

<http://www.pjbs.org>

**PJBS**

ISSN 1028-8880

# **Pakistan Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Antioxidant Activity and Total Phenolic Content of Endophytic Fungus *Fennellia nivea* NRRL 5504

<sup>1</sup>Vienna Saraswaty, <sup>1</sup>Yoice Srikandace, <sup>2</sup>N.A. Simbiyani, <sup>2</sup>Jasmansyah,  
<sup>3</sup>Henry Setiyanto and <sup>1</sup>Zalinar Udin

<sup>1</sup>Research Centre for Chemistry, Indonesian Institute of Sciences,  
Jl. Cisitu Sangkuriang Gd.50 Bandung, 40135, Indonesia

<sup>2</sup>Department of Chemistry, University of Jenderal Ahmad Yani,  
Jl. Terusan Jenderal Sudirman PO BOX 148, Cimahi, Indonesia

<sup>3</sup>Analytical Chemistry Research Group, Faculty of Mathematic and Sciences,  
Institute of Technology Bandung, Jl. Ganesha 10. Bandung, Indonesia

**Abstract:** Antioxidant is an interesting bioactivity since it has several correlations with disease such as Alzheimer, cancer, ageing and many others in which were promoted by free radicals. Investigation on our endophytic fungus collection namely *Fennellia nivea* NRRL 5504 revealed that the fermented medium has antioxidant property against DPPH through free radical scavenging effect. Fermentation in liquid medium i.e. Potatoes Dextrose Broth, resulted that the fermented medium reached about 86.51% ( $p < 0.05$ ) of free radical inhibition on the eighth day. Results of phytochemical screening from extracted fermentation medium showed the presence of terpenes, triterpenes, phenolic compound, tannin, flavonoid and also saponin. The highest antioxidant activity was showed by ethyl acetate extract. Calculation on total phenolic content of ethyl acetate crude extract was  $0.544 \text{ mg g}^{-1}$  equivalent to pyrogallol. By exposure above, we have alternative source of antioxidant that came from our endophytic fungus collection which we isolated previously from *Typhonium divaricatum* Lodd.

**Key words:** Antioxidant, endophytic, *Fennellia nivea*, fungus

### INTRODUCTION

Antioxidant substances can be found in healthy foods such as fruits, vegetables and also many herbal (Wootton-Beard and Ryan, 2011; Almeida *et al.*, 2011; Andarwulan *et al.*, 2010; Jungmin *et al.*, 2013; Arthur *et al.*, 2011; Naik *et al.*, 2003; Chang *et al.*, 2008; Schempp *et al.*, 2006). Antioxidant is an interesting bioactivity due to it is capacity to improve health such as bone health, anti aging and many other diseases which are promoted by free radical (Mackinnon, 2010; Zhang *et al.*, 2010; Di and Esposito, 2003).

Indonesia is a country with a rich of biodiversity. A lot of researchers have been performed to investigated bioactivity of plants (Ali *et al.*, 2008; Saraswaty *et al.*, 2012; Hanafi *et al.*, 2001; Syah *et al.*, 2002). Research in antioxidant have discovered that there were many potential antioxidant from Indonesian plants such as *Sauropus androgynus* (L) Merr, *Cosmos caudatus*, *Polyscias pinnata*, *Centella asiatica*, *Ocimum americanum* and many others (Andarwulan *et al.*, 2010).

Unfortunately, industrial impact had accelerated forest conversion. When deforestation came as the impact of industrialization of agriculture, 98% of Indonesia forest predicted will be destroyed by 2022 (Nelleman, 2007). For that, it is important to seek another alternative source for the research. Now, studies of endophytic fungi had been giving promising result, because we do not need to take a lot of plant as sample source (Strobel and Daisy, 2003).

Our preliminary investigation had revealed that our endophytic fungi collections had anticancer activity. For that, now we are seeking another bioactivity that can be found in our endophytic collections. *Aspergillus niveus* or *Fennellia nivea* is one of our endophytic collection which isolated from *Typhonium divaricatum* Lodd that grow in Indonesia. Scientific papers had been reported that *Aspergillus niveus* produced Aspochalamins A-D, Aspochalasin Z (Holtzel *et al.*, 2004) and several enzymes such as xylanase (Betini *et al.*, 2009), pectin lyase (Maller *et al.*, 2012), polygalacturonase (Maller *et al.*, 2011) and inulinase (De Souza-Motta *et al.*, 2005). Although, it had been mentioned that the fungus

produce several secondary metabolites and enzymes but the antioxidant activity of the fungus has not been investigated yet.

