Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 4| Issue 1 |JAN-MAR|2014|104-112

Research Article
Biological Sciences

EVALUATION OF ANTIOXIDANTS THROUGH SOLID STATE FERMENTATION FROM POMEGRANATE PEELS USING *ASPERGILLUS NIGER* AND IT'S ANTIBACTERIAL PROPERTIES

***Akhilesh Bind, Sandeep Kumar Singh, Veeru Prakash and Manish Kumar**

Department of Biochemistry & Biochemical Engineering

Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad-211007, India

***Corresponding Author Email: Akhileshbind@gmail.com**

ABSTRACT

Pomegranate peel is the by-product of the juice industry that can be targeted for production of value-added antioxidants. Experiments were conducted to determine the changes in the antioxidant properties and antibacterial activity of peel by solid-state fermentation using Aspergillus niger. The ability of Aspergillus niger to produce enhanced level of free phenolic compounds from pomegranate peel mixed with soy flour as potential nitrogen source was investigated. Two treatments were studied: peels without nitrogen source and peels with the nitrogen source. Antioxidant production was optimized at different substrate combination with nitrogen source, incubation period and pH. Results show increase in phenolic contents by addition of nitrogen source on 6th day of incubation period and at optimum pH of 6. DPPH (2, 2-diphenyl picrylhydrazyl) and β-Carotene oxidation i.e. Antioxidant Protection Factor (APF values) were maximum on 6th day with 46.21±3.99% and 1.49±0.18 respectively. Antibacterial activity of the extract was maximum in Klebsiella pneumoniae with 15 mm diameter and MIC (Minimum Inhibitory Concentration) at a concentration of 0.14 µg/ml.

KEY WORDS

Antioxidant, Antibacterial activity, Aspergillus niger, Solid-state fermentation, DPPH, β-carotene oxidation.

INTRODUCTION

Pomegranate, *Punica granatum* L., is an ancient, mystical, unique fruit borne on a small, long-living tree cultivated throughout the Mediterranean region, as far north as the Himalayas, California and Arizona in the United States. In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. The synergistic action of the pomegranate constituents appears to be superior to that of single constituent. In the past decade, numerous studies on the antioxidant, anti-carcinogenic and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage. Other potential applications include infant brain ischemia, male infertility, Alzheimer's disease, arthritis, and obesity [1].

Solid-state bio-processing consists of the utilization of water insoluble substrates for microbial growth and it is usually carried out in solid or semi-solid systems in the near absence of water. Biological conversion by processing of food wastes has been successfully converted into many value-added products through solid-state bio-processing. The fruit and vegetable solid wastes contain mainly soluble sugars and other hydrolysable materials and fibre. Disposal of such wastes at present poses a great challenge to environmentalist and direct disposal to soil or landfill may cause serious environmental problems. Thus, investigation and development of potential value-added product from these wastes can be highly attractive [2]. In the present study, an approach based on optimisation of solid-state fermentation with suitable

parameters of substrate, incubation period and pH by *Aspergillus niger* for enhanced level of antioxidant production.

MATERIALS & METHODS

COLLECTION OF CULTURE FOR ANTIOXIDANT PRODUCTION

Pure culture of *Aspergillus niger* (MCCB-0201) were procured from Microbial Culture Collection Bank of Department of Microbiology and Fermentation Technology, JSBB, SHIATS, Allahabad. Culture was maintained on PDA media and slants were grown at 28±2 ^{o}C for 7 days.

COLLECTION OF CULTURE FOR THE ANTIBACTERIAL ACTIVITY

Four bacterial strains were collected from Research laboratory, Department of Microbiology and Fermentation Technology, JSBB, SHIATS, Allahabad. The strains used were *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and cultured on nutrient agar medium at 37^{o}C.

VIABLE SPORE COUNT AND INOCULUMS PREPARATION

The total viable spore count on the PDA slant was determined with the help of Haemocytometer. 2 ml of distilled water containing 0.1% Tween- 80 was transferred to a sporulated (5-7 days old) PDA slant culture. The spores were dislodged using sterile needle. To prepare the counting chamber, surface of the haemocytometer and cover slip was cleaned and the cover slip was placed over the haemocytometer. Spore suspension (on each side) was introduced into the counting chamber by capillary action. The haemocytometer was then placed on the microscope stage and counting of the spores was done. Size of inoculum was 10^{6} spores/ml. Spores/ml = no. of spores counted on the middle square of the grid×10^{4}.

