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Research Article

ETHNOPHARMACOLOGICAL INVESTIGATION OF *PLEUROTUS OSTREATUS* FOR ANTI-OXIDATIVE AND ANTI-INFLAMMATORY ACTIVITY IN EXPERIMENTAL ANIMALS

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ABSTRACT

Objective: This particular study was aimed to evaluate the anti-oxidant effect of *Pleurotus ostreatus* using the *in vitro* method and also against inflammation.

Methods: A methanolic extract of *P. ostreatus* (MEPO) was prepared by the cold maceration technique. Different tests. *In vitro* anti-oxidant activity was investigated using the 1,1-diphenyl-1-picryl hydrazyl (DPPH) method. Wistar rats were chosen for the study; animals weighing 150–200 g were divided into six groups of six each (n=6). Control animals were grouped as I, group II was administered with approximately 100 µL of 1% suspension of carrageenan in saline and injected into the plantar surface of the right hind paw, group III, IV, V, and VI were given with carrageenan, followed by diclofenac sodium (150 mg/kg body weight, p.o.) and MEPO at the dose of 200, 400, and 800 mg/kg orally for 21 days. The paw volume and percentage of inhibition of the paw were measured in all animals.

Results: The IC_{50} values of the test extract in the DPPH free radical scavenging assay were found to be 44.02±0.09 as compared with the standard drug 35.01±0.12, which was considered significant (***p<0.001). The test extract at the dose of 400 and 800 mg/kg significantly decreased (**p<0.001) the paw volume thereby, the inflammation, the % percentage of inhibition in paw volume was compared to the positive control in the carrageenan-induced paw edema.

Conclusion: The study explored a potential source for anti-oxidants in the MEPO, which also seems to be effective in chemical-induced inflammation.

Keywords: *Pleurotus ostreatus*, Anti-oxidant effect, Reactive oxygen species, Inflammation.

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INTRODUCTION

A contemplated system which acts as a defense system of our body is named as inflammation, stands against harmful stimulants that cause injury to the tissues/cells, and subsides once healing begins; on the contrary, unrestricted inflammation is the root cause for several diseases such as allergic reactions, cardiovascular dysfunction, cancers, and autoimmune-related diseases. These have massive impact on individuals and also subsequently on the society [1]. A normal metabolism in the cells generates free radicals. A free radical has an unpaired or single electron in the valence shell and always has an odd number of electrons, which makes them unstable, fleeting, and extremely reactive. In many human diseases, the role of free radicals is highlighted as they cause damage to the cells and depend on the various inflammatory processes and mechanisms in the cells. With the impact of various physical and chemical conditions or pathological conditions, distinctive endogenous systems generate free radicals such as reactive oxygen species (ROS) and reactive nitrogen species [2]. Free radical production during chronic inflammation leads to an increase in the inflammation itself. The human body's numerous systems can be harmed by this ongoing vicious cycle. During the inflammation process, mast cell release proteases, release of ROS, and free radicals induce a cascade of redox events, which stand beneficial for signal transduction in cells and they also protect against pathogenic organisms but when uncontrolled becomes deleterious [3]. As a part of the innate immune system, the unregulated progression of proinflammatory activities is correlated to oxidative stress and other diseases such as cancer, diabetes, hypertension, and certain CNS disorders. Keeping in view the above considerations, control on the proinflammatory and chronic inflammation remained a cornerstone due to opportunities for lowering or eradicating severe neurodegenerative diseases [4].

To conquer the oxidative stress, the use of anti-oxidants becomes mandate as it is associated with a reduction in the genesis of ROS and lipid peroxides, a decrease in oxidative stress, post-translational modification of proteins, and damage to the DNA [5]. There exist several medications for regulating and repressing inflammation,suchassteroids, non-steroidal anti-inflammatory drugs, and immunosuppressants. In this context, depending on synthetic antioxidants used for regulating unwanted redox processes is dubious; imply to be expensive and frequently plagued by unavailability and negative effects. Alternatively, the majority of these protective antioxidants can be found in foods and plants. In clinical practice, a goal must be set for the minimum effective dose with more efficacy and fewest side effects.

