Research Article

In vitro antioxidative behaviour of ethanolic extract of *Russula albonigra*

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ABSTRACT

Ethanolic fraction from edible mushroom, Russula albonigra, was tested for in vitro antioxidant activity, namely, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, chelating effect on ferrous ions, reducing power and total antioxidant capacity assay and a quantitative estimation of putative antioxidant components like total phenol, flavonoid, β-carotene, lycopene and ascorbic acid was carried out. Findings showed that EC_{50} values were below 1 mg /ml except DPPH radical scavenging test. The extract exhibited 50% DPPH radical scavenging activity at only 1.8 mg /ml concentration. Estimated putative antioxidant components was in order of phenol > flavonoids > ascorbic acid > β-carotene> lycopene. Result implies that Russula albonigra can be a potential source of natural antioxidant which may be used as food suplement to treat various oxidative stress related diseases.

Keywords: Antioxidant activity, Antioxidant components, Chelating effect, Reducing power, Scavenging effect.
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INTRODUCTION

Deviation from normal metabolic processes create an imbalance between prooxidants and antioxidants in an organism that lead to oxidative stress and invites a number of degenerative diseases such as diabetes, lung diseases, neurological disorders, cardiovascular problems aging, rheumatoid arthritis etc.[1]. When the normal protective mechanisms are disrupted by various pathological processes, antioxidant supplements are vital to combat oxidative damage. So, the antioxidants can protect the human body from damage caused by reactive oxygen species (ROS) which are ultimately associated with lipid peroxidation, protein damage and DNA denaturation [2] and can prevent an individual from these killer diseases. Many synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) have been reported but they invite many side effects like hepatotoxicity, pneumotoxicity and cancer [3, 4]. Therefore, compounds from natural sources that possess antioxidant potential are being sought and naturally occurring substances mainly by supplementation of food having antioxidant property is becoming one of the most appealing modes of modern therapy. Among them, mushrooms or their derivatives or extracts occupy an elite position to perform this function [1, 5 - 9]. Owing to its geo-climatic exclusiveness, a large variety of mushrooms are found in the state of West Bengal, many of which are eaten by the local natives [10]. Extracts and purified compounds of wild edible mushrooms of West Bengal were analysed and have shown potential apoptogenic [11], cardioprotective [12], antidiabetic [13], hepatoprotective [14] antimicrobial [15, 16] antiparasitic [17] and immunomodulatory activity [18 - 20]. Our objective was to evaluate the antioxidant activity and determine the different bioactive components of the ethanolic extract of a wild edible mushroom *Russula albonigra* (Krombh) Fr.

EXPERIMENTAL SECTION

Materials:
The mushroom *Russula albonigra* was collected from the lateritic region of West Bengal, India. The fruit bodies were thoroughly cleaned and then dried. 25 g of the dried mushroom sample was extracted as suggested [21]. 100 ml

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ethanol was added to the dried and powdered mushroom and kept overnight at room temperature. After filtration using Whatman No. 2 filter paper, the residue was then extracted again with an additional portion of ethanol under the same conditions. The ethanolic extract was evaporated using a rotary evaporator at 50˚C for dryness. The dried extract was resolubilized in ethanol to obtain various concentrations of *EfraRal* (Ethanolic fraction of *R. albonigra*).

Chemicals:
BHT (butylated hydroxytoluene), L-ascorbic acid, quercetin, gallic acid, EDTA (ethylenediaminetetraacetic acid), potassium ferricyanide, ferrous chloride, Folin-Ciocalteu reagent, NBT (nitroblue tetrazolium), DPPH (2,2-diphenyl-1-picrylhydrazyl), TCA (trichlroacetic acid), ammonium molybdate and methionine were purchased from Sigma chemicals Co. (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Determination of total phenols:
Total phenols in the extract were measured using Folin-Ciocalteu reagent [22]. 1ml of ethanolic extract (100 mg/ml) was mixed with 1ml Folin-Ciocalteu reagent and incubated for 3 min at room temperature. After incubation, 1ml of 35 % saturated Na_2CO_3 solution was added in the reaction mixture, volume adjusted to 10 ml with distilled water and incubated in the dark for 90 min, after which the absorbance was monitored at 725 nm with a spectrophotometer. Gallic acid was used as standard. Total phenol content of the sample was expressed as mg of gallic acid equivalents per gram of extract.

