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Antioxidant and Anti-Inflammatory Activity of *Pleurotus tuber-regium* (Rumph. ex Fr.) Singer

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Abstract: Oxidative stress and inflammation are inextricably connected to each other. Phytochemicals are the major therapeutic components of ethnomycologically potent macrofungi which inhibit generation of free radicals and inflammatory agents in the body. In the present study among all the will be studied phytochemicals *mehtanolic* extract of *Pleurotus tuber-regium* contains alkaloids (30.54 ± 0.32 mg/100g) significantly (p<0.001) higher and tannins (3.21 ± 0.30mg/100g) content significantly (p<0.001) lower. The total antioxidant activity (TAC) (12.7% and 21.5% at concentration of 50µg/mL and 100µg/mL respectively) and hyaluronidase inhibition activity of aqueous extract (3.94% and 22.19% inhibition at concentration of 50µg/mL and 100µg/mL respectively) is significantly (p<0.001) higher than methanolic extract of *P. tuber-regium*.

Key words: Phytochemical · Antioxidant · Anti-inflammation · Free radical · *Pleurotus tuber-regium*

INTRODUCTION

Inflammation is one of the primary physiologic defence mechanisms that help body to protect against infection, burn, exposure to toxic chemicals, radiation, allergens, other noxious stimuli and chronic diseases [1, 2]. The inflammatory mechanisms can be attributed to various mediators such as histamine, serotonin, arachidonic acid metabolites and quinines etc. [3]. Suleyman *et al.* [4] reported that, complement system, fibrinolytic system and hyaluronidase enzyme are activated in plasma during inflammation. Inflammation is one of the manifestations of oxidative stress [5, 6]. Reactive oxygen species (ROS) and reactive nitrogen species (NOS) are produced under the stimulus of pro-inflammatory cytokines such as TNF-α, IL-1-β, IFN)-γ, IL-6 etc. through the activation of protein-kinases signalling in chronically inflamed tissue [7, 8].

Free radical includes, reactive oxygen species (ROS) and reactive nitrogen species (NOS) such as superoxide ($O_2\bullet$-), hydroxyl (OH$\bullet$), nitric oxide (NO$\bullet$), nitrogen dioxide ($NO_2\bullet$) etc. are continuously produced by the body via enzymatic and non-enzymatic reactions like respiratory chain reaction, the phagocytosis, prostaglandin synthesis, phosphorylation in the human body [9,10] but their excessive production during pathogenic attack, diseases and tissue injuries, exposure to radiation etc. [11,12].

Inflammation and oxidative stress are inextricably connected in physiologic as well as diseases and they are essential mediators of various diseases such as, cardiovascular disease and atherosclerosis [13-15] chronic renal disease [16], pulmonary disease [17], rheumatoid arthritis [18], cancers [19,20], metabolic syndrome, obesity and diabetes [21,22] etc.

Edible macrofungi or mushrooms belong to the phylum Basidiomycota which includes 80 families, 550 genera and 10,000 species among which approximately 700 species have been reported for their significant pharmacological activity [23, 24]. *Pleurotus tuber-regium* commonly edible gilled mushroom belonging to family Pleurotaceae has been use in pathogenic infection, renal and hepatic diseases, cancer, diabetes and other diseases [25, 26] but its use as antioxidant and antiinflammatory agent has not been reported.

Therefore, present study was undertaken to analyse the phytochemical composition, antioxidant activity and anti-inflammatory activity of edible macrofungi *Pleurotus tuber-regium.*

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MATERIALS AND METHODS

Collection of Macro Fungi: Fresh fruiting bodies of *Pleurotus tuber-regium* were collected from different sites of three National Parks (Orang National Park, Kaziranga National Park and Manas National Park) of Assam and were identified in laboratory of Department of Botany, Gauhati University, Guwahati, Assam and brought to Department of Zoology to evaluate the pharmacological potentiality.

Extract Preparation: Fresh mushrooms were washed and disinfected by treating with $HgCl_2$ and washed again. The mushrooms were dried in shade under room temperature for six to seven days, powdered and sieved [27]. 50g of the fine powder was subjected to extraction chamber of Soxhlet using methanol and distilled water separately for methanolic and aqueous extraction. The extracts obtained were filtered, concentrated and dried in rotary flash evaporator maintained at 45ºC for proper dehydration. Percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies [28,29].

Phytochemical Screening: Estimation of total phenol, flavonoids, tannins, saponins and alkaloids content of fungal extracts were done following Sofowara [30]. The details have been described elsewhere by Dandapat *et al*. [31, 32].