PREPARATION OF FRUIT WASTE MATERIAL

Pomegranates were purchased from the local market of Allahabad region. The peels were manually removed, washed with normal water and then with distilled water and dried in hot air oven 50±5^{o}C, after drying peels were grinded and further used for solid state fermentation.

SOLID STATE FERMENTATION

The fermentation was carried out in 250 ml conical flask containing 5 gm of solid substrate and 10ml of mineral solution containing the following (g/l) KH_2PO_4- 4.38, $NaNO_3$- 8.79, $MgSO_4.7H_2O$- 0.88, $CaCl_2.2H_2O$- 0.088, $MnCl.6H_2O$- 0.018, $NaMoO_4.2H_2O$- 0.008 and $FeSO_4.7H_2O$- 0.012 [3]. The content of the flask was mixed thoroughly and autoclaved at 121^{o}C for 20 minutes.

INOCULATION

After cooling the production media was inoculated with the 10^{6} spores/ml of inoculum and incubated under constant condition at 28±2^{o}C for 4 days.

WATER EXTRACTION

100 ml of water was added to the fungus pomace flask and then culture was homogenized for 30 minute using rotatory shaker at 250 rpm at room temperature , subsequently content was squeezed through the a distilled water wet muslin cloth. Filtrate was centrifuged at 5,000 rpm for 10 minutes at room temperature thereafter supernatant was filtered through a Whatman No. 1 filter paper.

TOTAL PHENOLIC ASSAY (TPC)

Total phenolic was estimated according to method given by Shetty [4].

TOTAL FLAVONOIDS CONTENT

Total flavonoids content was estimated according to method given by Chang [5].

DPPH RADICAL SCAVENGING ACTIVITY

DPPH radical scavenging activity was estimated according to method given by Cervato [6].

β - CAROTENE OXIDATION SYSTEM

β- Carotene oxidation was estimated according to method given by Hammerschmidt [7].

OPTIMIZATION

Effect of various concentration of substrate and nitrogen source i.e. pomegranate peel and soya bean flour taken for production. The fermentation was carried out at different pH i.e. 4, 6 and 8 to observe the effect of pH on the antioxidants production and antibacterial activity. The fermenting media was also incubated for 2, 4, 6 and 8 days to observe the effect of incubation time on the antioxidant production and antibacterial activity.

ANTIBACTERIAL ACTIVITY

Antibacterial activity was checked according to Dubey [8].

STATISTICAL ANALYSIS

Statistical analysis was done According to Fisher and Yates [9].

RESULTS

All the experiments have been performed in triplicates and all the values are given as the MEAN± SD.

TOTAL PHENOLIC CONTENT

Total phenol compounds determined by Folin Ciocalteu method, are reported as gallic acid equivalents by reference to standard curve. The water extractable phenolics after 4thday of Solid state fermentation were maximum. During first 2 days of growth, the concentration of total phenolics was constant; it increased from 3rd day and had maximum value on 4th day. Low phenolic content was observed during the early stages of growth suggesting that most natural phenolic were in bound form and only a relatively small part was in free phenolic form (**Table 1 & Figure 1**).

TOTAL FLAVONOID CONTENT

The total flavonoid contents were usually higher in peels ranging from 19.41 to 31.92 µg/ml (quercetin equivalent/ml) in the present experiment .The content of the flavonoids after the 4thday of fermentation were extracted with the distilled water and filtered through filter paper. The content of flavonoids gradually increased and was maximum at 4th day (**Table 1 & Figure 2**).

DPPH RADICAL SCAVENGING ACTIVITY

The DPPH radical formation was measured in water extracts on different days of fermentation. In the scavenging activity increased to 43.01% by 4th day compared with the initial value (Table 1 & figure 3).

β- CAROTENE OXIDATION SYSTEM

The APF of water extract did not change in first 2 days but showed significant increase on 3rd and 4th day (**Table 1 & Figure 4**).

