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Antioxident activity of the mangrove endophytic fungus (*Trichoderma* sp.)

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PEER REVIEW

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Comments

In this work, the authors have described the isolation of endophytic fungi from mangroves plants and the antioxidant activities from the isolates. It suggests a potential approach to seek novel antioxidants for pharmaceutical applications from endophytic fungi inhabiting in mangroves plants.
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ABSTRACT

Objective: To test antioxidant property of the endophytic *Trichoderma* species isolated from the leaves of 12 mangroves of Andaman Nicobar Islands.
Methods: Eight strains of *Trichoderma* species were found predominant and their crude extracts were assessed for antioxidant activity by using seven assays.
Results: Total antioxidant activity varied with the strains and it was maximum in *Trichoderma* EMFCAS8 and other strains also showed considerable activity.
Conclusions: This work concluded that mangroves are rich in endophytic *Trichoderma* species with potential for antioxidant activity.

KEYWORDS
Mangroves, Endophytic fungi, *Trichoderma* sp., Antioxidant activity

1. Introduction

Mangrove environments are a rich source for discovery of the new microorganisms with applications in pharmaceutical science[1-6]. Marine derived fungi are known to produce secondary metabolites higher than fungi of terrestrial origin[7]. Endophytic fungi derived secondary metabolites have a high antioxidant activity against foreign particles[8]. The marine fungi produce bioactive compounds with antibacterial, anticancer, antifungal, and antiviral and anti-inflammation properties to cure the human diseases[9-11]. Mangrove derived endophytic fungus, *Irpex hydnoides* is shown to have a significant cytotoxicity against Hep2 cell line[12]. Marine fungus of *Trichoderma* species is reportedly significant *in silico* anti cancer activity against human skin and breast cancer protein[13]. However, endophytic fungi are poorly investigated microorganisms for medicinal property[14]. Hence the present work was undertaken to test antioxidant property of mangrove derived endophytic *Trichoderma* species.

2. Materials and methods

2.1. Collection of the mangrove leaves

Healthy young mangrove leaves were collected from the 12 species of mangroves (*Lumnitzera littorea* (Jack) Voigt, *Scyphiphora hydrophyllacea (S. hydrophyllacea)*, *Xylocarpus mekongensis* Pierre, *Excoecaria agallocha* Linn,

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Bruguiera gymnorrhiza (L.) Lamk., *Rhizophora mucronata* (Lam.), *Aegiceras corniculatum* (L.) Blanco (*A. corniculatum*), *Rhizophora stylosa* Griff (*R. stylosa*), *Sonneratia griffithii* Kurz, *Ceriops tagal* (Perr.), *Avicennia marina* (Forstk.) Vierh (*A. marina*), *Bruguiera cylindrica* (L.) Blum), in Andaman and Nicobar Islands, India. The leaf samples were kept in sterile plastic bags separately and brought to the microbiological laboratory.

2.2. Isolation and maintenances of endophytic Trichoderma

The mangroves leaves were surface serialized by the method of Suryanarayanan *et al*[15]. Fresh leaf samples were washed five times with sterile distilled water. In each species, 1 g of leaf samples was taken and then crushed with 10 mL of sterile distilled water. A total of 1 mL of extract was serially diluted into 10^{-2} to 10^{-5} and inoculated in Petri dishes containing *Trichoderma* selective medium by spread plate method[16], and then incubated at 28 °C for 12 d for enumeration and the counts were calculated using the following formula and are expressed as colony forming units (CFU) in 1 g of fresh leaf tissue.

$$\text{Trichodema counts (CFU/ g of leaves fresh wt)} = \frac{\text{Total number of colonies}}{\text{Sample of volume plated (0.1mL)} \times \text{dilution}} \times \text{Total volume}$$

2.3. Extraction of crude secondary metabolic compounds and antioxidant assays

Trichoderma biomass was harvested and extracted in 80% methanol according to the method described by Saravankumar *et al*[13]. The crude extracts was assessed for antioxidant activity following seven assays: total phenol content assay[17], radical scavenging activity assay using 1, 2-diphenyl-1-picrylhydrazyl (DPPH)[18], total antioxidant capacity assay[19], measurement of reducing power[20], nitric oxide radical scavenging assay[21], and hydrogen peroxide radical inhibition assay[21,22].