Determination of total flavonoid content:
Flavonoid concentration was determined by the method as described [23]. Mushroom extract (100 mg/ml) was diluted with 4.3 ml of 80% aqueous methanol and 0.1 ml of 10% aluminium nitrate and 0.1 ml of 1 M aqueous potassium acetate were added to it. After 40 min at room temperature, the absorbance was determined spectrophotometrically at 415 nm. Total flavonoid concentration was calculated using quercetin as standard.

Determination of total ß-carotene and lycopene content:
ß-carotene and lycopene was determined by the processes as suggested [24]. In brief, 100 ml of mushroom extract (10 mg/ml) was vigorously shaken with 10 ml of acetone-hexane mixture (4:6) for 1 min and absorbance of the mixture was measured at 453, 505 and 663 nm. ß-carotene and lycopene contents were calculated according to the following equations:

Lycopene (mg/100mg) = $-0.0458A_{663} + 0.372A_{505} - 0.0806A_{453.}$

ß-carotene (mg/100mg) = $0.216A_{663} - 0.304A_{505} + 0.452A_{453}$

Determination of ascorbic acid content:
Ascorbic acid content was determined by a method as described [25] with a little 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 with a dye, 2, 6-dichlorophenol indophenol (21 mg sodium bicarbonate and 26 mg of dye in 100 ml water). The amount of dye consumed (V_1 ml) is equivalent to the amount of ascorbic acid. The sample (w µg/ml) was similarly titrated with the dye (V_2 ml). The amount of ascorbic acid was calculated using the formula,

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

Total antioxidant capacity:
The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH [26]. The tubes containing extract (1 mg/ml) and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95˚C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank. The antioxidant capacity was expressed as ascorbic acid equivalent (AAE).

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity
Different concentrations of *EfraRal* (0.5 to 2 mg) was added to 0.004% methanolic solution of DPPH [27]. The mixture was shaken vigorously and left to stand for 30 min in the dark. Absorbance was measured at 517 nm against a blank. EC_{50} value is the effective concentration of extract that scavenged DPPH radicals by 50% and it was obtained by interpolation from linear regression analysis.

Reducing power
Reducing power of *EfraRal* was determined following the method of Oyaizu, 1986 [28]. Variable concentrations of *EfraRal* were added to 2.5 ml 0.2 M phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture

was incubated at 50° C for 20 min. 2.5 ml of 10% trichloroacetic acid was added to the mixture and was centrifuged at 12000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml distilled water and 0.5 ml 0.1% ferric chloride and absorbance was measured at 700 nm. An increase in absorbance of the reaction mixture was taken to mean an increase in reducing power of the sample.

Chelating effect on ferrous ions
The ability of the extract of *EfraRal* to chelate ferrous ions was estimated by the method of Dinis et al, 1995 [29]. Briefly, the extract was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml), and the mixture was then shaken vigorously and left to stand at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated as $[(A_0 - A_1) / A_0] \times 100$, where A_0 was the absorbance of the control, and A_1 of the mixture containing the extract or the absorbance of a standard solution.

Superoxide radical scavenging assay
Superoxide radical scavenging activity was studied using the riboflavin-light-nitrobluetetrazolium (NBT) system suggested by Martinez et al. (2001) [30] with a little modification. Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, sample solution of various concentrations of *EfraRal*, 100 µM EDTA, 75 µM NBT and 2 µM riboflavin. One set of reaction mixtures were exposed to light for 10 min to activate the riboflavin-NBT and the absorbance of each mixture was measured at 560 nm against identical mixtures from another set kept in the dark fir the same duration. Butylated hydroxyanisole (BHA) was used as standard.

RESULTS AND DISCUSSION

Recovery percent and antioxidant components
In the present study, table 1 shows the percent yield, total phenol, flavonoids, ascorbic acid, beta-carotene and lycopene content in the *EfraRal*. Data shows that ascorbic acid, total phenols and flavonoids were the major antioxidant components whereas, β-carotene and lycopene were found in vestigial amounts.