Mushrooms have been prized for their distinctive flavor and delicate aroma in gourmet cuisine all over the world since ancient times [6]. Many species of mushrooms possessed and generated numerous of inexplicable biological properties [7]. The nutritive value of mushrooms was based on the presence of bioactive compounds. They stood as a remedy for plenty of diseases and also for nourishment [8]. Although ancient history narrated the mushrooms use in medicine, in the present time, few contemporary studies have promoted them in the maintenance of good health and vivacity. There are over 270,000 plants on the planet, out of which a tiny part has been explored phytochemically. Among the medicinal plants, mushrooms were found to possess approximately high amounts of carbohydrates, proteins, fiber, and low levels of fat; they are also rich in vitamins. They also contain bioactive phenolic compounds, carotenoids, and unsaturated fatty acids, all of which might help to combat medical conditions through a few properties such as anti-oxidant, anti-inflammatory, anti-fungal, anti-bacterial, anti-hypertensive, hepatoprotective, anti-allergic, anti-diabetic, and anti-cancer. The fungal species *Pleurotus ostreatus*, known as the oyster

mushroom, was noted for being an edible variant. During World War I, it was initially grown as a survival strategy in Germany [9]. Although it may be grown on straw and other materials, one of the more popular wild mushrooms is the oyster mushroom. It smells like benzaldehyde, just the same as bitter almonds [10].

Pleurotus species grow on sawdust, wood, and wet areas and require a temperature of 10–32°C for their growth, with medicinal benefits in the traditional system of medicine. Among the different varieties of mushrooms available, considering the medicinal potential of *P. ostreatus*, researchers focused and began to investigate the therapeutic efficacy [10]. Globally, *P. ostreatus* is the most grown and edible species among the mushrooms. In the present investigation, *in vitro* method was used to evaluate the anti-oxidant effect of the test drug. Ascorbic acid was used as a standard anti-oxidant agent, acts by participating in oxidation. Ascorbate gets oxidized into monodehydroascorbate and then dehydroascorbate in the presence of ROS; thereby, ROS gets reduced to water, while the oxidized form remains stable without causing damage to cells.

There is a release of many inflammatory and proinflammatory mediators during acute inflammation with an overabundance of free radicals, triggering complex enzymes. Novel anti-inflammatory compounds can be screened by a conventional model named the carrageenan-induced paw edema method. Carrageenan acts by release of amine autocoids and bradykinin, with a meager production of prostaglandins produced by cyclooxygenase enzymes (COX) followed by the infiltration of neutrophils [11]. Hence, it is always suggested that drugs targeting the COX enzyme, free radical generation, and proinflammation-related proteins might exhibit a greater control over the inflammation as compared to the other therapeutic agents [12]. The present study investigated the effect of the methanolic extract of *P. ostreatus* (MEPO) on oxidative stress and carrageenan-induced inflammation in Wistar rats.

METHODS

Plant collection and authentication

P. ostreatus was obtained from the local places of Tirupati, AP. *P. ostreatus* was authenticated by Dr. K. Madhava Chetty, M.Sc., M.Ed., M.Phil., Ph.D., PG DPD., Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh.

Samples preparation and extraction

The *P. ostreatus* fungus was thoroughly cleaned with water, evacuated, and then thinly sliced. Then dried at room temperature and strained through sieve mesh 60, again dried in the oven at 450°C. A cold maceration technique was used to extract 150 g with 500 ml of methanol for 72 h, along with agitation. The solution was filtered by means of a muslin cloth followed by Whatman No. 1 filter paper to obtain the test extract. Then, the extract was made concentrated in a rotary evaporator at a temperature of no more than 600°C. About 20 mL of the extract was collected and dried in an oven at 450°C, leaving behind a semi-liquid. The extract thus obtained was utilized for further testing and analysis [13].

