

Evaluation of Antioxidant Activity of Plant-parasitic Macrofungus: *Phellinus durissimus* (Lloyd) Roy

Suman K. Lahiri^{a,b,c}, Rina H. Gokani^a, Mrugesh D. Shukla^b, Hasmukh A. Modi^c, Devdas D. Santani^d, and Mamta B. Shah^{a,*}

^a Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad, Gujarat, India.

^b Department of Microbiology, M. G. Science Institute, Ahmedabad, Gujarat, India.

^c Department of Life Sciences, Gujarat University, Ahmedabad, Gujarat, India.

^d Department of Pharmacology, L. M. College of Pharmacy, Ahmedabad, Gujarat, India.

Received: 12 June 2009; Accepted: 28 October 2009

Abstract

Phellinus durissimus (Lloyd) Roy is a plant parasitic macrofungal drug, which is known to be used in traditional medicinal practices by tribes of South Gujarat. The methanol extract (PdM) was subjected to *in vitro* chemical assays like, DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical, superoxide radical, hydroxyl radical, nitric oxide scavenging activity, erythrocyte hemolysis test, β -carotene bleaching test, linoleic acid-ferric thiocyanate and lipid peroxidation. This was followed by the evaluation of the ethyl acetate soluble (PdEs) and insoluble (PdEi) fractions of PdM, by DPPH free radical scavenging activity, linoleic acid-ferric thiocyanate method and β -carotene bleaching test. PdM showed considerable antioxidant activity in most of the assays, yielding IC₅₀ values between 50 to 100 $\mu\text{g mL}^{-1}$, baring hydroxyl radical scavenging activity and erythrocyte hemolysis tests, of which its activity in the former assay was much lower and the later was higher than the rest of the assays. PdEs and PdEi showed substantial activities in either of the DPPH and lipid peroxidation assays n comparison to each other. The current work is the first report that establishes *P. durissimus* has significant antioxidant potential which is comparable to or better than other species of Hymenochaetaceae.

Keywords:

Phellinus durissimus; polypore; plant-parasite; antioxidant; traditional practice

1. Introduction

Researches related to natural antioxidants are fueled by rationales that encompass a broad vista, ramifies to various applications in the pharmaceutical, functional food, food supplements, nutraceuticals and cosmeceutical realms. The green tea extracts are one of the most popular natural antioxidant marketed as dietary supplements [1] or for topical use [2]. Off-late there has been a hype regarding the inclusion of natural free radical scavengers like phenolic compounds that would find place in anti-ageing and photoprotection cosmetic products [2,3]. Role of natural antioxidants as food preservatives are being considered as well, of which, use of rosemary and salvia extracts [4,5] are known. Moreover, hydrophilic extracts of *Flammulina velutipes* have been employed for preserving meat texture due to its antioxidant activity [6]. Moreover, efforts to make such aspects commercially viable by

* Corresponding Author

E-mail: mbshah2007@rediffmail.com

ISSN: 1306-3057

Moment Publication ©2010

recycling phenolic-rich agroindustrial and agricultural wastes, for antioxidant enhancement, have been rewarding [7]. Such facts portray a bright picture to be met in near future.

The most prominent aspect of all is the therapeutic effect of the antioxidants addressing several ailments regarding which research is done since several decades. Free radicals and reactive species like reactive oxygen species (ROS), hydroxyl radical, superoxide anion radical and hydrogen peroxide are primarily known to damage cell membrane. This along with other causes aggrandizes several pathodegenerative conditions like atherosclerosis, Alzheimer's disease, hepatopathic, nephropathic, retinopathic damage, cancer, adult respiratory distress syndrome, diabetes [8].

Ancient exploitation of macrofungi by the Indian and Oriental civilizations, for longevity and rejuvenation has been well documented [9]. The extent of macrofungal antioxidant potential can vary with geographical location, taxonomy, ecology, host, growth stages, parts of the fruiting body and even forms of products.

The variability in antioxidant effect from grass-root level to product manufacturing has been evaluated for *A. sylvaticus*, wherein freshly harvested, dehydrated, industrialized liquid suspension and tablets of the same were examined [10]. The results indicated preservation of antioxidant potential down the production line even at low concentrations of 0.5 to 1 mg/ml in product or raw form.

Variability in potential within the anatomical parts are known for some. Results indicated that the cap was found to be the most active part as reported for *Amanita rubescens* with high amount of organic acids and for *Russula cyanoxantha* and *Suillus granulatus* with substantial alkaloids, unlike *Boletus edulis* which had more alkaloids in stipe and was the most potent of all. Rather than the presence of phenolics like p-hydroxybenzoic acid, at high amounts in the cap of *A. rubescens*, the stipe with alkaloids [11] contributed to antioxidant activity.

Change in antioxidant potential along various growth stages revealed that the initial and latter stages were more effective than the middle stage of *Agaricus bisporus* [12].

Mushrooms belonging to different taxa, occupying diverse ecological niches have shown various degrees of antioxidant potentials. Those arising from direct ecological interactions with ants or termites as in case of *Termitomyces albuminosus* [13] or indirect ecological interactions as succession of several fungi and bacteria during degradation of foliage and down woods have shown varied degrees of antioxidant activities. Humus rich soil borne Basidiomycetes macrofungi like *Agaricus bisporus* [14], *Agrocybe aegerita* [15], *Coprinus comatus* [16] of order Agaricales and species of *Tremella* [17] of another subclass are known to have antioxidant activity.

Other saprophytes that grow directly on decaying or hardwoods without necessitating the presence of soil like *Lentinus edodes*, *Volvariella volvacea*, *Pleurotus cystidiosus*, *P. ostreatus*, *Flammulina velutipes* [18], and certain species of *Auricularia* [17] have shown evidence of antioxidant potential. Though all are saprobic, *Auricularia auricular* from a different sub class, alongwith two Agaricales, *Lentinula edodes* and *Flammulina velutipes* showed better activity than *Volvariella volvacea* [19], which was from the same order but a different Family. This may be due to variations in inherent degradation enzyme profiles.

