academicJournals

Vol. 7(17), pp. 1697-1702, 23 April, 2013
DOI: 10.5897/AJMR12.1065
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Anti oxidative and anti tumour activity of biomass extract of mycoprotein *Fusarium venenatum*

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Accepted 25 March, 2013

Fusarium venenatum **has been utilized as a mycoprotein source for human consumption in many countries for over a decade because of the rich source of high quality protein including essential amino acids and less fat. In the present study, anti oxidative and anticancer properties of biomass extract of *Fusarium venenatum* was studied. Biomass was obtained from *Fusarium venenatum* grown in Vogel's minerals medium and the biomass thus obtained was purified, extracted with distilled water and ethanol. The water and ethanol extracts thus prepared were evaluated for anti oxidative activity with DPPH radical scavenging activity whereas the antitumour activity was studied with Hep 2 cell line adopting MTT assay. Cytotoxic effect of both the extracts on vero cell line and human peripheral blood cells was also studied. Maximum free radical scavenging activity was recorded in 1000 µg/ml concentration of ethanol extract. The anti tumor activity against HEP2 cell lines by MTT assay reveals 1000 µg/ml concentration inhibited maximum viability followed by 800 µg/ml. In the case of vero cell lines viability was not affected at all tested concentrations The effect of extracts was studied over the human peripheral blood RBC in which the lysis, reduction and changes in morphology of blood cells was not recorded in any concentration. The study demonstrates the possible use of *Fusarium venenatum* biomass as the therapeutic agent associated with protein food supplement.**

Key words: Mycoprotein, *Fusarium venenatum,* anti oxidant, anti cancer, biomass.

INTRODUCTION

Owing to the insufficient source of proteins consumed by humans and animals the current study is focussed towards producing suitable, high-protein, microbial substitutes (Wiebe, 2002). Initially this research focused on the use of various yeasts. Subsequently, this interest spread to the use of both bacteria and filamentous fungi. A fungus rather than a yeast or bacterium was chosen for the project because (a) of the long history of man using fungi as food (b) it is possible to formulate food products from filamentous fungi which have the appropriate smell, taste and texture and (c) it is relatively easy to harvest fungal mycelia from culture broths (Edelman et al., 1983; Trinci, 1994; Wiebe, 2002; Hosseini et al., 2010). Mycoprotein may function as a prebiotic in the lower gut (Turnbull and Ward, 1995). *Fusarium venenatum* has been cultured as a mycoprotein source for human consumption in England for over a decade under the trade name of "Quorn". Rank Hovis McDougall (RHM) Company in England decided to produce its new protein-rich food from the filamentous fungus. Strain ATCC PTA-2684 *F. venenatum* was selected as the best organism for mycoprotein production and has many nutritive values like protein more than 41%, fat content 13% in dry biomass basis, dietary fiber 25%, minerals and antioxidants (Anderson and Solomons,

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1984; AOAC, 2005). It is also less energy dense than equivalent meat products and does not have animal fats and cholesterol.

Mycoprotein shows satiety and satiation properties which can be a solution for overweight by enabling people to achieve a healthier diet (low fat and high fiber (Wiebe, 2004). Now, use of microbes as natural source of anti oxidants and anti tumour agents is being encouraged in various parts of the world (Natrah et al., 2007). In the present study, the biomass derived from *Fusarium venenatum* grown in Vogel's minerals medium extracted with distilled water and ethanol, the extracts thus obtained is evaluated for the anti oxidant and anti tumour activity.

MATERIALS AND METHODS

Fungal strain

Fusarium venenatum was obtained from Fungal biodiversity centre, Netherland as lyophilized form and the fungi was activated in oats meal medium. Activated fungi was maintained on the oats meal agar slant as monosporic culture at 4°C.

Evaluation of media for biomass production

Potato dextrose broth, Sabouraud dextrose broth, Sabouraud maltose yeast extract broth, Czapek'Dox broth, yeast phosphate soluble starch, and Vogel's minerals medium were evaluated for the growth of the tested fungi. One hundred milliliter (100 ml) of each medium was poured in 250 ml capacity conical flasks and autoclaved at 15 psi pressure for 20 min. Five flasks of each medium was inoculated with 1 ml of spore suspension of *Fusarium venenatum* and incubated at 28°C for 7 days.

