Australian Journal of Basic and Applied Sciences, 6(1): 79-85, 2012
ISSN 1991-8178

Screening of antifungal activities from genera *Trametes* against growth of selected wood-degrading fungi from Malaysia.

Yi Peng, Teoh and Mat Don, Mashitah.

School of Chemical Engineering, University Sains Malaysia,
Seri Ampangan, 14300 Nibong Tebal, Penang, Malaysia.

Abstract: A total of 8 extracts from several species of the genera *Trametes* were evaluated for antifungal activities against the selected wood-degrading fungi. The minimum inhibitory concentrations of extracts determined by broth dilution method ranged from 0.3 to more than 5 μg/μL. The differences in antifungal activities observed in different fungal species suggested that the ability to produce bioactive compounds is not homogeneously distributed. 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP) was found as the major components in the methanol extracted of the *Trametes versicolor*, in which this flavonoid fraction is a complex natural products of current medicinal interest.

Key words: antifungal agent; *Trametes* sp.; wood-degrading fungi; minimum inhibitory concentration (MIC); 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP).

INTRODUCTION

Rubberwood (*Hevea brasiliensis*), which belongs to the family Euphorbiaceae, (Vis *et al*., 2006) is native of the Amazon valley of South Africa, was introduced to India in the latter half of the 19th century. (Edwin, and Ashraf, 2006) It is now widely cultivated in mostly twenty country of the world including Malaysia for natural rubber and wood panels production. (Akhter 2005) Rubber trees are replanted every 25-30 years when they are uneconomical for latex production. (FAO Food *et al*., 2010) Previous to the utilization of rubberwood for timber and timber based products, the felled trees were used as fuelwood. Nowadays, rubberwood can be used for making a wide range of products, such as rubberwood-based panels (e.g. particle board, plywood, and medium-density fibreboard), furniture and joinery products, floor tiles and parquet, and moldings. (Zhou 2007: Teoh *et al*., 2011)

Fungi occur ubiquitously and are well adapted to use a wide range of substrates as their carbon, nitrogen, and energy source. (Quiroga *et al*., 2001) Polyporaceae fungi are associated with major tree losses in rubber plantations in the equatorial and humid tropics. (Quiroga *et al*., 2001) Salmiah (Salmiah 1997) stated that the high carbohydrate, such as sugar and starch, reserves deposited in the parenchyma and the lack of phenolic compounds in the wood are the major factors governing the high decay susceptibility of rubberwood. Hence, there is a great demand for novel anti-fungal belonging to a wide range of structural classes, selectively acting on wood-degrading fungi with fewer side effects.

Generally, in Malaysia, there are several processes involved in treating rubberwood for long-term protection either in the form of logs or sawn timber, such as dip treatment, dip-diffusion process, pressure treatment, vacuum pressure process, oscillating pressure method, and double vacuum process. On the other hand, 3% sodium pentachlorophenoxide or 2% captafol in a bituminous compound may be applied for the temporary protection from staining of cut ends of logs. (Mohd Dahlan 1997) In conjunction, that treatment method is mostly applied to rubberwood using either preservative containing boron compounds or synthesis pyrethroids. (Mohd Dahlan 1997: Salamah 1993) The conventional chemical control has been a successful method of preserving wood-degrading fungi growth, but the effects of these chemicals are of concern because they create problems for the environment and public health. (Teoh *et al*., 2010)

Currently, chemical fungicides (e.g. methyl bromide) are commonly used to control the growth of wood-degrading fungi. (Matan *et al*., 2009) Besides, commercial fungicides for agricultural uses can be used for rubberwood temporary protection, such as chlorothalonil, copper oxine, carbendazim, benomyl, isothiszolinone, propiconazole, etc. On the other hand, sodium pentachlorophenol is not recommended due to its high toxicity and it also contributes to severe corrosion of the drying kiln component and thus turning wood into brown color. (Zhou *et al*., 2007)

Antifungal secondary metabolites isolated from the heartwood of plants have been considered to contribute to tree resistance against wood-degrading fungi. (Quiroga 2001) There is a paucity of information on the use of the extracts of herbs for the control of wood degradation by wood-rot fungi. Verma *et al.,* 2007. claimed that *Trichoderma* spp. is currently the most extensively investigated biocontrol fungi for forest product preservation, particularly in protecting wood against basidiomycetes.