A study revealed lipid peroxidation activity of ethanol extracts in decreasing order as *Agaricus bisporus* > *Hypsizigus marmoreus* > *Volvariella volvacea* > *Flammulina velutipes* > *Pleurotus eryngii* > *Pleurotus ostreatus* > *Hericium erinaceus* > *Lentinula edodes*, for some East-Asian edible saprobic mushrooms [20]. Here only the most potent doesn't fruit on wood directly but needs it to be extensively degraded in soil but rest of the following species are all

dead wood degrading saprobes. *Agaricus bisporus* and *Ganoderma lucidum* fruiting-bodies aqueous extracts arrested H₂O₂-induced oxidative damage to cellular DNA using the single-cell gel electrophoresis, but *Flammulina velutipes*, *Auricularia auricula*, *Hypsizygus marmoreus*, *Lentinula edodes*, *Pleurotus sajor-caju*, and *Volvariella volvacea* surprisingly showed no activity [21]. Of both the active species, one is soil borne and another is parasitic indicating possibility of a greater battery of antioxidant molecules.

Malaysian wood saprobic Agaricales like, *Pleurotus porrigens*, *Hygrocybe conica*, *Xerula furfuracea* and *Schizophyllum commune* are said to contain antioxidant capacity equivalent to the commercial mushroom *Pleurotus florida*, wherein the non-polar extract excelled in DPPH radical scavenging but the methanol extract did well in reducing power and ferrous ions chelating ability [22].

Generally growing at the wood soil junction, saprobic or parasitic, *Grifola frondosa* [13,23] and *Hericium erinaceus*, are known to cause soft rot and its mycelia and fruiting body have exhibited antioxidant effects.

Numerous mushrooms that grow above soil and are mycorrhizal like *Dictyophora indusiata*, *Tricholoma giganteum* [23], *Lactarius deterrimus* [24] are known to yield antioxidant extracts. Other mycorrhizal mushrooms of the order Thelephorales like *Thelephora vialis* [25,26], *Thelephora ganbajun*, *Thelephora aurantiotinct* and *Boletopsis grisea* [27] have been reported to contain several potent antioxidant molecules apart from those belonging to Bolatales, *Suillus collitinus*, *Boletus eduli*, and *B. chrysenteron* [24].

Certain European mushrooms and their molecules are reported to have considerable antioxidant activities [28]. Aqueous extracts of few European edible mushrooms, *Boletus edulis*, *Lentinus edodes* and *Amanita cesarean* showed better antioxidant activity than *Lactarius deliciosus* and *Cantharellus cibarius*, of which except the 2nd one all are soil borne with mycorrhizal associations [29].

Some saprophytes of the order Phallales like the *Geastrum saccatum* and *Ramaria flava* have shown antioxidant capabilities. *G. saccatum* known as terrestrial star is a Brazilian traditional medicine used for eye infections and asthma. It is reported to possess anti-oxidant activities and is related to high molecular weight compounds [30]. *R. flava* ethanol extract is known to possess DPPH free radical scavenging, and anti-lipid peroxidation qualities attributed to flavonoids and phenolics [31]. Some Portuguese saprobes like *Cantharellus cibarius*, *Hypholoma fasciculare*, *Lepista nuda*, *Lycoperdon molle*, *L. perlatum*, *Macrolepiota procera*, *Tricholoma acerbum* and *R. botrytis* and many others are reported to have moderate to high antioxidant activity wherein the last one is said to have highest concentration of phenolic compounds [32,33].

Ascomyceteous soil-borne mushrooms like *Morechella* are also reported to have antioxidant activities. Mycelia of *M. esculenta* have been shown to contain greater phenolic compounds than *Grifola frondosa* and *Termitomyces albuminosus* [13]. Some Turkish species like *M. rotunda*, *M. crassipes*, *M. esculenta*, *M. umbrina*, *M. deliciosa*, *M. elata*, *M. angusticeps* and *M. conica* have shown varied levels of antioxidant capacity [34].

Several Ascomycetes and Basidiomycetes truffles or related species from North America like *Elaphomyces granulatus*, *E. muricatus*, *Geopora clausa*, *Hymenogaster subalpinus*, *Melanogaster tuberiformis*, *Rhizopogon couchii*, *R. nigrescens*, *R. pedicellus*, *R. subaustralis*, *R. subgelatinosus*, and *Scleroderma leave*, were found to be antioxidant or had activities [35]. These are mycorrhizal and grow under the soil occupying a completely different ecological niche.

Aphylophores such as *Lenzites betulina*, *Sterum hirsutum*, *Cryptoporus volvatus* and *Ganoderma lucidum* [36] have exhibited diverse kinds of antioxidant activities. Methanolic extracts of Asian medicinal mushrooms like *Ganoderma lucidum*, *G. lucidum* antler and *Ganoderma tsugae* of Ganodermataceae was found to perform better than *Coriolus versicolor* from Polyporaceae [37]. Polyporale members, *Polyporus tenuiculus* [38] and *Laetiporus sulphureus* (ethanol extracts), showed potent anti-DPPH free radical-scavenging, β -carotene/linoleic acid oxidation effect [39].

Macrofungi growing on dead or live wood need to degrade them and those producing soft rot especially degrade lignin and cellulose both. The lignin itself is polyphenolic and its enzymatic degradation produces further phenolic byproducts. The presence of these metabolites significantly contributes to antioxidant activities and correlates widely. But the parasitic fungi have to degrade and have to sustain assaults from the host and overcome its immune response as well. This makes it a better contender for antioxidant research.

