Journal of Medicinal Plants Studies
Year:**2013**,Volume:**1**,Issue:**3**
First page: (**166**) Last page: (**175**)
ISSN: **2320-3862**
Online Available at **www.plantsjournal.com**

Journal of Medicinal Plants Studies

Antioxidant and Antimicrobial Activity of *Pleurotus Florida* Cultivated in Bangladesh

Mohammad Mijanur Rahman [1], Asiqur Rahaman [1], Taslima Nahar [1], Borhan Uddin [1], Mafroz Ahmed Basunia [1], Shahdat Hossain [1*]

1. Laboratory of Alternative Medicine & Behavioral Neurosciences, Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.
[E-mail: shahdat@dhaka.net]

Mushroom, a new food item in Bangladesh, has remained largely obscure about its nutritional and medicinal values. Evaluation of antioxidant and antimicrobial activity of the *Pleurotus florida* ethanol extract was the purpose of the present study. Crude *P. florida* extract was qualitatively screened for the presence of phytochemicals while total polyphenol content was measured quantitatively. DPPH-free radical scavenging activity, total reducing power and ability of inhibiting *in vitro* Fenton's reagent-induced oxidative stress were used to evaluate the antioxidant potentials of the *P. florida* extract. Minimum inhibitory concentration and antibiogram of the *P. florida* extract was assessed against six human pathogenic bacteria. Ethanol extract of *P. florida* possesses appreciable antioxidant activity, as indicated by the polyphenol contents, DPPH scavenging activity, reducing power and anti-lipid peroxide effect. The extract exhibited antimicrobial activity against all of the microorganisms tested.

Keyword: Antioxidant activity, Antimicrobial activity, Phytochemicals, Lipid peroxidation.

1. Introduction

The mushroom *P. florida* is one of three of *Pleurotus* species which is recently being cultivated in Bangladesh. 100 grams fresh *P. florida* contains 2.5~2.75 g of proteins, 0.5~0.6 g of lipids, 2.9~3.1 g of fiber and 5.0~5.6 g of carbohydrate[1]. *P. florida* provides essential amino acids, including cystein and methionin, unsaturated fatty acid (87%), including linoleic acid, and minerals[2,3]. In addition, *P. florida* is more digestible and energetically superior than *P. ostreatus* and *P. sajor-caj*[4]. Extract of *P. florida* fruiting bodies showed antitumor, immunomodulatory, anti-inflammatory, antiplatelet-aggregating, hyoplipidemic and hepatoprotective activity[5,6].

Reactive oxygen species (ROS) that are generated during the normal course of oxidative metabolism in the body can attack cellular components, including polyunsaturated fatty acid residues of phospholipids, side chains of all amino acid residues of proteins, and DNA[7,8,9]. Thus, ROS are involved in oxidative stress that have been implicated in the pathophysiology of many clinical conditions and disorders, including ischemia, myocardial infarction, neurodegeneration, cancer, atherosclerosis, hemorrhagic shock and diabetes mellitus. In most cases, ROS are secondary to the diseases while in some instances they are causal[10]. Therefore, dietary antioxidant supplementation is a promising mean to strengthen the antioxidant defense and repair systems of the body to reduce such fatal oxidative damage, as these systems are insufficient to prevent the damage entirely.

In addition, a spectrum of untreatable bacterial infections is emerging due to increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of antibiotic resistance strains while orthodox modern

antibiotics are cost effective and inaccessibility to especially rural/3rd world country[11]. Another problem with orthodox modern antibiotics is the toxicity on the host tissue. Still now about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care[12]. Therefore, searching of alternative or complementary medicine is more rational. In this investigation, we therefore studied whether the ethanol extract of the *P. florida* could act as a natural antioxidant and antimicrobial agent.

2. Materials and methods

2.1 Chemicals

All the chemicals used in this present experiment were of analytical grade. Among these absolute ethanol, Sodium dodecylsulfate (SDS), Hydrogen peroxide, Folin reagent, Trichloroacetic acid, Potassium ferricyanide, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Butylated hydroxytoluene (BHT), Vitamin E and Tetracycline (as acetate) were products of Merck (Darmstadt, Germany). Pyridine, n-Butanol, Thiobarbituric acid (TBA), 1, 1, 3, 3-tetraethoxypropane (MDA) were purchased from Sigma Chemical Co. (St. Louis, USA).