Antioxidant activity can be analyzed using several method i.e., TRAP, TEAC, ORAC, TOSC, FRAP, Follin-Ciocalteu Total Phenolic Assay and DPPH (Mermelstein, 2008; Mates, 2000). DPPH or 2,2-Diphenyl-1-picrylhydrazyl is a stable radical. It commonly used for investigation of antioxidant property. The characteristic of oxidant reduction was measured by use of spectrophotometer at  $\lambda$  517 nm. By use DPPH as antioxidant assay, the reduction form of DPPH radical became DPPH-H under electron transformation mechanism can be monitored.

Our paper presented antioxidant activity from the extract of liquid fermentation medium from our collection endophytic fungus Tf.10 f namely *Fennelia nivea* NRRL 5504 against free radical DPPH and the total of phenolic content.

## MATERIALS AND METHODS

**Material:** Endophytic fungus Tf.10f (*Fennelia nivea* NRRL 5504) was isolated from *Typhonium divaricatum* Lodd. and determined by Research Centre for Biology Bogor, Indonesia. DPPH is from Sigma (p.a grade), Potatoes Dextrose Broth (PDB) is from HiMedia. Organic solvent i.e. methanol is from Merck (p.a grade), n-hexane, dichloromethane and ethyl acetate which were used for extraction are technical grade and destilated before use.  $\text{Na}_2\text{CO}_3$ , Folin Ciocalteu, HCl,  $\text{AlCl}_3$  and Pyrogallol are from eMerck (pro analysis grade). Quercetin is from Sigma.

**Fermentation:** Endophytic fungi was regenerated for 7 days on PDA slant, then one slant endophytic fungus was suspended in 20 mL of sterile aqua DM then transformed into 200 mL of PDB for activation (48 h, RT, 100 rpm horizontal shaker). After activation about 10% (v/v) suspension of endophytic fungus was fermented in 2 L of Potato Dextrose Broth (PDB) for 8 days (RT, 100 rpm, horizontal shaker). The biomass and supernatant were separated using Whatman paper No. 1. Every one day the supernatant was monitored for antioxidant activity using DPPH (Ho-H8).

**Extraction:** Extraction of liquid fermentation medium was performed using liquid-liquid partition method. An equal volume of organic solvent was added to supernatant of liquid fermentation medium (1:1). First the non polar organic solvent (n-hexane) was added to the supernatant and mixed. The layer of n-hexane extract was collected and

evaporated under vacuum. Then the residue was added by other organic solvent i.e. dichloromethane and ethyl acetate respectively. Evaporation of organic extract and residue was performed using Heidolph Rotary vapor (Germany) at 45-50°C.

### Antioxidant assay (Free radical scavenging effect):

About 100  $\mu\text{L}$  of sample with various concentration (1, 0.5, 0.25 and 0.125%) was diluted with 1800  $\mu\text{L}$  methanol p.a then was mixed with 200  $\mu\text{L}$  of DPPH (1000 ppm). Incubation of mixed reagent and samples were performed for 30 min, at room temperature. Then the absorbance was measured using Hitachi spectrophotometer U 2000 at 517 nm.

**Total phenolic content:** Total phenolic contents of extract were determined by using Folin-Ciocalteu reagent and external calibration with pyrogallol. Briefly, 100  $\mu\text{L}$  of extract (1 % w/v) was mixed with 4.5 mL of aquadest then added with 100  $\mu\text{L}$  of Folin-Ciocalteu reagent and the reagents were mixed throughly. After three minutes 300  $\mu\text{L}$  of  $\text{Na}_2\text{CO}_3$  was added and then the mixtures were allowed to stand in room temperature for 2 h. The absorbance was measured at 760 nm using a HITACHI, U-2000 spectrophotometer. The estimation of total phenolic in the extract was determined as equivalent mg  $\text{g}^{-1}$  of pyrogallol through an equation obtained from pyrogallol calibration curve ( $y = 2.5957x + 0.0784$ ,  $R^2 = 0.9857$ ).