Phytochemical screening

The MEPO was subjected to the identification of various phytoconstituents such as flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, and carbohydrates [13,14].

Evaluation of oxidative stress by *in vitro* method

2,2-Diphenyl 1-picryl Hydrazyl) assay (DPPH) [15]:

This assay was used to evaluate the anti-oxidant effect of MEPO. It is a violet-colored free radical with a spare electron. A decrease in the absorbance at 517 nm was noted with the addition of the antioxidants to the DPPH solution in methanol. When a substance was mixed with DPPH, a yellow-colored compound was formed.

$$(DPPH)+(H\text{-}A)\longrightarrow DPPH\text{–}H+(A)$$

(Purple) (Antioxidant) (Yellow)

Anti-oxidant agents reduce DPPH to DPPH-H. The free radical scavenging activity of the extract was determined by decreased absorbance in this reaction. The percentage inhibition was calculated by the following formula:

$$\text{Radical scavenging activity}\ (\%) = \frac{ODControl - ODSample}{ODControl} \times 100$$

$$\frac{ODControl - ODSample}{ODControl} x100$$

IC_{50} is the concentration of sample required to scavenge 50% of the free radicals. IC_{50} was calculated from the equation obtained by plotting a graph of concentration versus % inhibition.

Added 1.9 mg of DPPH to methanol to obtain a concentration of 0.1 mm and volume was made up to 100 mL with methanol. The reaction was completed when the solution was kept in darkness for 30 min. A stock solution of 1000 µg/mL concentration was prepared by dissolving a quantity of 25 mg of the test extract and ascorbic acid separately in methanol, and the final volume was made up to 25 mL. Different concentrations of 50, 100, 200, 300, 400, and 500 µg/mL were prepared from the stock solution by diluting it with methanol. 1 ml was taken in each test tube, and 3 mL of a methanolic solution of DPPH was added to these. They were incubated for 20 min at 37°C. The absorbance was recorded at 517 nm, and the radical scavenging property in the form of % was calculated. Ascorbic acid was used as a standard agent. The concentrations of the test extract were recorded in triplicate, and the average result was noted; the IC_{50} was calculated [16].

Acute toxicity tests

According to OECD Guidelines No. 423, toxicity studies were carried out. Animals were grouped into eight groups, each group with six rats (n=6), administered with an initial dose of 50–2000 mg/kg body weight. Observations for toxicity were noted after 48 h for behavioral changes, nervous problems, and any lethality [17].

Experimental animals

Wistar rats weighing 150–200 g were brought from the lab bearing a valid registration number to Hyderabad, India. With the maintenance of room temperature, animals were housed in cages with water and food *ad libitum*, and the animals were kept at a constant temperature of 20±1°C on a 12-h light/dark cycle. The protocol was approved in the Institutional Animal Ethics Committee as per CCSEA, and it was accepted with no. 1447/PO/Re/S/11/CCSEA-65/A.

Experimental design

The animals were divided into six groups of six rats each (n=6)

- Group I: Control
- Group II: Carrageenan-induced (CI, 1% p.o.)
- Group III: CI+Diclofenac sodium (150 mg/kg body weight)
- Group IV: CI+MEPO (200 mg/kg, p.o.)
- Group V: CI+MEPO (400 mg/kg, p.o.)
- Group VI: CI+MEPO (800 mg/kg, p.o.)

Anti-inflammatory activity by the carrageenan-induced rat paw edema method

In the present study, approximately 100 µL of a 1% suspension of carrageenan in saline was prepared 1 h before the experiment and was injected into the plantar surface of the right hind paw of animals from groups II to VI. Animals of groups from III to VI were administered with a single dose of the standard and test drugs at their respective doses for 21 days, 30 min before the carrageenan injection. Immediately after carrageenan injection at 0, 1, 2, 3, and 4 h, the paw volume was measured using a plethysmometer. The percentage inhibition in paw volume was calculated using the formula [18]:

$$\%\ Inhibition\ in\ paw\ volume = \frac{Paw\ volume\ (Control) - Paw\ volume\ (Test}{Paw\ volume\ (Control)} \times 100$$