2.4. Gas chromatography-mass spectrometer (GC-MS) analysis of crude secondary metabolic compound of Trichoderma EMFCAS8

GCmate II GC-MS (Agilent) was used for the analysis of the crude secondary metabolites present in the *Trichoderma* EMFCAS8 extract by using HP-5 capillary column. About 1 μL of the extract was injected in the injection port with the temperature of 220 °C and helium as the carrier gas. Compounds were identified by matching with known compounds in library of the instrument.

3. Results

Counts of endophytic *Trichoderma* species from 12 mangrove species are shown in Figure 1. The counts significantly varied among the species (P<0.01). The high count was recorded as 256.5 CFU×10^3/g in *A. corniculatum*, whereas low count was recorded in *S. hydrophyllacea*, *R. stylosa* and *A. marina*.

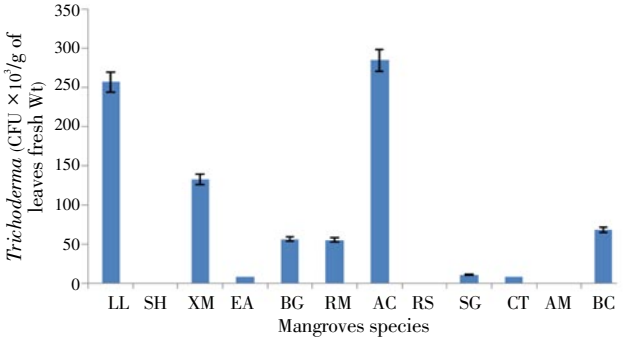

Figure 1. Counts of the mangrove derived endophytic *Trichoderma* (CFU×10^3/g of fresh leaves).
LL-*Lumnitzera littorea*, SH- *S. hydrophyllacea*, XM-*Xylocarpus mekongensis*, EA-*Excoecaria agallocha*, BG-*Bruguiera gymnorrhiza*, RM-*Rhizophora mucronata*, AC-*A. corniculatum*, RS-*R. stylosa*, SG-*Sonneratia griffithii*, CT-*Ceriops tagal*, AM-*A. marina*, BC-*Bruguiera cylindrica*.

3.1. Antioxidants assays

The mangroves derived crude extracts were tested for total phenol, total antioxidant activity, DPPH radical scavenging activity, NO_2 radical scavenging activity, H_2O_2 radical scavenging activity and total reducing power. Total phenol content significantly varied among the fungal strains or concentrations of the extracts (P<0.01). *Trichoderma* strain of EMFCAS8 was rich in total phenol content and other strains were considerable in the content which also varied with concentrations of the extracts (Figure 2).

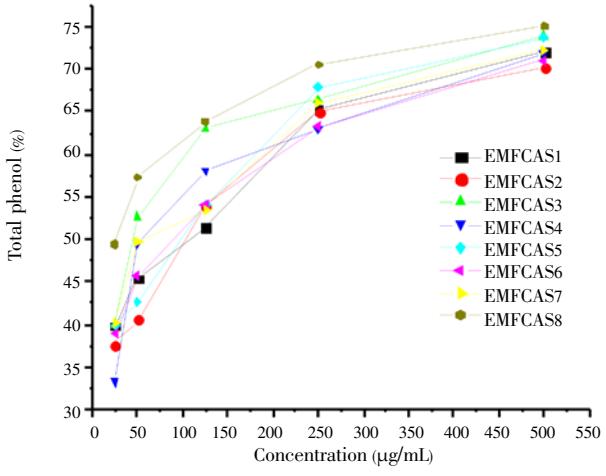

Figure 2. Total phenol (%) content in mangrove derived endophytic *Trichoderma*.

Total antioxidant activity and DPPH radical scavenging activity varied significantly (P<0.01) among the extract concentrations or fungal strains. *Trichoderma* strain EMFCAS8 showed the high antioxidant activity and DPPH radical scavenging activity as compared to other *Trichoderma* strains tested (Figure 3 and 4). NO_2 radical scavenging activity, H_2O_2 radical scavenging activity and total reducing power varied significantly (P<0.01) among

fungal strains or concentrations of extracts. The higher NO_2 radical scavenging activity, H_2O_2 radical scavenging activity and total reducing power were observed to be high in the EMFCAS8 strain at high concentration and in the EMFCAS6 strain at lower concentration. Other strains also showed the significant activities (Figures 5 and 6). The total reducing power was higher in the EMFCAS8 and lower in the EMFCAS3 (Figure 7).

