Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(9):100-107

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Evaluation of antioxidative activity of ethanolic extract from *Russula delica*: An *in vitro* study

Somanjana Khatua[a], Snigdha Paul[a], Ananya Chatterjee[a], Debal Ray[b], Anirban Roy[b] and Krishnendu Acharya[a]*

[a]*Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany, University of Calcutta, Kolkata, West Bengal, India*
[b]*West Bengal Biodiversity Board, Paribesh Bhawan, 10A, LA Block, Sec-VIII, Saltlake City, Kolkata, West Bengal, India*

ABSTRACT

Russula delica, a wild edible mushroom, is commonly available at lateritic zone of West Bengal. The macrofungus was subjected to ethanolic extract and its antioxidant properties using multimechanistic assays were studied. The extract exhibited excellent activities in superoxide radical scavenging (EC_{50}= 0.465 mg/ml), reducing power (EC_{50}= 0.56 mg/ml) and chelating ability of ferrous ion assays (EC_{50}= 0.59 mg/ml) than inhibition of β-carotene bleaching (EC_{50}= 0.965 mg/ml) and DPPH radical scavenging methods (EC_{50}= 1.2 mg/ml). Total polyphenols were the major naturally occurring antioxidant component found in the extract, whereas ascorbic acid was present in vestigial amount. Antioxidant activity in all the assays were established to be highly correlated with total phenols (R^2= 0.987) and flavonoids (R^2= 0.953) implying that the polyphenols was partly responsible for the antioxidant activities. Thus the study scientifically demonstrated use of R. delica as a potential source of natural antioxidant.

Keywords: Antioxidant activity, correlation, edible mushroom, flavonoid, phenol, reactive oxygen species

---

INTRODUCTION

Reactive oxygen species (ROS) are produced as a result of normal consequence of biochemical processes in body or due to increased exposure to environmental xenobiotics. Evidences indicate that harmful ROS plays an important role in most major health problems such as cancer, cardiovascular diseases, rheumatoid arthritis, cataract, Alzheimer`s disease and other degenerative diseases associated with aging which can be reduced by intake of antioxidants [1]. In market many synthetic antioxidants are available but there is a growing interest for natural antioxidants because of their potentially lower toxicity [2]. On the other hand, natural antioxidants such as phenols, flavonoids, vitamins and others are not synthesized in the human body. This fact emphasizes the importance of diets enriched in antioxidants [3].

As a health food, edible mushrooms are widely consumed by people all over the world because they are low in calories and high in vegetable proteins, vitamins, fiber and minerals. Medicinal mushrooms have a long tradition in Asian countries, whereas their use in Western nations has only slightly increased during the last decade [4]. Mushrooms are becoming more and more important in our diet owing to their nutritional and pharmacological characteristics [5-10]. Recently, much attention has been paid to the therapeutic value of mushroom based on its antioxidative property and interest in the role of antioxidants in human health has prompted research in the fields of food science [11].

Russula delica Fr., commonly known as milk-white brittlegill placed under the family Russulaceae, is an edible ectomycorrhizal wild mushroom distributed throughout the lateritic region of West Bengal [12]. However, very little is known about the medicinal application of this mushroom. In the course of our ongoing search we studied the antioxidant property of ethanolic fraction of *R.delica* and evaluated presence of bioactive components.

EXPERIMENTAL SECTION

Mushroom sampling

Basidiocarps of *R. delica* were collected in the month of July from the lateritic region of West Bengal and identified by comparing morphological features using standard literature [13]. The voucher specimen (AMFH 600) was deposited in the mycological herbarium of the University of Calcutta, Kolkata, West Bengal, India.

Chemicals

All chemicals used were of analytical grade. L-methionine, nitro-blue tetrazolium (NBT), riboflavin, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ferrous chloride, ferrozine, potassium ferricyanide, β-carotene, Tween 20, linoleic acid, Folin-ciocalteu reagent, standards such as L-ascorbic acid, ethylenediamine tetraacetic acid (EDTA), butylated hydroxyanisole (BHA), gallic acid, quercetin were purchased from Sigma Chemicals Co. (St. Louis, MO, USA).

