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## ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF *FOMITOPSIS DOCHMIUS* (BERK. & BROOME) RYVARDEN

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### Keywords:

*Fomitopsis dochmius*,  
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**ABSTRACT:** The antioxidant and anti-inflammatory activities of various solvent extracts of *Fomitopsis dochmius*, a polypore found in Western Ghats of Kerala, India were investigated. Petroleum ether (PE), ethyl acetate (EA), 70 % aqueous ethanol (ETH) and hot water (AQ) extracts of the fruiting bodies of the mushroom were examined for their antioxidant activities. PE was found to show activity against hydroxyl radicals, lipid peroxidation and ABTS<sup>+</sup> radicals. EA extract was able to scavenge DPPH, hydroxyl, and ABTS<sup>+</sup> radicals and also showed ferric ion reducing and lipid peroxidation inhibition properties. ETH and AQ extracts were active against all the above mentioned radicals besides superoxide and nitric oxide radicals. Since ETH extract showed higher antioxidant activity than other solvent extracts, its anti-inflammatory property was examined. The extract, at a dose of 500 mg/kg body weight, was found as effective as the standard anti-inflammatory drug diclofenac (10 mg/kg body weight). The preliminary phytochemical analysis of the four extracts indicated that the fruiting body of the mushroom contained steroids, terpenes, coumarins, phenols, alkaloids, saponins, anthraquinones and polysaccharides.

**INTRODUCTION:** Mushrooms remain an inadequately studied natural source for their therapeutic potentials. Of the 140, 000 estimated species of mushrooms, only 10 % are known<sup>1, 2</sup> and among them, a few have been studied adequately for their medicinal value. Several studies have established that mushrooms contain compounds having a wide spectrum of bioactivities such as antioxidant, anti-inflammatory, anticancer, antimicrobial, cardioprotective, hepatoprotective and immunomodulatory activities<sup>3, 4, 5, 6</sup>.

Among the less studied mushrooms, but suspected to have significant medicinal properties are the various species of the genus *Fomitopsis*, belonging to polyporaceae family. The Greek physician, Dioscorides described the use of *F. officinalis* for the treatment against tuberculosis in 65 AD<sup>7</sup>. One of the well studied species is *F. pinicola* having antimicrobial activity<sup>2</sup>, and strong inhibitory action on vascular endothelial growth factor- induced tube formation. The ethanolic extract of this mushroom showed activity to suppress production of the interferon (IFN)- $\gamma$ -induced inflammation marker, IP-10<sup>8</sup>, antihyperglycemic activity<sup>9</sup> and antifungal property<sup>10</sup>. Other members of the genus having medicinal value are *F. rosea* with antibacterial activity<sup>11</sup>, *Fomitopsis nigra* which contain fomitoid-K that can induce apoptosis<sup>12</sup> and *F. officinalis* having antipox properties<sup>7</sup>.

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*Fomitopsis dochmius* is commonly found in Africa and Asia<sup>13</sup>. This species is widely distributed in Western Ghats of Kerala, India<sup>14</sup>. However, the mushroom has not been studied adequately for its medicinal properties. We examined the antioxidant and anti-inflammatory properties of *F. dochmius* and the findings are reported in this communication.

## MATERIALS AND METHODS:

### Animals:

Female Swiss albino mice weighing  $25 \pm 3$  g and 6-8 weeks old were purchased from Small Animal Breeding Station, Agricultural University, Mannuthy, Thrissur, Kerala, India. They were maintained under standard environmental conditions and fed with standard mice feed (Sai Durga Feeds & Food, Bangalore, India) and water *ad libitum*. Animal experiments were conducted as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg. No. 149/1999/ CPCSEA) and with the approval of the Institutional Animal Ethics Committee.