Fruiting body and mycelia of an entomoparasitic *Cordyceps sinensis* have shown anti-oxidation activity mainly attributed to its polysaccharides [40], which on high temperature hydrolysis by sulphuric acid showed 30-80% enhancement in radical scavenging activity [41].

Phellinus durissimus (Lloyd) Roy is a plant parasite employed by the tribes of South Gujarat in India. In plants, pathogens are known to induce hypersensitive response by generation of superoxide and hydrogen peroxide [42] involved in local oxidative burst mediated coup, which is then propagated to distant sites as secondary microbursts imparting systemic acquired resistance [43]. Thus to manifest itself, the insurgent, has to overcome such assaults launched by the host. This phenomenon itself relays the rationale that paves the platform for our work investigating about existence of such antioxidant molecules. Prioritized by an analysis of the tribal traditional ethnomedicinal practices regarding few macrofungi (communicated elsewhere), we report here antioxidant activities of *P. durissimus* examined by well established assays.

2. Materials and methods

2.1. Materials

DPPH (1,1-diphenyl-2-picrylhydrazyl) and nitroblue tetrazolium, were from Sigma Chemicals Ltd., rest of the chemicals were from either Merck India Ltd. or Ranbaxy fine chemicals Ltd., β -carotene and α -tocopherol were gifted by Cadila Pharmaceuticals Ltd.

2.2. Fruiting body material

The fruiting bodies of *Phellinus durissimus* were collected from *Acacia catechu*, *Terminalia alta* and several others hosts. They are engaged in traditional practices by tribals of Dang district of South-Gujarat in Westerns India (communicated elsewhere). The specimens were kindly identified by, Dr. A. B. De, Raj College, Burdhan. The voucher specimen HC10-46882 was deposited in the Plant Pathology herbarium of Indian Agriculture Research Institute, New Delhi.

2.3. Preparation of fractions

The fruiting bodies were cleaned, dried, powdered and extracted exhaustively at 65 ± 3 °C with methanol, to give the methanol fraction (PdM, yield – 3.976%). The methanol fraction (PdM) was further partitioned in ethyl acetate and water (10% methanol) to give reddish-orange colored ethyl acetate soluble fraction (PdEs, 53.35% of PdM or yeild -

2.121%) and yellowish-brown colored ethyl acetate insoluble fraction (PdEi, 46.64% of PdM or yeild - 1.854%).

2.4. Mycochemical screening, antioxidant assays and data analysis

Phenolics, flavonoids, alkaloids, sterols, triterpenes and anthraquinones [44], were detected from the active parent extract.

The DPPH free radical scavenging activity, erythrocyte membrane stabilizing activity [45], β -carotene bleaching assay [46], Erythrocyte membrane stabilizing activity [47], superoxide radical scavenging activity [48], hydroxyl radical scavenging activity [49, 50], linoleic acid-ferric thiocynate method [51], rat liver lipid peroxidation assay [52] and nitric oxide scavenging activity [53] were done with test samples as reported earlier. The activity was expressed as median effective or inhibitory concentration (EC_{50} or IC_{50}), deduced from % inhibition calculated as $[1 - \text{mean absorbance of extract} / \text{mean absorbance of control}] \times 100$. % Protection for erythrocyte membrane stabilizing activity was calculated as $[\text{mean absorbance of extract} / \text{mean absorbance of control}] \times 100$. % Relative protection was calculated for β -carotene bleaching test as $[1 - (\text{mean absorbance of control} / \text{mean absorbance of test})] \times 100$. Nano mole of malondialdehyde production in rat liver lipid peroxidation assay was calculated as $[(\text{OD Test}) / (\text{Control}) \times 0.025 \times 10^9] / 1.56 \times 10^5$. Total phenol and flavonoid [54] content of PdM was estimated as gallic acid and rutin equivalent/g dry weight. Linear regression correlation analysis was done to realize the probable activity contributor.

Dose dependent activity was evaluated by regression analysis of % inhibition. Bonferroni t-test against control was done for absorbance of test and standard groups against absorbance of control group ($n = 3$), where $p < 0.05$ was considered significant. Rat liver lipid peroxidation assay was approved by institutional animal ethical committee.

3. Results and discussion

Excited states and other reactive species collectively known as ROS are known to damage membranes of various cell and cellular organells consisting of polyunsaturated fatty acids by lipid peroxidation and their byproducts [55]. At intracellular level accumulation of high ROS concentration contribute to cell death, as in case of T-lymphocytes undergoing hydrogen peroxide mediated CD95 independent apoptosis, via NF- κ B activation [56]. The mRNAs of *c-fos* and *c-jun* are induced by relatively small amounts of hydrogen peroxide, superoxide, NO, and other inducers of oxidative stress [57]. Thus neutralization of ROS (reactive oxygen species) RNS (Reactive nitrogen species) upstream or down stream of signal cascades can ameliorate deleterious responses.

Medicinal macrofungi occurring in Southern-India namely *Ganoderma lucidum*, *Phellinus rimosus*, *Pleurotus florida* and *Pleurotus pulmonaris* are also reported to possess palpable antioxidant and antitumor activities [55, 58].

Immunomodulatory and antiinflammatory animal studies carried out earlier in our laboratory revealed PdM as the active extract (communicated elsewhere). To evaluate its antioxidant potential certain *in vitro* antioxidant assays were engaged. PdM gave positive results for the presence of terpenoids, phenolics along with, flavonoids and anthraquinones. PdM was found to contain 0.854% phenol and 1.22% flavonoid. PdEs was found to have pattern of pharmacological activities similar to its mother extract, PdM (communicated elsewhere). Thus, in order to identify the daughter fraction inheriting parent activity pattern, PdEs and PdEi were evaluated by DPPH, linoleic acid-ferric thiocynate and β -carotene bleaching assays.