2.2 Mushroom collection and crude extract preparation

Fruit bodies of *P. florida* mushrooms were procured from the National Mushroom Development and Extension Center, Department of Agricultural Extension, Ministry of Agricultural, Government of the people's Republic of Bangladesh. Freshly harvested mushrooms were washed with water and cut into small pieces. *P. florida* was then dried, powdered and subjected to exhaustive extraction with 100% ethanol. The extract was then filtered, evaporated using oven to a thick residue at 40°C and stored at 4ºC until use.

2.2.1 Estimation total polyphenols

The total polyphenols of the extracts were determined following Rahman *et al.* (2013) using pyrogallol as standard[12]. The concentration of total phenol compounds in extracts was determined as pyrogallol equivalents (µg of PE/mg of extract).

2.2.2 Qualitative phytochemical screening

Qualitative phytochemical screening was performed for polyphenols, flavonoid, tannin, alkaloid, glycoside, terpenoid and steroid following Rahman *et al.* (2013)[13].

2.2.3 Quantitative estimation of antioxidant activity

Evaluation of antioxidant activity of *P. florida* was conducted by the following assays: i. DPPH scavenging activity, ii. Total reducing power, and iii. The ability of inhibiting Fenton's reagent-induced *in vitro* oxidative stress.

2.2.4 DPPH radical scavenging activity

DPPH-free radical scavenging activity of *P. florida* extract was measured by the method of Braca *et al.* (2002)[14]. DPPH-free radical scavenging activity of *P. florida* was calculated as % of radical inhibition and expressed as IC_{50} that is the concentration of *P. florida* required to scavenge 50% of DPPH used. Butylated hydroxytoluene (BHT) was used as positive control. Percentage (%) of redical inhibition was calculated from the following equation:

$$\% \text{ Radical Inhibition} = \{(\text{Control OD} - \text{Sample OD}) / \text{Control OD}\} X\ 100$$

Furthermore, to directly visualize the antioxidant activity of the extract, aliquots of 8 µl (0.4 mM) DPPH solution were subjected to thin layer chromatographic (TLC) plate. After air dry, 8 µl of each extract and BHT was re-subjected to the DPPH spots. After 30 min of incubation, the spots were photographed and analyzed by ImageJ.

2.2.5 Total Reducing Power

The reducing power of the extracts was estimated following Oyaizu (1986) using vitamin C as standard[15]. The reducing power of the extract was calculated as ascorbic acid equivalents (µg of AE/mg of extract).

2.3 Fenton's reagents-induced *In vitro* oxidative stress

Three male Wistar adult rats of ~40 weeks age (255g-275g) were sacrificed. Then blood was removed rapidly and the brain was perfused with normal cold saline. The brain was removed from the body; cerebral cortex was separated and homogenated into phosphate buffer saline (50 mM; pH 7.2). The homogenate was centrifuged (800 rpm; 10 min) to remove unruptured tissue. This tissue homogenate was used in subsequent experiments. Fenton's reagent [H_2O_2 (45 mM) +

$FeSO_4$ (2mM)] were used to induce *in vitro* oxidative stress in the brain cortical homogenates without (control) or with (test) *P. florida* extract at a final concentration of 1000 µg/ml. Vitamin E (as acetate) was used as positive control. Malondialdehyde content in the homogenates was determined by previously described (Hossain *et al,* 2012) method, as an indicator of oxidative stress[16]. Lipid peroxidation of the brain was calculated as nmol/mg of protein and total protein in the tissue homogenate was estimated by the Lowry method[17].

2.4 Antimicrobial activity

Antimicrobial activity of ethanol extract of *P. florida* was studied against six human pathogenic bacteria, pure culture of whose were procured from Bangladesh Institute for Research and Rehabilitation in Diabetes (BIRDEM) and Metabolic Disorders, Shahbag, Dhaka, Bangladesh. These bacteria were *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi-A* and *Serretia marcescens.* Bacterial strains were cultivated at 37°C and maintained on nutrient agar slant at 4°C and subcultures in nutrient Broth at 37°C, prior to each antimicrobial test.