Table 1: Percentage inhibition of MEPO in DPPH free radical scavenging assay with IC_{50} values

Groups	Concentration (µg/mL)	% inhibition	IC_{50} values
Ascorbic acid	50	6.00±1.15	38.76±0.52
	100	13.9±1.2	
	200	29.05±0.91	
	300	49.01±1.73	
	400	57.35±1.34	
	500	82.9±1.73	
MEPO	50	10.09±0.57	44.02±0.09**
	100	18.80±0.52	
	200	34.12±0.58	
	300	50.05±1.15	
	400	78.68±0.57	
	500	82.66±0.58	

**p<0.001 considered as significant; compared with corresponding standard

Statistical analysis

The data values were compiled and represented in mean±SEM, analyzed by one-way ANOVA followed by Dunnett's test in Graph Pad Prism 5. The groups were compared to that of control. Values were considered significant at p values: ***p<0.001, **p<0.01, *p<0.05, NS-Non-significance.

RESULTS AND DISCUSSION

Preliminary screening of phytochemical constituents

The presence of various phytoconstituents such as flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, and carbohydrates was observed in MEPO.

Acute toxicity studies

No toxicity signs were observed in acute toxicity tests, and the MEPO was found to be safe for the current investigation. The maximum tolerated dose of MEPO was 2000 mg/kg; the test doses were selected in a geometric pattern. Hence, three doses of MEPO (200, 400, and 800 mg/kg body weight, p.o.) were chosen for the study.

In the DPPH assay, the % inhibition of the test extract at different concentrations was dose-dependent. The test extract showed 50% inhibition (IC_{50}) at 44.02±0.09 as compared to ascorbic acid (35.01±0.12).

In carrageenan-induced paw edema in rats, the effect of a methanolic extract of *P.ostreatus* was evaluated. The anti-inflammatory activity was investigated by paw volume. The test extract and standard (150 mg/kg) were administered at their respective doses. When carrageenan was injected in rats and the reading was taken, there was an increase in volume at different time intervals, which represented an inflammatory reaction. The test extract at the dose of 400 mg/kg showed a significant reduction in the volume (**p<0.001) with 1.61, 1.65, 1.99, and 1.73 mL at 1, 2, 3, and 4 h, respectively. Furthermore, the test extract at the dose of 800 mg/kg showed a significant reduction in the volume (**p<0.001) with 1.48, 1.42, 1.40, and 1.35 ml at 1, 2, 3, and 4 h. The % inhibition recorded at 4 h produced by diclofenac sodium was found to be 24.27%. After the treatment for 21 days, the test extract at the dose of 400 and 800 mg/kg produced about 22 % of inhibition in paw volume.

Free radicals are produced continuously in living systems and can significantly harm tissues and biomolecules, resulting in a variety of diseases, including degenerative disorders, and causing significant lysis [19]. About the different treatment options, there are several synthetic drugs available to combat oxidative damage but present adverse effects [20]. Hence, to resolve this problem, it is always better to consume anti-oxidants naturally from the regular