The IC₅₀ of PdM against DPPH free radical (Table 1) was comparable to the reference standard ascorbic acid but showed lack of dose dependent activity. Both the daughter fractions of PdM had considerable activity, of which PdEs performed 2.8 times better than ascorbic acid. PdEi, which landed up closer to PdM had a lower dose dependent activity than its sister fraction, PdEs. Direct ethyl acetate extract of *Phellinus rimosus* is reported to be more potent than the methanolic extracts of *Pleurotus florida*, *P. sajour-caju*, and *G. lucidum* [59] apart from other extracts of *Pleurotus florida*, *P. sajour-caju*, and *G. lucidum* [59] and other extracts of *Phellinus gilvus* and *P. merrillii* [60] with IC₅₀ of 13.34 and 660 µg mL⁻¹, respectively in DPPH assay. This implies that the activity of PdEs is closer to *P. gilvus* and at the same time better than *P. merrillii*.

Table 1. The results of IC₅₀ of PdM against DPPH free radical

DPPH free radical scavenging activity (%Inhibition)						IC ₅₀ µg mL ⁻¹	R ²	y
Dose (µg mL ⁻¹)	10	20	30	40	50			
Ascorbic acid	15.55*	28.52*	31.72*	47.99*	57.67*	43.22	0.97	1.0371x + 5.1802
Dose	25	50	75	100	150			
PdM	27.85*	63.22*	62.26*	66.66*	73.01*	50.57	0.63	0.2924x + 35.214
PdEi	26.18*	58.45*	68.63*	84.45*	85.36*	47.24	0.78	0.4462x + 28.919
Dose	25	50	75	100	125			
PdEs	45.37*	69.78*	79.42*	84.75*	86.25*	15.25	0.83	0.3869x + 44.097
Superoxide radical scavenging activity (%Inhibition)								
Dose	5	20	30	40	50			
Ascorbic acid	22.32	26.76	50.59*	61.57*	73.53*	31.48	0.94	1.2258x + 11.408
dose	10	20	30	40	50			
PdM	23.84	24.94	40.55	48.79	57.73†	41.81	0.96	0.9163x + 11.684
Erythrocyte membrane stabilizing activity (% Protection)						EC ₅₀		
Dose	10	25	50	75	100			
Hydrocortisone	16.57*	22.40*	36.90*	55.51*	62.78*	72.41	0.98	0.547x + 10.391
Dose	100	150	200	250	300			
PdM	20.71*	39.19*	50.86*	58.98*	64.74*	214.36	0.95	0.2157x + 3.7656
Hydroxyl radical scavenging activity (%Inhibition)						IC ₅₀		
Dose	10	20	30	40	50			
PdM	33.02‡	35.83†	53.62*	81.76*	84.59*	24.79	0.92	1.4907x + 13.046
Liver lipid peroxidation assay (%Inhibition)								
Dose	10	25	50	75	100			
α tocopherol	43.084	47.70	55.59†	64.87‡	79.14*	31.32	0.98	0.3907x + 37.764
Dose	10	20	40	80	100			
PdM	23.74	32.76	43.97	56.31†	74.04*	57.62	0.97	0.5032x + 21.008
Nitric oxide scavenging activity (%Inhibition)								
Dose	10	25	50	75	100			
PdM	13.63	22.24	39.29*	48.19*	54.38*	83.24	0.96	0.4628x + 11.483
Linoleic acid - ferric thiocyanate method (%Inhibition)								
Dose	10	20	30	40	50			
PdM	11.90	31.03	38.15	60.74*	74.07*	34.43	0.98	1.5405x - 3.0356
PdEi	29.32	36.09†	49.90*	64.39*	75.11*	29.2	0.99	1.199x + 14.995
PdEs	4.85	13.33	17.85	21.14	24.13	102.74	0.95	0.4638x + 2.3504

The p value marked † are < 0.05, ‡ are < 0.016 to 0.01, * are < 0.007 to 0.001, respectively, those unmarked are insignificant.

Against superoxide anions though only the last concentration was found to be statistically significant, it can be broadly said that PdM, being an extract, seems to be almost as good as ascorbic acid with only 1.23 times lesser (Table 1) activity. A direct ethyl acetate extract of defatted fruiting body of *P. rimosus* is reported to have an IC_{50} of $22 \pm 1 \text{ mg mL}^{-1}$ [55], which is much farther than the activity of *P. durissimus*, being sensitive at c.a. 500 times lower concentration.

Evaluation that connects superoxide generation by cell membrane mediated lipid peroxidation is comparatively more biologically relevant. Highly unsaturated lipids in the cell membrane of RBCs are capable of initiating lipid peroxidation with hemoglobin, when stimulated like illumination, which in turn causes aggregation of membrane proteins, inactivation of enzymes and transport carriers and eventually hemolysis [61]. PdM had lower but sustainable efficacy than the chemical assay, which being dose dependent gave one third protection in comparison to hydrocortisone (Table 1).

Superoxide anion reacts with water to form H_2O_2 that in turn results into hydroxyl radical causing necrotic changes in the tissue inflicted by lipid peroxidation. Ferrous or cuprous like reduced transition metal-ions also form super-reactive hydroxyl radical from hydrogen peroxide [62]. DNA exposed to hydroxyl radical can cause strand breakage, deoxy-sugar fragmentation or base modification directly [63]. PdM showed dose dependent activity with seemingly best IC_{50} (Table 1) in comparison to most of the assays. PdM out-marked methanol (0.33 mg mL^{-1}) and hot water (0.21 mg mL^{-1}) extracts of *P. baumii* with high phenolics [64] and direct ethyl acetate extract of *P. rimosus* giving an IC_{50} of $68 \pm 4.1 \text{ mg mL}^{-1}$ [65].