### Preparation of extract:

Fruiting bodies of *F. dochmius* were dried under sunlight and then at  $50^{\circ}\text{C}$  for 48 hours and powdered. One hundred gram (100 g) samples of powdered material were extracted with solvents of increasing polarity, petroleum ether, ethyl acetate, 70 % aqueous ethanol and water for 8-10 hours. The extracts were filtered through Whatman No.1 filter paper. The filtrates were concentrated by a rotary vacuum evaporator and then dried at  $40^{\circ}\text{C}$ . The yields for PE, EA, ETH and AQ were 1.0g, 4.01g, 1.2g and 0.80 g % respectively.

### Estimation of *in-vitro* antioxidant activity:

#### DPPH radical scavenging assay:

In this method a commercially available stable free radical DPPH $\cdot$  (2,2-diphenyl-1-picryl hydrazyl) which is soluble in methanol was used. 100 $\mu\text{L}$  of different concentrations of the extracts were added to freshly prepared DPPH solution (150  $\mu\text{M}$ ) to make a final volume of 2ml and kept under dim light. After 20 minutes, absorbance of the test samples was measured at 517 nm against methanol. The ability of the extracts to scavenge DPPH radical was calculated by the comparison of

absorbance value of the test with that of control. The reduction in absorbance was expressed in percentage<sup>15</sup>.

#### Superoxide scavenging activity:

Assay is based on the ability of the extracts to inhibit or scavenge the super oxide radical generated from the photoreduction of riboflavin. The reaction mixture contained various concentrations of the extracts in a final volume of 3 ml. The tubes containing the reaction mixture were illuminated under an incandescent lamp for 15 min. The optical density (O.D) at 560 nm was measured before and after illumination against distilled water. The inhibition of the superoxide radical generation was found out by comparing the absorbance values of the treated with that of control. The reduction in absorbance was expressed in percentage<sup>16,17</sup>.

#### Hydroxyl radical scavenging activity:

Hydroxyl radicals were generated by Fenton's reaction. The reaction mixture contained various concentrations of the extracts in a final volume of 1 ml. Hydroxyl radical scavenging activity of extracts was determined by the method of Ohkawa et al (1979). The hydroxyl radical scavenging activity was determined by comparing absorbance of reaction mixture containing the extract with that of control. The difference in OD was expressed as percentage of inhibition<sup>18</sup>.

#### Inhibition of lipid peroxidation:

Lipid peroxidation was induced by  $\text{Fe}^{2+}$  - ascorbate system in the rat brain homogenate in the presence and absence of extracts<sup>19</sup>. The reaction mixture contained various concentrations of the extracts in a final volume of 0.5ml. The TBARS formed was measured according to the method of Ohkawa et al (1979).

#### ABTS<sup>+</sup> radical scavenging assay:

In this assay the extracts were allowed to react with ABTS<sup>+</sup>, a model stable - free radical derived from 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid). Ammonium persulphate (2.45 mM, final concentration) was added to a solution of ABTS (7 mM) and allowed to react for more than 16 hrs in dark at room temperature. ABTS and persulphate react with each other leading to the incomplete oxidation of ABTS to generate ABTS<sup>•+</sup> radical. The

ABTS<sup>+</sup> radical solution was diluted to an absorbance of 0.75 at 734 nm using ethanol. Different concentrations of the extracts were added to 2 ml of ABTS<sup>+</sup> radical solution. The decrease in the absorbance was measured against ethanol by a spectrophotometer after 6 minutes of initial mixing and expressed as percentage of reduction by comparing to the control. The reaction mixture without extracts was served as control<sup>20</sup>.

**Ferric reducing antioxidant power (FRAP) assay:** The ferric reducing ability of the extracts was measured at low pH. The FRAP reagent was prepared by mixing acetate buffer (300 mM, pH-3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and ferric chloride (20 mM) in a ratio of 10:1:1. Different concentrations of the extracts were added into the reagent solution and incubated in dark for 30 minutes. The intense blue colour developed was measured at 593 nm. The reducing power of the extracts was calculated in terms of number of ferric ions reduced from a standard graph plotted by using different concentrations of FeSO<sub>4</sub>. 7H<sub>2</sub>O (10-100µM in distilled water) in the reagent solution<sup>21</sup>.