Collection and processing of biomass

After the incubation period, the broth was filtered through Whatman No.1 filter paper, the filtrate was discarded. One hundred grams (100 g) of the collected mycelial biomass was washed with milipore water and the washed biomass transferred to pre dried Whatman No.1 filter paper, kept in sterile Petri plate, dried using an oven at 60°C to a constant weight. According to the standard safety method, the RNA content of biomass was reduced by subjecting the biomass to heat shock at 64 -65°C for 20-30 min (Wiebe, 2002). After the heat processing, RNA of the biomass was determined adopting Ahangi et al. (2008) method. Kjeldahl technique was used for the crude protein. The media which supported maximum biomass production was selected for further studies.

Inocula preparation

Inocula of the tested fungi was prepared in Vogel's minerals medium. Vogel medium consisted of: 10 g glucose, 2.6 g $Na_3C_6H_5O_7{\cdot}2H_2O$, 2.52 g KNO_3, 2.88 g $(NH_4)H_2PO_4$, 1.6 g KH_2PO_4,0.2 g $MgSO_4{\cdot}7H_2O$, 0.1 g, $CaCl_2{\cdot}2H_2O$, 2.5 mL of biotin solution and 5 mL of trace elements per liter. The trace elements solution consisted of 0.1 g citric acid, 0.1 g $ZnSO4{\cdot}7H_2O$, 0.02 g $FeSO_4{\cdot}(NH_4)_2SO_4{\cdot}6H_2O$, 5 mg $CuSO_4{\cdot}5H_2O$, 1 mg $MnSO_4{\cdot}H_2O$,1 mg H_3BO_3, 1 mg $Na_2MoO_4{\cdot}2H_2O$ per 100 mL. The pH of the medium was adjusted to 5.8. One hundred milliliter (100 ml) of the media with the above mentioned nutrient composition was prepared in 250 ml conical flask. One milliliter (1ml) of the spore suspension (10^8 spores/ml) was inoculated into the flask. Fungal spore was collected from 15 days old culture by scrapping off with a sterilized glass rod. A homogenous spore suspension was prepared in sterile distilled water by adding a few drops of the wetting agent Tween80 (0.01%). The spore concentration of the suspension was determined using an improved Neubauer haaemocytometer (Germany).

Preparation of biomass extracts

The processed biomass (100g) thus obtained was extracted with double the volume of distilled water and ethanol. Respective extracts were then concentrated using a flash evaporator, the concentrated extracts were dissolved in respective solvents and used for bioassays with the final concentration of 10, 50, 100, 500 and 1000 µg/ml.

Anti oxidant activity

DPPH radical scavenging activity

The DPPH free radical scavenging assay was carried out for the evaluation of the antioxidant activity. This assay measures the free radical scavenging capacity of the different concentration of aqueous and ethanol extracts. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple colour typical for free DPPH radical decays, and the absorbance change at λ = 517 nm is measured. This test provides information on the ability of a compound to donate a hydrogen atom, on the number of electrons a given molecule can donate, and on the mechanism of antioxidant action. The method was carried out as previously described by Brand et al (1995). The aqueous and ethanolic extracts were redissolved in methanol and 5% ethanol, respectively and various concentrations (10, 50, 100, 500 and 1000 µg/ml) of each extract were used. Similar concentrations of ascorbic acid were used as positive control. The assay mixture contained in a total volume of 1 ml, 500 µl of the extract, 125 µl prepared DPPH (1 mM in methanol) and 375 µl solvent (methanol or 5% ethanol). After 30 min incubation at 25°C, the decrease in absorbance was measured at λ = 517 nm. The radical scavenging activity was calculated from the equation:

$$\% \text{ of radical scavenging activity} = Abs_{control} - Abs_{sample}/Abs_{control} \times 100$$

Anti tumor activity against HEP 2 cell line and non target cytotoxic effect on vero cell line