Corresponding Author: M.D. Mashitah, School of Chemical Engineering, University Sains Malaysia,Seri Ampangan, 14300 Nibong Tebal, Penang, Malaysia.
Tel: +604-5996468:
Email: chmashitah@eng.usm.my

Trametes versicolor is the most common species of the family Polyporaceae. It was normally found in the Hawaiian forest. In addition, several biological activities was found from its extract, such as antibacterial, antifungal, antioxidant, antitumor, antiviral, kidney and liver tonic and also immune support. (Farghali and Masek 1998: Hsieh and Wu, 2001) Furthermore, Nyanhongo *et al.,* 2007. reported that there is a number of evidence that showing *Trametes* is among the most versatile of white-rotters with ongoing intensive research into bioremediation application.

In this study, we present results from a screening for antifungal activities from the genera *Trametes* to inhibit the growth of wood-degrading fungi of rubberwood in Malaysia.

MATERIALS AND METHODS

Fungal Strain:

Fungal belonging to several species of the genera *Trametes* (Table 1) were obtained from the Biocomposite and Protection of Timber Forest Products Laboratory, Forest Research Institute Malaysia (FRIM), Kepong, Malaysia. All stock cultures were grown on malt extract agar (MEA) at 30^{o}C and maintained on agar slant prior for subsequent studies.

Table 1: Species of the genera *Trametes* used in this study.

No.	Fungal strain used
1.	*Trametes versicolor*
2.	*Trametes feei*
3.	*Trametes menziezi*
4.	*Trametes elegans*

Wood-degrading Fungi Used:

Selected wood-degrading fungal strains of *Schizophyllum commune*, *Pycnoporus sanguineus*, *Lentinus strigosus*, *Lentinus* sp., *Lentinus sajor-caju*, *Microporus affinis*, and *Microporus xanthopus* were obtained from the Biocomposite and Protection of Timber Forest Products Laboratory, Forest Research Institute Malaysia (FRIM), Kepong, Malaysia. All stock cultures were grown on malt extract agar (MEA) at 30^{o}C and maintained on agar slant prior for subsequent studies.

Mycelia Suspension Preparation:

Mycelia suspension was prepared by suspending mycelia discs from 7-days-old culture plate in sampling bottle containing sterilized distilled water and 0.1% (v/v) Tween 80. The disc of 5 mm diameter was cut on the mycelia mats of the agar plate using a sterilized cork borer. A total of 10 discs for every 10 mL of sterilized water were vortexed for 5 mins in order to make the mycelia suspensions became homogenous.

Crude Extract Preparation:

10 mL (10% v/v) of the mycelia suspension was added to 90 mL of medium containing different typical compositions (Table 2) in 250 mL Erlenmeyer flasks. The medium was autoclaved at 121^{o}C for 15 mins before transferring the mycelia suspension into the culture media. The culture was shaken at 200 rpm in an incubator shaker at 30±2^{o}C for 5 days. The culture broth was then harvested and centrifuged at 4000 x g for 15 mins. The residues (also known as mycelia biomass) was then dried and homogenized prior to extraction process.

Table 2: Composition of different media for growth of genera *Trametes* in shake flask culture.

Composition	Medium 1 (g/L)	Medium 2 (g/L)	Medium 3 (g/L)	Medium 4 (g/L)
Malt extract	6.0	10.0	17.0	17.0
Yeast extract	1.2	10.0	-	-
Peptone	-	-	3.0	-
Dextrose	6.0	-	-	-
Glucose	-	20.0	-	-
Maltose	1.8	-	-	-

Batch Solvent Extraction Process:

Dry residues (100 g) obtained from the mycelia were boiled in water and methanol (ratio 1 g : 20 mL) for 48 hrs, respectively. Then, the crude extract obtained was dried and kept at 4^{o}C for further analysis.