Antiradical activity using β -carotene bleaching test is an important assay for determination of the activity of extract that could be either direct by neutralizing degrading products or indirect by intervening downstream cascades after lipid peroxidation of erythrocyte membrane causing haemolysis. Activity of PdM in this assay is clear and confirms that it can directly act on linoleic acid degradation products effectively. The parent attribute was justified better by the activity of PdEi than PdEs, though the assay didn't tell them apart very clearly. In comparison to control the absorbance of all the tests were statistically significant ($p < 0.002$). The %Relative protection estimated for PdM ($56.21, 3 \text{ mg mL}^{-1}$; $53.48, 1.5 \text{ mg mL}^{-1}$) resembled PdEs ($61.32, 3 \text{ mg mL}^{-1}$; $56.65, 1.5 \text{ mg mL}^{-1}$), rather than PdEi ($64.91, 3 \text{ mg mL}^{-1}$; $57.52, 1.5 \text{ mg/mL}^{-1}$), yet was greater than both. Minor change between $3 - 1.5 \text{ mg mL}^{-1}$ suggested major activity was in lower concentration resurfacing in the next assay. The linoleic acid-ferric thiocynate assay seemed to amplify the activities witnessed in β -carotene bleaching test. PdM was significant at last two doses, which bought it an IC_{50} mirrored in PdEi with lower value and statistically significant at all the first concentration. Herein the activity of PdEs sagged without statistical significance and higher but considerable IC_{50} .

Hydroxyl radicals are known to cross react with the lipid constituents of the cell membranes, forming 4-OH-2,3-transnaneal, reactive with sulfhydryl group of proteins and malonaldehyde cross-linking protein amino groups [65] or mutating DNA bases [63]. The activity of PdM extract was almost half the standard drug, α tocopherol, both of which had dose dependent activity (Table 1). Extracts of *P. rimosus* of the same genus, is also reported to be active against lipid peroxidation by two major forms of ROS formed by radiation and AAPH (2'Azobis(2-amidopropane) dihydrochloride) induced lipid peroxidation in rat liver and brain mitochondria [55]. Direct ethyl acetate extract of *P. rimosus*, is known to have an IC_{50} value of $162 \pm 7 \text{ mg mL}^{-1}$ [65], which is lower than that of *P. durissimus*.

Physiologically normal level of NO (nitric oxide) produced by phagocytes is beneficial for the host's defense against microorganism, parasites and tumor cells but the over production of the same is implicated in inflammation, cancer, and other pathological conditions [66]. IC₅₀ value (Table 1) of PdM was from a dose dependent response that was around 4 times of curcumin (21.59 µg mL⁻¹) reported by [67], but almost 1/4th of direct ethyl acetate extract of *P. rimosus* having an IC₅₀ of 438 mg mL⁻¹ [65].

The linear regression correlation analysis revealed that both phenol and flavonoid had similar significant correlation coefficients throughout. The antioxidant potential can be attributed to either the high- or low-molecular-weight compounds or their combinations. The phenolic compounds are most widely assigned such properties, which may owe their presence in metabolome due to of oxidative enzymes, such as peroxidases or polyphenol oxidases [68, 69]. The dietary phenolic compounds are known to avert DNA damage and its downstream effects [70]. It is articulated that the antioxidant property of the phenolic compounds may be direct [71] or indirect wherein quinines formed by diphenol oxidation can react with other phenolic molecules in the extract to produce diverse potent molecules like dopachrome, indole, catechol dimmers, or other polyphenolic polymers [29]. Significant correlations between antioxidant activity and concentration of the phenolic compounds are reported for plants [72] and mushrooms [73, 39]. At the same time insignificant correlations for plants [74-76] and mushrooms [34] are reported as well. This is associated with difference in polarity of extraction solvents as it is seen that higher phenolics containing acetone extract (lipophilic) is less active than the 70% acetone in water extract (hydrophilic) with lesser phenolics [76]. The lack of correlation is also attributed to presence of other non-phenolic compounds like tocopherols and β-carotene [34].

Linoleic acid-ferric thiocynate assay had highest R² value (0.98), followed by liver lipid peroxidation (0.969) and superoxide radical scavenging (0.957) assay. Erythrocyte membrane stabilizing (0.949) and hydroxyl radical scavenging activity (0.924) had lower values. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity had lowest correlation (0.63) of all the assays. The flavonoids are known to participate in DPPH mediated H atoms transfer to radicals [77]. Good correlation in linoleic acid-ferric thiocynate and liver lipid peroxidation assays may be due to phenolic and flavonoidal compounds which on fractionation, will concentrate in PdEi owing to higher polarity. This reinforces the fact that a similar pattern of good activity is observed for PdEi in these assays. Poor correlation for DPPH radical scavenging assay may signify presence of other classes as important role players. These compounds may be comparatively non-polar contained in PdEs observed to efficiently contributing to DPPH scavenging, which would be lacking polar polyphenols and flavonoids in PdEi, thus justifying lower correlation.

Hispidin at 0.1 and 1.0 mM [78] and several oxygenated and unsaturated metabolites of hispidin isolated from *Inonotus* and *Phellinus*, are said to possess significant scavenging activity against the superoxide and DPPH radicals [79]. *P. durissimus* is inferred to have phenols, flavonoids, terpinoids and anthraquinones, which may contain several of the compounds, mentioned above, and can be the prime reason for the antioxidant activity of the mushroom.

4. Conclusion

PdM was found to be active in the following decreasing order, hydroxyl radical scavenging > linoleic acid by ferric thiocynate > superoxide radical scavenging > DPPH free radical scavenging > rat liver lipid peroxidation > Nitric oxide scavenging > erythrocyte membrane stabilizing > β-Carotene bleaching. In most of the assays IC₅₀ of PdM was below 100 µg mL⁻¹, suggesting that it has significant antioxidant activity. PdEs demonstrated

excellent DPPH free radical scavenging, whereas PdEi performed better in β -carotene bleaching and linoleic acid-ferric thiocynate assay. Though both showed potent antioxidant activity, PdEs was not better than PdEi in arresting lipid peroxidation. In most of the assays the level of performance was found to be better than other reports of macrofungi of the same or allied genus. This also seems to profusely influence the immunomodulatory and anti-inflammatory activities of this species. This is the first report on the study of antioxidant potential of *Phellinus durissimus* establishing it as a strong contender. We are now aiming at isolation of proactive mycochemicals which render such antioxidant and related activities that are of prime interest.