**Statistical analysis:** All procedure are performed triplicate. Statistical analysis was performed using software SPSS 10.

**Phytochemical screening:** Phytochemical screening assay was performed as described by Harborne (1987).

## RESULTS AND DISCUSSION

### Antioxidant activity of liquid fermentation medium:

Antioxidant activity of liquid medium which is fermented by *Fennelia nivea* NRRL 5504 was monitored using DPPH and the results are as follow (Table 1).

Table 1: Antioxidant activity of liquid fermentation medium

Day	Inhibition (%)
0	61.45
1	61.56
2	66.37
3	72.86
4	69.98
5	72.96
6	71.81
7	73.17
8	86.51
Ascorbic acid (0.25% w/v)	97.28

Antioxidant activity of liquid fermentation medium was presented in Table 1. It showed that the fermentation medium at H0 had already giving bioactivity as antioxidant with 61.45% inhibition. It might because of the fungus was activated in PDB before fermentation for 48 h and the bioactive constituent might have already produced in activation medium even though the fermentation process have not begun. And after 24 h (H1), antioxidant activity of fermentation medium only increased about 0.11%. So, we suggested that from H0 to H1, it was the preliminary process of production antioxidant substances.

Antioxidant activity on the fourth and sixth day is lower than the day before, this is still possible and might because of the different bioactive constituents were being synthesized within the day. Another bioactive constituent which were produced on third day might being used for biosynthesis process of compounds which can be revealed on the fourth day. The same opinion and argumentation we argued for what happening on the sixth day. On the eighth day, we can see that the antioxidant activity of fermented medium raise rapidly, it reach about 86.51% of inhibition, compare to H0, it had been increased about 25% higher than before fermentation. From this activity we expected that the amount of bioactive constituents increased.

**Extraction and antioxidant activity:** The results of antioxidant activity from extracted liquid fermentation medium which was inoculated with *Fennellia nivea* NRRL 5504 can be seen in Table 2. The yields are weight of each fraction from 2 L of fermentation medium.

From Table 2, it showed that the highest weight after extraction was resulted in residue. Meanwhile the most active extract was ethyl acetate with  $IC_{50}$  of 0.83 mg mL<sup>-1</sup>. This result showed that among others the bioactive constituent dominantly found in this extract. And although the residue yielded the highest result, but the antioxidant activity of this extract was lower than ethyl acetate extract. According to it we expected that the polarity of bioactive constituent was higher than ethyl acetate but lower than water.

#### Phytochemical screening and total phenol content:

Phytochemical screening assay showed the presence of phenolic compounds, tannin, terpenoid, triterpenoid and flavonoids in n-hexane, dichloromethane and ethyl acetate extract. Meanwhile the residue only showed the presence of saponin and steroid compound.

Further investigation on the levels of total phenolic content based on the relative capacity of standard used in assay (pyrogallol) could be seen in Table 3. From the

Table 2: Yield and antioxidant activity from fraction of liquid fermentation medium

Extract	Yield (g)	Antioxidant activity ( $IC_{50}$ mg mL <sup>-1</sup> )
N-hexane	0.0184	10.60
Dichloromethane	0.1116	6.20
Ethyl acetate	0.1659	0.83
Residue	5.2	5.40

Table 3: Total phenolic content

Extract	Total phenolic (mg g <sup>-1</sup> )
Dichloromethane	0.821
Ethyl acetate	0.544

table, value of total phenolic content of dichloromethane extract was higher than ethyl acetate extract. Although, we found that dichloromethane extract has a higher total of phenolic content than ethyl acetate extract, but the activity of antioxidant properties was lower than ethyl acetate extract (Table 2). This result may caused by the different substituent pattern from chromophore group of phenolic compound. Substituent such as rhamnose sugar will decreased the bioactivity due to the blockade of hydroxyl group by glycosidic linkage (Materska and Perucka, 2005). Beside that another important factor is also the position and total of hydroxyl group. Second hydroxyl group at position meta will resulted lower antioxidant activity than at position of ortho and para. This due to electronic effects of OH group related to the radical cation stability. Total of hydroxyl group will also affect to antioxidant activity. The higher number of hydroxyl group will also increased the bioactivity that means polyphenolic compounds have better bioactivity than monophenolic compound. Moreover, methoxylic group that substituted in monophenolic compound bioactivity will increase antioxidant property (Cuvelier *et al.*, 2000; Merkl *et al.*, 2010).