Cell line Hep2 and Vero were obtained from KINGS institute, Chennai with passage number 2 were grown in Minimum Eagles Medium (MEM) contained 10% heat inactivated Fetal Calf Serum (FCS) and 100 units/ml Penicillin G and 100 µg/ml. Streptomycin at a 37°C in a humidified 5% CO_2 incubator and the cell lines were treated with different concentration of biomass extracts. Colorimetric MTT assay was performed to evaluate anti tumour activity. MTT is reduced to purple formazan by mitochondrial succinate dehydrogenase of living cells. Stock MTT (10x), was prepared by dissolving tetrazolium in PBS (phosphate buffer saline) at a concentration of 5 mg/ml and filtering through 0.45 mm filter. The medium of the confluent cells was removed, and then 100 µl of 2X MTT was added to each well. Following incubation at 37°C with 5% CO_2 for 4 h, 100 µl of acidic Isopropanol was added and mixed to release the color from the cells. MTT is removed and washed with 2 drop of Phosphate Buffer saline. Optical density was measured at 540 nm using UV-Vis spectrophotometer (Namasivayam, 2011).

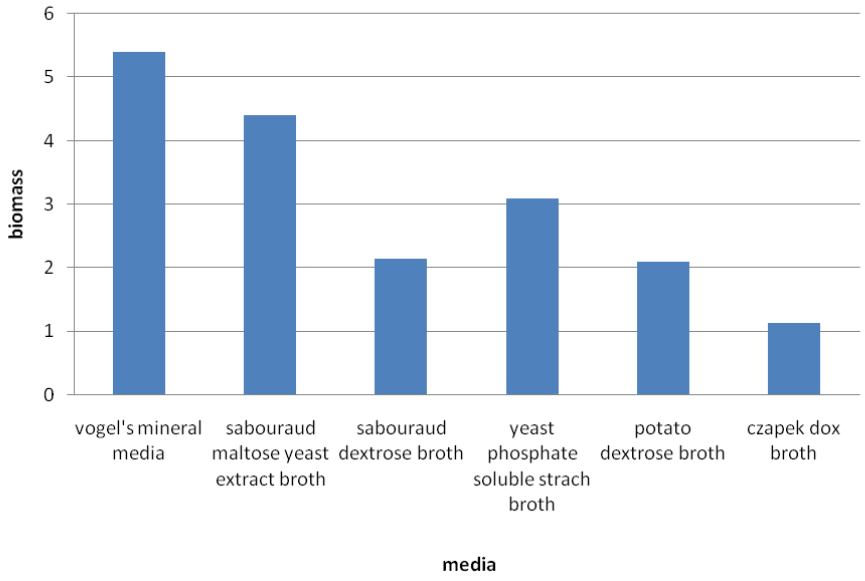

Figure 1. Effect of media on biomass (g) of *Fusarium venenatum*.