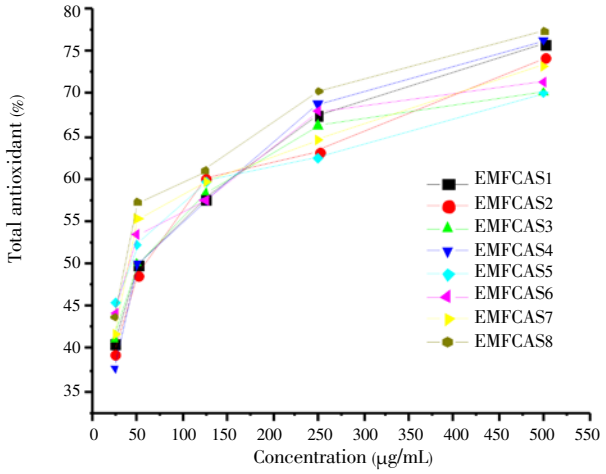

Figure 3. Total antioxidant activity of mangrove derived endophytic *Trichoderma*.

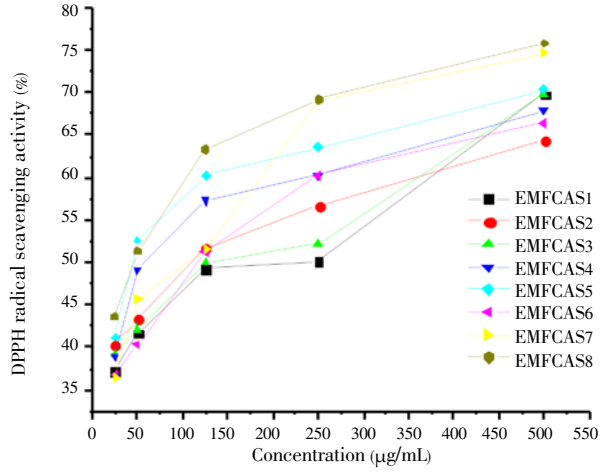

Figure 4. DPPH radical scavenging activity of mangrove derived endophytic *Trichoderma*.

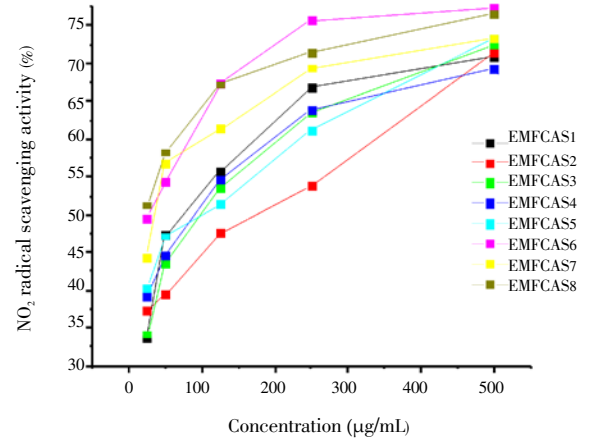

Figure 5. NO_2 radical scavenging activity of mangrove derived endophytic *Trichoderma*.

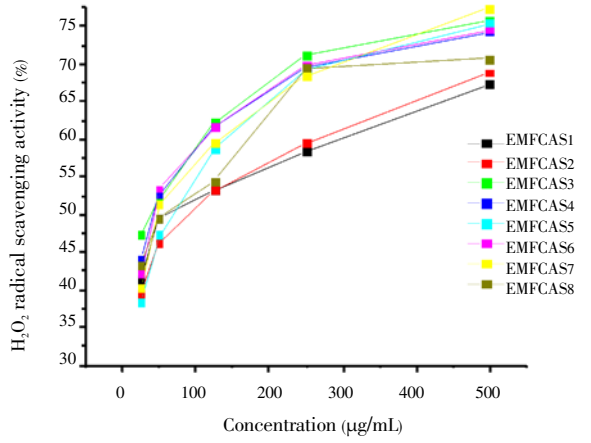

Figure 6. H_2O_2 radical scavenging activity of mangrove derived endophytic *Trichoderma*.