Minimum Inhibitory Concentration (MIC) Assay:

MIC was defined as the lowest concentration of fungal mycelia extract to which no growth of wood degrading fungi was observed after incubation period. (Teoh *et al*., 2011) A broth dilution method using a 96-well microtitre plate was employed for MIC determination. Six different concentrations of each of extracts were prepared ranging from 0.1 to 5 µg/µL. The wood-degrading fungi mycelia suspension of each strain was

prepared and standardized according to 0.5 McFarland standard turbidity. Then, the crude extract obtained was dissolved into 5% dimthyl sulfoxide (DMSO). Each well contained malt extract broth with 0.09 mL volume of serially diluted crude extracts. Each well was inoculated with the mycelia suspension of wood-degrading fungi in a final volume of 0.1 mL. Incubations were then performed at 30^{o}C for 48 hrs. The effectiveness of inhibition was quantified by adding 0.02 mL of the yellow tetrazolium MTT reagent into each well. The results were obtained after an incubation period of 2 hrs. The clear colour wells indicated the presence of growth inhibition whereas the dark bluish color wells indicated the absence of growth inhibition.

Analytical Method:

In this study, gas chromatography mass spectrometry (GC-MS) was used to analyze the sample qualitatively by referring to the molecular weight of the compounds. The gas chromatography analyses were performed using Perkin Elmer Clarus 600 gas chromatograph equipped with an ELITE-5 column. The gas chromatography was coupled to the Perkin Elmer 600T mass spectrometer. The oven temperature was programmed at 65^{o}C for 4 mins and then increased to 280^{o}C at a rate of 8^{o}C/min.

RESULTS AND DISCUSSION

Effect of Media Composition on Mycelia Growth in Shake Flask Culture:

Fig. 1 shows the effect of different media on the growth of *Trametes* spp. in shake flask culture. The highest biomass was recorded in Medium 1 and therefore used throughout the study. Herewith, the result was in good agreement with the studies undertaken by Manjunathan and Kaviyarasan 2010: and Teoh *et al.,* 2011. Manjunathan and Kaviyarasan (Manjinathan and Kaviyarasan 2010) reported that dextrose was an excellent carbon source for growth of Lentinus tuberregium, and this was followed by maltose. Teoh *et al.,*2011. observed that dextrose and maltose was important carbon source for antifungal production by *Pycnoporus sanguineus*. On the other hand, malt and yeast extract served as the key component in the media for growth of most microorganisms. (Yahaya 2008) Yeast extract was the best supplement for nitrogen source and provide convenient growth factors for most microorganisms, while the simulating effect of malt extract may attribute to its carbon and protein components in the media. (Teoh *et al*., 2011: Yahaya 2008: Cheng 1991).

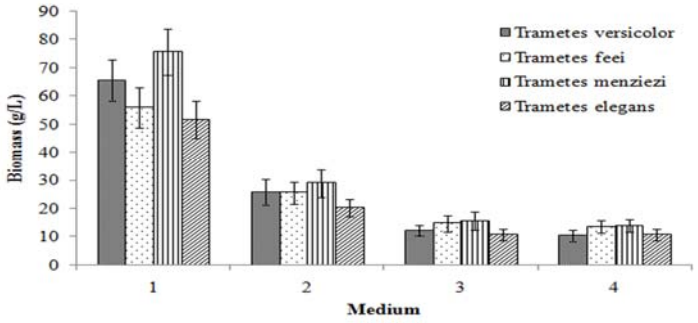

Fig. 1: Biomass produced of genera *Trametes* in different production medium.

Antifungal Activity:

Antifungal activity from *Trametes* spp. was assayed against seven wood-degrading fungi isolated locally. The antifungal activity was evaluated using minimum inhibitory concentration (MIC) assay as shown in Table 3. It is clear that the MIC values were depended mainly on the types of solvent used and the tested fungal strains. Results showed MIC values ranging from 0.1 to more than 5 µg/µL. As reported by Getha *et al.*, 2009. several different polysaccharide antitumour drugs have been developed from the fruiting bodies, mycelia, and culture media of *T. versicolor*. This might be due to the fact that most basidiomycetes were capable to produce a great variety of antimicrobial activity. (Anke 1989).

