PRODUCTION OF ANTI-FUNGAL AGENT BY *SCHIZOPHYLLUM COMMUNE* AGAINST WOOD-DEGRADING FUNGI OF RUBBERWOOD

TEOH YI PENG @ TENG YI PENG

UNIVERSITI SAINS MALAYSIA

2014

PRODUCTION OF ANTI-FUNGAL AGENT BY *SCHIZOPHYLLUM COMMUNE* AGAINST WOOD-DEGRADING FUNGI OF RUBBERWOOD

by

TEOH YI PENG @ TENG YI PENG

Thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

FEBRUARY 2014

ACKNOWLEDGEMENT

First of all, I would like to express my deepest gratitude to my beloved parents, Teng Ah Ba and Kee Mooi Choo for their endless love, and tolerance. Not to forget my sister, brother, and loved one, Zhong Xian for their persevering support, encouragement and unconditional support all this while.

I am greatly indebted to my dedicated supervisor, Assoc. Prof. Dr. Mashitah Mat Don for her support, guidance, encouragement, advice, and also generosity in sharing knowledge during period of my PhD degree. Special thanks for the willingness to spare her time and energy in correcting my paper and thesis in the midst of her business and unhealthiness. Sincere thanks also to my site-supervisor, Dr. Salmiah Ujang for her friendly help and support without any reluctance.

Not forgetting to thank the dean of School of Chemical Engineering (USM), Prof. Dr. Azlina and technical staff especially to Ms. Yusnadia, Mr. Shamsul, Mr. Roqib, and Ms. Latifah. This project would have never been completed without their valuable support and advice during period of my studies. Furthermore, I also wish to acknowledge to NSF scheme for the financial support.

Last but not least, I would like to thank my dear friends Stephanie, Kamila, Kak Yus, Kiew Ling, and Peck Loo that support behind me and give me advices and suggestions to conduct a proper experiment throughout the studies. To all the people, who have helped me throughout my PhD research, directly or indirectly; your contribution shall not be forgotten. Thank you very much!!!

Teoh Yi Peng

February 2014

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AI	Artificial intelligence
ANN	Artificial neural network
ANOVA	Analysis of variance
ASM	Antimicrobial susceptibility method
BB	Boric acid borax
BBD	Box-Behnken design
C:N	Carbon : Nitrogen
CCA	Copper-chromium-arsenic
CCD	Central Composite design
CCLF	Combined Continuous Logistic and Fermi
CCLFMLP	Combined Continuous Logistic and Fermi incorporated Modified Luedeking-Piret
CMLLP	Chavez-Parga’s Modified Logistic incorporated Luedeking-Piret
CPML	Chavez-Parga’s Modified Logistic
DDMP	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
DMSO	Dimetyl sulfoxide
DNS	Dintrosalicyclic acid
DO	Dissolved oxygen
DoE	Design of experiment
EAs	Evolutionary algorithms
ED_{50}	Effective dose at 50% inhibition

EPS	Exopolysaccharides
FL	Fuzzy logic
FRIM	Forest Research Institute Malaysia
GA	Genetic algorithm
GC-MS	Gas chromatography-mass spectrometry
HPLC	High performance liquid chromatograph
ITA	Investment Tax Allowance
LLP	Logistic incorporated Luedeking-Piret
L-M	Levenberg-Marquardt
LMLP	Logistic incorporated Modified Luedeking-Piret
LPTD	Luedeking-Piret with Time Delay
LZMLP	Logistic incorporated Zhang's Modified Luedeking-Piret
MDF	Medium-density fiberboard
MEA	Malt extract agar
MIC	Minimum inhibitory concentration
MLLP	Modified Logistic incorporated Luedeking-Piret
MLMLP	Modified Logistic incorporated Modified Luedeking-Piret
MMLP	Monod incorporated Modified Luedeking-Piret
MOE	Modulus of elasticity
MOR	Modulus of rupture
MTT	3-(4,5-dimetylthylthiazolyl-2)-2,5-diphenyltetrazolium bromide
NAP	National Agricultural Policy
NIST	National Institute Standard and Technology

OD	Optical density
OFAT	One-factor-at-a-time
OPM	Oscillating pressure method
OTR	Oxygen transfer rate
OUR	Oxygen uptake rate
PBD	Plackett-Burman design
PIA	Promotion of Investment Act
pO_2	Polarographic dissolved oxygen
PPO	Polyphenol oxidase
PS	Pioneer Status
RISDA	Rubber Industry Smallholders Development Authority
RMSD	Root mean square deviation
RRIM	Rubber Research Institute of Malaysia
RSM	Response surface methodology
TFC	Total flavonoid content

LIST OF SYMBOLS

A_i	Extraction kinetic parameter related to parabolic diffusion model ($1/min^{0.5}$)
A_0	Washing coefficient related to parabolic diffusion model ($1/min^{0.5}$)
A_1	Diffusion rate constant related to parabolic diffusion model ($1/min^{0.5}$)
α	Coefficients represent the growth related term (g/g)
B	Parameter of the power law model incorporating the characteristics of the extraction system ($1/min^n$)
β	Coefficients represent the non-growth related term (g/g.h)
C^*	Saturation dissolved oxygen concentration (mg/l)
C_L	Dissolved oxygen concentration (mg/l)
C_{LSS}	Dissolved oxygen concentration at steady state (mg/l)
E_0, E_1	Extraction kinetic parameters of Elovich's model
k	Kinetic parameter related to initial specific growth rate
k_La	Volumetric oxygen transfer coefficient (1/s)
K_1	Peleg's rate constant (min.g/mg)
K_2	Peleg's capacity constant (g/mg)
K_I	Inhibition constant
K_s	Saturation constant (g/l)
μ	Specific growth rate (1/h)
μ_F	Decline rate constant (1/h)
μ_L	Unrestricted growth rate (1/h)
μ_o	Initial specific growth rate (1/h)