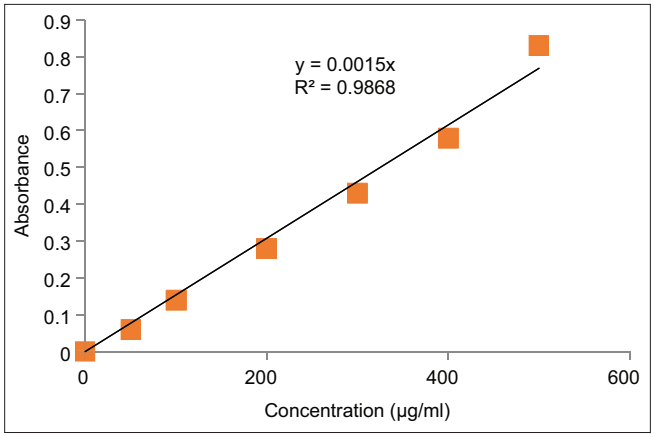

Fig. 1: Standard graph of ascorbic acid in DPPH radical scavenging assay

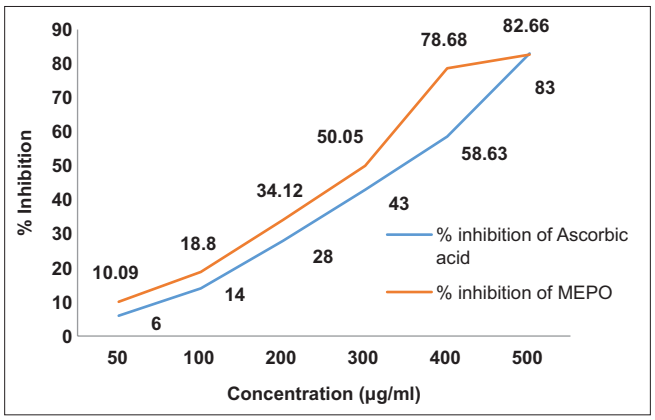

Fig. 2: Effect of methanolic extract of *P. ostreatus* and ascorbic acid on % inhibition in DPPH assay

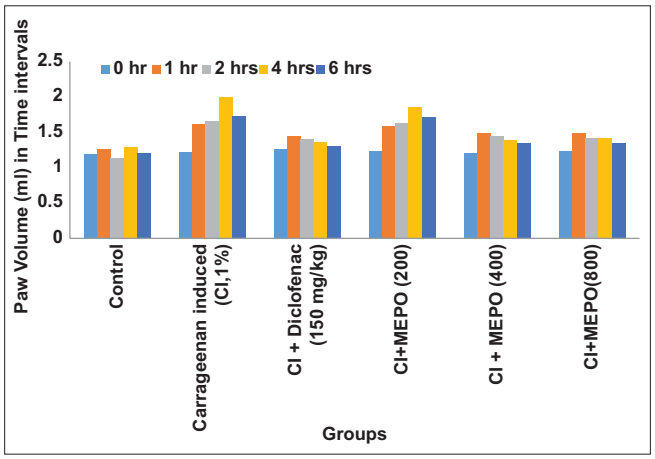

Fig. 3: Effect of methanolic extract of *P. ostreatus* on carrageenan-induced paw edema in rats

diet and might act as medicine obtained from a plant source. Many natural antioxidants have recently been recognized from various herbs. In this context, the oyster mushroom (*P. ostreatus*) is a culinary mushroom with a potential source of a plethora of essential nutrients and also the presence of bioactive compounds with their therapeutic effects. The bioactive compounds include peptides, polysaccharides, liposaccharides, glycoproteins, lectins, triterpenoids, fatty acids, essential amino acids, and nucleosides. Globally, as mushrooms can be cultivated commercially, it is quite easy for mankind to include them in

Table 2: Effect of methanolic extract of *P. ostreatus* on carrageenan-induced paw edema in rats

Treatment	Paw volume (ml) at different hours				
	0 h	1 h	2 h	3 h	4 h
Control	1.19±0.16	1.26±0.23	1.13 ± 0.01	1.29±0.7	1.21±0.5
Carrageenan-induced (CI) (1%)	1.22±0.02	1.61±0.9**	1.65±0.14**	1.99±0.8**	1.73±0.8**
CI+Diclofenac sodium (150 mg/kg)	1.26±0.20	1.45±0.12**	1.40±0.22**	1.36±0.14**	1.31±0.6** (24.27%)
CI+MEPO (200 mg/kg)	1.23±0.02	1.59±0.01	1.63±0.04	1.85±0.01	1.71±0.21** (11.49%)
CI+MEPO (400 mg/kg)	1.21±0.01	1.48±0.12**	1.45±0.1**	1.39±0.17**	1.34±0.15** (22.54%)
CI+MEPO (800 mg/kg)	1.24±0.01	1.49±0.012**	1.42±0.1**	1.41±0.21**	1.35±0.15** (22%)