Table 1: Daily variation of antioxidants production

Days	TPC (µg/ml)	Flavonoids (µg/ml)	DPPH	APF
1	13.21±0.10	19.41±0.58	16.88±1.69	1.05±0.20
2	13.26±0.20	20.06±2.01	19.66±0.90	1.06±0.10
3	14.75±0.23	22.54±1.38	21.69±1.23	1.19±0.09
4	15.66±0.25	31.92±1.62	43.01±1.89	1.35±0.11

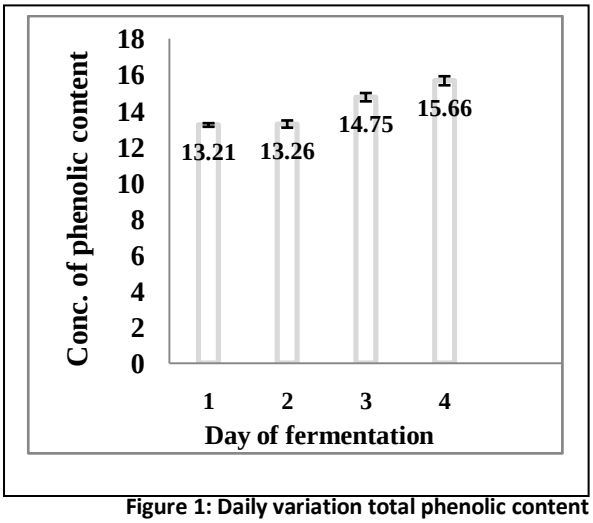

Figure 1: Daily variation total phenolic content

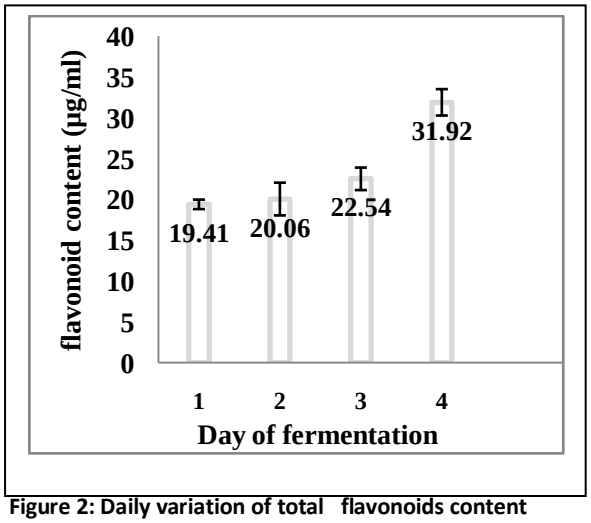

Figure 2: Daily variation of total flavonoids content

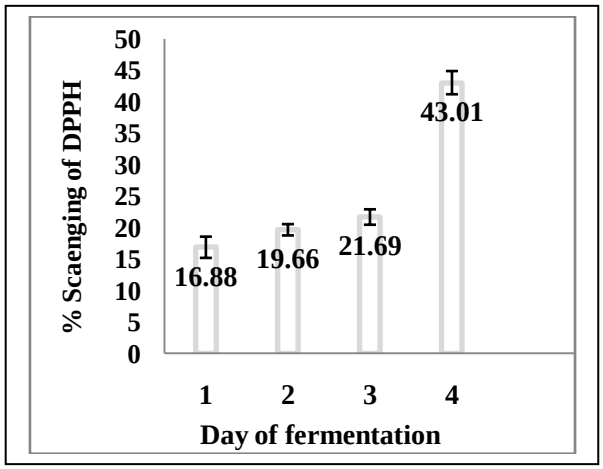

Figure 3: Daily variation in free radical Scavenging by DPPH

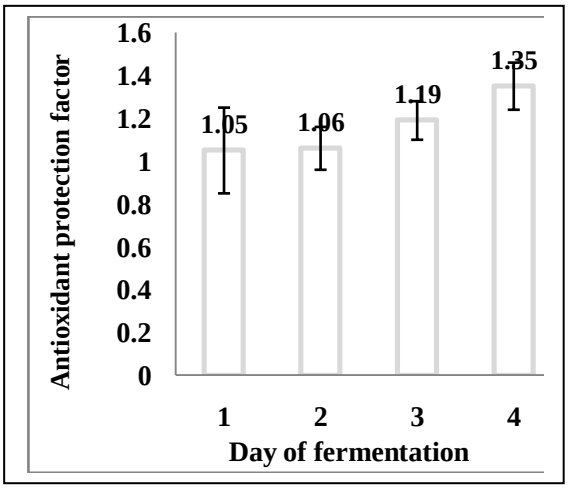

Figure 4: Daily variation in antioxidant protection factor (APF)

OPTIMIZATION OF ANTIOXIDANT PRODUCTION

Optimization for maximum production of antioxidants was done with different parameters like substrate concentration, nitrogen source, pH and incubation time.