Preparation of extract

The fruiting bodies of *R. delica* were cleaned to remove residual compost and dried to eliminate moisture content. Desiccated mushroom samples were ground to obtain fine particles. 10 gm of mushroom powder was soaked in 200 ml ethanol and stirred at 25ºC for 1 day at 150 rpm. Subsequently the solvent was separated through Whatman No. 1 filter paper and the entire extraction process was repeated on the residue. After filtration, the combined solvent was rotary evaporated at 40ºC under vacuum to acquire ethanolic fraction of *R. delica* (EfraRud) and stored in amber coated bottle at 4ºC until further analysis [14].

Superoxide radical scavenging assay

The scavenging potential of EfraRud for superoxide radical was analyzed as described by Martinez *et al* [15] with some modification in the riboflavin-light-NBT system. Each 3 ml reaction mixture sequentially contained 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, various concentrations (0.3, 0.5 and 0.7 mg/ml) of EfraRud, 100 μM EDTA, 75 μM NBT and 2 μM riboflavin. Reaction was started by illuminating sample with light and the increased absorbance was measured at 560 nm after 10 min of illumination. Identical tubes with the reaction mixture were kept in dark and served as blank. BHA was used as a positive control. The degree of scavenging was calculated by the following equation:

Scavenging effect (%) = $\{(A_0-A_1)/ A_0\} \times 100$

A_0 and A_1 were the absorbance of the control and absorbance in presence of sample respectively.

DPPH radical scavenging assay

Radical scavenging activity was determined using a DPPH assay as described by Shimada *et al* [16]. Various concentrations of EfraRud (0.8, 1.1 and 1.4 mg/ml) were added to 2ml of 0.004% methanol solution of DPPH (w/v). After 30 min incubation at room temperature in dark, the absorbance was read against a methanol blank at 517 nm. EC_{50} value is the effective concentration at which DPPH radicals were scavenged by 50%. Ascorbic acid was used for standard. The degree of scavenging was calculated by the following equation:

Scavenging effect (%) = $\{(A_0-A_1)/ A_0\} \times 100$

A_0 and A_1 were the absorbance of the control and absorbance in presence of sample respectively.

Chelating ability of ferrous ions

Chelating ability was determined according to the method of Dinis *et al* [17]. Different concentrations of EfraRud (0.2, 0.6 and 0.8 mg/ml) were mixed with 0.1 ml of 2 mM ferrous chloride. The reaction was initiated by addition of 0.2 ml of 5 mM ferrozine. After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm against a blank. EDTA was used as positive control. EC_{50} value is the effective concentration at which ferrous ions were chelated by 50%. The percentage of inhibition of ferrozine- Fe^{2+} complex formation is given by this formula:

Scavenging effect (%) = $\{(A_0-A_1)/ A_0\} \times 100$

A_0 was the absorbance of the control and A_1 was the absorbance in presence of sample.

Determination of reducing power
The reducing power of EfraRud was determined according to the method of Oyaizu [18]. Various concentrations of the fraction (0.1, 0.5 and 1 mg/ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50ºC for 20 min and then 2.5 ml of TCA (10%) was added to the mixture. 2.5 ml of the solution was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The reaction mixture was incubated for 15 min and absorbance was measured at 700 nm. A higher absorbance indicates a higher reductive capability. Ascorbic acid was used as standard. EC_{50} value is the effective concentration at which the absorbance was 0.5 for reducing power.

Inhibition of β-carotene bleaching assay
The antioxidant activity of EfraRud was also evaluated by the β-carotene linoleate model system [19]. Reaction mixture consisted of 0.5 mg β-carotene in 1ml HPLC grade chloroform, 25 µl linoleic acid and 200 mg Tween 40. Chloroform was completely evaporated. Then 50 ml distilled water was added with vigorous shaking. Aliquots (2ml) of this emulsion were transferred into different tubes containing diverse concentrations of (0.6, 0.8 and 1 mg/ml) and absorbance was read at 490 nm. The tubes were placed at 50°C for 2 h and again absorbance was taken. BHA was used as positive control. The antioxidant activity as percent inhibition rate of β-carotene bleaching relative to control at 2 h was calculated using the equation as follows:

Scavenging effect (%) = $\{(D_0-D_1)/ D_0\} \times 100$

Where D_0 was the β carotene content after 2 h in case of control and D_1 was the β carotene content after 2 h in the presence of sample.