Based on the data listed in Table 3, the methanolic extract of *T. versicolor* showed better inhibition on the test wood-degrading fungi, particularly against *Lentinus* sp. with MIC 0.3 µg/µL. Meanwhile, Teoh *et al.,* 2011. reported that *Pycnoporus sanguineus* could inhibit the growth of *Lentinus* sp. with lower MIC 0.1 µg/µL. However, Fagade and Oyelade (Fagade and Oyelade 2009) reported that the ethanolic extract of *T. versicolor* showed no inhibition against of test bacteria, such as *Escherichia coli*, *Flavobacterium* sp., and *Bacillus cereus*. This phenomenon might be due to the fact that some antifungal proteins have a specificity of action against only certain fungal species. (Pushpa and Purushothama, 2010).

*The symbol *TV*, *TF*, *TM*, and *TE* denote the fungal strains of *T. versicolor*, *T. feei*, *T. menziezi*, and *T. elegans*, respectively. The superscript m and w denote the extraction carried out by methanol and water,

respectively. In this study, the tested concentration was 5 µg/µL. Activity concentration: weak activity: MIC > 5 µg/µL; moderate activity: 1 µg/µL < MIC ≤ 5 µg/µL; strong activity: MIC ≤ 1 µg/µL.

According to the review studied by Zjawiony, (Zjawiony 2004) the use of hot water extracts of medicinally important basidiomycetes as a remedy against cancer is known in the folk medicine of many countries, such as Japan, Korea, China, Russia, and other eastern countries. This was due to the fact that the water extracts of the basidiocarps contain predominantly polysaccharides, which being the principal components of the fungal cell walls. These high molecular weight compounds exhibit the immunoprotective activity, which are able to protect from a variety infectious diseases. However, the result established in Table 3 summarized that the methanolic extracts gave better antifungal activity compared to those of aqueous water extracts. Although the result was contradict with the previous report done by Zjawiony 2004. but it was in good agreement with the study done by Parthasarathy *et al.,* 2009 This phenomenon was due to the fact that the alcohol extract provides a more complete extraction, in which less polar compounds formed possess antifungal properties and hence provided wider spectrum on antimicrobial activity. (Parthasarathy *et al*., 2009)

Table 3: Antifungal activity of the *Trametes* spp. crude extract towards selected wood-degrading fungi.

Wood-degrading fungi	Minimum inhibitory concentration, MIC (µg/µL)							
	TV^m	TV^w	TF^m	TF^w	TM^m	TM^w	TE^m	TE^w
Schizophyllum commune	>5	>5	>5	>5	>5	>5	>5	>5
Pycnoporus sanguineus	>5	>5	>5	>5	>5	>5	>5	>5
Lentinus strigosus	1.2	5.0	1.2	2.5	2.5	>5	>5	>5
Lentinus sp.	0.3	0.6	1.2	5.0	1.2	2.5	0.3	>5
Lentinus sajor-caju	1.2	1.2	1.2	5.0	1.2	>5	>5	>5
Microporus affinis	0.3	0.6	2.5	>5	0.6	2.5	0.3	0.6
Microporus xanthopus	0.3	1.2	5.0	>5	>5	>5	0.6	>5

GC-MS Analysis:

The interpretation of the mass spectra obtained by the GC-MS method was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown component from sample was compared with the spectrum of the known components stored in the NIST library version 2.0. On this basic, the name, molecular weight and structure of the components of the tested materials were ascertained. Concerning the effect of anti-fungal activity towards wood-degrading fungi tested, this study involved only the methanol extract from mycelia of *T. versicolor*, which performed much better as compared to those others *Trametes* spp. used. Fig. 2 shows the GC-MS chromatogram of the methanol extract from *T. versicolor* mycelia. The corresponding major components present in the extract of this fungus based on GC-MS spectrum are recorded in Table 4.