EFFECT OF SUBSTRATE CONCENTRATION COMBINATION WITH NITROGEN SOURCES

In the present study, pomegranate peel substrate was used and supplemented with soy flour, which were used in different combinations (**Table 2**). Number of inoculums was kept constant throughout all experiment.

Table 2: Effect of substrate and nitrogen combinations on antioxidants production

Groups	Peel (g)	Nitrogen source (g)	TPC (µg/ml)	Flavonoids (µg/ml)	DPPH	APF
A_1	4	1	15.2±1.0	22.36±0.05	24.67±2.90	1.05±0.20
A_2	4	2	16.2±2.51	22.94±1.02	16.24±0.61	1.06±0.04
A_3	4	3	17.0±2.01	22.70±2.02	17.22±1.11	1.09±0.05
B_1	6	1	17.9±2.61	22.29±2.04	36.63±3.97	1.04±0.14
B_2	6	2	16.8±1.15	22.25±2.04	32.05±0.38	1.08±0.02
B_3	6	3	16.6±1.52	22.66±2.03	32.38±0.07	1.19±0.08
C_1	8	1	18.1±1.73	22.66±2.07	45.41±4.97	1.10±0.16
C_2	8	2	17.2±3.05	22.96±2.9	44.12±0.48	1.14±0.06
C_3	8	3	16.9±2.08	22.86±2.03	44.97±0.06	1.28±0.09

It was observed from the experiment, that if the pomegranate peel was kept constant i.e. 4 g and if the nitrogen source was increased then the value of the total phenolic increased but as we have increased the substrate in group B the phenolic content decreased. In group C, the C_1 had the maximum phenolic content in comparison with other two. Consequently keeping Nitrogen source constant and varying substrate, C_1 was found to have maximum polyphenolic content. So it has been finally optimized that if we used peel 8 g and Nitrogen source 1 g then we get the maximum TPC value (Polyphenols).

EFFECT OF pH

The pH of the mineral solution has very important role in the growth of the fungus strain. It has been reported that *Aspergillus niger* strains possess tannin protein complex degrading activity at a pH range of 3.5–6.5 [10]. Production of antioxidants was investigated at

three different pH 4, 6 and 8.The best result was obtained at pH 6 (**Table 3**).

Table 3: Effect of pH in production of antioxidant

pH	TPC (µg/ml)	Flavonoids (µg/ml)	DPPH (%)	APF
4	16.33±2.11	22.25±1.90	17.07%±1.67	1.45±0.15
6	19.50±.1.54	23.82±2.78	25.10%±2.76	1.56±0.16
8	17.70±.2.01	22.51±1.56	19.09%±1.87	1.35±0.12

EFFECT OF INCUBATION

In the final production of antioxidants, the parameters were pH at 6; substrate 8 g, nitrogen 1g and tested the sample at different incubation period. The best result was obtained in 6th days as shown in the **Table 4**.

Table 4: Effect of incubation on antioxidant production

Incubation time (Days)	TPC (µg/ml)	Flavonoids (µg/ml)	DPPH	APF
2	14.09±1.36	22.69±1.24	9.73±0.98	1.36±0.12
4	16.47±1.87	34.26±3.98	21.86±2.72	1.31±0.16
6	20.82±2.11	38.33±2.09	46.21±3.99	1.49±0.18
8	15.09±1.98	37.64±2.64	28.78±2.09	1.42±0.17

ANTIBACTERIAL ACTIVITY OF FERMENTED PEEL EXTRACT

The results obtained from the 6th day, solid state fermentation is the best. This contain maximum amount of polyphenolic content which is responsible for the antibacterial activity. Tannins are molecules of low biodegradation and represent the main chemical group of natural antimicrobial occurring in the plants [10]. The polyphenolic content especially the hydrolysable tannins ellagitannins and gallotannins are the major component present in the pomegranate peel. These compounds produced after the microbial biodegradation of tannins (gallotannins) to produce the antioxidant gallic acid [3] and from ellagitannins to ellagicacid. This bioprocess includes the use of microbial cultures to induce the biosynthesis of the tannin acyl hydrolase (EC 3.1.1.20) generally referred to as tannase, an esterase which hydrolyses ester bonds present in tannins [11].