Total polyphenol content determination
The content of total phenolic compounds in EfraRud was estimated according to Singleton and Rossi [20]. 1 ml of extract solution was mixed with 1 ml of Folin-ciocalteu reagent. After 3 min incubation, 35% sodium carbonate solution (1 ml) was added to the mixture and it was adjusted to 10 ml by water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm against blank. Gallic acid (10 – 40 µg) was used to calculate the standard curve. Estimation of phenolic compound was carried out in triplicate. The results were expressed as µg of gallic acid equivalents per mg of extract.

Total flavonoid estimation
Total flavonoid content was determined according to Park *et al* [21]. 1 ml extract was diluted with 4.1 ml of 80% aqueous ethanol, 0.1 ml of 10% aluminium nitrate and 0.1 ml of 1M potassium acetate. After 40 min incubation at room temperature the absorbance was measured at 415 nm. Quercetin (5 – 20 µg) was used to calculate the standard curve. Estimation of flavonoids was carried out in triplicate. The results were expressed as µg of quercetin equivalents per mg of extract.

β-carotene and lycopene estimation
β-carotene and lycopene were estimated according to the method of Nagata and Yamashita [22]. 100 mg EfraRud was mixed with 10 ml acetone-hexane mixture (4:6) for 1 min and filtered through Whatman no 4. Absorbance was measured at 453, 505, 663 nm. The assays were carried out in triplicate. Contents of β-carotene and lycopene were calculated according to the following equations:

β-carotene (mg/100 ml): $0.216\ A_{663} – 0.304\ A_{505} + 0.452\ A_{453}$.

Lycopene (mg/100 ml): $-0.0458\ A_{663} + 0.372\ A_{505} – 0.0806\ A_{453}$.

Ascorbic acid determination
Ascorbic acid was determined by titration according to the method described by Rekha *et al* [23] with some modification. Standard ascorbic acid (100 µg/ml) was taken in a conical flask and made up to 10 ml by 0.6% oxalic acid. It was titrated against 2, 6-dichlorophenol indophenol dye which was prepared by adding 21 mg sodium bicarbonate and 26 mg dye in 100 ml water. The amount of dye consumed (V_1 ml) is equivalent to the amount of ascorbic acid. Similarly, sample (concentration w µg/ml) was also titrated against dye (V_2 ml). The amount of ascorbic acid was calculated using the formula,

Ascorbic acid (µg/mg) = $[\{(10\ \mu g/V_1 ml) \times V_2\ ml\} \times w\ \mu g] \times 1000$.

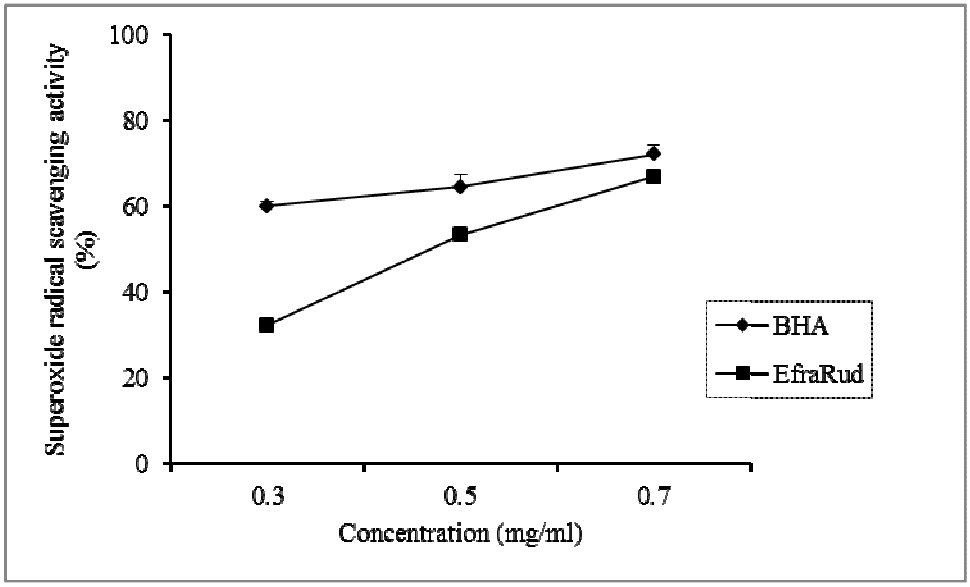
Statistical analysis
Statistical analysis was done using Excel for Windows Software. A regression analysis, R^2, was established between phenolic and flavonoid contents of the extract using the same software.