Table 1: yield percentage and antioxidant components of ethanolic extract of *Russula albonigra* (*EfraRal*)
Values are the mean ± standard deviation of three separate experiments, each in triplicate. Total phenols are expressed in gallic acid equivalent (GAE) and flavonoids as quercetin equivalent (QE).

	Flavonoids	Total phenols	Ascorbic acid	β- carotene	Lycopene
Yield %	(µg/mg)				
1.8 ± 0.3%	6.375 ± 0.37	9.53 ± 0.45	2.77 ± 0.416	0.873 ± 0.006	0.499 ± 0.0093

Phenolic compounds are known to be powerful chain-breaking antioxidants and they possess scavenging ability due to their hydroxyl groups. The phenolic compounds contribute directly to the antioxidative action. In the present study, the total phenolic content of *EfraRal* (9.53 ± 0.45 µg/mg) was found to be higher than that of the ethanolic extract of *Pleurotus* ostreatus, which was reported to be 5.49 µg/mg [31], but was comparatively less than *Pleurotus squarrosulus*, which was 18.1 µg/mg [32] and relatively similar to *Pleurotus citrinopileatus*, that being 8.62 µg/mg [33].

Ascorbic acid is reported to interact directly with radicals such as O_2^- and OH . in plasma, thus preventing damage to red cell membranes, it also assists α-tocopherol in inhibition of lipid peroxydase by recycling the tocopherol radical [34]. In the present study, the ascorbic acid content of *R. albonigra* extract was high (2.77 ± 0.416 µg/mg) when compared to the values reported from other species such as *P.* ostreatus (25 ng/1 mg) [31] and *P. citrinopileatus* (31 ng/mg) [33]. Many other naturally occurring antioxidant components, including *β*-carotene, lycopene and flavonoids are known to possess strong antioxidative characteristics [35]. In this study *β*-carotene and lycopene were found in vestigial amounts, i.e. 0.873 ± 0.006 µg/mg and 0.499 ± 0.0093 µg/mg respectively, which are higher than that of the methanolic extract of *P. squarrosulus*, which were 570 ng and 225 ng per mg respectively [32], and *P. ostreatus* [31]. *β*-carotene was not detected in the ethanolic extract of *P.* citrinopileatus [33]. The estimated flavonoid content of *EfraRal* is 6.375 ± 0.37 µg/mg which is higher than *P. squarrosulus*, whereit was reported to be 3.07 µg/mg [32] and also *P. citrinopileatus*, 71.2 ng/mg [33]. Total phenols, ascorbic acid and flavonoids were the major naturally occurring antioxidant components estimated in this study. The higher amounts of these components in this extract might explain its more effectiveness in antioxidant properties.

Total antioxidant capacity
Total antioxidant capacity of *EfraRal* was determined by the formation of green phosphomolybdenum complex. The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the

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formation of green phosphate/Mo (V) complex with the maximal absorption at 695 nm. Total antioxidant activity of the extract was estimated, using ascorbic acid as standard. Analysing the data, it was found that 1mg of extract is as functional as approximately 17 ± 3.7 µg of ascorbic acid, expressed as 17 µg AAE. The total antioxidant capacity of *EfraRal* may be attributed to their chemical composition and phenolic acid content. A recent study [36] showed that some bioactive compounds from citrus fruits had strong total antioxidant activity, which was probably due to the presence of flavonoids, carotenoids and ascorbic acid.