2.5 Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was determined by previously described broth dilution method with slight modification[18]. In brief, to a 2.0 ml of broth culture containing various concentrations of extract 0.01ml of inoculums of test bacteria with turbidity of 0.5 McFarland standard was added. Tubes containing bacterial cultures were then incubated at 37ºC for 18 hours in the incubator. After incubation each of the tubes were examined for microbial growth by observing for turbidity. The lowest concentration of extract which inhibited the growth was considered as MIC.

2.6 Antibiogram of *P. florida* extract

Antibiogram of ethanol extract of *P. florida* was studied against six human pathogenic bacteriaby disk diffusion methodusing Muller Hinton agar media[19]. Prior to inoculation, bacterial cultures were adjusted to 0.5 McFarland turbidity standard using sterile saline. Then, 100 µml of inoculums was poured and spread onto Muller Hinton agar plates with the aid of sterile spreader. The dried crude mushroom extract was dissolved in sterilized distilled water and sterilized by filtration through a syringe filter (0.22µm). In case of disk diffusion method, filter paper discs (Whitman No. 1) of 6 mm diameter were loaded with 5.0 mg crude extracts and place at the center of Muller Hinton agar media. The plates were incubated for 24 hours at 37°C in the incubator and zone of inhibition was measured in mm under the surface of the Petri dish by using a transparent scale. Tetracycline (30µgm/disk) for disk diffusion was used as standard. The result of antimicrobial activity was expressed in term of the zone of inhibition around each disc.

2.6.1 Statistical analysis

The results are expressed as mean ± SEM (Standard error of mean). All parameters for inter-group differences were analyzed by one-way ANOVA followed by Tukey's least square differences test for post hoc comparisons. The kinetic data were evaluated by nonlinear regression analysis. The statistical programs used were StatView® 4.01 (MindVision Software, Abacus Concepts, Inc., Berkeley, CA, USA) and GRAPHPAD PRISM® (version 4.00; GraphPad Software Inc., San Diego, CA, USA). A level of $P < 0.05$ was considered statistically significant.

3. Results

3.1 Qualitative phytochemical screening

Preliminary photochemical analysis of ethanol extract of *P. florida* showed the presence of phenolic compound, flavonoids, terpenoid, tannin, glycoside, steroid, saponin but not alkaloid (Table 1).

3.2 Total Polyphenol Content

The amount of the total polyphenol content of *P. florida* ethanol extract was 0.404±0.005µg of GAE/mg of fresh mushroom.

3.3 Total Reducing Power

The reducing power of extracts of *P. florida* expressed as µg of ascorbic acid equivalents. The reducing power of extracts of *P. florida* was 68.69±1.87µg of ascorbet equivalent/mg of extract.

Table 1. Qualitative photochemical analyses of *P. florida* ethanol extract.

Photochemical identified	Indication
Phenolic compounds	+
Flavonoids	+
Terpenoid	+
Tannin	+
Glycoside	+
Alkaloid	-
Steroid	+
Saponin	+

'+' indicates the presence of phytochemicals while '-' indicates the absence.

3.4 DPPH Radical Scavenging Activity

DPPH scavenging activity of *P. florida* extract was two-fold of that of the BHT, as indicated by IC_{50} values (Fig 3). The IC_{50} of *P. florida* ethanol extract was 0.066 ± 0.012mM GAE, while it was 0.154 ± 0.064 mM for BHT.