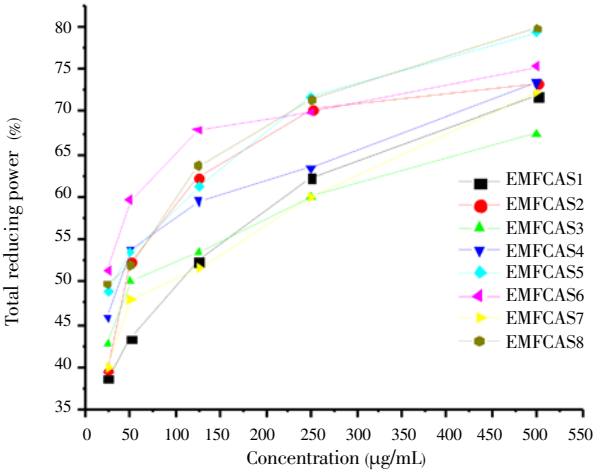

Figure 7. Total reducing power of mangrove derived endophytic *Trichoderma*.

3.2. GC-MS analysis

The potent antioxidants isolate *Trichoderma* EMFCAS8 derived secondary metabolites was analyzed by using GC. GC chromatogram of EMFCAS 8 showed the four pecks (Figure 8) indicating the presence of Pregnane-3,20β-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)], diacetate; 4-piperidineacetic acid,1- acetyl-5-ethyl-2-[3-(2-hydroxyethyl)-1-H-indol-2-yl]-a- methyl, methyl ester; Corynan-17-ol, 18,19-didehydro-10-methoxy and oleic acids.

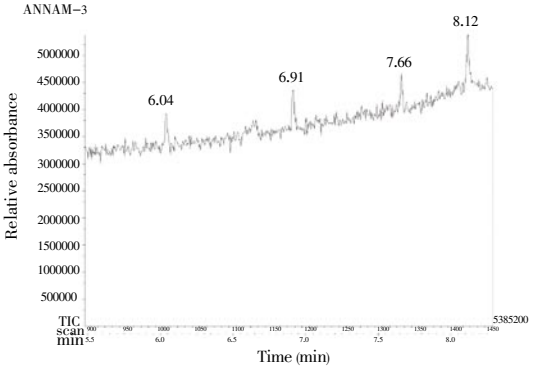

Figure 8. GC-MS chromatogram of EMFCAS8.

4. Discussion

In the present study the endophytic colonization of *Trichoderma* was found higher in the mangrove leaves of *A. corniculatum* than the other mangroves. This result can be attributed to the biochemical variations in the mangrove species, as the endophytic fungal colonizations are highly dependent on the biochemical characteristics of host leaves[23]. The ecological roles of endophytes are diverse, and they play a protective role against insect herbivory and serve as potential producers of antimicrobial metabolites[24].

Trichoderma strain EMFCAS8 exhibited the maximum antioxidant activities. In general, antioxidants activities in removal of toxic free radicals are positively related to the content of phenolic derivatives[25-28]. There is a positive relationship between total phenol content and antioxidants activity[25]. This is in support of the present study that *Trichoderma* strain EMFCAS8 which was rich in total phenol content showed the maximum antioxidant activity.

DPPH is an excellent simple method for identification of the potential antioxidants compounds and also it is not affected by metals and enzyme inhibition[29]. *Trichoderma* extract displayed high free radicals scavenging activity. Hydroxyl radicals are sensitive and bring the damage to the adjacent molecules[29,30]. *Trichoderma* showed increasing hydroxyl scavenging activity with increasing concentration of the extract. Thus hydroxyl scavenging activity is highly dependent on concentrations of the crude extract. Mangrove-derived endophytic *Trichoderma* species also showed the significant reducing sugar activity. The presence of pregnane-3,20β-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)], diacetate; 4-piperidineacetic acid,1- acetyl-5-ethyl-2-[3-(2-hydroxyethyl)-1-H-indol-2-yl]-a- methyl, methyl ester; corynan-17-ol, 18,19-didehydro-10-methoxy and oleic acids in the trchodemra EMFCAS8 was confirmed by GC-MS analysis, These metabolites may be a reason for the higher antioxidant activity of strain EMFCAS8. One of the metabolites, pregnane-3,20β-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)], diacetate is reported as potential hepatoprotective compound[31]. This study revealed that mangroves derived endophytic *Trichoderma* was a potent source for the discovery of the antioxidant compounds.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

It has been proved that some microorganisms, including fungi and bacteria, have antioxidant activities. Certain plant endophytic fungi derived secondary metabolites have antioxidant activities, which can be used as a source of novel antioxidants for pharmaceutical applications.