DPPH radical-scavenging activity
DPPH is a stable free radical that has a characteristic absorption at 517 nm, which decreases significantly on exposure to radical scavengers by providing hydrogen atom or electron to become a stable diamagnetic molecule. The use of stable DPPH radical has the advantage of being unaffected by side reactions such as enzyme inhibition and metal chelation [6, 37]. Such reactivity has been widely used to test the ability of a compound or extract as free radical scavengers. Upon treatment with an increasing concentration of the *EfraRal*, a marked decrease in absorption was observed, indicating a potent DPPH scavenging ability of the extract (Fig. 1). EC_{50} of DPPH radical scavenging activity was 1.7 ± 0.02 mg/ml, similar to the ethanolic extract of *Pleurotus flabellatus* [21], while being lower than that of *Calocybe gambosa* [38], *Clitocybe odora* [38] and *Coprinus cometus* [38] and significantly higher than that of *Armillaria mellea* [39]. In our earlier investigations, the EC_{50} value for the ethanolic extract of *Meripilus giganteus* [6], *Ramaria aurea* [7] showed very low EC_{50} value. Therefore, the DPPH radical scavenging activity of the ethanolic extract from different basidiocarps were in decending order *M. giganteus ~ R. aurea > A. mellea > R. albonigra > P. flabellatus > C. cometus > C. odora > C. gambosa*. In a related study, the edible mushroom *Volvariella volvaceae*, showed 57.8% DPPH scavenging at a concentration of 9 mg/ml [40]. Thus it can be said that the ethanolic extract of *R. albonigra* has significant DPPH radical scavenging ability.
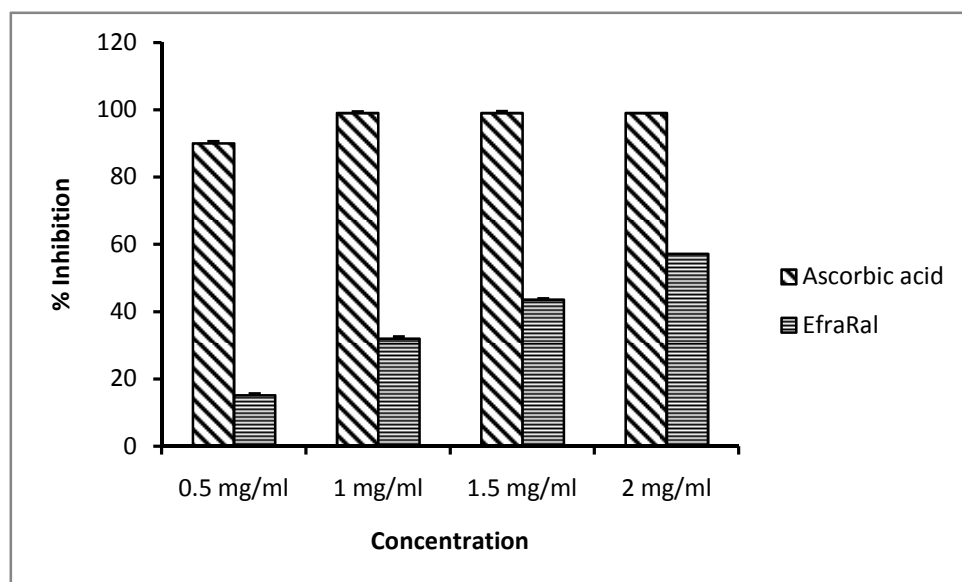

Fig. 1: DPPH radical scavenging activity of *EfraRal* compared with that of the standard, ascorbic acid
Values are the mean ± standard deviation of three separate experiments, each in triplicate.