TEST FOR SENSITIVITY OF PEEL EXTRACT

Four microorganisms were tested for their sensitivity of extract. The antimicrobial potency was determined by the agar well-diffusion method. Table 5 shows diameters of inhibition zones exerted by the various extracts towards challenged microorganisms. Clear zones against *Klebsiella pneumoniae* and *Escherichia coli* but in *Pseudomonas aeruginosa* showed the hazy zones i.e. not clear.

Table 5: Zone of inhibition

Micro-organisms	Inhibition zone (mm)
Staphylococcus aureus	7.0±0.2
Escherichia coli	10±0.2
Klebsiella pneumonia	15±0.3
Pseudomonas aeruginosa	8±0.2

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

Quantitative evaluation of the activity was carried out against selected microorganisms according to the [8]. The MICs, in 9 mg/ml, of the most active extract are presented in **Table 6**. The Gram-negative bacteria,

Pseudomonas aeruginosa and *Staphylococcus aureus* were found to be most sensitive to 0.14 mg/ml (**Table 6**).

Table 6: Minimum Inhibitory Concentration on various microorganisms

Test tube no.	Extract conc.	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Klebsiella pneumoniae*
1	2.25	1.242	0.706	1.123	0.419
2	0.56	1.131	0.529	0.959	0.079
3	0.14	0.778	0.170	0.542	0.122
4	0.035	0.961	0.334	0.420	0.150
5	0.0087	0.980	0.340	0.564	0.167
6 (Blank)	0.000	0.000	0.000	0.000	0.000

DISCUSSIONS

TOTAL PHENOLIC AND FLAVONOID CONTENT

The period of fermentation depends upon the nature of medium, fermenting organisms, concentration of nutrients and the process physiological conditions. The water extractable phenolic for both group (A_3, C_1) showed a similar trend in the result that the maximum production of free phenolic and flavonoids in the optimization process of substrate and nitrogen combination. If we discuss about the final maximum production conditions, C_1 group, pH 6 and incubation period 6th day were the best parameters. Since the data with respect to these parameters are statistically significant, polyphenolic content (F_{cal}144.32 >F_{tab}4.07 at 5% level of significance) and the flavonoids content (F_{cal}366.8 >F_{tab}4.07 at 5% level of significance) [12]. This low phenolic content observed during the early stages of growth suggests that most natural phenolic were in bound form and only a relatively small part was in free phenolic form (soluble). The total phenolic content for this condition was significant; this enhancement is likely to be due to the higher nitrogen source from soy flour. It may be said that peel contain less nitrogen content but when we add nitrogen from another source phenolic compound increases. Peel residue has low protein content and this nutritional limitation could act as a barrier for free phenolic production. If a media contains an appropriate amino acid combination, the energy need for protein biosynthesis will be considerably reduced and this can result in an increased growth rate [13]. Phenolic mobilization is likely to be the product of secondary metabolism, so this nutritional impairment can reflect directly in reduced efficiency of phenolic mobilization under nitrogen limitation. A further decrease in the phenolic during the later stages of growth could be due to polymerization of the released phenolic due to stress induced on the fungus for both.

ANTIOXIDANT ACTIVITY

Lately it has been reported that reactive oxygen species (ROS) are implicated in a large number of human diseases. When an imbalance between antioxidants and generation of ROS occurs, oxidative damage can occur and generate a large number of health problems [14]. The DPPH method measures the ability of certain extract photochemical to scavenge free radicals and in doing so, it is a useful analysis to understand whether the antioxidant enriched extract can block the oxidation initiation phase by the ability to neutralize or inhibit the formation of radical species [6]. β- Carotene assay quantifies the ability of the antioxidant to act at a lipid–water interface. The antioxidant protection factor directly quantifies the capacity of the antioxidant enriched extract to prevent the oxidation of β- carotene catalyzed by the presence of H_2O_2 [15].

DPPH RADICAL SCAVENGING ACTIVITY

The result of the present study showed that the extract of pomegranate peel, which contain highest amount of flavonoids and polyphenolic compounds, exhibited the