**Nitric oxide scavenging activity:** The nitric oxide scavenging activity was measured according to the method of Sreejayan and Rao (1997). Immediately before the experiment, 10 mM solution of sodium nitroprusside was prepared in PBS (pH 7.4). Sodium nitroprusside solution with and without the extracts in a final volume of 3 ml was incubated at 25°C for 150min. After incubation, 0.5 ml of the reaction solution was removed and mixed with 0.5 ml of Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% N - (1- naphthyl) ethylenediamine dihydrochloride). The absorbance of the chromophore was read immediately at 546 nm against reagent blank. The nitric oxide scavenging activity was determined by comparing the absorbance of treated with that of control<sup>22</sup>.

#### **Preliminary phytochemical analysis:**

Phytochemical analysis of the extracts was carried out by standard methods.

#### **Determination of anti-inflammatory activity:**

Since the interdependence of oxidative stress and inflammation is well established,<sup>23, 24</sup> we selected

ETH extract which showed higher antioxidant activity than other extracts, for studying the anti-inflammatory activity.

#### **Acute model:**

Female Swiss albino mice weighing 25±3 g were used for the study. Animals were divided into five groups containing six animals in each group. Animals of group 1 were maintained as untreated control which received no drug and those of group 2 were orally administered with diclofenac at 10 mg/Kg b. wt dose. ETH extract was given orally to all the animals of 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> groups at 500, 250 and 100 mg/Kg b. wt dose respectively.

After one hour of medication, edema was induced in all the animals of all the groups on hind leg paw by subcutaneous injection of 20µL of 1% carrageenan in 0.01% carboxymethyl cellulose. Paw thickness of all the animals was measured before carrageenan injection using Vernier Caliper and it was taken as initial paw thickness. After three hours of edema induction, paw thickness was again measured and noted as final paw thickness. The inhibition of paw edema was quantified by comparing the difference in paw thickness of treated animals with that of untreated animals and expressed as percentage of reduction using the formula,  $((UP - TP)/UP) \times 100$ . Where UP is mean value of net paw thickness of the untreated group, TP is mean value of net paw thickness of treated groups.

#### **Chronic model:**

The procedure was same as that of acute inflammation model except the inflammation was induced by injecting 2% formalin and the final paw thickness was measured on the seventh day after six days of consecutive drug/extract administration.

#### **Statistical analysis for anti-inflammatory result:**

All the results were expressed as mean ± SD. Statistical analysis of the data on anti-inflammatory activity was done by unpaired t test with Welch correction.

## **RESULTS:**

#### **Antioxidant activities:**

**DPPH radical scavenging activity:** All the extracts except PE extract were able to scavenge

DPPH radical. Although 1000, 500, and 100 µg/mL concentrations of the extracts were able to scavenge the radical to a detectable level, the ETH extract was found to have highest activity reaching upto 80 % reduction (Fig. 1).

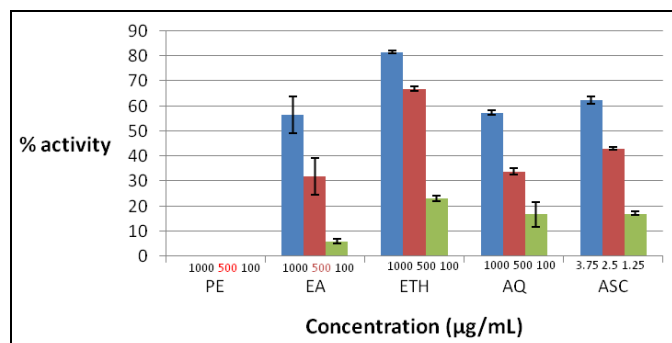


FIG. 1: DPPH RADICAL SCAVENGING ASSAY : THE REDUCTION IN COLOR CAUSED BY THE EXTRACTS COMPARED TO THE CONTROL WAS EXPRESSED IN PERCENTAGE ASCORBIC ACID WAS USED AS STANDARD