Reducing power
Reducing power of a compound may serve as a significant indicator of its potential antioxidant activity [28]. The presence of reducers (i.e. antioxidants) causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. In the reducing power assay the presence of antioxidants in the *EfraRal* would effect in the reduction of Fe^{3+} to Fe^{2+} by the donation of an electron. The increasing absorbance at 700 nm by measuring the formation of Perl's Prussian Blue indicates an increase in reductive ability. A steady increase in reducing power was observed (fig 3). Results showed that EC_{50} for the reducing power of *EfraRal* was of 0.69 ± 0.03 mg/ml. Compared with the reducing powers of previously studied edible mushrooms from previously reported studies, the ethanolic extract of *R. albonigra* was an excellent reducer of ferric ions. The reducing power of ethanolic extracts of different edible mushrooms in descending order are *H. marmoreus* [43] > *C. gambosa* [38] > *A. mellea* [39] > *C. odora* [38] > *T. giganteum* [45] > *C. comatus* [38] > *R. albonigra* > *P. flabellatus* [21]. The ethanolic extract of *R. albonigra* can thus be considered as an excellent reducing agent.
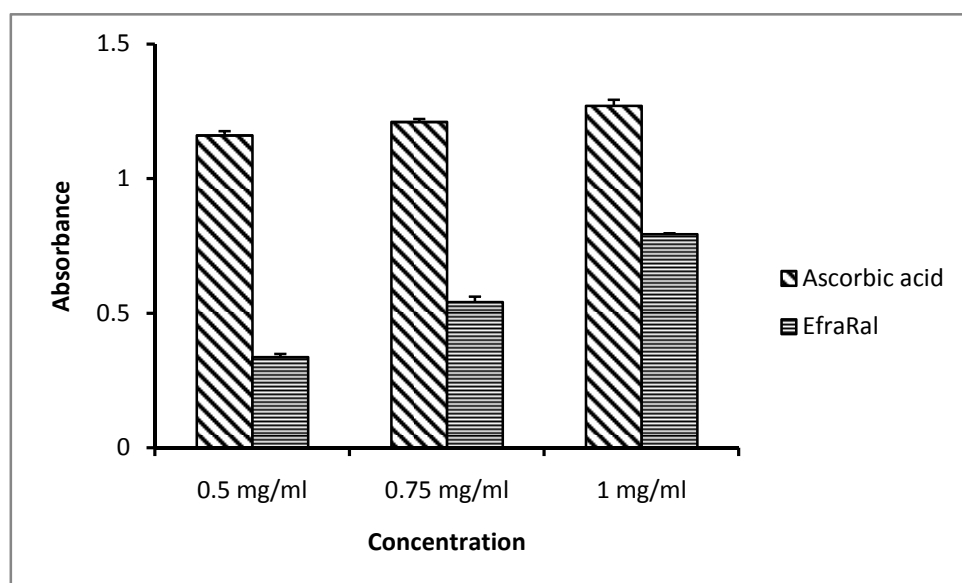
Fig 3: Reducing power of *EfraRal* with respect to that of ascorbic acid used as standard
Values are the mean ± standard deviation of three separate experiments, each in triplicate.

Chelating effect on ferrous ions

Like many transition metals, Ferrous ions in a biological system could catalyse Heber-Weiss and Fenton-type reactions leading to the formation of hydroxyl radicals. Antioxidants chelate these transition metal ions resulting in the suppression of hydroxyl radical generation and inhibition of peroxidation process of biomolecules. The range and the mean of Fe^{2+} chelating capacity is directly related with antioxidant potentiality. At 300 – 900 µg/ml the chelating effects of the *EfraRal* was between 22.6 and 62.7% (fig 2). At the same concentration range, the chelating effects of the known metal chelator EDTA, was between 69% - 90%. It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential thereby stabilising the oxidised forms of metal ions [41]. Iron can stimulate lipid peroxidation by the Fenton reaction and accelerate peroxidation by decomposing lipid peroxide into peroxyl and alcoxyl radical that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation. The main mechanism of the ferrous ion chelating activity is the ability to deactivate and/or chelate Fe^{2+} which can promote Fenton reaction and hydroperoxide decomposition. Iron toxicity is associated with an increased risk of free radical damage and cancer. Chelation therapy may possibly reduce iron related free radical damage and increase the overall survival in cardiovascular diseases [42]. The ferrous ion chelating ability of *EfraRal* was effective and the EC_{50} value was found to be 0.81 mg/ml. Previous investigators have shown that the EC_{50} value of the ethanolic extract for *Russula delica* [43] and *Hypsizigus marmoreus* [44] were more than 3mg/ml which is much higher than that of *EfraRal*. In our earlier investigations, the EC_{50} value of the ethanolic extract of *Pleurotus flabellatus* was much lower than *EfraRal* [21], and the EC_{50} value of the ethanolic extract of *Tricholoma giganteum* was very close to 1 mg/ml [45]. Hence, the studied mushroom extract shows high interference with the formation of ferrous and ferrozine complex and can be considered as a good chelator of ferrous ions.