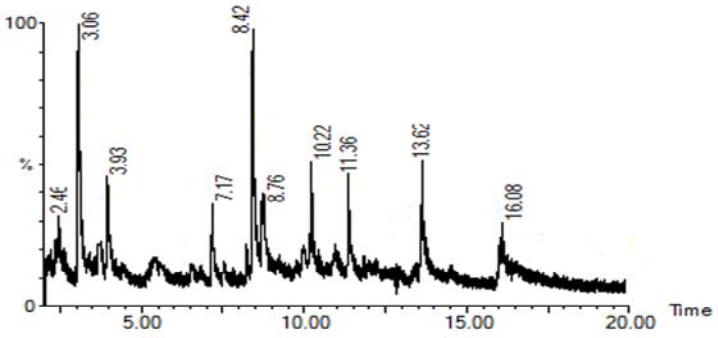

Fig. 2: Chromatogram for the methanol extract from *Trametes versicolor.*

As reported by Zjawiony, (Parthasarathy *et al*., 2009) the crude extract of the wood-rotting fungus, *T. versicolor*, contains cytotoxic, polyoxygenated ergosterol derivatives. As shown in Fig. 2 and Table 4, the major components present in the methanol extracted of the *T. versicolor* was propanal,2,3-dihydroxy- (RT: 3.06) and 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (RT: 8.42). The unique sugar residue, 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (called DDMP), was first identified from *Phaseolus coccineus*. (Yoshiki Kim and Okubo 1994) Based on previous studies, several important biological activities of DDMP were observed by several researchers, such as anti-mutagenic activity against arylamine and 2-acetoxyacetylaminofluorene (2AAAF)-induced DNA damage in Chinese hamster ovary cells, (Berhow *et al*., 2000) anti-alpha-glucosidase activity in patients with diabetes mellitus, (Quan *et al*., 2003) reactive oxygen-scavenging activity, (Takara *et al*., 2007) and also anti-tumour activity. (Ban *et al*., 2007) In addition, DDMP composition including the flavonoid fraction, a complex natural product of current medicinal interest, in which

its anti-microbial, anti-oxidant and anti-inflammatory have been, recognized. (Teoh *et al*., 2011: Kumar and Maneemegalai 2008: Kumar *et al*., 2010) Thus, these results indicate that the presence of chemical compounds in *T. versicolor* might be the reason for it to have anti-fungal activities against the wood-degrading fungi.

Table 4: Composition of the methanol extract from *Trametes versicolor*.

Retention time (mins)	Chemical composition
2.46	Pyrimidine-2,4(1H,3H)-dione,5-amino-6-nitroso-
3.06	Propanal,2,3-dihydroxy-
3.93	2-Propanone,1,3-dihydroxy-
7.17	4,5-Diamino-6-hydroxy pyrimidine
8.42	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-
8.76	2(3H)-Furanone,dihydro-4-hydroxy-
10.22	1,2,3-Propanetriol, monoacetate
11.36	1,2-Ethanediol,1-phenyl-
13.62	Sucrose
16.08	3-Deoxy-d-mannoic lactone

Conclusion:

To the best of our knowledge, this study was to investigate the antifungal activities of *Trametes* spp. under submerged culture condition. It is worthily mentioning that the extracts obtained from various solvents used in this study had anti-fungal activity. From this study, it can be partly concluded that methanolic extract of *Trametes versicolor* can serve as an antifungal agent to inhibit the growth of wood-degrading fungi of rubberwood.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the National Science Fellowship (NSF) from the Ministry of Science, Technology and Innovation (MOSTI) of Malaysia and the Research University (RU) grant (Grant Account No.: 814057) provided by University Sains Malaysia to support this research work. Authors would also like to extend their gratitude to Dr. Salmiah Ujang of the Forest Research Institute of Malaysia (FRIM) for supplying the stock culture of fungus.

REFERENCES

Akhter, K., 2005. Preservative treatment of rubberwood (*Hevea brasiliensis*) to increase its service life. 36th Annual Meeting, The International Research Group in Wood Protection, Section 4-Processes and Properties, IRG Secretariat SE-100 44 Stockholm, Sweden.

Anke, T., 1989. Basidiomycetes: A source for new bioactive secondary metabolites. Progress in Industrial Microbiology, 27: 51-66.