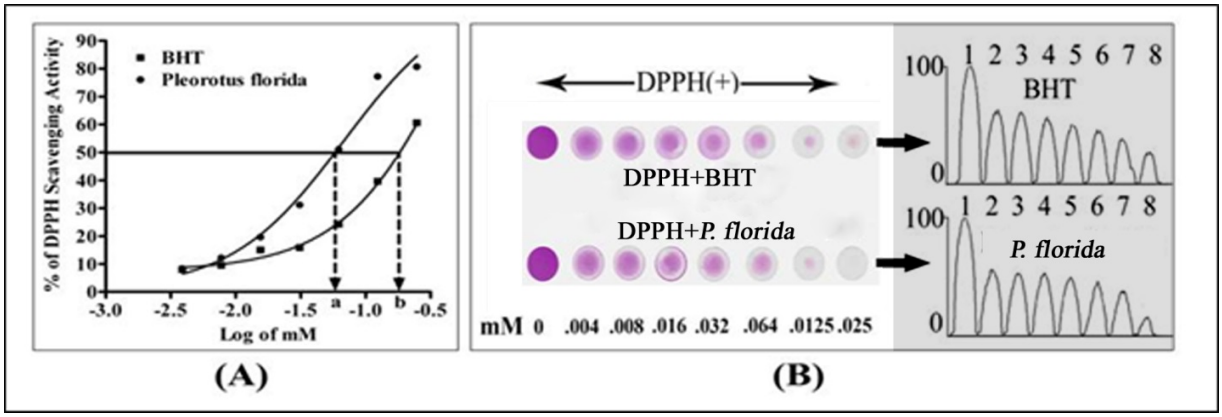

Fig 1: DPPH-scavenging activity of *P. florida* . (A). The IC_{50} values of *P. florida* . Data are the mean ± SEM of triplicate determinations. IC_{50} values were calculated by fitting the data to nonlinear regression analysis equitation [dose-response]. (B). Comperative DPPH- deactivating activity of BHT and *P. florida* . TLC plate was stained initially with 8.0 µl solution DPPH. After air dry the spots were reloaded with 8 µl volume of butylated hydroxyl toluene (BHT) and *P. florida* extract at equimolar concentration. The stable DPPH free radicals were clearly deactivated by the antioxdants (BHT and extract), as indicated by the gradual lightening of the DPPH's purple color of the spots. The scavenging effects were dose dependent. The color of the spots was digitized and calculated by using NIH ImageJ analyzer (Right panel).

3.5 Effect on *In vitro* Oxidative Stress

In vitro anti-oxidative activity of *P. florida* ethanol extract was evaluated by monitoring its ability to withstand the Fenton's reagents induced *in vitro* oxidative stress in the rat brain cortical tissue homogenates. As expected, the Fenton's reagents significantly increased the oxidative stress, as compared to those of the control samples. The results are consistent with the increased levels of lipid peroxide (LPO) in the Fenton's

reagents-induced brain samples (Fig. 2). As expected, vitamin E, which was used as positive control, significantly reduced the levels of LPO. Co-treatment of *P. florida* extract with Fenton's reagent also significantly reduced the levels of LPO. Ethnol extract of *P. florida* alone also significantly decreased the levels of LPO in the absence of oxidative stress (data not shown). The effect was again about 50% of that of the vitamin E (data not shown). These results thus indicate that *P. florida* ethanol extract is able to withstand the Fenton's reagents induced production of free radicals in the biological samples.

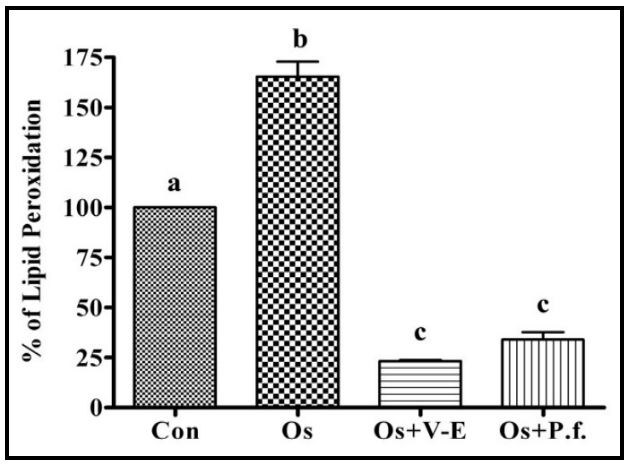

Fig 2: The effect of *P. florida* on in vitro oxidative stress in the brain tissues. The bars represent the mean ± SEM (n=3). Con = Control; Os = Fenton's reagent-induced oxidative stress; Os + V-E = Vitamin E plus Fenton's reagent-induced oxidative stress; Os+ P.f.= *P. florida* extract plus Fenton's reagent-induced oxidative stress. Data were analyzed by One-way ANOVA followed by Tukey's least square differences test for post hoc comparisons. Bars with different alphabets are significantly different at $P<0.05$ level.

3.7 Minimum Inhibitory Concentration

Extract concentrations ranging from 400 to 1400 µgm/ml were used in the determination of minimum inhibitory concentration (Table 2). *Salmonella paratyphi-A* had the highest (1300 µgm/ml) MIC while, *Serretia marcescens* and *Staphylococcus aureus* had the lowest MIC (1000 µgm/ml).