RESULTS AND DISCUSSION

Superoxide radical scavenging assay
In the human body, superoxide anion, a primary ROS, can be generated by auto-oxidative processes. Although it is a week oxidant, it gives rise to generation of powerful and secondary ROS such as hydroxyl radicals, singlet oxygen; both of which contribute to the oxidative stress [24]. Therefore, it is important to characterize scavenging ability of superoxide radical of antioxidants.

The method used by Martinez *et al* (2001) is based on generation of superoxide radical by auto-oxidation of riboflavin which in turn reduces yellow dye NBT to produce blue formazon in presence of light. Decrease in absorbance at 560 nm with antioxidants designates the consumption of superoxide anion [15]. Figure 1 shows the scavenging activity on superoxide radical in EfraRud. For EfraRud EC_{50} value was 0.465 ± 0.01 mg/ml which is highly comparable with standard, BHA. Ethanol extract of *Tricholoma giganteum* showed similar type of scavenging activity on superoxide radical (EC_{50}= 0.551 mg/ml) [25]. In the present test a significant correlation exists between total phenolics and superoxide radical scavenging activity (R^2= 0.986) and between flavonoids and superoxide radical scavenging activity (R^2= 0.972).

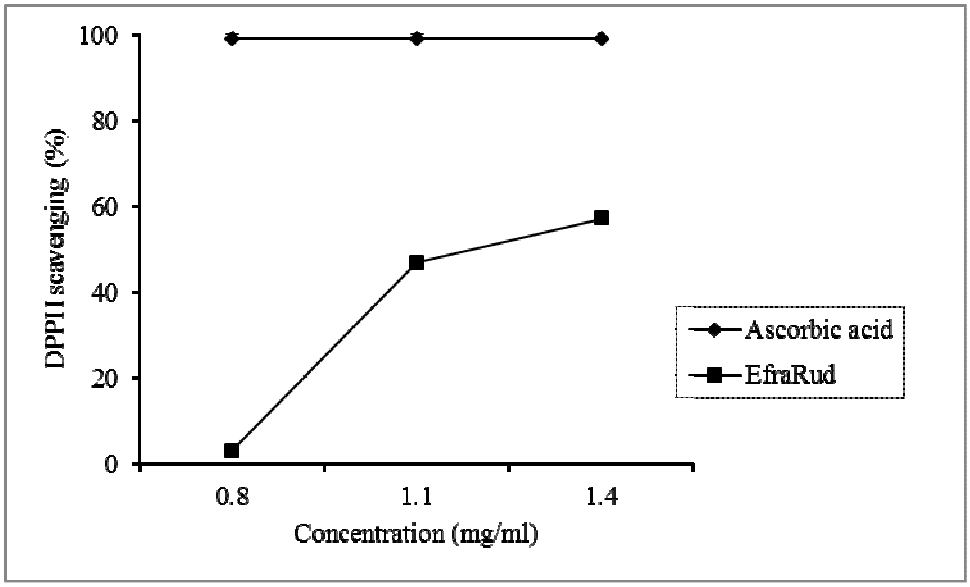
Figure 1: Superoxide radical scavenging activity of ethanol fraction of *Russula delica* (EfraRud)
Results are the mean ±SD of three separate experiments, each in triplicate



DPPH radical scavenging assay
DPPH assay has been widely used to test free radical scavenging ability of various food samples. DPPH is a stable N_2-centered free radical which accepts an electron/ hydrogen to gain stability . Antioxidant has ability to donate electron can neutralize $DPPH^{\cdot}$ by transfer of an electron or hydrogen atom [26].

In methanol solution DPPH produces violet colour. Suitable reducing agent donate electron to DPPH and the solution loses colour depending upon the number of electron taken up. Colour changes from purple to yellow and the reduction capacity of $DPPH^{\cdot}$ is determined by decrease in its absorbance at 517 nm [27]. With regard to scavenging ability of DPPH radicals, EfraRud performed well as evidenced by its low EC_{50} value (1.2 ± 0.05 mg/ml) (figure 2). Ascorbic acid was established to be an excellent scavenger (EC_{50} 4.3 ± 0.3 µg/ml). Chen *et al* [28] determined antioxidant activity of methanol extract of pileus and stipe of *Russula griseocarnosa* separately. In DPPH radical scavenging assay, pileus and stipe showed EC_{50} value at 11.65 mg/ml and 13.88 mg/ml concentration respectively, both of which are very high than EfraRud. In the present assay a significant correlation exists between total phenolics and DPPH scavenging activity (R^2= 0.971) and between flavonoids and DPPH scavenging activity (R^2= 0.939).