Acknowledgements

The authors are thankful to Gujarat Council on Science and Technology for funding the project (GUJCOST/MRP/2004-05/10013) and Department of Forest for permitting and facilitating the collection of fruiting bodies.

References

1. Buetler TM, Renard M, Oford EA, Schneider H and Ruegg UT (2002) Green tea extract decreases muscle necrosis in mdx mice and protects against reactive oxygen species. *American Journal of Clinical Nutrition*, 75: 749–753.
2. Katiyar SK and Elmets CA (2001) Green tea polyphenolic antioxidants and skin photoprotection. *International Journal of Oncology* 18: 1307–1313.
3. Lupo MP (2001) Antioxidants and vitamins in cosmetics. *Clinics in Dermatology* 19: 467–473.
4. Karpinska M, Borowski J and Danowska-Oziewicz M (2000) Antioxidative activity of rosemary extract in lipid fraction of minced meat balls during storage in a freezer. *Nahrung* 44: 38–41.
5. Zupko I, Hohmann J, Redei D, Falkay G, Janicsak G and Mathe I (2001) Antioxidant activity of leaves of *Salvia* species in enzyme-dependent and enzyme-independent systems of lipid peroxidation and their phenolic constituents. *Planta Medica* 67: 366–368.
6. Bao ND, Ushio H and Ohshima T (2009) Antioxidative activities of mushroom (*Flammulina velutipes*) extract Added to bigeye tuna meat: dose-dependent efficacy and comparison with other biological antioxidants *Journal of Food Science* 74: C162-C169.
7. Peschel W, Sanchez-Rabaneda F, Diekmann W, Plescher A, Gartzia I, Jimenez D, Lamuela-Raventos R, Buxaderas S and Codina C (2006) An industrial approach in the search of natural antioxidants from vegetable and fruit wastes *Food Chemistry* 97: 137–150.
8. Sies H (1985) Oxidative stress: introductory remarks. In: Sies, H. (Ed.), *Oxidative Stress*. Academic Press, Orlando, FL, pp. 1-8.
9. Wasson RG (1967) In *Soma The Divine Mushroom of Immortality*. Harvest special, *Ethnomycology Studies* No. 1, Harcourt Brace Inc., USA.
10. Percario S, Naufal AS, Gennari M S and Gennari JL (2009) antioxidant activity of edible blushing wood mushroom, *Agaricus sylvaticus* Schaeff. (Agaricomycetideae) in vitro. *International Journal of Medicinal Mushrooms* 11: 2.

11. Ribeiro B, Lopes R, Andrade PB, Seabra RM, Goncalves RF, Baptista P, Quelhas I and Valentao P (2008) Comparative study of phytochemicals and antioxidant potential of wild edible mushroom caps and stipes Food Chemistry 110: 47–56.
12. Tsai SY, Wu TP, Huang SJ and Mau JL (2008) Antioxidant properties of ethanolic extracts from culinary-medicinal button mushroom *Agaricus bisporus* (J. Lange) Imbach (Agaricomycetidae) harvested at different stages of maturity International Journal of Medicinal Mushrooms 10: 2.
13. Mau JL, Chang CN, Huang SJ and Chen CC (2004) Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia Food Chemistry 87: 111–118.
14. Yang JH, Lin HC and Mau JL (2002) Antioxidant properties of several commercial mushrooms Food Chemistry 77: 229–235.
15. Lo KM and Cheung PCK (2005) Antioxidant activity of extracts from the fruiting bodies of *Agrocybe aegerita* var. Alba Food Chemistry 89: 533–539.
16. Tsai SY, Tsai HL and Mau JL (2009) Antioxidant properties of *Coprinus comatus*. Journal of Food Biochemistry 33: 368-389.
17. Mau JL, Chao GR and Wu KT (2001) Antioxidant properties of methanolic extracts from several ear mushrooms. Journal of Agricultural and Food Chemistry 49: 5461–5467.
18. Yang JH, Lin H C and Mau JL (2002) Antioxidant properties of several commercial mushrooms Food Chemistry 77: 229–235.
19. Bor JY, Chen HY and Yen GC (2006) Evaluation of Antioxidant Activity and Inhibitory Effect on Nitric Oxide Production of Some Common Vegetables. Journal of Agriculture and Food Chemistry 54: 1680-1686.
20. Fui HY, Shieh DE and Ho CT (2002) Antioxidant and free radical scavenging activities of edible mushrooms Journal of Food Lipids 9: 35–46.
21. Shi Y, James AE, Benzie IFF and Buswell JA (2002) Mushroom-derived preparations in the prevention of H₂O₂-induced oxidative damage to cellular DNA Teratogenesis Carcinogenesis and Mutagenesis 22: 103-111.
22. Wong JY and Chye FY (2009) Antioxidant properties of selected tropical wild edible mushrooms. Journal of Food Composition and Analysis 22: 269–277.
23. Mau JL, Lin HC and Chen CC (2002) Antioxidant Properties of Several Medicinal Mushrooms. Journal of Agriculture and Food Chemistry 50: 6072-6077.
24. Sarikurkcu C, Tepe B and Yamac M (2008) Evaluation of the antioxidant activity of four edible mushrooms from the Central Anatolia, Eskisehir - Turkey: *Lactarius deterrimus*, *Suillus collitinus*, *Boletus edulis*, *Xerocomus chrysenteron* Bioresource Technology 99: 6651–6655.
25. Tsukamoto S, Macabalang AD, Abe T, Hirota H and Ohta T (2002) Thelephorin A: a new radical scavenger from the mushroom *Thelephora vialis* Tetrahedron. 58:1103-5.
26. Xie C, Koshino H, Esumi Y, Takahashi S, Yoshikawa K and Abe N (2005) Vialinin A, a novel 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenger from an edible mushroom in China Bioscience Biotechnology and Biochemistry 69: 2326-32.