**Superoxide scavenging activity:**

PE and EA extracts were not effective in scavenging superoxide radicals generated in the reaction while ETH and AQ extracts efficiently scavenged the superoxides. AQ extract showed slightly more activity compared to ETH extract. However, the AQ extract at two different concentrations (250 and 500µg/mL) showed almost the similar quantum of activity (Fig. 2).

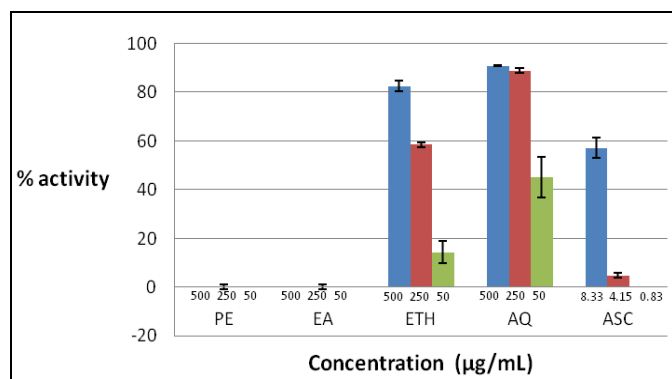


FIG. 2: SUPEROXIDE SCAVENGING ASSAY: SUPEROXIDE SCAVENGING ACTIVITY OF THE EXTRACTS SHOWN AS PERCENTAGE COMPARED TO THE CONTROL. ASCORBIC ACID WAS USED AS STANDARD

**Hydroxyl radical scavenging activity:**

All the extracts were able to scavenge the hydroxyl radicals generated in the reaction mixture. EA extract was the most efficient scavenger among the four extracts tested followed by ETH, PE and AQ

extracts respectively. There was only a little difference in the activity among the PE, EA and ETH extracts where as AQ extract showed a lower activity (Fig. 3).

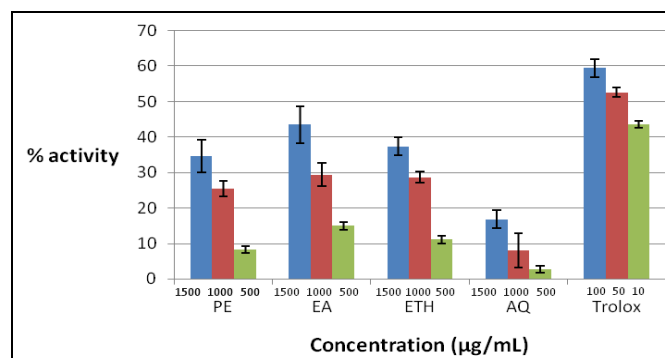


FIG. 3: HYDROXYL RADICAL SCAVENGING ASSAY: HYDROXYL RADICAL SCAVENGING ACTIVITY OF THE DIFERENT EXTRACTS EXPRESSED IN PERCENTAGE. TROLOX WAS USED AS STANDARD

**Lipid peroxidation inhibition activity:**

Inhibitory activity of the extracts on lipid peroxidation was almost in the same pattern as that of hydroxyl radical scavenging activity of the extracts. The three extracts, PE, EA and ETH, are shown to be highly active in decreasing lipid peroxidation with ETH extract showing highest activity followed by EA and PE extracts respectively. AQ extract was found to have least activity compared to that of other extracts (Fig. 4).