greatest antioxidant activity. The high scavenging property may be due to hydroxyl groups existing in the phenolic compound that can provide the necessary component as a radical scavenger [16]. The DPPH radical scavenging activity of group C_1 was maximum as compared to other. Both treatments show significant changes in scavenging activity. We see that, in the DPPH system, the radical inhibition capacity was highest on 4th day (without nitrogen source) and 6th day in the water extracts of group C_1 in final optimized condition, respectively. When soya bean flour was used as the nitrogen source the % of free radical scavenging activity peaked on day 6 suggesting a rapid synthesis and release of antioxidants. The data was found to be statistically significant (F_{cal}166.4 >F_{tab}4.07 at 5% level of significance). A delayed increase in the DRI capacity when nitrogen supplement was used suggests a metabolic lag in the utilization of soya bean flour as the nitrogen source because of the time taken for the gradual physiological adaptation by the fungus to synthesize the complex machinery required to assimilate nitrogen as amino acids into its protein. This may have induced a delayed nitrogen stress on the fungus only during the later stages of the growth when protein synthesis saturates. A significant DPPH radical scavenging capacity was observed in water extractable and decreased release of ethanol extractable phenolic during the growth of the fungus [17].

β- CAROTENE OXIDATION

The APF directly measures the ability of the antioxidant/extract to prevent the H_2O_2 catalyzed oxidation of β-carotene. The β-carotene system potentially quantifies the ability of the antioxidant to function at a lipid water interface and, therefore, the antioxidant has to be a partially hydrophobic in nature. In the β-carotene system, for both treatment the APF increased during the fungal growth. The maximum APF of the/fungus extract was, however, more when soya bean flour was used as a nitrogen source. A steady increase in the APF in the supplemented peel could be because of the lower nitrogen depletion stress induced on the fungus and its gradual metabolic adaptation for growth on the substrate. The highest APF in the water extracts after optimization was observed on 6th day. The increase in APF was higher in flour containing medium than in the without nitrogen during the whole growth period. A rapid decrease in APF may be due to dimerization and trimerization of simple mono phenolic during the later stages of growth. This process may lead to initiation of a polymerization by fungal growth in order to form tannins or similar polymers against stress arising due to nutrient depletions. The cleavage of lignin-like compounds via fungal-mediated degradation could lead to the decreased antioxidant activity [2].

TEST FOR SENSITIVITY OF PEEL EXTRACT

This was complete agreement with the finding regarding the correlation between total phenolic and the antibacterial activity of pomegranate peels extract then possessed only a strong activity (clear zones) against *Staphylococcus aureus* and *Escherichia coli.* The antibacterial activity of pomegranate peels may be indicative of the presence of some metabolic toxins or broad spectrum antibiotic compounds [18]. In pomegranate fruits, tannins are substantial constituents representing as high as 25% of peels [19].

MINIMUM INHIBITORY CONCENTRATION (MIC)

MIC of water extract of peels was 0.14 mg/ml against the *Staphylococcus aureus* showing less growth and effective against two strains *Staphylococcus aureus* and *Pseudomonas*. Finally we may say that fermented peel works as the antibacterial against *Staphylococcus aureus.*

CONCLUSION

The objectives of the present study were to extract the important antioxidants and its antibacterial activity from pomegranate peel (waste) through solid state fermentation by *Aspergillus niger*. Antioxidant production media was optimized for incubation time, pH and Nitrogen source for maximum production by pomegranate peel and is found to be on 6th day and at pH 6. A significant effect on the antioxidant activity with nitrogen source i.e. soybeans flour in the substrate as the supplement was observed as and found to be: total phenolic content, flavonoid content, DPPH, antioxidant protection factor were 20.82 µg/ml, 38.33 µg/ml, 46.21% , 1.49 respectively. The data was found to be statistically significant for all assays except antioxidant protection factor. The antibacterial activity of extracts was maximum in *Klebsiella pneumoniae* with 15 mm diameter and MIC with O.D. value of 0.122 which conc. 0.14 µg/ml. It is an effort in the accumulation of the

antioxidant phenolic compounds by biodegradation of tannins present in pomegranate peels, and further studies to optimize the process in solid-state fermentation indicates that *Aspergillus niger* was found to utilize fruit waste for production of antioxidants through solid state fermentation. The pomegranate peels found to be an appropriate substrate for antioxidant production along with antibacterial activity. However, thorough characterization of selected isolates is next step before taking up scale up studies for final use in food industry. This area has great potential to expand in near future due to the increased consumer desire to improve health through food human pathogens in the environment showed sensitivity to peel extracts in vitro (agar diffusion). Added-value items from by products of food could provide health benefits to humans and may be employed in food preservation and pharmaceutical purposes.

REFERENCES

[1] Jurenka, J., Therapeutic Applications of Pomegranate (Punica granatum L.): A Review, Alternative Medicine Review, 13 (2008).