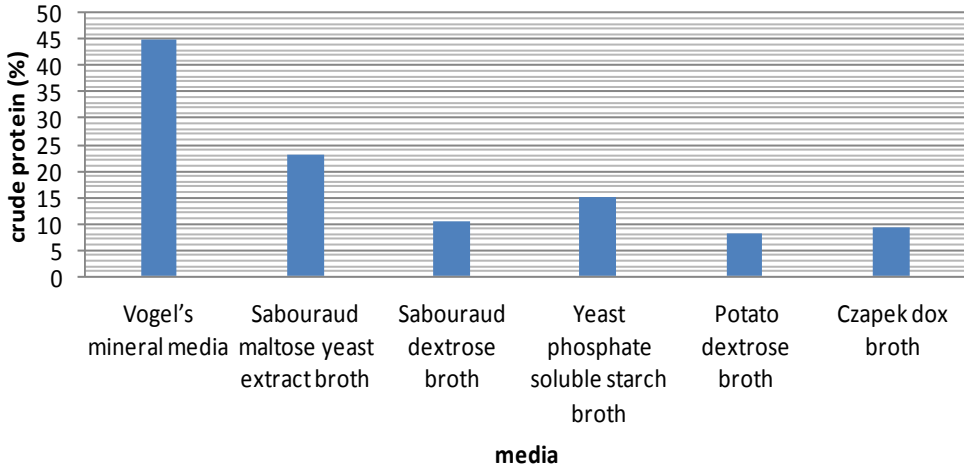

Figure 2. Effect of media on crude protein (%) of *Fusarium venenatum.*

Cytotoxicity assay against Human RBC

Using 10 ml sterile syringe a peripheral blood was collected in sterile 15 ml centrifuge tube containing 0.1% EDTA and the collected blood was centrifuged at 2500 rpm for 15 min. The supernatant was discarded and the collected RBC was washed with sterile PBS. 0.1 ml of the washed cell suspension mixed with 0.9 ml of PBS was incubated with respective concentration of the biomass extracts. The mixture was incubated at 37°C for 12-24 h and RBC count was made using Hemocytometer and microscopic examination with Leishmann stain was done to detect any morphological changes on RBC.

RESULTS

Effect of media on biomass production

All the media tested in the present study supported growth of the fungi and maximum biomass production was recorded in Vogel's mineral broth with 5.40 g /l mycelial dry weight followed by Sabouraud maltose yeast extract broth and yeast phosphate soluble broth which revealed 4.40 and 3.0 g/l respectively (Figure 1). 2.21 and 2.04 g/l of biomass was obtained in Sabouraud dextrose broth and potato dextrose broth respectively. Least biomass production was recorded in Czapek dox broth recorded 1.14 g/l. Total protein of the biomass was also determined by Kjeldahl method (Figure 2). Maximum protein content was recorded in biomass derived from Vogel's mineral broth (45.12%), Sabouraud maltose yeast extract broth (23.11%) and yeast phosphate soluble broth (15.0%). 10.0, 9.12 and 8.75% total protein was recorded in biomass from Czapek's Dox broth, Sabouraud dextrose and potato dextrose broth respectively.

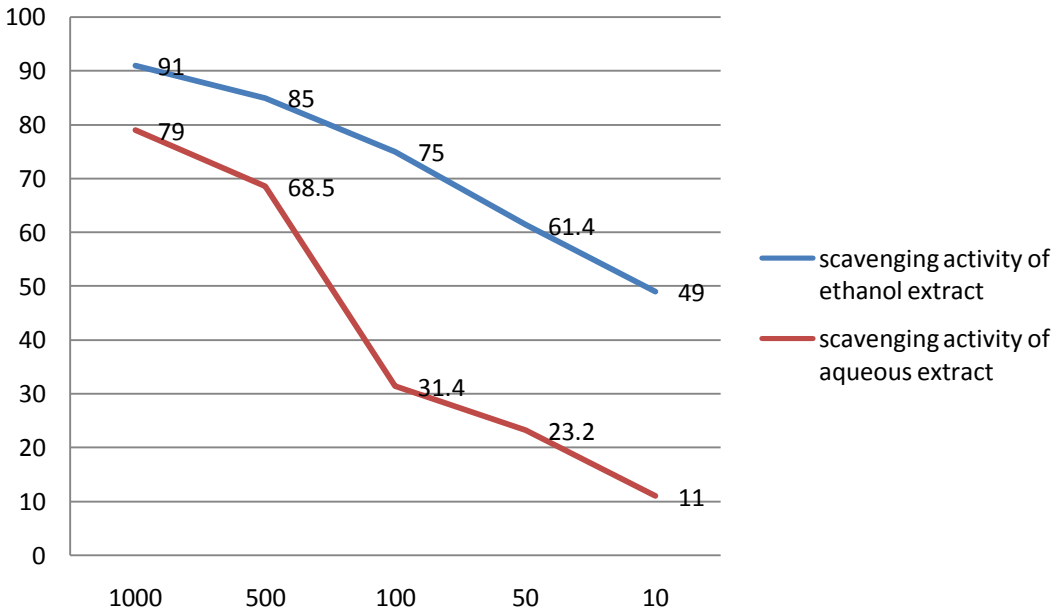

Figure 3. Anti oxidative activity (%) of biomass extracts of *Fusarium venenatum.*

Anti oxidant activity

Anti oxidant activity was carried out with aqueous and ethanol extract of fungal biomass derived from Vogel's mineral broth as dose dependent manner. Maximum scavenging activity was recorded in 1000 µg/ml of ethanol extract with 91.0% followed by 500 µg/ml with 85.0 of scavenging activity. 75.0, 61.4 and 49.0 % activity was recorded in 100, 50 and 10 µg/ml concentration. Aqueous extracts with 1000 and 500 µg/ml reveals 79.0 and 68.5% scavenging activity whereas 31.4, 23.2 and 11.0% activity was recorded in 100, 50 and 10 µg/ml concentration (Figure 3).