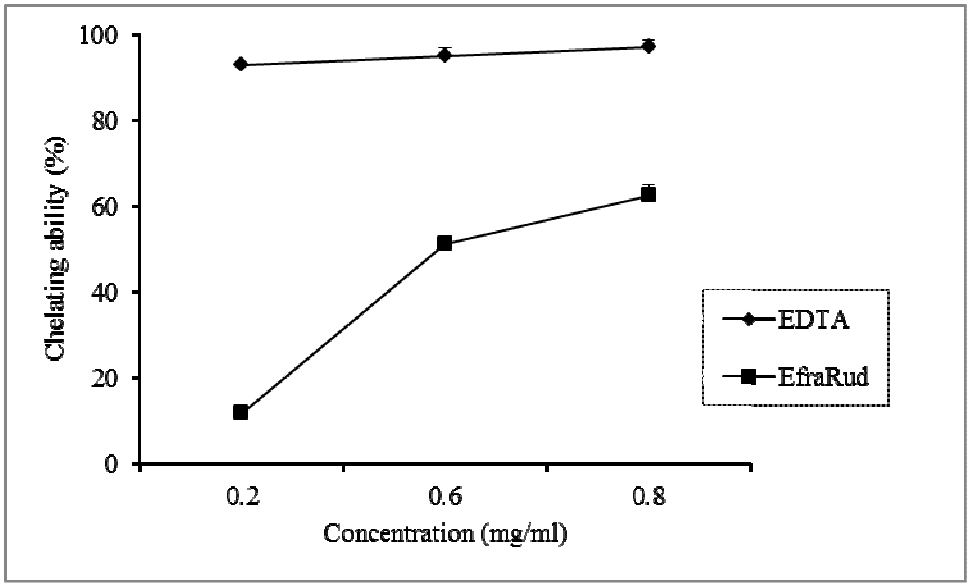
Figure 2: DPPH radical scavenging activity of ethanol fraction from *Russula delica* (EfraRud)
Results are the mean ±SD of three separate experiments, each in triplicate



Chelating ability of ferrous ions
Transition metals have been reported as catalyst to initiate radical formation. Ferrous ion, a transition metal, has capacity to reduce oxygen to superoxide radical and it can also catalyze decomposition of peroxide. It helps in generation of hydroxyl radical from hydrogen peroxide by Fenton`s reaction [29].

As shown in figure 3, extract exhibited dose-dependent chelating abilities on ferrous ions. EfraRud at 0.59 ± 0.02 mg/ml revealed good chelating potencies of 50%. However, EDTA showed the strongest chelating ability. Interestingly, ethanolic extract of *R. delica* collected from Turkey displayed chelating effect much lower than EfraRud as evidenced by high EC_{50} value i.e. 4 mg/ml [30]. EfraRud presented significant correlation between total phenolics and ferrous ion chelating activity (R^2 = 0.993) and flavonoids and ferrous ion chelating activity (R^2 = 0.959).

Figure 3: Ferrous ion chelating ability of ethanol fraction of *Russula delica* (EfraRud)
Results are the mean ±SD of three separate experiments, each in triplicate