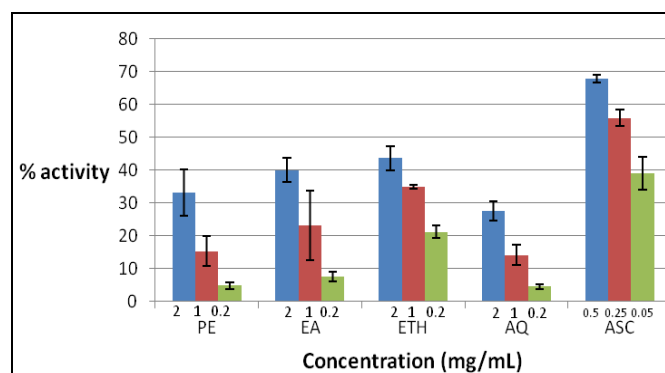


FIG. 4: LIPID PEROXIDATION INHIBITION ASSAY: PROTECTION AGAINST LIPID PEROXIDATION BY THE EXTRACTS AS COMPARED TO THE CONTROL. ASCORBIC ACID WAS USED AS STANDARD

**ABTS<sup>+</sup> scavenging activity:**

Though all the extracts were able to reduce ABTS<sup>+</sup> ions, ETH extract showed highest activity followed by AQ extract. PE and EA extracts showed less than 50% activity compared to that of the other two extracts (Fig. 5).



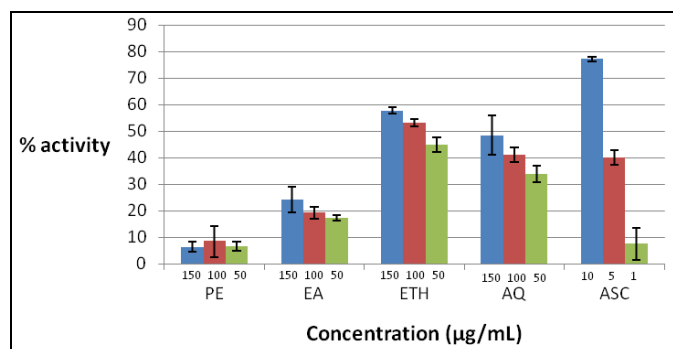


FIG. 5: ABTS.+ RADICAL SCAVENGING ACTIVITY: QUANTITY OF ABTS.+RADICALS SCAVENGED IS EXPRESSED AS PERCENTAGE COMPARED TO THE CONTROL. ASCORBIC ACID WAS USED AS STANDARD

**FRAP activity:**

All the extracts except PE extract showed ferric ion reducing activity. AQ extract showed higher reduction than the EA and ETH extracts (Fig. 6).

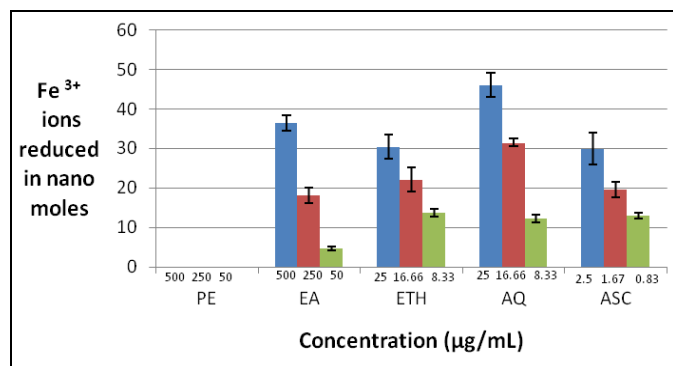


FIG. 6: FRAP ASSAY: ACTIVITY OF DIFFERENT EXTRACTS IN REDUCING FERRIC IONS. ASCORBIC ACID WAS USED AS STANDARD

**Nitric oxide scavenging activity:**

PE and EA extracts were unable to scavenge nitric oxide radicals. All the three concentrations, 500, 250 and 50µg/mL, of ETH and AQ extracts scavenged the radical with ETH extract possessing slightly more activity than the AQ extract (Fig. 7).