Total Antioxidant Capacity: Total antioxidant activity (TAC) of aqueous and methanolic extracts of *Pleurotus tuber-regium* were measured following Prieto *et al.* [33]. 10µg/mL, 50µg/mL and 100µg/mL concentrations of samples (aqueous and methanolic extracts) were taken in a series of test tubes and added 1.9mL of reagent solution was added. (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 minutes and allowed to cool then absorbances of the both samples were measured at 695 nm against blank. Antioxidant capacities are expressed as equivalents of ascorbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. Butylated hydroxy anisole (BHA) is a standard synthetic antioxidant was used to compare the efficacy of the TAC of the extracts. The values were expressed as ascorbic acid equivalents in µg/mL of extract.

Anti Inflammatory Analysis: Anti inflammatory analysis of aqueous and methanolic extracts of *Pleurotus tuber-regium* was estimated by hyaluronidase inhibition activity following Ling *et al.* [34]. The assay medium consisting of 3 - 5U hyaluronidase (from Sigma –Aldrich, Bangalore) in 100µL of 20mM sodium phosphate buffer (pH 7.0) with 77mM sodium chloride, 0.01% BSA was preincubated with different concentrations (10µg, 50µg and 100 µg) of the test compound for 15 min at 37 °C. Then the assay was commenced by adding 100µL hyaluronic acid (from Sigma –Aldrich, Bangalore; 0.03% in 300mM sodium phosphate, pH 5.35) to the incubation mixture and incubated for a further 45 min at 37 °C. The undigested hyaluronic acid was precipitated with 1mL acid albumin solution made up of 0.1% bovine serum albumin in 24mM sodium acetate and 79mM acetic acid, (pH 3.75). After standing at room temperature for 10 min, the absorbance of the reaction mixture was measured at 600 nm. The absorbance in the absence of enzyme was used as the reference value for maximum inhibition. The inhibitory activity of test compound was calculated as the percentage ratio of the absorbance in the presence of test compound *vs.* absorbance in the absence of enzyme. The enzyme activity was checked by control experiment run simultaneously, in which the enzyme was preincubated with 5µL DMSO instead and followed by the assay procedures described above. Compound was tested in a range of 10µg -100µg in the reaction mixture. Indomethacin (Indo) was used as reference standard.

Statistical Analysis: All results were expressed as mean ± standard error of mean (S. E. M.). Data were analysed using Student's t-test, $p<0.05$ considered as statistically significant.

RESULTS AND DISCUSSION

Phytochemical Screening: Results of phytochemical screening of aqueous and methanolic extract (Figure-1A and 1B) of *P. tuber-regium* are presented in table 1. The results revealed that methanolic extract of *P. tuber-regium* contain alkaloids (30.54 ± 0.32 mg/100g) and tannins (3.21 ± 0.30mg/100g) significantly ($p<0.001$) in higher quantity and saponins (20.19 ± 0.21mg/100g) content of aqueous extract of *P. tuber-regium* significantly ($p<0.001$) higher among all the studied phytochemicals.

Table 1: Phytochemical composition of aqueous and methanolic extract of *P. tuber-regium* (M ± SD; n=6).

Phytochemicals (mg/100g)	Aqueous	Methanolic
Alkaloids	28.14 ± 0.50[a]	30.54 ± 0.32[b]
Saponins	20.19 ± 0.21[a]	16.34 ± 0.27[a]
Flavonoids	13.78 ± 0.52[a]	19.67 ± 0.22[a]
Tannins	2.74 ± 0.28[a]	3.21 ± 0.30[a]
Phenols	20.19 ± 0.25[a]	19.50 ± 0.31[c]

a = (p<0.001); b= (p<0.005); c = (p<0.05)

Fig. 1: (A) Aqueous extract and (B) Methanolic extract of *P. tuber-regium.*

Udu-Ibiam *et al.* [35] reported 64.12 ± 1.2 mg/g phenols, 0.016 ± 0.001 mg/g flavanoid, 0.28 ± 0.04mg/g saponins, 0.1 ± 0.04% alkaloids and 0.014 ± 0.003 % tannins in *Tricholoma nudum* and 6.012 ± 0.91 mg/g phenols, 0.031 ± 0.02 mg/g flavanoids, 0.27 ± 0.008mg/g saponins, 2.0 ± 0.01% alkaloids and 0.014 ± 0.001 % tannins in *Psalliota campestris.* In the present study phytochemical composition of *P. tuber-regium* is higher than the studied mushrooms. Antioxidant compounds such as phytophenols, flavonoids, tannins, saponins etc. minimize the generation of free radicals, act as anti-inflammatory agents and minimize the risk of diseases oxidative stress and inflammation [36-42].