Results were expressed in Mean±SEM, (n=6), was found to be significant as compared with positive control (**p<0.001)

their regular diet, thus can be preferred as food and medicine. It also contains proteins, carbohydrates, vitamins, amino acids, lipids, and fibers. *P. ostreatus* is produced commercially for its culinary, nutritive, and therapeutic benefits. Its ethnobotanical uses emphasize the presence of nutrients such as vitamins B1 (thiamin), B2 (riboflavin), B3 (niacin), B9 (folic acid), and ascorbic acid, also it contains that internal polysaccharides and exopolysaccharides were found to possess anti-oxidant properties. *P. ostreatus* has a huge potential to generate unique, value-added products that promote health. In the current investigation, the preliminary phytochemical screening of *P. ostreatus* revealed the presence of various phytoconstituents such as flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, and carbohydrates in MEPO. The test extract expressed an *in vitro* anti-oxidant effect, which plays a crucial role in radical scavenging activity, thus counteracting with cancer. A recognized method for evaluation of the anti-oxidant activity of plant extracts was the DPPH free radical scavenging method. In the present study, the anti-oxidant effect was investigated using the DPPH method. The decolorization of the methanol solution of DPPH was used for the determination of the hydrogen atom-donating ability of the plant extract [21]. This method involved molecules that act as anti-oxidants if they were able to reduce and scavenge DPPH free radicals were detected by a distinctive color shift from blue to yellow, measured at 517 nm. In addition, the test drug was a robust anti-oxidant agent, as the IC_{50} value was less than 50% and % inhibition was concentration-dependent [22]. The anti-oxidant property was attributed to the presence of phenolic substances. Phenolic compounds are metabolites of plants responsible for color, nutritional benefits, and anti-oxidant properties [23]. It has been reported that there is a strong relationship between phenolic content and the anti-oxidant effect. Some therapeutic plants and herbs' anti-inflammatory effects have been shown effective, while others are ineffective. Alternative therapeutic modalities are required because the use of current anti-inflammatory drugs is frequently associated with severe side effects. For the past few decades, globally population has relied on herbal medicines for primary healthcare due to acceptance with the human system and possesses minor side effects [24]. To validate the efficacy and clarify the safety profile of such traditional or herbal therapies for their anti-inflammatory potential, additional scientific study as well as traditional knowledge must be combined. Hence, the present study was selected for investigation of the anti-inflammatory activity of the methanolic extract of *P. ostreatus* in carrageenan-induced paw edema in experimental rats. Carrageenan was used for the induction of acute inflammation in experimental animals for the determination of anti-inflammatory activity. According to past studies, it is a challenge to cause inflammation in experimental animal models. The majority of research found that a wide range of factors contribute to the variable ways in which inflammation is induced [25]. In the present study, carrageenan injection induced the inflammation process which was evident from the increased paw volume at 1, 2, 3, and 4 h, respectively. With the treatment of the test drug, the paw volume was reduced at the dose of 400 and 800 mg/kg, and the % inhibition was about 22%, which indicated an anti-inflammatory effect. This particular effect was attributed to the presence of flavonoids in the test extract, which is one of the phenolic compounds found in the plants. These flavonoids possess anti-inflammatory properties and act by inhibiting enzymes that take part in the inflammation process [26]. Correspondingly, flavonoids serve as potent anti-oxidants to reduce the generation and scavenge the free radicals. Hence, they manifest an important role in the immune mechanisms to combat inflammation and related ailments. In this context, phenolic substances were also found to be critical in oxidative stress and were found to be with reductive and oxidative capacities that conquer the effects of free radicals. The test extracts anti-oxidant and anti-inflammatory activities due to the presence of constituents responsible for the same [27]. These molecules illustrated a potential effect on the scavenging system to destroy radicals or neutralize them. Herbal medicine is considered predominant as a complementary medicine, also prescribed by practitioners as a traditional medicine for various illnesses. Herbal treatments were used to treat mild to moderate illnesses or as a first line of therapy before the start of conventional medicines [28]. Hence, treatments with traditional medicines were found to be trustworthy, with fewer side effects, cost-effective, and explicit patient compliance. In addition, consuming herbs in daily regular food or as a supplement fetches a great use in conquering many ailments, such as diabetes, cancer, and diseases related to inflammation.