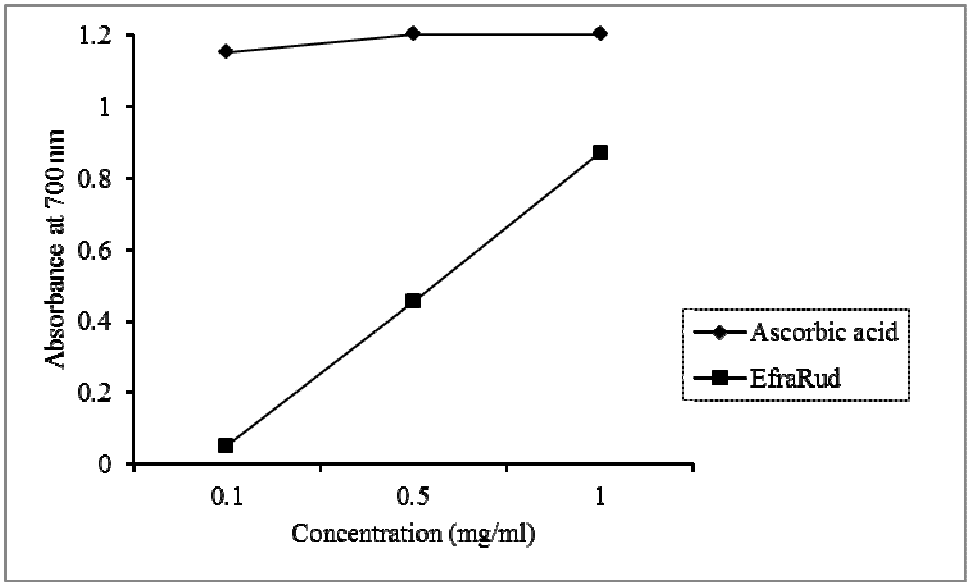


Determination of reducing power
Reducing properties of antioxidants are associated with hydrogen atom donation abilities. Antioxidant can break free radical chains by donating hydrogen atoms and can also react with certain precursors of peroxide to prevent

peroxide formation [31]. For the measurements of the reductive ability, $Fe^{3+} \rightarrow Fe^{2+}$ transformation, in the presence of EfraRud was investigated.

Reducing power of EfraRud was compared to BHA (figure 4). It is clear that BHA, synthetic antioxidant, has better reductive capacity than EfraRud. EfraRud showed EC_{50} value at the extract concentration of 0.56 ± 0.01 mg/ml which is much lower than ethanol extract of *Armillaria mellea*, *Calocybe gambosa*, *Clitocybe odora* and *Coprinus comatus* (EC_{50} value at 17.13, 11.46, 3.63 and 1.47 mg/ml concentration respectively) [32]. Correlation evaluation showed high association between phenol and reducing power (R^2 = 0.996) as well as between flavonoid and reducing power (R^2= 0.989).

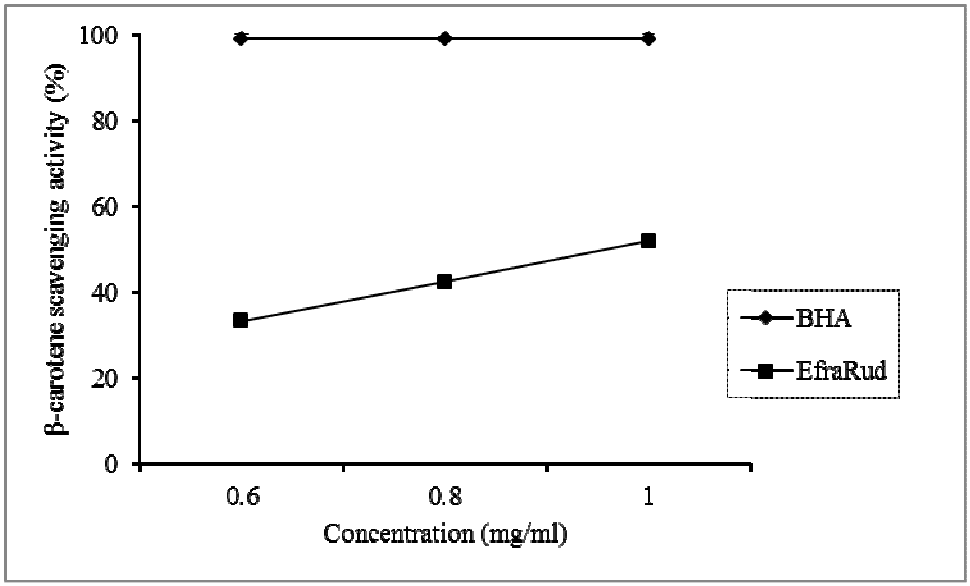
Figure 4: Reducing power of ethanol fraction of *Russula delica* (EfraRud)
Results are the mean ±SD of three separate experiments, each in triplicate



Inhibition of β-carotene bleaching assay

Inhibition of lipid peroxidation by donation of a hydrogen atom is the basis of β-carotene /linoleic acid bleaching test. Oxidation of linoleic acid releases linoleic acid peroxide as free radical that oxidizes β-carotene. As a result, carotene is broken down in part, resulting in discoloration and thereby decreasing absorbance value [33].