27. Liu JK, Hu L, Dong ZJ and Hu Q (2004) DPPH radical scavenging activity of ten natural *p*-terphenyl derivatives obtained from three edible mushrooms indigenous to China. *Chemistry and Biodiversity* 1: 601-605.
28. Kalac P (2009) Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry* 113: 9–16.
29. Ramirez-Anguiano AC, Santoyo S, Reglero G and Soler-Rivas C (2007) Radical scavenging activities, endogenous oxidative enzymes and total phenols in edible mushrooms commonly consumed in Europe *Journal of the Science of Food and Agriculture* 87: 2272–2278.
30. Dore CMPG, Azevedo TCG, de Souza MCR, Rego LA, de Dantas JCM, Silva FRF, Rocha HAO, Baseia IG and Leite EL (2007) Antiinflammatory, antioxidant and cytotoxic actions of β -glucan-rich extract from *Geastrum saccatum* mushroom *International Immunopharmacology* 7: 1160–1169.
31. Gezer K, Duru M E, Kivrak I, Turkoglu A, Mercan N, Turkoglu H and Gulcan S (2006) Free-radical scavenging capacity and antimicrobial activity of wild edible mushroom from Turkey. *African Journal of Biotechnology* 5: 1924-1928.
32. Barros L, Dueñas M and Ferreira ICFR, Baptista P, Santos-Buelga C (2009) Phenolic acids determination by HPLC-DAD-ESI/MS in sixteen different Portuguese wild mushrooms species. *Food Chem Toxicol.*; 47: 1076-1079.
33. Barros L, Venturini BA, Baptista P, Estevinho LM and Ferreira I C F R (2008) Chemical composition and biological properties of portuguese wild mushrooms: a comprehensive study. *Journal of Agriculture and Food Chemistry* 28: 56:3856-62.
34. Gursoy N, Sarikurkcü C, Cengiz M and Solak MH (2009) Antioxidant activities, metal contents, total phenolics and flavonoids of seven *Morchella* species *Food and Chemical Toxicology* 47: 2381–2388.
35. Stanikunaite R, Trappe JM, Khan SI and Ross SA (2007) Evaluation of therapeutic activity of hypogeous Ascomycetes and Basidiomycetes from North America. *International Journal of Medicinal Mushrooms* 9: 1.
36. Zjawiony J (2004) Biologically active compounds from Aphyllophorales (Polypore) fungi. *Journal of Natural Products* 67: 300–310.
37. Mau JL, Lin HC and Chen CC (2002) Antioxidant Properties of Several Medicinal Mushrooms. *Journal of Agriculture and Food Chemistry* 50: 6072-6077.
38. Wong JY and Chye FY (2009) Antioxidant properties of selected tropical wild edible mushrooms. *Journal of Food Composition and Analysis* 22: 269–277.
39. Turkoglu A, Duru ME, Mercan N, Kivrak I and Gezer K (2007) Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill *Food Chemistry* 101: 267–273.
40. Li SP, Dong TTX and Tsim KWK (2001) Anti-oxidation activity of different types of natural *Cordyceps sinensis* and cultured *Cordyceps* mycelia *Phytomedicine* 8: 207–212.
41. Yan J K, Li L, Wang Z M, Leung P H, Wang W Q and Wu J Y (2009) Acidic degradation and enhanced antioxidant activities of exopolysaccharides from *Cordyceps sinensis* mycelial culture *Food Chemistry* 117: 641–646.
42. Lamb C, and Dixon RA (1997) The oxidative burst in plant disease resistance. *Annual Review of Plant Physiology and Plant Molecular Biology* 8: 251–275.

43. Alvarez M, Pennell RI, Meijer PJ, Ishikawa A, Dixon RA, and Lamb C (1998) Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. *Cell* 92: 773–784.
44. Raaman N (2006). In *Phytochemical Techniques, Qualitative Phytochemical Screening*, New India Publishing Agency, Delhi, India p. 19-24.
45. Navarro MC, Montilla MP, Martin A, Jiemenz J and Utrilla MP (1992) Free radical scavenging activity and antihepatotoxic activity of *Rosmarinus tomentosus*. *Planta Medica* 59: 312-318.
46. Taga MS, Miller EE and Pratt DE (1984) Chia seeds as a source of natural lipid antioxidants. *Journal of American Oil Chemical Society* 61: 928-930.
47. Cheung LM, Cheung PCK and Ooi VEC (2003) Antioxidant activity and total phenolics of edible mushroom extracts *Food Chemistry* 81: 249–255.
48. Fontana M, Mosca L and Rosei MA (2001) Interaction of enkephalines with oxyradicals. *Biochemical Pharmacology* 61: 1253–1257.
49. Klein SM, Cohen G and Cederbaum AL (1981) Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical generating systems. *Biochemistry* 20: 606.
50. Rekka E, Kolstee J, Timmermen H and Bast A (1989) The effect of some H₂- receptor antagonists on rat hepatic microsomal cytochrome P-450 and lipid peroxidation in vitro. *European Journal of Medicinal Chemistry* 24: 43-54.
51. Kikuzaki H, and Nakatani N (1993) Antioxidant effects of some ginger constituents. *Journal of Food Science* 58: 1407-1409.
52. Farina M, Soares FA, Feoli A, Roehring C, Brusque AM, Rotta L, Perry ML, Souza D O and Rocha JBT (2003) In vitro effects of selenite and mercuric chloride on liver thiobarbituric acid–reactive substances and non-protein thiols from rats: influences of dietary cholesterol and polyunsaturated and saturated fatty acids. *Nutrition* 19: 531–535.
53. Marcocci L, Lpacker L, Droy-Lefaiz MT, Sekaki A, and Albert FM (1994) In *Methods in Enzymology* 234: pp. 462-464.
54. Barros L, Baptista P and Ferreira ICFR (2007) Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. *Food Chemistry and Toxicology* 45: 1731-1737.
55. Lakshmi B, Tilak JC, Adhikari S, Devasagayam TPA, and Janardhanan KK (2005) Inhibition of lipid peroxidation induced by gamma-radiation and AAPH in rat liver and brain mitochondria by mushrooms. *Current Science* 88: 484-488.
56. Dumont A, Hehner SP, Hofmann TG, Ueffing M, Droge W, Schmitz ML (1999) Hydrogen peroxide-induced apoptosis is CD95-independent, requires the release of mitochondria-derived reactive oxygen species and the activation of NF- κ B. *Oncogene* 18: 747–757.
57. Janssen YM, Matalon S, and Mossman BT (1997) Differential induction of *c-fos*, *c-jun*, and apoptosis in lung epithelial cells exposed to ROS or RNS. *American Journal of Physiology and Lung Cell Molecular Physiology* 273: L789–L796.
58. Ajith TA, Janardhanan KK (2007) Indian medicinal mushrooms as a source of antioxidant and antitumor agents. *Journal of Clinical Biochemistry and Nutrition* 40: 157–162.