Anti tumour activity

Anti tumour activity was studied using MTT assay on HEP 2 cell line which revealed both the extracts inhibited viability as dose dependent manner (Figure 4). Viability was not recorded at 1000 µg/ml of both the aqueous and ethanol extracts. Distinct effect on viability was also observed in further concentration. 4.5, 18.2, 43.4 and 51.2% was noticed in 500, 100, 50 and 10 µg/ml of aqueous extract. Ethanol extracts revealed similar effect. A linear decrease in viability was recorded in increased concentration of ethanol extract. 2.3, 11.2, 41.2 and 49.4% viability was recorded in 500, 100, 50 and 10 µg/ml respectively.

Cytotoxic effect on Vero cell line and human peripheral red blood cells

Cytotoxicity study was carried out using Vero cell line and human peripheral red blood cells. Both the biomass extracts did not affect the viability of the cell lines at all the tested concentrations (Figure 5). Maximum viability was found to be 100% at all the concentration of aqueous and 10, 50, 100 µg/ml of ethanol biomass extracts. 95.0 and 93.1% viability was recorded in 500 and 1000 µg/ml of ethanol biomass extracts. The cytotoxic assay using human blood cells mainly RBC reveals no distinct cytotoxicity in all tested concentrations of both the biomass extracts. Moreover, lysis, structural changes and reduction was not observed. RBC count was found to be stable as in the control (Table 1).

DISCUSSION

Fusarium venenatum has been used as a mycoprotein source for human consumption in various parts of the world for over a decade due to the high protein, fiber and less fat. In the present study, anti oxidative and anti tumour activity of biomass extracts of the fungi was discussed. Biomass was obtained from Vogel's media which supported maximum biomass with 5.40 g/l dry weight and 45.12% total protein. Followed by Vogel's media, Sabouraud maltose yeast extract broth and yeast phosphate soluble broth reveals maximum biomass production. Least biomass production was recorded in potato dextrose broth and Czapek Dox broth. Cultivation of the fungi for the maximum biomass production with simple carbohydrate and nitrogen source under fermentation condition reported by many workers clearly reveals the organism requires simple utilizable sugars and simple nitrogen source (Hosseini and Darani, 2011).

Supply of carbon and nitrogen sources determines the growth and sporulation of fungi (Li and Holdom, 1995).

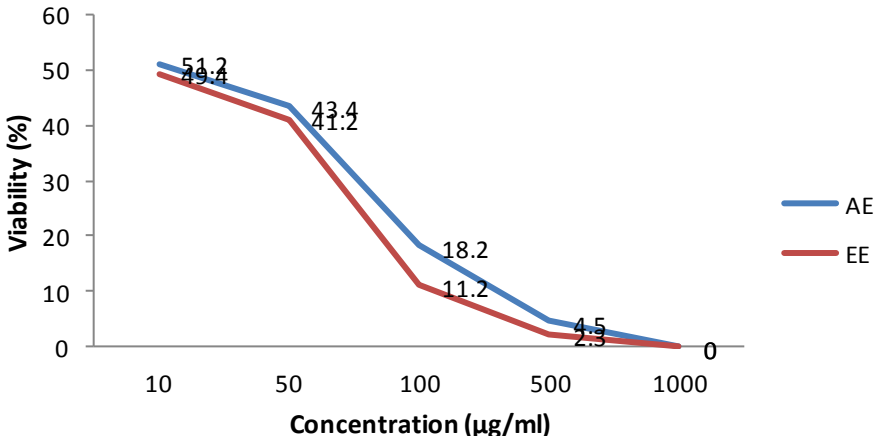

Figure 4. Viability (%) of Hep 2 cell line treated with biomass extracts.