Figure 5: Inhibition of β-carotene bleaching activity of ethanol fraction of *Russula delica* (EfraRud)
Results are the mean ±SD of three separate experiments, each in triplicate



EfraRud showed significant prevention of bleaching of carotene in a dose dependent manner (figure 5). At concentration of 0.965 ± 0.015 mg/ml EfraRud was able to inhibit 50% carotene bleaching. Ethanol extract of *Armillaria mellea*, *Calocybe gambosa*, *Clitocybe odora* and *Coprinus comatus* showed lower activity than EfraRud as indicated by their high EC_{50} (8.94, 7.57, 1.36 and 1.26 mg/ml respectively) [32]. The peroxidation inhibition by EfraRud shows a moderate level of correlation between total phenolics (R^2= 0.988) and flavonoid content (R^2= 0.907).

Table 1: Correlation coefficients for relationships between antioxidant activities and bioactive compounds such as phenol and flavonoid in ethanol fraction of *R. delica* (EfraRud)

Assay	Phenol	Flavonoid
Superoxide radical scavenging	0.986	0.972
DPPH scavenging	0.971	0.939
Chelating effect of ferrous ion	0.993	0.959
Reducing power	0.996	0.989
β-carotene bleaching	0.988	0.907
Mean	0.987	0.953

Antioxidant components

In the present study, table 2 demonstrates total phenol, flavonoid, ascorbic acid, β-carotene and lycopene content in EfraRud. Data shows that phenol and flavonoid as the major antioxidant components. This is in agreement with ethanolic extract of *R. delica* collected from Turkey which was found to be consisted of phenol as major compound (6.23 mg/g) and ascorbic acid in small amount (2.93 mg/g). The extract was also found to be consisted of vestigial amount of β-carotene and lycopene (0.11 mg/g and 0.03 mg/g) [30].

Table 2: Total phenol, flavonoid, ascorbic acid, β-carotene and lycopene contents of ethanol fraction of *Russula delica* (EfraRud)
Values are mean ±SD of three separate experiments each in triplicate. Total phenols are expressed in gallic acid equivalent (GAE), and flavonoids as quercetin equivalent (QAE). Nd= not determined

Phenol (µg /mg)	Flavonoid (µg /mg)	β-carotene (µg /mg)	Lycopene (µg /mg)	Ascorbic acid(µg /mg)
12.54 ± 2.84	0.387 ± 0.01	Nd	Nd	0.516 ± 0.08

CONCLUSION

In the current study, ethanolic extract of *R. delica* (EfraRud) have been evaluated for their antioxidant capacities on the basis of various antioxidant assays. The extract showed admirable superoxide radical scavenging activity, chelating ability of ferrous ion and reducing power. EfraRud was also able to inhibit β-carotene bleaching and DPPH radical scavenging. EC_{50} values of the extract for all the assays ranged from 0.465 mg/ml to 1.2 mg/ml. EfraRud contained mixture of bioactive components which is in the order of phenol< flavonoid< ascorbic acid whereas β-carotene and lycopene were undetermined. The antioxidant properties of the extract were mainly influenced by polyphenols as phenol and flavonoid showed strong association with those activities. Thus it can be suggested that EfraRud may be used as a natural additive in food and pharmaceutical industries.

Acknowledgments

The authors (A. C., D. R., A. R. and K. A.) are grateful to Department of Environment, Government of West Bengal for financial assistance.