Research frontiers

The present research work describes the isolation of endophytic *Trichoderma* strains from leaves of different species of mangroves and the assessments of the crude extracts from the endophytic *Trichoderma* strains for antioxidant activities.

Related reports

It is reported that plant endophytic fungi can produce pharmaceutically important and valuable compounds like glycoside digoxin. Some of the endophytic fungi like *Phyllosticta* sp. and *Trichoderma* sp. have been found to show antioxidant activities.

Innovations and breakthroughs

This study depicts that the endophytic fungi isolated from mangroves plant exhibit antioxidant activities, which indicates that mangroves plant derived endophytic fungi may be a sources of compounds with antioxidant activities.

Applications

The research work suggests a potential approach to seek novel antioxidants from endophytic fungi inhabiting in mangroves plants.

Peer review

In this work, the authors have described the isolation of endophytic fungi from mangroves plants and the antioxidant activities from the isolates. It suggests a potential approach to seek novel antioxidants for pharmaceutical applications from endophytic fungi inhabiting in mangroves plants.

References

[1] Gayathri S, Saravanan D, Radhakrishnan M, Balagurunathan R,

Kathiresan K. Bioprospecting potential of fast growing endophytic bacteria from leaves of mangrove and salt marsh plant species. *Indian J Biotechnol* 2010; **9**: 397-402.

[2] Boopathy NS, Kathiresan K. Anticancer drugs from marine flora: an overview. *J Oncol* 2010; doi:10.1155/2010/214186.

[3] Lin Y, Wu X, Feng S, Jiang G, Luo J, Zhou S, et al. Five unique compounds: xyloketals from the mangrove fungus *Xylaria* sp. from the South China Sea coast. *J Org Chem* 2001; **66**: 6252-6256.

[4] Grant WD, Atkinson M, Burke B, Molly C. Chitinolysis by the marine ascomycete *Corollospora maritim*a Werdermann: purification and properties of chitobiosidase. *Botanica Marina* 1996; **39**: 177-186.

[5] Pointing SB, Hyde KD. Lignocellulose-degrading marine fungi. *Biofouling* 2000; **15**: 221-229.

[6] Atri NS, Sharma SK. Qualitative estimation of cellulases and lignin modifying enzymes in five wild *Lentinus* species selected from North West India. *Acad J Plant Sci* 2012; **4**(4): 105-109.

[7] Manimegalai K, Asha Devi NK, Padmavathy S. Marine fungi as a source of secondary metabolites of antibiotics. *Int J Biotechnol Bioeng Res* 2013; **4**(3): 275-282.

[8] Kaul S, Ahmed M, Zargar K, Sharma P, Dhar MK. Prospecting endophytic fungal assemblage of *Digitalis lanata* Ehrh. (foxglove) as a novel source of digoxin: a cardiac glycoside. *3 Biotech* 2013; **3**: 335-340.

[9] Namikoshi M, Akano K, Kobayashi H, Koike Y, Kitazawa A, Rondonuwu AB, et al. Distribution of marine filamentous fungi associated with marine sponges in coral reefs of Palau and Bunaken Island, Indonesia. *J Tokyo Univ Fish* 2002; **88**: 15-20.

[10] Jasti S, Sieracki ME, Poulton NJ, Giewat MW, Rooney-Varga JN. Phylogenetic diversity and specificity of bacteria closely associated with *Alexandrium* spp. and other phytoplankton. *Appl Environ Microbiol* 2005; **71**(7): 3483-3494.

[11] Samuel JB, Stanley JA, Princess RA, Shanthi P, Sebastian MS. Gestational cadmium exposure-induced ovotoxicity delays puberty through oxidative stress and impaired steroid hormone levels. *J Med Toxicol* 2011; **7**(3): 195-204.