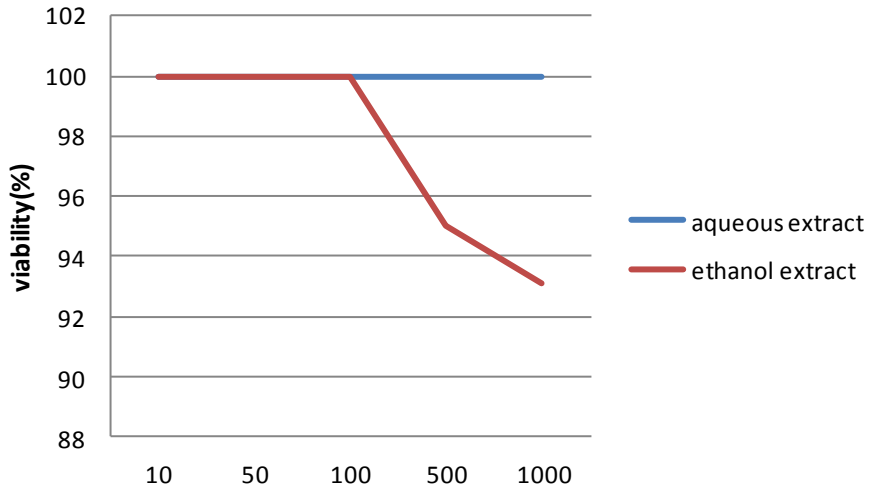

Figure 5. Viability (%) of biomass extracts on vero cell line.

Table 1. RBC count after the treatment with biomass extracts.

Concentration (µg/ml)	RBC count (X10 ^{6})	
	12 h	24 h
10	1.98	1.98
50	1.98	1.98
100	1.98	1.98
500	1.98	1.98
1000	1.98	1.98
Control	1.98	1.98

Hussein (2011) cultivated *Fusarium venenatum* in Vogel.s media supplemented with dates syrup and the total protein in the final dried biomass was 47.34% such that the improved production of protein content might be the various optimum condition provided by the authors. In the present study, our final biomass contain 45.12% of crude protein that was not significant than the crude protein obtained by Hosseini (2011) ($P>0.05$). Biomass derived from Vogel's mineral media was extracted with distilled water and ethanol, the extracts thus obtained used to study anti oxidative, anti tumour and non toxic cytotoxic effect. Maximum scavenging activity was recorded in 1000 µg/ml of ethanol extract. Now, there is increasing interest in using microbes as natural antioxidants source for cosmetics (example sunprotecting) and functional food/nutraceuticals (Gouveia et al., 2008). Natrah et al. (2007) reported a stronger antioxidant activity exhibited by methanolic microalgal crude extracts from *Isochrysis galbana*, *Chlorella vulgaris*, *Nannochloropsis oculata, Tetraselmis tetrathele*, *Chaetoceros calcitrans* when compared with a tocopherol, but lower than the synthetic antioxidant BHT. However BHT and BHA synthetic antioxidants, are questionable in terms of their safe use, since they are believed to be carcinogenic and tumorigenic if given in high doses

(Schildermann et al., 1995; Aruoma, 2003). Anti tumour activity was also recorded in both the extracts. The cytotoxicity of biomass extracts was performed over VERO cell line by MTT assay method. Both the biomass extracts did not affect the viability of the cell lines at all the tested concentrations. The cytotoxic assay using human blood cells mainly RBC reveals no distinct cytotoxicity in all tested concentrations of both the biomass extracts. Moreover, lysis, structural changes and reduction was not observed. RBC count was found to be stable as in control (Table 1). The present study clearly reveals the biomass extracts of *Fusarium venenatum* derived from biomass of the fungi grown in Vogel's mineral media shows anti oxidant and anti tumour activity without causing effect on RBC and vero cell line. Though, the biomass extracts recorded anti oxidant and anti tumour activity, further study with animal model will be essential to reveal anti oxidant and anti tumour activity which suggest the possible use of *Fusarium venenatum* biomass as the therapeutic agent associated with protein food supplement.

ACKNOWLEDGEMENT

We acknowledge Fungal Biodiversity centre, Netherland for providing fungal culture. We also thank the Management of Sathyabama University for the encouragement and opportunity to carry out this work.

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