Superoxide radical scavenging assay

Superoxide radical is known to be very harmful to cellular components as a percursor of more reactive species. One risk of the superoxide generation is related to its interaction with nitric oxide to form peroxinitrite which is a potent oxidant that causes nitrosative stress in the organ systems [9]. In the present study, *EfraRal* was found to be a notable scavenger of superoxide radicals generated in riboflavin- nitrobluetetrazolium (NBT) light system (Fig.4). The EC_{50} value of the fraction was 0.74 mg/ml. Compared with an previously reported studies, the EC_{50} value of *EfraRal* was higher than that of *Astraeus hygrometricus* [11], *Macrocybe gigantea* [46] and *Tricholoma giganteum* [45].
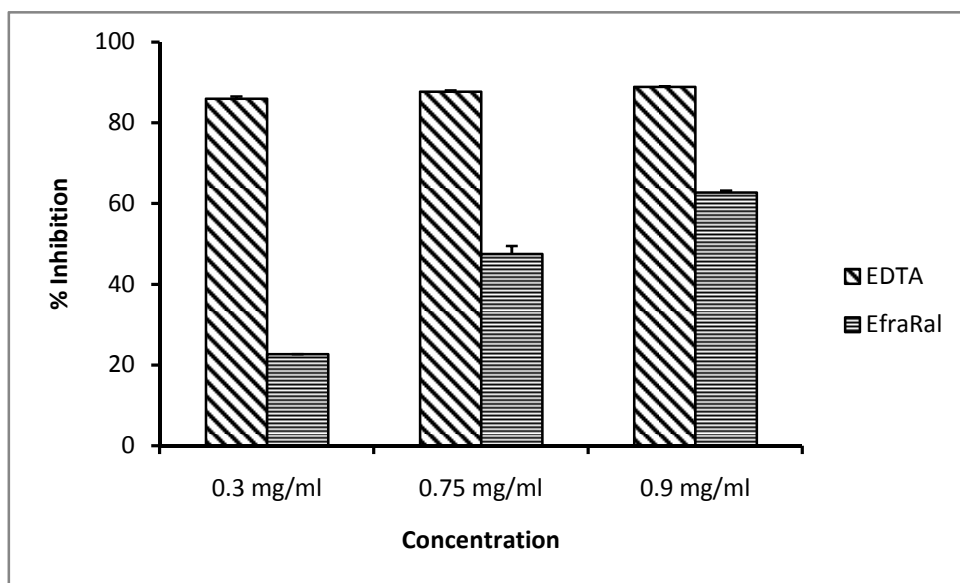
Fig 2: Chelating effects of *EfraRal* on ferrous ions compared with that of EDTA, used as standard
Values are the mean ± standard deviation of three separate experiments, each in triplicate.
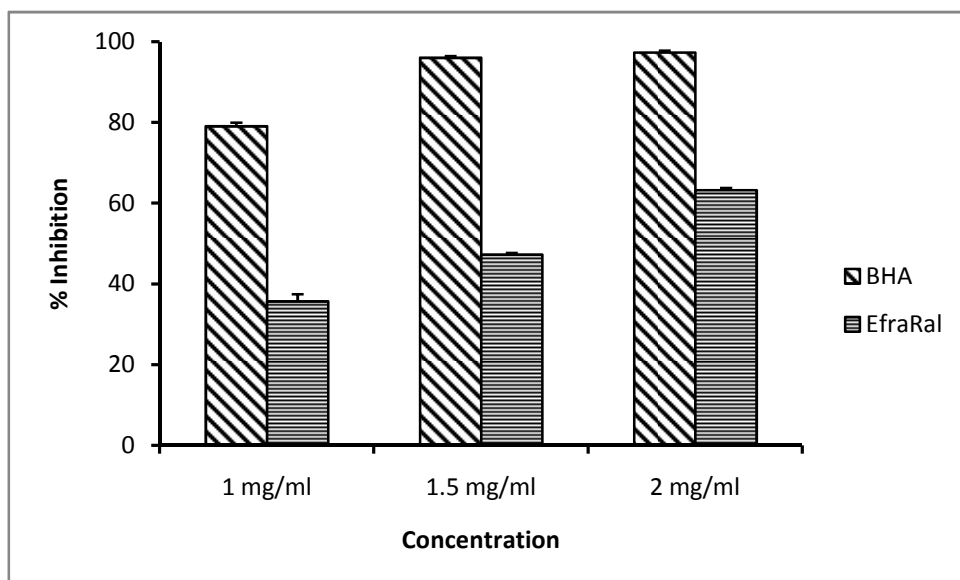

Fig 4: Superoxide radical scavenging activity of *EfraRal* compared to that of the standard BHA
Values are the mean ± standard deviation of three separate experiments, each in triplicate.