[2] Roberta, T.P., Correia, Patrick McCue, Margarida, M.A., Magalhães and Gorete, R., Production of phenolic antioxidants by the solid-state bioconversion of pineapple waste mixed with soy flour using Rhizopus oligosporus, Process Biochem, 39: 2167–2172 (2004).

[3] Aguilar, C.N., Rodríguez, R., Gutierrez-Sanchez, G., Augur, C., Favela-Torres, E., Prado-Barragán, L.A., Ramírez-Coronel, A. and Contreras-Esquivel, J.C., Microbial tannases: advances and perspectives, Appl Microbiol Biotechnol, 76: 47–59, (2007).

[4] Shetty, K., Curtis, O.F., Levin, R.E., Witkowsky, R., and Ang, W., Prevention of verification associated with in vitro shoot culture of oregano (Origanum vulgare) by Pseudomonas spp, J Plant Physiol, 147: 447– 451 (1995).

[5] Chang, C., Yang, M., Wen, H. and Chern, J., Estimation of total flavonoid content in propolis by two complementary colorimetric methods, J Food Drug Anal, 10: 178-182 (2002).

[6] Cervato, G., Carabelli, M., Gervasio, S., Cittera, A., Cazzola, R. and Cestaro, B., Antioxidant properties of oregano (Odganum vulgare) leaf extracts, J Food Biochem, 24: 453–465 (2000).

[7] Hammerschmidt, P.A. and Pratt, D.E., Phenolic antioxidants of dried soybeans, J Food Sci, 43: 556-559 (1978).

[8] Dubey, R.C., Practical microbiology 2nd Ed., 174-175 (2006).

[9] Fisher, R.A. and Yates, F., Analysis of variance, A handbook of biostatistics, Oliver and Boyd, London 2nd ed, 254-258 (1938).

[10] Aguilera-Carbo, A., Hernandez-Rivera, J.S., Prado-Barragán, L.A., Augur, C., Favela-Torres, E. and Aguilar, C.N., Ellagic acid production by solid state culture using a Punica granatum husk aqueous extract as culture broth. In: Proceedings of the 5th International Congress on Food Technology, Thessaloniki, (2007).

[11] Aguilar, C.N. & Gutiérrez-Sánchez, G., Review sources, properties, applications and potential uses of tannin acyl hydrolase, Food Sci Technol Int, 7: 373–382, (2001).

[12] Patil, S.R. and Dayanand, A., Production of pectinase from deseeded sunflower head by Aspergillus niger in submerged and solid state conditions, Bioresour technol, 97: 2054-2058 (2006).

[13] Braga, G., Destefano, R.H.R. and Messias, C.L., Oxygen consumption by Metarhizium anisopllae during germination and growth on different carbon sources, J Invertebr Pathol, 74: 112–119 (1999).

[14] Okawa, M., Kinjo, J., Nohara, T. and Ono, M., DPPH (1,1-diphenyl-2- picrylhydrazyl) radical scavenging activity of flavonoids obtained from some medicinal plants, Biol Pharm Bull, 24: 1202–1209 (2001).

[15] Vattem, D.A. and Shetty, K., Solid-state production of phenolic antioxidants from cranberry pomace by Rhizopus oligosporus, Food Biotechnol, 16: 189–210 (2002).

[16] Pourmorad, F., Hosseinimehr, S.J., and Shahabimajd, N., Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants, African Journal of Biotechnology, 5: 1142-1145 (2006).

[17] Vattem, D.A. and Shetty, K., Ellagic acid production and phenolic antioxidant activity in cranberry pomace (Vaccinium macrocarpon) mediated by Lentinus edodes using a solid-state system, Process Biochemistry, 39: 367-379 (2003).

[18] Voravuthikunchai, S., Lortheeranuwat, A., Jeeju, W., Sririrak, T., Phongpaichit, S., and Supawita, T., Effective medicinal plants against entero haemorrhagic Escherichia coli O157:H7, Journal of Ethnopharmacology, 94: 49–54 (2004).

[19] Voravuthikunchai, S., Sririrak, T., Limsuwan, S., Supawita, T., Iida, T., and Honda, T., Inhibitory effects of active compounds from Punica granatum pericarp on verocytotoxin production by enterohemorrhagic Escherichia coli O157: H7, Journal of Health Sciences, 51: 590–596 (2005).

*Corresponding Author:
Akhileshbind@gmail.com