CONCLUSION

Numerous studies have demonstrated that medicinal plants possess active constituents that are in charge of their anti-oxidant action. Many chronic diseases can be treated with phytochemical constituents, which are contained in medicinal plants. Several healing plants have sprouted up in recent years with many potent biomolecules. The present study explored the effectiveness of *P. ostreatus* as an anti-oxidant and anti-inflammatory agent. After the treatment with *P. ostreatus* in chemically induced inflammation, the results showed a tremendous and significant effect against inflammation, hence suggesting effective herbal medicine and functional foods against inflammation.

AUTHORS' CONTRIBUTIONS

All authors contributed for the study. D.B., designed and involved in the readiness of the manuscript. L.C. conducted the experiment, analyzed and compiled the results, and prepared the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interests.

REFERENCES

1. Mansouri MT, Hemmati AA, Naghizadeh B, Mard SA, Rezaie A, Ghorbanzadeh B. A study of the mechanisms underlying the anti-inflammatory effect of ellagic acid in carrageenan-induced paw edema in rats. Indian J Pharmacol. 2015 May-Jun;47(3):292-8. doi: 10.4103/0253-7613.157127. PMID: 26069367; PMCID: PMC4450555
2. Duan L, Rao X, Sigdel KR. Regulation of inflammation in autoimmune disease. J Immunol Res. 2019 Feb 28;2019:7403796. doi: 10.1155/2019/7403796. PMID: 30944837; PMCID: PMC6421792
3. Chen Z, Bozec A, Ramming A, Schett G. Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. Nat Rev Rheumatol. 2019 Jan;15(1):9-17. doi: 10.1038/s41584-018-0109-2. PMID: 30341437
4. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn

Rev. 2010 Jul;4(8):118-26. doi: 10.4103/0973-7847.70902. PMID: 22228951; PMCID: PMC3249911