The scavenging potential of samples can be seen from the degree of discolouration from  $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydrazyl (deep violet colour) become  $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydrazine (yellow). As mentioned by Aluko *et al.* (2013), the ability of polyphenolic compound to act as antioxidant based on their potential as hydrogen atom donor. By exposure above, we suggested that bioactive constituent that came from phenolic compound of ethyl acetate crude extract also possessed donating hydrogen atom.

Although, it had been determined that the bioactive compound from ethyl acetate crude extract could classified in phenolic compound, the structure of active component still needed to be investigated. So far, isolation and identification of bioactive compounds is needed for a better understanding of antioxidant mechanism. This investigation is still underway to go in our laboratory.

## CONCLUSION

In conclusion, we have found another bioactivity of *Fennelia nivea* NRRL 5504 i.e., antioxidant. The highest antioxidant activity was revealed in ethyl acetate extract with value of total phenolic content was 0.544 mg g<sup>-1</sup> extract.

## ACKNOWLEDGMENT

We acknowledge the financial support of DIPA-Indonesian Institute of Sciences, under funding year of 2010-2013. Author also thank to Mrs. Nina Artanti M.Sc (Research Centre for Chemistry, Indonesian Institute of Sciences) for her help to provide DPPH material, Prof. Dr. Sc. M. Hanafi (Research Centre for Chemistry, Indonesian Institute of Sciences) for his suggestion and discussion in this research.

## REFERENCES

- Ali, S.S., N. Kasoju, A. Luthra, A. Singh, H. Sharanabasava, A. Sahu and U. Bora, 2008. Indian medicinal herbs as sources of antioxidants. Food Res. Int., 41: 1-15.
- Almeida, M.M.B., P.H.M. de Sousa, A.M.C. Arriaga, G.M. do Prado, C.E. de Carvalho Magalhaes, G.A. Maia and T.L.G. de Lemos, 2011. Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil. Food Res. Int., 44: 2155-2159.
- Aluko, B.T., O.I. Oloyede and A.J. Afolayan, 2013. Polyphenolic contents and free radical scavenging potential of extracts from leaves of *Ocimum americanum* L. Pak. J. Biol. Sci., 16: 22-30.
- Andarwulan, N., R. Batari, D.A. Sandrasari, B. Bolling and H. Wijaya, 2010. Flavonoid content and antioxidant activity of vegetables from Indonesia. Food Chem., 121: 1231-1235.
- Arthur, H., E. Joubert, D. de Beer, C.J. Malherbe and R.C. Witthuhn, 2011. Phenylethanoid glycosides as major antioxidants in *Lippia multiflora* herbal infusion and their stability during steam pasteurisation of plant material. Food Chem., 127: 581-588.
- Betini, J.H.A., M. Michelin, S.C. Peixoto-Nogueira, J.A. Jorge, H.F. Terenzi and M.L.T.M. Polizeli, 2009. Xylanases from *Aspergillus niger*, *Aspergillus niveus* and *Aspergillus ochraceus* produced under solid-state fermentation and their application in cellulose pulp bleaching. Bioproc. Biosyst. Eng., 32: 819-824.
- Chang, Y.X., X.P. Ding, J. Qi, J. Cao and L.Y. Kang *et al.*, 2008. The antioxidant-activity-integrated fingerprint: An advantageous tool for the evaluation of quality of herbal medicines. J. Chromatogr. A, 1208: 76-82.
- Cuvelier, M.E., V. Bondet and C. Berset, 2000. Behavior of phenolic antioxidants in partitioned medium: Structure-activity relationship. J. Am. Oil Chem. Soc., 77: 819-824.
- De Souza-Motta, C.M., M. A.Q. Cavalcanti, A.L.F. Porto, K.A. Moreira and J.L.L. Filho, 2005. *Aspergillus niveus* Blochwitz 4128URM: New source for Inulinase production. Braz. Arch. Biol. Technol., 48: 343-350.
- Di, M.V. and E. Esposito, 2003. Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Curr. Drug Targets: CNS Neurol. Disord., 2: 95-97.
- Hanafi, M., L. Sutedja, R. Muldar and C. Braybook, 2001. Isolation and identification of chemical constituent from the rhizome of *Zingiber zerumbet*. Proceedings of the International Seminar on Natural Product Chemistry and Utilization of Natural Resources, June 5-7, 2001, Depok.
- Harborne, J.B., 1987. Phytochemistry Method. 2nd Edn., ITB Bandung, USA.
- Holtzel, A., D.G. Schmid, G.J. Nicholson, P. Krastel and A. Zeeck, *et al.*, 2004. Aspochalamins A-D and aspochalasin Z produced by the endosymbiotic fungus *Aspergillus niveus* LU 9575. II. Structure elucidation. J. Antibiot., 57: 715-720.
- Jungmin, O., J. Heonjoo, A.R. Cho, S.J. Kim and J. Han, 2013. Antioxidant and antimicrobial activities of various leafy herbal teas. Food Control, 31: 403-409.
- Mackinnon, E.S., 2010. The role of carotenoid lycopene as an antioxidant to decrease osteoporosis risk in women: Clinical and *In vitro* studies. Ph.D. Thesis, Institute of Medical Science, University of Toronto.
- Maller, A., A.R.L. Damasio, T.M. da Silva, J.A. Jorge, H.F. Terenzi and M.L.T. Moraes Polizeli, 2011. Biotechnological potential of agro-industrial wastes as a carbon source to thermostable polygalacturonase production in *Aspergillus niveus*. Enzyme Res.
- Maller, A., T.M. da Silva, A.R.L. Damasi, V.R.A. Reis, J.A. Jorge and M.L.T.M. Polizeli, 2012. Production of pectin lyase by *Aspergillus niveus* under submerged and solid state fermentations using agro-industrial residues as carbon sources. Int. Res. J. Microbiol., 3: 29-35.