Table 2: EC$_{50}$ values of the extract for different antioxidant properties
Values are the mean ± standard deviation of three separate experimental data, each in triplicate

	Chelating	DPPH	Reducing
EC$_{50}$ (mg/ml)	0.81 ± .007	1.7 ± .02	0.69 ± .03

CONCLUSION

As a result of the study, the ethanolic extract of *Russula albonigra* was found to be an effective antioxidant in different *in vitro* assays including Ferrous iron chelating, ferric iron reducing, DPPH free radical scavenging and total antioxidant activity, and can be suggested as a natural additive in food and pharmaceutical industries.

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REFERENCES

[1] S Khatua; S Paul; K Acharya. *Res. J. Pharm. Tech.*, **2013**, 6(5), 496-505.
[2] M Rai, G Biswas, SC Mandal, K Acharya. New Central Book Agency (P) Ltd., Kolkata, India, **2011**; 479-496.
[3] HM Lin; FL Yen; LT Ng; CC Lin. *J. Ethnopharmacol.*, **2007**, 111(1), 129-136.
[4] SF Nabavi; MA Ebrahimzadeh; SM Nabavi; B Eslami. *Grasas y Aceites.*, **2010**, 61(3), 244-250.
[5] P Mitra; S Khatua; K Acharya. *Asian J. Pharm. Clin. Res.*, **2013**, 6(3), 67-70.
[6] K Acharya; M Rai. *Int. J. Pharm. Pharm. Sci.*, **2013**, 5(1), 67-72.
[7] M Rai; K Acharya. *Res. J. Pharm. Tech.*, **2012**, 5(11), 1421-1427.
[8] M Rai; K Acharya. *Int. J. Pharm. Pharm. Sci.*, **2012**, 4(4), 460-463.
[9] K Acharya; S Chatterjee; S Ghosh. *Pharmacologyonline*, **2011**, 1, 440-450.
[10] P Pradhan; AK Dutta; A Roy; SK Basu; K Acharya. *Biodiversity*, **2012**, 13(2), 88-99.
[11] G Biswas; S Chatterjee; S Sarkar; K Acharya. *Int. J. Biomed. Pharm.* Sci., **2010**, 4, 21-26.
[12] G Biswas; S Rana; S Sarkar; K Acharya. *Pharmacologyonline*, **2011**, 2, 808-817.
[13] G Biswas; K Acharya. *Int. J. Pharm. Pharm. Sci.*, **2013**, 5 (1), 391-394.
[14] S Chatterjee; R Datta; A Dey; P Pradhan; K Acharya. *Res. J. Pharm. Tech.*, **2012**, 5(8), 1034-1038.
[15] S Giri; G Biswas; P Pradhan; SC Mandal; K Acharya. *Int. J. PharmTech. Res.*, **2012**, 4(4),1554-1560.
[16] M Rai; S Sen; K Acharya. *Int. J. PharmTech. Res.*, **2013**, 5(3), 949 – 956.
[17] S Mallick; A Dutta; S Dey; J Ghosh; D Mukherjee; S S Sultana; S Mandal; S Paloi; S Khatua; K Acharya; C Pal. *Exp. Parasitol.*, **2014**, 138, 9 – 17.
[18] P Patra; S K Bhanja; I K Sen; A K Nandi; S Samanta; D Das; K Sanjana; P Devi; T K Maiti; K Acharya; S S Islam. *Carbohydrate Res.*, **2012**, 362, 1 – 7.
[19] A K Nandi; I K Sen; S Samanta; K Maity; K Sanjana; P Devi; S Mukherjee; T K Maiti; K Acharya**;** S S Islam. *Carbohydr. Res.*, **2012,** 363, 43-50**.**