59. Lakshmi B, Tilak JC, Adhikari S, Devasagayam TPA, and Janardhanan KK (2004) Evaluation of antioxidant activity of selected Indian mushrooms. *Pharmaceutical Biology* 42: 179-185.
60. Chang ZQ, Hwang MH, Rhee MH, Kim KS, Kim JC, Lee SP, Jo WS, Park SC (2008) The in vitro anti-platelet, antioxidant and cellular immunity activity of *Phellinus gilvus* fractional extracts. *World Journal of Microbiology and Biotechnology* 24: 181-187.
61. Halliwell B and Gutteridge JMC (1989) In *Free Radicals in Biology and Medicine* (2nd ed.). Oxford, UK, Clarendon, pp. 58-62.
62. Chance B, Sies H and Boveris A (1979). Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* 59: 527–605.
63. Russo A, Izzo AA, Cardile V, Borrelli F and Vanella A (2001). Indian medicinal plants as antiradicals and DNA cleavage protectors. *Phytomedicine* 8: 125-132.
64. Shon MY, Kim TH, and Sung NJ (2003) Antioxidants and free radical scavenging activity of *Phellinus baumii* (*Phellinus* of *Hymenochaetaceae*) extracts. *Food Chemistry* 82: 593-597.
65. Janardhan KK, Ajith TA (2002) Antioxidant and antihepatotoxic activities of *Phellinus rimosus* (Berk) Pilat. *Journal of Ethnopharmacology* 81: 387-391.
66. Moncada A, Palmer RMJ and Higgs EA (1991) Nitric oxide: Physiology, pathophysiology and pharmacology. *Pharmacological Review* 43: 109-142.
67. Bafna AR and Mishra SH (2005) In vitro antioxidant activity of methanol extract of rhizomes of *Curculigo orchoides* Gaertn. *Ars Pharmaceutica* 46: 125-138.
68. Lee EJ and Jang HD (2004) Antioxidant activity and protective effect of five edible mushrooms on oxidative DNA damage *Food Science and Biotechnology* 13: 443–449.
Cheung LM and Cheung PCK (2005) Mushroom extracts with antioxidant activity against lipid peroxidation *Food Chemistry* 89: 403–409.
69. Cheung LM and Cheung PCK (2005) Mushroom extracts with antioxidant activity against lipid peroxidation *Food Chemistry* 89: 403–409.
70. Tanaka M, Kuei CW, Nagashima Y, Taguchi T (1998) Application of antioxidative maillard reaction-products from histidine and glucose to sardine products. *Nippon Suisan Gakkaishil* 54: 1409–1414.
71. Duh PD, Tu YY and Yen GC (1999) Antioxidant activity of water extract of *Harng Jyur* (*Chrysanthemum morifolium* Ramat). *Lebensm-Wiss Technology* 32: 269–277.
72. Velioglu YS, Mazza G, Gao L and Oomah BD (1998) Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products *Journal of Agricultural and Food Chemistry*, 46: 4113–4117.
73. Cheung LM, Cheung PCK and Ooi VEC (2003) Antioxidant activity and total phenolics of edible mushroom extracts *Food Chemistry* 81: 249–255.
74. Bocco A, Cuvelier ME, Richard H and Berset C (1998) Antioxidant activity and phenolic composition of citrus peel and seed extracts *Journal of Agricultural and Food Chemistry* 46: 2123–2129.
75. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K and Kujala TS (1999) Antioxidant activity of plant extracts containing phenolic compounds *Journal of Agricultural and Food Chemistry*, 47: 3954-3962.

76. Demiray S, Pintado ME and Castro PML (2009) Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: *Tilia argentea*, *Crataegi folium* leaves and *Polygonum bistorta* roots World Academy of Science Engineering and Technology 54: 312-317.
77. Sanchez-Moreno C, Larrauri JA and Saura-Calixto FA (1998) Procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture 76: 270–276.
78. Park IH, Chung SK, Lee KB, Yoo YC, Kim SK, Kim GS, and Song KS (2004) An antioxidant hispidin from the mycelial cultures of *Phellinus linteus*. Archives of Pharmaceutical Research 27: 615-618.
79. Lee IK, and Yun BS (2007) Highly oxygenated and unsaturated metabolites providing a diversity of hispidin class antioxidants in the medicinal mushrooms *Inonotus* and *Phellinus*. Bioorganic and Medicinal Chemistry 15: 3309-3314.