- Materska, M. and I. Perucka, 2005. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). *J. Agric. Food Chem.*, 53: 1750-1756.
- Mates, J.M., 2000. Effect of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology*, 153: 83-104.
- Merkl, R., I. Hradkova, V. Filip and J. Smidrkal, 2010. Antimicrobial and antioxidant properties of Phenolic acids alkyl esters. *Czech J. Food Sci.*, 28: 275-279.
- Mermelstein, N.H., 2008. Determining antioxidant activity. *Laboratory Food Technol.*, 11: 63-66.
- Naik, G.H., K.I. Priyadarshini, J.G. Satav, M.M. Banavalikar, D.P. Sohoni, M.K. Biyani and H. Mohan, 2003. Comparative antioxidant activity of individual herbal components used in ayurvedic medicine. *Phytochemistry*, 63: 97-104.
- Nelleman, C., 2007. The Last Stand of the Orangutan: State of Emergency: Illegal Logging, Fire and Palm Oil in Indonesia's National Parks. UNEP/Earthprint, UK., ISBN: 9788277010434, Pages: 49.
- Saraswaty, V., H. Setiyanto and J. Nurhajati, 2012. Antibacterial activity from *Croton argyratus* stem bark extract. *Int. J. Pharm. Tech. Res.*, 4: 190-193.
- Schempp, H., D. Weiser, O. Kelber and E.F. Elstner, 2006. Radical scavenging and antiinflammatory properties of STW 5 (Iberogast) and its components. *Phytomedicine*, 13: 36-44.
- Strobel, G. and B. Daisy, 2003. Bioprospecting for microbial endophytes and their natural products microbiol. *Microbiol. Mol. Biol. Rev.*, 67: 491-502.
- Syah, Y.M., S.A. Achad, E.L. Ghisalberti, E.H. Hakim, L. Makmur and D. Mujahidin, 2002. Artoindonesianin M: suatu flavon terpenilasi baru dari *Artocarpus champeden* spreng. *Bull. Soc. Nat. Prod. Chem.*, 2: 31-36.
- Wootton-Beard, P.C. and L. Ryan, 2011. Improving public health?: The role of antioxidant-rich fruit and vegetable beverages. *Food Res. Int. J.*, 44: 3135-3148.
- Zhang, W.H., Y. Yang, C.J. Lin and Q. Wang, 2010. Antioxidant attenuatin of ROS-involved ytotoxicity induced by Paraquat on HL-60 cells. *Health*, 2: 253-261.