[12] Bhimba BV, Franco DA, Jose GM, Mathew JM, Joel EL. Characterization of cytotoxic compound from mangrove derived fungi *Irpex hydnoides* VB4. *Asian Pac J Trop Biomed* 2011; **1**: 223-226.

[13] Saravanakumar K, Sahu SK, Kathiresan K. *In silico* studies on fungal metabolite against skin cancer protein (4,5-Diarylisoxazole HSP90 chaperone). *ISRN Dermatol* 2012; doi: 10.5402/2012/626214.

[14] Srinivasan K, Jagadish LK, Shenbhagaraman R, Muthumary J. Antioxidant activity of endophytic fungus *Phyllosticta* sp. isolated from *Guazuma tomentosa*. *J Phytol* 2010; **2**(6): 37-41.

[15] Suryanarayanan TS, Kumaresan V, Johnson JA. Foliar fungal endophytes from two species of the mangrove *Rhizophora*. *Can J Microbiol* 1998; **44**: 1003-1006.

[16] Askew DJ, Laing MD. An adapted selective medium for the quantitative isolation of *Trichoderma* species. *Plant Pathol* 1993; **42**: 686-690.

[17] Slinkard K, Slingleton VL. Total phenolic analyses: automation and comparison with manual method. *Am J Enol Vitic* 1977; **28**: 49-55.

[18] Duan XJ, Zhang WW, Li XM, Wang BG. Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. *Food Chem* 2006; **95**: 37-43.

[19] Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem* 1999; **269**: 337-341.

[20] Azzahra LF, Fouzia H, Mohammed L, Noureddine B. Antioxidant response of *Camellia sinensis* and *Rosmarinus officinalis* aqueous extracts toward H_2O_2 stressed mice. *J Appl Pharm Sci* 2012; **2**: 70-76.

[21] Govindarajan R, Rastogi S, Vijayakumar M, Shirwaikar A, Rawat AK, Mehrotra S, et al. Studies on the antioxidant activities of *Desmodium gangetium*. *Biol Pharm Bull* 2003; **26**: 1424-1427.

[22] Gulcin I, Sat IG, Beydemi S, Kufrevioglu OI. Evaluation of the *in vitro* antioxidant properties of Broccoli extracts (*Brassica oleracea* L.). *Ital J Food Sci* 2004; **16**: 17-30.

[23] Coley PD, Barone JA. Herbivory and plant defenses in tropical forests. *Annu Rev Ecol Syst* 1996; **27**: 305-335.

[24] Fisher PJ, Anson AE. Novel antibiotic activity of an endophytic *Cryptosporiopsis* sp. isolated from *Vaccinium myrtillus*. *Trans Br Mycol Soc* 1984; **83**: 145-148.

[25] Liu F, Ng TB. Antioxidative and free radical scavenging activities of selected medicinal herbs. *Life Sci* 2000; **66**: 725-735.

[26] Zafra-Rojas QY, Cruz-Cansino N, Ramírez-Moreno E, Delgado-Olivares L, Villanueva-Sánchez J, Alanís-García E. Effects of ultrasound treatment in purple cactus pear (*Opuntia ficus-indica*) juice. *Ultrason Sonochem* 2013; **20**: 1283-1288.

[27] Kasote DM. Flaxseed phenolics as natural antioxidants. *Int Food Res J* 2013; **20**(1): 27-34.

[28] Ranković BR, Kosanić MM, Stanojković TP. Antioxidant, antimicrobial and anticancer activity of the lichens *Cladonia furcata*, *Lecanora atra* and *Lecanora muralis*. *BMC Complement Altern Med* 2011; **11**: 97.

[29] Zanwar AA, Hegde MV, Bodhankar SL. *In vitro* antioxidant activity of ethanolic extract of *Linum usitatissimum*. *Pharmacologyonline* 2010; **1**: 683-696.

[30] Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. *Physiol Rev* 1979; **59**: 527-605.

[31] Al-Olayan EM, El-Khadragy MF, Aref AM, Othman MS, Kassab RB, Abdel Moneim AE. The potential protective effect of *Physalis peruviana* L. against carbon tetrachloride-induced hepatotoxicity in rats is mediated by suppression of oxidative stress and downregulation of MMP-9 expression. *Oxid Med Cell Longev* 2014; doi: 10.1155/2014/381413.