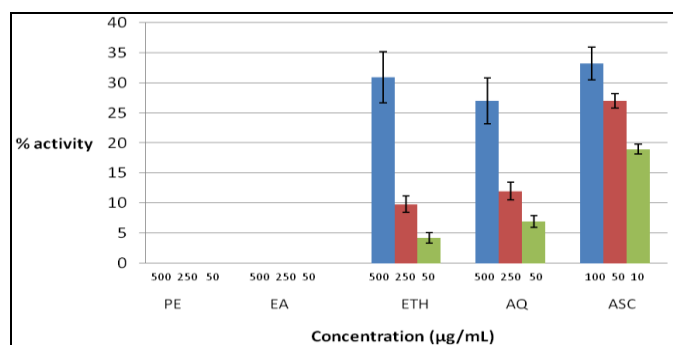


FIG. 7: NITRIC OXIDE SCAVENGING ASSAY: REDUCTION IN NITRIC OXIDE RADICALS DUE TO THE EXTRACTS EXPRESSED AS PERCENTAGE COMPARED TO THE CONTROL

**Phytochemical analysis:**

Phytochemical analysis indicated the presence of steroids in PE extract. EA extract was found to contain coumarins and terpenoids. ETH extract contained phenol, alkaloids, saponins, coumarins, and polysaccharide. Phytochemical principles of AQ extract were- almost similar to that of ETH extract with the difference that coumarins were not present but anthraquinones were present in it.

**Anti-inflammatory activity of ETH extract:**

Since, the ETH extract showed overall higher antioxidant activity than other extracts, it was selected for evaluating anti-inflammatory activity. The ETH extract was effective in reducing both the acute and chronic inflammations. The anti-inflammatory effect of the extract was found dose dependent. The extract at a dose of 500 mg/Kg b.wt was able to reduce the paw edema by 47.06 and 52.38 % in acute and chronic inflammations respectively (Fig. 8 & 9). Statistical analysis showed that the anti-inflammatory activity of ETH extract at 500mg/Kg b. wt dose, was as effective as that of the standard drug diclofenac (10 mg/ Kg b. wt).

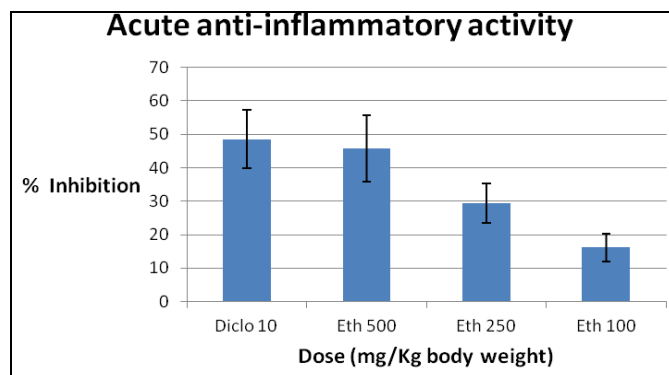


FIG. 8: ANTI-INFLAMMATORY EFFECT OF ETH EXTRACT AGAINST ACUTE INFLAMMATION

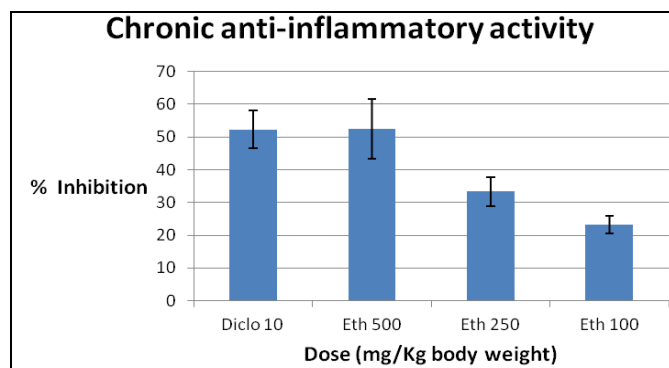


FIG. 9: ANTI-INFLAMMATORY EFFECT OF ETH EXTRACT AGAINST CHRONIC INFLAMMATION

**DISCUSSION:** Oxidative stress has been shown to be the fundamental cause of many diseases. Powerful oxidative agents often extract electrons from biomolecules which are important for normal functioning of the system, thereby destructing their structure that results in impaired function.