Ban, J.O., I.G. Hwang, T.M. Kim, B.Y. Hwang, U.S. Lee, H.S. Jeong, Y.W. Yoon, D.J. Kim and J.T. Hong, 2007. Anti-proliferate and pro-apoptotic effects of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyranone through inactivation of NF-$_K$B in human colon cancer cells. Archives of Pharmacal Research, 30(11): 1455-1463.

Berhow, M.A., E.D. Wagner, S.F. Vaughn and M.J. Plewa, 2000. Characterization and antimutagenic activity of soybean saponins. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 448(1): 11-22.

Chaturvedi, A., S. Singh and T.N. Nag, 2010. Antimicrobial activity of flavonoids from *in vitro* tissue culture and seeds of *Gossypium* species. Romanian Biotechnological Letters, 15(1): 4959-4963.

Cheng, P., R.E. Mueller, S. Jaeger, R. Bajpai and E.L. Iannotti, 1991. Lactic acid production from enzyme-thinned corn starch using *Lactobacillus amylovorus*. Journal of Microbiology, 7: 27-34.

De Vis, R.M.J., G.J. De Moraes and M.R. Bellini, 2006. Mites (*Acari*) of rubber trees (*Hevea brasiliensis* Muell. Arg., Euphorbiaceae) in Piracicabi, State of Sao Paulo, Brazil. Neotropical Entomology, 35(1): 112-120.

Edwin, L. and P.M. Ashraf, 2006. Assessment of biodeterioration of rubberwood exposed to field conditions. International Biodeterioration & Biodegradation, 57: 31-36.

Fagade, O.E. and A.A. Oyelade, 2009. A comparative study of the antibacterial activities of some wood-decay fungi to synthetis antibiotic discs. Electronic Journal of Environmental, Agricultural and Food Chemistry, 8(3): 184-188.

FAO Food and Agricultural Organization of the United Nations, 2010. Crop – rubber, http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor (Accessed 25 Oct 2010).

Farghali, H. and K. Masek, 1998. Immunopharmacologic agents in the amelioration of hepatic injuries. International Journal Immunopharmacology, 20(4-5): 125-139.

Getha, K., M. Hatsu, H.J. Wong and S.S. Lee, 2009. Submerged cultivation of basidiomycete fungi associated with root diseases for production of valuable bioactive metabolites. Journal of Tropical Forest Science, 21(1): 1-7.

Hsieh, T.C. and J.M. Wu, 2001. Cell growth and gene modulatory activities of Yunzhi (Windsor Wunxi) from mushroom *Trametes versicolor* in androgen-dependent and androgen0insensitive human prostate cancer cells. International Journal of Oncology, 18(1): 81-88.

Kumar, M.S. and S. Maneemegalai, 2008. Evaluation of larvicidal effect of *Lantana camara Linn* against mosquito species *Aedes aegypti* and *Culex quinquefasciatus*. Advances in Biological Research, 2(3-4): 39-43.

Kumar, P.P., S. Kumaravel and C. Lalitha, 2010. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. African Journal of Biochemistry Research, 47(7): 191-195.

Manjinathan, J. and V. Kaviyarasan, 2010. Studies on the growth requirements of *Lentinus tuberregium* (Fr.), an edible mushroom. Middle-East Journal of Scientific Research, 5(2): 81-85.

Matan N., W. Saengkrajang and N. Matan, 2009. Inhibition of *Aspergillus niger* on tea box packaging made of rubberwood treated with vapour of peppermint oil. Asian Journal of Food and Agro-Industry, 2(4): 767-772.

Mohd Dahlan, J., L.T. Hong, M. Azlan and A.H.H. Wong, 1997. Preservation of rubberwood." In: Rubberwood – Processing and Utilization, Eds., Hong, L.T. and H.C. Sim. Forest Research Institute Malaysia (FRIM), Kepong, Malaysia.

Nyanhongo, G.S., G. Gubitz, P. Sukyai, C. Leitner, D. Haltrich and R. Ludwig, 2007. Oxidoreductases from *Trametes* spp. in Biotechnology: A wealth of catalytis activity. Food Technology and Biotechnology, 45(3): 250-268.