REFERENCES

[1] S Khatua; S Paul; K Acharya. *Res J Pharm Technol*, **2013**, 6(5), 496-505.
[2] M Spiteller; T Ozen; A Šmelcerović; S Zuehlke; N Mimica-Dukić. *Fitoterapia*, **2008**, 79, 191-193.
[3] P Sandro; SN Audrey; SG Marcelo; LG Jorge. *Int J MedMushrooms*, **2009**; 11(2), 133-140.
[4] N Sharma. *Ethnobotan*, **2003**, 15, 97-99.
[5] G Biswas; S Chatterjee; K Acharya. *Dig J Nanomater Bios*, **2012**, 7, 185-191.
[6] S Chatterjee; G Biswas; S Chandra; GK Saha; K Acharya. *Bioprocess Biosyst Eng*, **2013**, 36, 101-107.
[7] G Biswas; S Rana; S Sarkar; K Acharya. *Pharmacologyonline*, **2011**, 2, 808-817.
[8] G Biswas; K Acharya. *Int J Pharm Pharm Sci*, **2013**, 5, 391-394.
[9] S Chatterjee; R Datta; A Dey; P Pradhan; K Acharya. *Res J Pharm Technol*, **2012**, 5, 1034-1038.
[10] TK Lai; G Biswas; S Chatterjee; A Dutta; C Pal; J Banerji; N Bhuvanesh; JH Reibenspies; K Acharya. *Chem Biodivers*, **2012**, 9(8), 1517-1524.
[11] P Ashwini; M Krishnamoorthy. *Int J Res Ayurveda Pharm*, **2011**, 2(1), 250-252.
[12] P Pradhan; AK Dutta; A Roy; SK Basu; K Acharya. *Biodiversity*, **2012**, 13(2), 88-99.

[13] K Das; JR Sharma. Russulaceae of Kumaon Himalaya. Botanical Survey of India, Ministry of Environment and Forest, Kolkata; **2005**; 176-228.
[14] S Giri; G Biswas; P Pradhan; SC Mandal; K Acharya. *Int J Pharmtech Res*, **2012**, 4, 1554-1560.
[15] AC Martinez; EL Marcelo; AO Marco; M Moacyr. *Plant Sci*, **2001**, 160, 505-515.
[16] K Shimada; K Fujikawa; K Yahara; T Nakamura. *J Agri Food Chem*, **1992**, 40, 945-948.
[17] TCP Dinis; VMC Mudaira; LM Alnicida. *Arch Biochem Biophys*, **1994**, 315, 161-169.
[18] M Oyaizu. *Jpn J Nutr* **1986**, 44, 307-315.
[19] A Dapkevicius; R Venskutonis; TA Van Beek; PH Linssen. *J Sci Food Agr*, **1998**, 77, 140-146.
[20] VL Singleton; JA Rossi Jr. *Am J Enol Viticult*, **1965**, 16, 144-158.
[21] YK Park; MH Koo; M Ikegaki; JL Contado. *Arq Biol Tecnol*, **1997**, 40, 97-106.
[22] M Nagata; I Yamashita. *Nippon Shokuhin Kogyo Gakkaishi*, **1992**, 39, 925-928.
[23] C Rekha; G Poornima; M Manasa; V Abhipsa; DJ Pavithra; KHT Vijay; TRP Kekuda. *Chem Sci Trans*, **2012**, 1, 303-310.
[24] SS Vidović; IO Mujić; ZP Zeković; ŽD Lepojević; VT Tumbas; AI Mujić. *Food Biophys*, **2010**, 5, 49-58.
[25] S Chatterjee; GK Saha; K Acharya. *Pharmacologyonline*, **2011**, 3, 88-97.
[26] CP Anokwuru; I Esiaba; O Ajibaye; AO Adesuyi. *Res J Medicinal Plant*, **2011**, 5(5), 557-566.
[27] T Seal; K Chaudhuri; B Pillai. *J Chem Pharm Res*, **2013**, 5(1), 276-282.[28] X-H Chen; L-X Xia; H-B Zhou; G-Z Qiu. *J Agric Food Chem*, **2010**, 58, 6966-6971.
[29] Z Zekovic; S Vidović; I Mujić. *Croat J Food Sci Technol*, **2010**, 2(2), 16-21.
[30] T Yaltirak; B Aslima; S Ozturkb; H Alli. *Food Chem Toxicol*, **2009**, 47, 2052-2056.
[31] M-Y Lung; Y-C Chang. *Int J Mol Sci*, **2011**, 12, 6367-6384.
[32] J Vaz; L Barros; A Martins; C Santos-Buelgaf; MH Vasconcelos; ICFR Ferreira. *Food Chem*, **2011**, 126(2), 610-616.
[33] RH Gokani; MA Rachchh; TP Patel; SK Lahiri; DD Santani; MB Shah. *J Herbal Med Toxicol*, **2011**, 5, 47-53.