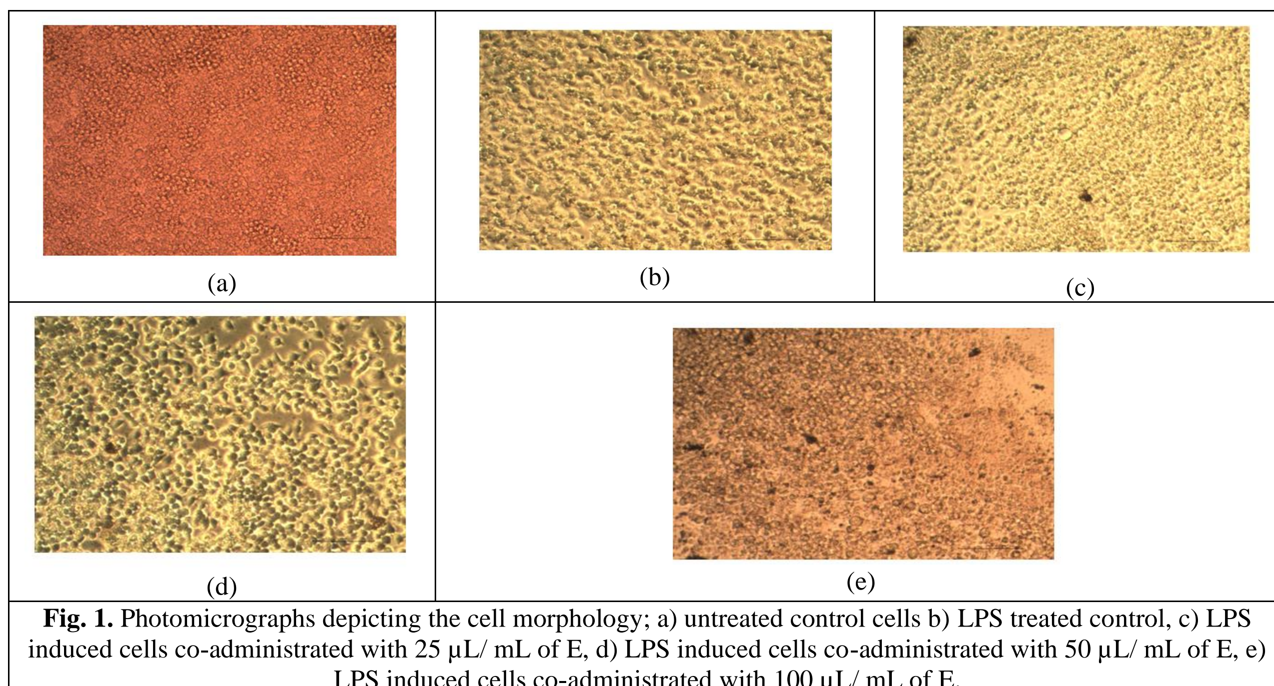

Fig. 1. Photomicrographs depicting the cell morphology; a) untreated control cells b) LPS treated control, c) LPS induced cells co-administrated with 25 µL/ mL of E, d) LPS induced cells co-administrated with 50 µL/ mL of E, e) LPS induced cells co-administrated with 100 µL/ mL of E.

DISCUSSION

The phenolic concentrations (162.72 + 8.77, 124.01 + 0.80, 103.34 + 2.39, 88.65 + 5.58) mg/g tannic acid equivalents for the various mushrooms *Piccnoporrus cinnabarinus, Pleurotus eryngii, Termitomyces robustus*, as well as *Pleurotus ostreatus* demonstrated the phenolics are present in significant amounts in the mushrooms. Their studies and investigations that phenolics are a substantial source in some mushroom species. The hydroxyl groups in the phenolics may be the cause of the mushroom extracts' antioxidant action (Hatano *et al.,* 1989). A similar conclusion was made for the extracts of *Eucopia ulmoides* and *Acacia confusa*, which showed that their antioxidant activity and enhanced phenolics corresponded well (Yen & Hsieh1998; Chang *et al.,* 2001).

According to Gonzalez-Nunez *et al.* (2001), flavonoids have a cortisone-like action on tissues, reduce capillary fragility, and defend against cancer and heart disease (Filippos *et al.,* 2007). The therapeutic effect (antihypertensive) of certain mushroom species previously described may be due to the high flavonoid content in the mushroom extracts. *P. ostreatus* extract had the greatest concentration of flavonoids, followed by *P. cinnabarinus* extract, while P. *eryngii* and *T. robustus* extracts had the lowest concentrations of flavonoids (Hatano *et al.,* 1989; Gonzalez-Nunez *et al.,* 2001; Ogbonnia *et al.,* 2008).