The results of the current investigation indicate that *F. dochmius* possess profound antioxidant activity. Among the various extracts tested, all the extracts except PE were able to donate electrons to the DPPH radical. ETH was found to be the most efficient extract in donating electrons to the DPPH radical. The inability of PE to reduce DPPH radical may be explained on the basis of its phytochemical constituents. Steroids were the only class of compounds detected in PE extract. Steroids, in general, are not that efficient electron donors. EA extract was able to reduce the DPPH radical. This extract contained terpenoids and coumarins. Both the classes of compounds are known for their antioxidant activity. ETH extract showed highest activity. The phytochemical analysis of ETH showed that it contained phenols, coumarins, polysaccharides, alkaloids and saponins. The above classes of compounds are diverse molecules with antioxidant activity.

ETH and AQ extracts prevented the reduction of NBT by superoxide anions as revealed from the reduced color development. It is known that phenolic compounds can scavenge superoxide anions effectively<sup>25</sup>. Hydroxyl groups on the carbon ring structure of the compounds increase the superoxide scavenging ability of the compounds and the more the number of hydroxyl groups on benzene ring structure, the more pronounced is the activity<sup>26</sup>. Our results show that ETH extract have concentration dependent superoxide scavenging activity, while AQ extract did not show such a concentration dependent activity beyond 250µg/mL concentration.

All the four extracts were able to scavenge hydroxyl radicals. Previous study has shown that a steroid with hydroxyl groups on its aliphatic side chain has a strong quenching activity on hydroxyl radicals<sup>27</sup>. In our study also PE extract which was shown to contain steroids gave strong quenching activity against hydroxyl radicals. The terpenoids

and coumarins are able to scavenge hydroxyl radicals, hence, the EA extract showed strong activity. In the experiment hydroxyl radicals are generated by inducing Fenton reaction. Here the interaction between  $Fe^{2+}$  and  $H_2O_2$  is necessary for the production hydroxyl radicals. A previous study has pointed out that many  $OH\cdot$  scavengers interact with the metal ions there by making the  $Fe^{2+} - H_2O_2$  interaction less likely<sup>28</sup>. This may perhaps impaire  $OH\cdot$  radical production. Hence many phytochemicals that appear to be scavengers of  $OH\cdot$  radicals, in reality may not be sequestering the radicals rather they may be hindering the production of these radicals.

In FRAP assay, except PE extract all the extracts caused a reduction of FRAP radical. AQ extract was found to be the most active in scavenging FRAP radicals. It is a common observation in a number of studies that aqueous/ methanol extracts showed strongest activity in FRAP assay. This shows the nature of the compound/s is responsible for the antioxidant activity. Obviously the compound/s responsible for this, must be highly polar and that the polarity has some contributory effect on the antioxidant activity.

ABTS<sup>+</sup> scavenging activity was found with all the extracts. Highest activity was with ETH extract closely followed by AQ extract. PE extract showed least activity. Various phenolic compounds and compounds containing thiol group have been reported to have ABTS<sup>+</sup> scavenging property. It is known that the reduction of ABTS<sup>+</sup> radicals is carried out in a biphasic manner with one phase is very fast and the other is time consuming. In our assay we have followed the most common procedure of allowing 6 minute time for the completion of the reaction. Since there is a slow reacting step in the scavenging process,<sup>29</sup> it is necessary to allow a longer time for a more accurate analysis.