[20] A K Nandi; S Samanta; I K Sen; K Sanjana; P Devi; T K Maiti; K Acharya; S S Islam. *Carbohydr. Polym.*, **2013,** 94(2), 918– 926.
[21] A Dasgupta; M Rai; K Acharya. *Int. J. PharmTech. Res***., 2013,** 5(4), 1655-1663.
[22] VL Singleton; JA Rossi. *Am. J. Enol. Vitic.*, **1965**, 16(3), 144-158.
[23] EA Adebayo; JK Oloke; AA Ayandele; CO Adegunlola. *J. Microbiol. Biotech. Res.*, **2012**, 2(2), 366-374.
[24] M Nagata; I Yamashita. *Nippon Shokuhin Kogyo Gakkaishi.*, **1992**, 39(10), 925-928.
[25] C Rekha; G Poornima; M Manasa; V Abhipsa; DJ Pavithra; KHT Vijay; KTR Prashith. *Chem. Sci. Trans.*, **2012**, 1(2), 303-310.
[26] P Prieto; M Pineda; M Aguilar. *Anal. Biochem.*, **1999**, 269, 337–341.
[27] K Shimada; K Fujikawa; K Yahara; T Nakamura. *J. Agric. Food Chem.*, **1992**, 40(6), 945-948.
[28] M Oyaizu. *Jpn. J. Nutr.*, **1986**, 44, 307-315.
[29] TCP Dinis; VMC Madeira; LM Almeida. *Arch. Biochem. Biophys.*, **1994**, 315, 161-169.
[30] AC Martinez; EL Marcelo; AO Marco; M Moacyr. *Plant Sci.*, **2001**, 160(3), 505-515.
[31] T Jayakumar; PA Thomas; P Geraldine. *Innovative Food Sci. Emerg. Technol.*, **2009**, 10(2), 228-234.
[32] YL Lee; GW Huang; ZC Liang; J.L Mau. *LWT - Food Sci. Technol.*, **2007**, 40(5), 823-833.
[33] J Pal; S Ganguly; KS Tahsin; K Acharya. *Indian J. Exp. Biol.*, **2010**, 48(12), 1210-1218.
[34] WN Beyer; E Conner; S Gerould. *J. Wildl. Manage.* **1994**, 58, 375-382.
[35] SZ Dziedzic; GJF Hudson. *Food Chem.*, **1983**, 12, 205-212.
[36] GK Jayaprakasha; B Girennavar; BS Patil. *Bioresour. Technol.*, **2008**, 99(10), 4484-4494.
[37] M Waltasinghe; F Shahidi. *Food Chem.*, **1999**, 63(4), 399-414.
[38] J Vaz; L Barrosa; A Martinse; C Santos-Buelgaf; MH Vasconcelosb; ICFR Ferreira. *Food Chem.*, **2011**, 126(2), 610-616.
[39] M Rai; G Biswas; S Chatterjee; SC Mandal; K Acharya**.** *J. Biol. Sci.*, **2009**, 1, 39-45**.**
[40] LM Cheung; PCK Cheung; VEC Ooi. *Food Chem.*, **2003**, 81(2), 249-255.
[41] K Sowndhararajan; JM Joseph; S Manian. *Int. J. Food Prop.*, **2013**, 16(8), 1717-1729.
[42] B Halliwell; JMC Gutteridge. *Methods Enzymol.*, **1990**, 86, 1-85.
[43] T Yaltirak; B Aslim; S Ozturk; H Alli. *Food Chem. Toxicol.*, **2009**, 47(8), 2052-2056.
[44] YL Lee; MT Yen; JL Mau. *Food Chem.*, **2007**, 104(1), 1-9.
[45] S Chatterjee; G Biswas; SK Basu; K Acharya. *Aust. J. Crop Sci.*, **2011**, 5, 904-911.
[46] A Banerjee; G Biswas; M Rai; GK Saha; K Acharya. *Global J. Biotechnol. Biochem.*, **2007**, 2(2)**,** 40-44.