The ability of some medicinal plants can reduce their ability to fight free radicals. According to a study by Nenadis *et al.* (2004), reducing properties may constitute a unique antioxidative defense mechanism. This is possible because the antioxidant compound has the ability to reduce transition metals. The redox characteristics of phenolics, which enable them to function as reducing agents, hydrogen donors, and singlet oxygen quenchers, are what give them their antioxidant capabilities. The findings of the tests for the different mushrooms extract showed that *P. cinnabarinus* extract had the lowest reducing power with increasing concentration, whereas *T. robustus* extract had the highest reducing power as the concentration increased (Rice-Evans *et al.,* 1996).

According to Singh *et al.* (2012), the methanol *Centella asiatica* extract used in the research of nitric oxide had positive findings, reaching a maximum activity of 50% at 200 µg/ml, compared to ascorbic acid's 47.3% inhibition at the same dosage. In my research, the mushrooms' ability to scavenge nitric oxide radicals was shown. In all three concentrations, the mushroom extract exhibited a similar capability for reduction. However, it was found that ethyl acetate had the highest capacity to fix or scavenge nitric radicals (65% at a concentration of 100 µg/ml), while *B. berkeleyi* extract had the lowest capacity of the mushroom extracts to convert hydroxyl radicals to safe water molecules (22% at a concentration of 25 µg/ml). The fundamental idea behind the phosphomolybdenum test, according to Khan *et al.* (2012), is the conversion of Mo (VI) to Mo (V) by plant extracts that include antioxidant chemicals. In the current investigation, inclusion of the different *Launaea procumbens* fractions revealed that the methanolic extract of *L. procumbens* (IC5064.27 2.1 g/ml) was more successful in reducing Mo (VI) to Mo (V), but the hexane extract of *L. procumbens* (123 3.09 g/ml) had the least effects. Ascorbic acid (IC50 72.3 2.2 g/ml) was administered to convert Mo (VI) to Mo (V), which indicated the existence of potent antioxidants in different *L. procumbens* fractions. In his investigation, ethyl acetate extract exceeded hexane extract in terms of results. Similarly, in our work, an ethyl acetate extract of *B. berkeleyi* at the concentration of 100 µg/ml showed promising result of 65%.

He *et al.* (2018) found that the amount of AKP that leaked out of groups that were treated with corylifolinin

at the MIC level seems to be much higher than that of the control group. The amount of AKP that leaked out was linked to the amount of corylifolinin. The more AKP that could be found, the higher the concentration. In the same way, in our experiment, polypore mushroom extract made bacterial strains' cell walls more permeable in a relatively short period of time.

Diao *et al*. (2014) investigation showed that adding linalool made macromolecules leak out of cells. So, linalool may act on the cell membrane and modify the way it works. When this happens, nucleic acids and proteins leak out of the cell through the damaged membrane, and more macromolecules leak out of the cell. In the same way, when the protein leakage in *Proteus vulgaris* was treated with polypore mushroom extract at the MIC level and 3MIC level, the soluble protein leakage was higher than in the control group, especially after 5 hours.

Research by Ejelonu *et al.* (2013) showed that the four mushroom extracts can bind to metals. The *P. ostreatus* extract showed that 400 g/ml had the highest value (53.78%). The lowest metal chelating capacity of the four mushroom species was possessed by *T. robustus*, which also displayed the lowest metal chelating ability value (25.18% at 10 g/ml). Researchers have discovered that antioxidants which attach to metal ions may also reverse the harm done by other pro-oxidant metal ions like Cu that are less well-known but just as detrimental (Halliwell, 2001). In my study, crude samples of *B. berkeleyi* mushrooms showed a high range of metal-binding ability, with 65% of metals being bound in 100 µg/ml.

CONCLUSIONS

In summary, our research showed that the mushroom in concern possessed anti-inflammatory capabilities. This suggests that the mushroom could be considered a functional food with anti-inflammatory qualities. Now that we have a clearer grasp of the biological activity, we must turn our attention to identifying the molecules responsible for it. However, further research is required to learn how exactly these bioactive components and other crucial nutrients affect and interact with one another. To sum up, the aforementioned obstacles must be addressed through further study before macrofungi can be universally recognised as one of the most important biofactories for the manufacturing of anti-inflammatory medications.

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Conflict of Interest. None.

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