Total Antioxidant Capacity: Total antioxidant capacity (TAC) is the free radical scavenging capacity of the bioactive constituents present in the test sample [43] Result of total antioxidant activity is presented in figure 2, which revealed the TAC of standard BHA is significantly (p<0.005) higher than aqueous and methanolic extract. However, the TAC of aqueous extract significantly (p<0.005) higher than methanolic exctract of *P. tuber-regium* (Figure 2).

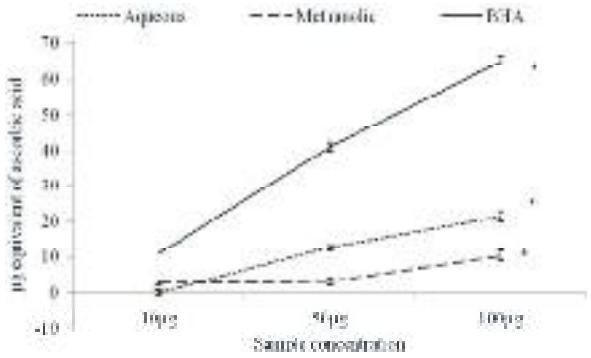

Fig. 2: Total antioxidant capacity (TAC) of aqueous and methanolic extract of *P. tuber-regium* (M ± SD; n=6); (*= p<0.005).

Barros *et al*. [44] studied total antioxidant activity of some edible fungi of *Agaricus* sp. and reported 15.85 ± 0.27%, 9.61 ± 0.07%, 6.22 ± 0.10%, 5.37 ± 0.06% and 6.39 ± 0.16% total antioxidant activity of *A. arvensis, A. bisporus, A. romagnesii, A. silvaticus* and *A. silvicola* respectively. On the basis of above results it can be concluded that TAC of *P. tuber-regium* possess good antioxidant activity.

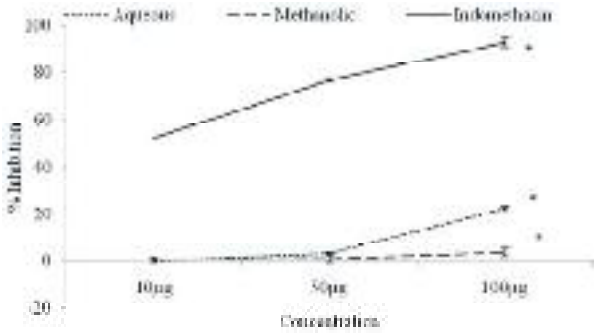

Fig. 3: Anti-inflammatory activity of aqueous and methanolic extract of *P. tuber-regium* (M ± SD; n=6); (*= p<0.001).

Anti-Inflammatory Activity: Results of anti-inflammatory activity by Hyaluronidase inhibition is presented in figure 3. The results revealed that both aqueous and methanolic extract of *P. tuber-regium* significantly (p<0.005) shows good hyaluronidase inhibition. However, the standard indomethacin shows significantly (p<0.005) higher hyaluronidase inhibition activity than aqueous and methanolic extract of *P. tuber-regium*.

Yahaya and Don [45] studied anti-inflammatory activity of macro fungus *Trametes lactinea* and rported supernatant of extract 80% inhibited the activities of hyaluronidase. Kamiyama *et al.* [46] reported studied the anti-inflammatory activity of mushroom *Trametes versicolor* and reported anti-inflammatory activity increases (37%, 55.6% and 76.4%) with increase in concentration (100µg/mL, 200µg/mL and 500µg/mL). Procida *et al.* [47] reported hyaluronidase activity in blood is increased during inflammation and the decrease in inflammation parallels a decrease in hyaluronidase activity. According to the above results it can be concluded that, anti-inflammatory activity by hyaluronidase inhibition of *P. tuber-regium* extracts is much higher than studied fungal extract.

CONCLUSION

In the present study ethnomycologically edible macrofungi *P. tuber-regium* contains high phytochemical constituents. The high concentration of phytochemicals can reduces the activity of hyaluronidase an essential partner of inflammation and oxidative stress. The high antioxidant capacity and anti-inflammatory activity of *P. tuber-regium* is beneficial to the body affected by oxidative stress, inflammation and oxidative stress associated diseases. Therefore, *P. tuber-regium* can be used as an antioxidant and inti-inflammatory supplement.

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