Table 2: Minimum inhibitory concentration (MIC) of *P. florida* ethanol extract.

Extract(mg/ml)	Bacteria					
	E. coli	*Ps. aerugnosa*	*S. typhi*	*S. marcescens*	*S. aureas*	*S. paratyphi-A*
400	+	+	+	+	+	+
600	+	+	+	+	+	+
800	+	+	+	+	+	+
1000	+	+	+	-	-	+
1200	-	+	+	-	-	+
1300	-	-	-	-	-	+
1400	-	-	-	-	-	-

'+' indicates the growth of bacteria, while '-' indicates no-growth.

3.8 Antibiogram of *P. florida* extract

The results of antimicrobial activity of *P. florida* ethanol extract is shown in Table 3. The order of bacteria according to increased degree of susceptibility to ethanol extract of *P. florida* was following: *S. aureas*> *S. marcescens*> *S. typhi*> *E. coli*> *S. paratyphi-A*> *Ps. aerugnosa*.

Table 3: Antibiogram of *P. florida* ethanolic extract against tetracycline.

Bacteria	**Tetracycline disk** (30µg)	***P. florida* disk** (5mg)
E. coli	21.0 ± 1.0	14.0 ± 0.5
Ps. aerugnosa	14.0 ± 0.5	9.20 ± 1.0
S. typhi	21.2 ±1.30	15.0 ± 1.5
S. marcescens	19.0 ± 0.3	17.0 ± 0.5
S. aureas	21.0 ±1.20	17.0 ± 0.5
S. paratyphi A	20.5 ± 0.76	12.6 ± 0.2

Values are mean inhibition zone (mm) ± SEM of three replicates.

4. Discussion

Data on the phytochemical analysis of *P. florida* fruit body is lacking in Bangladesh, hence the present study is the first to describe its phytochemical analysis which revealed the presence of phenolic compound, saponin, terpenoid, tannin, glycoside, steroid, flavonoids but not alkaloid and glycoside (Table 1). Recently, much attention has been paid to natural antioxidants as most commonly used synthetic antioxidants (butylatedhydoxyanisole, butylatedhydoxy-toluene, propylgallat and butylated-hydroquinone) have side effects such as liver damage and carcinogenesis[20]. The *P. florida* extract had a substantial amount of total polyphenol, as measured by the gallic acid standard. The results are consistent with the fact that it significantly scavenged the free radicals of DPPH (Fig. 1). DPPH is a nitrogen-centered free radical, the color of which changes from violate-purple to yellow upon reduction by the process of hydrogenation. A substance which is able scavenge DPPH is considered as an antioxidant and radical scavenger[21]. Due to redox properties phenolic compounds are considered as the principal antioxidants present in natural products. Phenolic compounds are composed of phenolic acids and flavonoids those are potent radical terminators. Thus they donate hydrogen to radicals and neutralize them[22]. The results of the present investigation indicate that *P. florida* could act as an antioxidant like BHT. More importantly, *P. florida* extract is a better antioxidant than BHT, as suggested by the lower IC_{50} value of the extract than that of the BHT. The results are further authenticated by the reducing power of *P. florida*. The reducing power of the *P. florida* extract was 68.69±1.87µg of ascorbet equivalent/mg of extract. After these biochemical evidence at hand, we directly visualized the free radical scavenging activity of *P. florida* by loading the extract on the spots of the DPPH in thin layer chromatographic plates. The violate-purple

color of the DPPH-spots disappeared gradually thus providing a direct evidence of its free radical scavenging potential.