Phenols are known to possess nitric oxide scavenging property<sup>30</sup>. We observed nitric oxide scavenging activity for the ETH and AQ extracts. These extracts are found to contain phenols.

Lipid peroxidation can be inhibited either by preventing the Fenton reaction or by scavenging

the hydroxyl radicals generated in the Fenton reaction. In lipid peroxidation, methylene (RH) bridge in the lipid is the most crucial target site because of the presence of an adjacent double bond to the RH group<sup>31</sup>. Most of the antioxidants from natural sources have a conjugated ring structure. So, presumably, the delocalized electrons of these compounds might be acting as an alternative source for electrons and thereby protecting lipids from oxidation. This may be the reason why all the other three extracts gave stronger results than that of the AQ extract because compounds with a benzene ring structure are always preferentially extracted with solvents with less polarity.

Since free radicals contribute to the onset and progression of acute and chronic inflammation, many compounds showing anti-inflammatory activity are strong antioxidants. In our study ETH extract was chosen from the four extracts to study its anti-inflammatory activity because the extract showed strong antioxidant activity against all of the tested free radicals. The extract showed a dose dependent anti-inflammatory activity in both the acute and chronic anti-inflammatory activity assays. In acute and chronic inflammations, significant reduction in edema was found at a dose of 250 mg/ Kg body weight and at 500 mg/ Kg b. wt dose, the edema was reduced to the same extent as that of the standard drug, diclofenac (10 mg/ Kg boy weight).

Injection of carrageenan results in injury to mast cells<sup>32</sup> and injection of formalin damages cell membranes thereby intensifying inflammatory damage<sup>33</sup>. Formalin also causes change in the relative composition of mucopolysaccharides, glycoprotein, hexosamine, hydroxyproline and sialic acid<sup>34</sup>. These changes result in the activation of many mediators involved in inflammatory process and end up in inflammation. Since nitric oxide, superoxide anion and hydroxyl radicals are mediators in inflammation, compounds that can scavenge these ROS, are potential agents for anti-inflammatory activity.

In our experiments, the ETH extract was able to scavenge all the above mentioned ROS and subsequently shown to have anti-inflammatory activity. A previous study has shown that ethanolic

extract of *F. pinicola* reduces inflammation by suppressing the production of the interferon (IFN)- $\gamma$ -induced inflammation marker, IP-10<sup>35</sup>, and this finding suggests a mechanism of the anti-inflammatory activity of the mushroom.

Phenols are known to inhibit both COX-1 and COX-2 enzymes and prostanoid product generation via the LO pathway. Terpenes have been reported to prevent COX-2 and iNOS expression in RAW 264.7 macrophages and reduce inflammatory response. Many terpenes inhibit COX-2 by inhibiting NF- $\kappa$ B. Alkaloids also have been seen to reduce inflammation by blocking the production of several metabolites of Arachidonic acid, TXB<sub>2</sub> and 6-ketoPGF<sub>1 $\alpha$</sub> , as well as by decreasing the activity of both COX. Certain alkaloids activate Ca<sup>2+</sup>-nitric oxide-cGMP pathway resulting in vasodilation<sup>36</sup>. All the different classes of compounds discussed above are present in the ETH extract. Medicinal properties of *Fomitopsis* species have not been investigated adequately. However, the current investigations demonstrate that *F. dochmius* possesses significant antioxidant and anti-inflammatory activities.

**CONCLUSION:** Medicinal properties of *F. Docmius* was investigated for the first time. The mushroom possessed profound antioxidant and significant anti-inflammatory activities. Preliminary phytochemical analysis revealed that this mushroom contains many pharmacologically important classes of compounds such as steroids, terpenes, coumarins, phenols, alkaloids, saponins, anthraquinones and polysaccharides. Though preliminary in nature, this study suggests that *F. dochmius* has important medicinal properties.

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**CONFLICT OF INTEREST:** The authors of this article declare no conflict of interest in this study.

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