Parthasarathy, S., J.B. Azizi, S. Ramanathan, S. Ismail, S. Sasidharan, M.I.M. Said and S.M. Mansor, 2009. Evaluation of antioxidant and antibacterial activities of aqueous, methanolic and alkaloid extracts from *Mitragyna speciosa* (Rubuaceae family) leaves. Molecules, 14: 3964-3974.

Pushpa, H. and K.B. Purushothama, 2010. Antimicrobial activity of *Lyophyllum decastes* an Edible Wild Mushroom. World Journal of Agricultural Sciences, 6(5): 506-509.

Quan, J., X. Yin, M. Jin and M. Shen, 2003. Study on the inhibition of alpha-glucosidase by soyasaponins. Zhong Yao Cai, 26(9): 654-656.

Quiroga, E.N., A.R. Sampietro and M.A. Vattuone, 2001. Screening antifungal activities of selected medicinal plants. Journal of Enthnopharmacology, 74: 89-96.

Salamah, S., A.I. Wan, M. Habibah and A. Faezah, 1993. Determination of Cypermethrin content in oscillating pressure treated rubberwood. Journal of Tropical Forest Science, 5(3): 342-352.

Salmiah, U., 1997. Basidiomycota in Forest Reserves and Plantation Forest in Peninsular Malaysia. PhD thesis, University of Portsmouth, United Kingdom.

Schmidt, O., 2000. Wood and Tree Fungi – Biology Damage, Protection and Usage, Springer, Germany.

Siti, H.A.H., 2008. Evaluation on the Presence of Hazardous Material from Medium Density Fiberboard. MSc thesis, University Sains Malaysia, Malaysia.

Takara, K., K. Otsuka, K. Wada, H. Iwasaki and M. Yamashita, 2007. 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity and tyrosinase inhibitory effects of constituents of sugarcane molasses. Bioscience, Biotechnology, and Biochemistry, 71(1): 183-191.

Taylor, W.G., D.H. Sutherland and K.W. Richards, 2009. Soyasaponins and related glycosides of *Desmodium canadense* and *Desmodium illinoense*. The Open Natural Products Journal, 2: 59-67.

Teoh, Y.P. and M. Mat Don, 2010. Screening of selected fungi for biological control agent potential towards wood-degrading fungi of Malaysia. Malaysian Journal of Pharmaceutical Sciences, Supplement No. 1: 1.

Teoh, Y.P., M. Mat Don and S. Ujang, 2011. Assessment of the properties, utilization, and preservation of rubberwood (Hevea brasiliensis): a case study in Malaysia. Journal of Wood Science, doi 10.1007/s10086-011-1173-2.

Teoh, Y.P., M. Mat Don and S. Ujang, 2011. Media selection for mycelia growth, antifungal activity against wood-degrading fungi, and GC-MS study by *Pycnoporus sanguineus*. Bioresources, 6(3): 2719-2731.

Verma, M., S.K. Brar, R.D. Tyagi, R.Y. Surampalli and J.R. Valero, 2007. Antagonistic fungi, *Trichoderma* spp.: Panoly of biological control. Biochemical Engineering Journal, 37: 1-20.

Yahaya, Y.A., 2008. Biosorption of Heavy Metals by Free and Immobilized *Pycnoporus sanguineus*: Batch and Column Studies. MCs thesis, Universiti Sains Malaysia, Malaysia.

Yoshiki, K., J.H. Kim and K. Okubo, 1994. Saponins conjugated with 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one from *Phaseolus coccineus*. Phytochemistry, 36(4): 1009-1012.

Zhao, Y., 2008. Promotion of rubberwood processing technology in the Asia-Pasific Region. In the Proceedings of the ITTO/CFC International Rubberwood Workshop, Haikou, China.

Zhou, Y.D., M.L. Jiang, R.Q. Gao and X.L. Li, 2007. Rubberwood Processing Manual. Demonstration of Rubberwood Processing Technology and Promotion of Sustainable Development in China and other Asian Countries. Research Institute of Wood Industry, Chinese Academy of Forestry, Beijing, China.

Zjawiony, J.K., 2004. Biologically active compounds from Aphyllophorales (polypore) fungi. Journal of Natural Product, 67: 300-310.