During Fenton's reagents-induced brain lipid peroxidation, iron salts are thought to react with H_2O_2 called the Fenton's reaction, to make hydroxyl radicals ($^{\bullet}OH$), which bringabout peroxide reaction of lipids and lead to the formation of malonaldehyde (MDA)[23]. The •OH radical is the most potent lone pair reactive oxygen species that brings about a severe detrimental change in membrane structure leading to an increased permeability of membrane and damage to biological component molecules[24]. Therefore, we induced the production of hydroxyl (•OH) radicals by incubating the brain tissues with Fenton's H_2O_2+Fe_2SO_4 reagents and examined whether the oxidative stress (of $^{\bullet}OH$) is reduced in the presence of *P. florida* extract (Fig 2). The levels of lipid peroxide were used as an indicator of oxidative stress. The levels of LPO were increased by ~75%, as compared to those of the controls. This suggests that we were able to generate free radicals including $^{\bullet}OH$ radicals in the biological tissues. The presence of vitamin E (a-tocopherol), a well known antioxidant, significantly inhibited the production of free radicals. The *P. florida* extract also inhibited ($p<0.05$) the oxidative potentials in the *in vitro* oxidatively-stressed brain samples, though the inhibition was weaker than that conferred by the vitamin E. Our results are thus consistent with the fact that *P. florida* extract might act as a good antioxidant whose efficacy is between the BHT and vitamin E. However the mechanism(s) of actions remains to be resolved. Localization of flavonoids within the artificial and biological membranes and interactions of flavonoids at the surface of bilayers through hydrogen bonding has been already documented that can act to reduce the access of deleterious molecules (i.e. oxidants) by altering cell membrane structures. Thus, flavonoids protect the structure and function of membranes[25,26]. Therefore, flavonoids, a class of phenolic compounds present in *P. florida* ethanol extract, might contribute to the antioxidant potential of the extract. In addition to flavonoids, antioxidant activity of terpenoid and several types of glycoside has also been documented. The presence of terpenoid and glycoside may also be a candidate to the antioxidant activity of the extract.

Antimicrobial activity of ethanol extract of *P. florida* was studied against six human pathogenic bacteria including *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi-A* and *Serretia marcescen*. The highest antibiogram was recorded in both *S. marcescens* and *S. aureas* measured 17 mm while the lowest antibiogram was recorded in *Ps. aerugnosa* measured 9 mm. Crude extract could be considered as potential useful therapeutics if they have MIC value < 8mg/ml[27] and any chemicals that have antimicrobial activity with a zone of inhibition of 7 mm and above can be considered as potentially usefull therapeutics[28]. Therefore, *P. florida* ethanol extract can be considered as potential antimicrobial therapeutics.

5. Conclusion

Mushroom as a food item is new in Bangladesh. Mass population has remained largely obscure about its nutritional as well as medicinal values. Recently, mushroom of about 20 species are being cultivated and *P. florida* is popular one. The results of the present investigation clearly demonstrate that *P.florida* fruit body could be used as a good food supplements that would support for both natural antioxidant and

antimicrobial actions. Such researches will certainly contribute to the attention of the cultivator, consumers and above all mitigation of nutritional demand in an alternative mean.

6. Acknowledgements

This study was supported, in part, by a Grant from the University Grant Commision (UGC), Dhaka, Bangladesh. Authors also are grateful to the National Mushroom Development and Extension Center (NMDEC), Savar, Dhaka Bangladesh; and Bangladesh Institute for Research and Rehabilitation in Diabetes and Metabolic Disorders (BIRDEM) for their cordial cooperation.