5. Aruoma OI. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutat Res. 2003 Feb-Mar;523-524:9-20. doi: 10.1016/s0027-5107(02)00317-2. PMID: 12628499
6. Sarangi I, Ghosh D, Bhutia SK, Mallick SK, Maiti TK. Anti-tumor and immunomodulating effects of *Pleurotus ostreatus* mycelia-derived proteoglycans. Int Immunopharmacol. 2006 Aug;6(8):1287-97. doi: 10.1016/j.intimp.2006.04.002, PMID: 16782541
7. Liu RH. Health benefits of fruits and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr. 2003;78:517S-S520.
8. Sullivan R, Smith JE, Rowan NJ. Medicinal mushrooms and cancer therapy: Translating a traditional practice into Western medicine. Perspect Biol Med. 2006 Spring;49(2):159-70. doi: 10.1353/pbm.2006.0034. PMID: 16702701
9. Barh A, Sharma VP, Annepu SK, Kamal S, Sharma S, Bhatt P. Genetic improvement in *Pleurotus* (oyster mushroom): A review. 3 Biotech. 2019 Sep;9(9):322. doi: 10.1007/s13205-019-1854-x, 2019 Aug 6. PMID: 31406644; PMCID: PMC6684725
10. Akcay C, Ceylan F, Arslan R. Production of oyster mushroom (*Pleurotus ostreatus*) from some waste lignocellulosic materials and FTIR characterization of structural changes. Sci Rep. 2023 Aug 9;13(1):12897. doi: 10.1038/s41598-023-40200-x. PMID: 37558821; PMCID: PMC10412599
11. Arawwawala M, Thabrew I, Arambewela L, Handunnetti S. Anti-inflammatory activity of *Trichosanthes cucumerina* Linn. in rats. J Ethnopharmacol. 2010 Oct 5;131(3):538-43. doi: 10.1016/j.jep.2010.07.028. PMID: 20654707
12. Domínguez M, Avila JG, Nieto A, Céspedes CL. Anti-inflammatory activity of *Penstemon gentianoides* and *Penstemon campanulatus*. Pharm Biol. 2011 Feb;49(2):118-24. doi: 10.3109/13880209.2010.503708. Epub 2010 Oct 28. PMID: 20979542
13. NagalingamS, Sasikumar CS, CherianKM. Extraction and preliminary phytochemical screening of active compounds in *Morinda citrifolia* fruit. Asian J Pharm Clin Res. 2012;5(2):179-81.
14. Hinneburg I, Damien Dorman HJ, Hiltunen R. Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem. 2006;97:122-9.
15. Garg D, Shaikh A, Muley A, Marar T. *In vitro* antioxidant activity and phytochemical analysis in extracts of *Hibiscus rosasinensis* stem and leaves. Free Radic Antioxid. 2012;2(3):41-6. doi: 10.5530/ax.2012.3.6
16. Rajesh KD, Vasantha S, Panneerselvam A, Valsala Rajesh NV, Jeyathilakan N. Phytochemical analysis, *in vitro* antioxidant potential and gas chromatography-mass spectrometry studies of *Dicranopteris linearis*. Asian J Pharm Clin Res. 2016;9(2):1-6. doi: 10.22159/ajpcr.2016.v9s2.13636.14
17. Kennedy GL Jr. Acute toxicity studies with oxamyl. Fundam Appl Toxicol. 1986 Apr 1;6(3):423-9. doi: 10.1016/0272-0590(86)90215-0, PMID 3699328
18. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and antiinflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharm Sci. 2010;2:146-55.
19. Reshma, Arun KP, Brindha P. *In vitro* anti-inflammatory, antioxidant and nephroprotective studies on leaves of *Aegle marmelos* and *Ocimum sanctum*. Asian J Pharm Clin Res. 2014;7:121-9.
20. Iqbal Z, Iqbal MS, Mishra K. Screening of antioxidant property in medicinal plants belonging to the family *Apocynaceae.* Asian J Pharm Clin Res. 2017;10:415-8.
21. Anoop MV, Bindu AR. *In-vitro* anti-inflammatory activity studies on *Syzygium zeylanicum* (L.) DC leaves. Int J Pharm Res Rev. 2015;4:18-27.
22. Leelaprakash G, Caroline RJ, Mohan DS. *In vitro* anti-inflammatory activity of *Momordica charantia* by inhibition of lipoxygenase enzyme. Int J Pharm Pharm Sci. 2012;4:148-52.
23. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009 Nov-Dec;2(5):270-8. doi: 10.4161/oxim.2.5.9498. PMID: 20716914; PMCID: PMC2835915
24. Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutat Res. 2005;579:200-13.
25. Kaur N, Kishore K. Antioxidant activity of methanolic extract of *Phaseolus trilobus* root powder. Int J Pharm Pharm Sci. 2012;4:271-5.
26. Zhang L, Ravipati AS, Koyyalamudi SR, Jeong SC, Reddy N, Smith PT, *et al*. Antioxidant and anti-inflammatory activities of selected medicinal plants containing phenolic and flavonoid compounds. J Agric Food Chem. 2011;59:12361-7.
27. Talhouk RS, Karam C, Fostok S, El-Jouni W, Barbour EK. Anti-inflammatory bioactivities in plant extracts. J Med Food. 2007;10:1-0.
28. Wang C, Levis GB, Lee EB, Levis WR, Lee DW, Kim BS, *et al*. Platycodin D and D3 isolated from the root of *Platycodon grandiflorum* modulate the production of nitric oxide and secretion of TNF-[alpha] in activated RAW 264.7 cells. Int J Immunopharmacol. 2004;4:1039-49.