7. References

1. Alam N, Amin R, Khan A, Ara I, Shim MJ, Lee MW et al. Nutritional Analysis of Cultivated Mushrooms in Bangladesh – *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus florida* and *Calocybeindica*. Mycobiology 2008; 36(4): 228-232.
2. Bano Z, Bhagya S, Srinivasan SK. Essential amino acid composition and proximate analysis of the mushrooms, *Pleurotuseous* and *Pleurotus florida*a. Mushroom newslatter for the Tropics 1981; 1:6-10.
3. Khanna PH, Garcha HS. Nutritional value of mushroom *Pleurotus florida*. Mushroom Science. XI1981; 561-572.
4. Khanna PH, Garcha HS. Pleurotus mushroom-A source of food protein. Mushroom news latter for the Tropics 1986; 4: 9-14.
5. Gregori A, Svagelj M, Pohleven J. Cultivation Techniques and Medicinal Properties of Pleurotus spp. Food Technology and Biotechnology 2007;45 (3): 238-249.
6. Muthulingam M, Savio PD, Seeli TS, Indra N, Sethupathy S. Theraputic role of edible mushroom *Pleurotus florida* (Mont.) on thioacetamideinduced Hepatotoxicity in rats. International Journal of Current Research 2010; 5: 41-46.
7. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine, 3rd ed., Oxford University Press, Oxford, UK, 1999, 415-421.
8. Stadtman ER. Role of oxidant species in aging. Current Medical Chemistry 2004; 11: 1105-1112. PMID: 15134509.
9. Siems WG, Grune T, Esterbauer H. 4-Hydroxynonenal formation during ischemia and reperfusion of rat small- intestine. Life Science 1995; 57:785–789. PMID: 7637552.
10. Aajith T, Janardhanan KK. Indian mushroom as source of antioxident and antitumour agents. Journalof ClinicalBiochemistry and Nutrition2007; 40(3): 157-162. doi: 10.3164/jcbn.40.157
11. Sieradzki K, Robart RB, Haber SW, Tomasz A. The development of vancomycin resistance in a patient with methicillin-resistant *Staphylococcus aureus* infection. The New England Journal of Medicine 1999; 340: 517-523. PMID: 10021472.
12. Rahman M, Hossain S, Rahaman S, Fatima N, Nahar T, Borhan Uddin B et al. Antioxidant activity of *Centella asiatica*(linn.) urban: impact of extraction solvent polarity 2013; 1 (6): 27-32.
13. Rahman M, Rahaman A, Basunia MA, Fatima N, Hossain S. Antihemolytic activity of *Clerodendrum viscosum*vent. is mediated by its antioxidant effect. European Journal of Medicinal Plants 2013; 3(1): 127-134.
14. Braca A, Sortino C, Politi M. Antioxidant activity of flavonoids from *Licanialicaniaeflora*. Journal of Ethnopharmacology 2002; 79: 379-381.
15. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition 1986; 44:307-315.
16. Hossain. S. Rahman. A. Nahar. T. Basunia. MA. Mowsumi. FR. Uddin. B. Sahriar. M. Mahmud. I. (2011) *Syzygium cumini*(L.) skeels seed ameliorates in vitro and in vivo oxidative potential of brain cerebral cortex of alcoholtreated rats. Orient Phermacology and Experimental Medicine. 2011; 12:59-66.
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin reagent. Journal of Biological Chemistry. 1951; 193: 265-275. PMID:14907713
18. Doughari JH. Antimicrobial Activity of *Tamarindus indica* Linn. Tropical Journal of Pharmaceutical Research 2006; 5(2): 597-603.
19. Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller

(Lamiaceae). Food Chemistry 2005: 90: 333–340.
20. Buxiang S, Fukuhara M. Effects of coadministration of butylatedhydroxytoluene, butylatedhydroxyanisole and flavonoide on the activation of mutagens and drug- metabolizing enzymes in mice. Toxicology 1997; 12: 61-72.
21. Brand-Williams W, Cuvelier M, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensmittel- Wissenschaft und – Technologie 1995; 28: 25-30.
22. Gülcin I, Beydemir S, Alici HA, Elmastaş M, Büyküokurôlu ME. In vitro antioxidant properties of morphine. Pharmacological Research 2004; 49: 59-66. PMID:14597153
23. Duh PD. Antioxidant activity of burdock (*Arctiumlappa*Linne): It's scavenging effect on free radical and active oxygen. Journal of the American Oil Chemists' Society 1998; 75(4): 455–461.
24. de Beer D, Joubert E, Gelderblom WCA, Manley M. Phenolic compound: a review of their possible role as in vivo antioxidants' of wine. South African Journal for Enology and Viticulture 2002; 23(2): 48-61.
25. Arora A, Byrem TM, Nair MG, Strasburg GM. Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. Archives of Biochemistry and Biophysics2000; 373: 102-109. PMID: 10620328
26. Joseph JA, Denisova N, Fisher D, Shukitt-Hale B, Prior R, Cao G. Membrane and receptor medications of oxidative stress vulnerability in aging. Nutritional considerations. Annals of the New York Academy of Sciences 1998;854: 268-276.
27. Fabry W, Okemo PO, Ansorg R. Antimicrobial activity of East African medicinal plant. Journal of Ethnopharmacology 1998; 60:79-84.
28. Gislene GF, Locatelli J, Preitis PC, Silva GL. Antimicrobial activity of plant extract and Phytochemicals on antibiotic resistant bacteria. Brazilian Journal of Microbiology 2000;31 (40): 20-33.