μ_{max}	Maximum growth rate (1/h)
m_s	Maintenance coefficient (g/g.h)
n	Diffusion exponent of the power law model
P	Product concentration (g/l)
P value	Mean relative percentage deviation (%)
r	Index of the inhibitory effect
R^2	Linear correlation coefficient
S	Substrate concentration (g/l)
S_m	Critical inhibition concentration (g/l)
t	Time (h)
t_c	Time to reach 50% survival (h)
T	Temperature (^oC)
X	Biomass concentration (g/l)
X_c	Critical cell concentration (g/l)
y	Product concentration (µg/mg)
$Y_{P/S}$	Product yield coefficient (g/g)
$Y_{X/S}$	Biomass yield coefficient (g/g)
Δ	Energy spilling-associated coefficient of substrate consumption
σ	Variance

PENGHASILAN AGEN ANTI-KULAT OLEH *SCHIZOPHYLLUM COMMUNE* KE ATAS KULAT PEREPUT KAYU BAGI POKOK GETAH

ABSTRAK

Kulat pereput kayu adalah merupakan suatu ancaman yang serius kepada kayu getah. Penggunaa pengawet kimia oleh industri pemuliharaan kayu getah perlulah berhati-hati kerana ia memberikan kesan kepada kesihatan dan masalah alam sekitar. Pengawalan secara biologi adalah merupakan cara alternatif untuk menyelesaikan masalah ini. Di dalam kajian ini, dua belas spesies kulat pereput kayu tempatan yang liar telah dipencil dan disaring akan keupayaannya untuk menghasilkan agen antikulat. Keputusan menunjukkan ekstrak metanol bagi biojisim *Schizophyllum commune* memberikan aktiviti antikulat yang tinggi diantara kulat-kulat yang diuji dengan kepekatan rencatan minimum (KMM) dalam julat dari 0.1 ke 5.0 µg/µl. Sebatian kimia yang terdapat di dalam ekstrak biojisim telah dianalisa melalui kromatografi gas-permeteran spektrum jisim (GC-MS) dan kromatografi cecair berprestasi tinggi (HPLC). 4H-pyran-4-one, 2,3-dihidro-3,5-dihidroksi-6-metil- (DDMP), satu pecahan flavonoid, telah didapati di dalam ekstrak biojisim. Strategi pengoptimuman berdasarkan kaedah satu-faktor-di-satu-masa (OFAT) dan statistik telah diguna untuk mengoptimumkan pertumbuhan *S. commune* di dalam kultur kelalang goncang. Berdasarkan rekabentuk "Plackett-Burman", pembolehubah-pembolehubah seperti ekstrak yis, glukosa, dan $MgSO_4 \cdot 7H_2O$ memberikan kesan yang signifikan bagi pertumbuhan kulat ini. Nilai-nilai optimum bagi pembolehubah proses ini telah diperolehi menggunakan metodologi permukaan sambutan yang bergandingan dengan rekabentuk "Box-Behnken". Bagi pengekstrakan agen antikulat, keadaan optimumnya ialah pada 70.75% metanol, 29 °C, dan 145 putaran/min. Kesemua kajian pengoptimuman telah disah dan didapati data eksperimen

adalah berpadanan dengan model-model terpilih dengan peratusan ralat kurang daripada 1%. Kinetik pengesktrakan juga telah dikaji dengan menggunakan model resapan “Parabolic”, model hukum Tenaga, model Pelag, dan model Elovich. Kesemua model adalah berpadanan dengan data eksperimen, dimana hukum Tenaga memberikan nilai R^2 tertinggi dan RMSD yang terendah. Untuk mempertingkatkan penghasilan bagi biojisim, flavonoid total (TF), dan DDMP yang lebih tinggi, prestasi bagi 2 l bioreaktor turus gelembung telah dikaji. Keputusan menunjukkan penghasilan biojisim (32.38 g/l), TF (1.33 µg QE/mg sampel), and DDMP (1.28 µg/mg sampel) yang tertinggi telah diperolehi di dalam kultur yang tumbuh pada 30 ^{o}C. Lanjutan daripada itu, pekali pemindahan oksigen (k_La) bagi *S. commune* juga telah dikaji untuk mengetahui keupayaan pemindahan oksigen di dalam bioreaktor dengan mengambil kira kesan kadar pengudaraan. Didapati bahawa biojisim dan penghasilan agen antikulat yang maximum adalah pada 4 l/min dengan nilai k_La 0.04 1/s. Beberapa model tak-berstruktur bagi pertumbuhan, produk, penggunaan dan perencatan substrat bagi kulat ini telah dikaji pada kepekatan glukosa di julat 20-100 g/l. Di antara pelbagai model kinetik yang diuji, model Logistic untuk pertumbuhan mikrob, model “Logistic incorporated Zhang’s Modified Luedeking-Piret” untuk produk dan penggunaan substrat, serta model Mulchandani untuk perencatan substrat memberikan jangkaan kinetik fermentasi yang tepat dengan nilai R^2 yang tinggi dan RMSD yang rendah. Keputusan juga menunjukkan penghasilan agen antikulat oleh *S. commune* dengan menggunakan glukosa sebagai substrat adalah perkaitan pertumbuhan-produk campuran dengan α dan $\beta \neq 0$. Ekstrak biojisim yang terhasil kemudiannya telah tindakbalaskan ke atas blok kayu getah untuk menguji akan keberkesanannya. Keputusan menunjukkan ekstrak biojisim yang mengandungi agen antikulat, DDMP berkeupayaan merencat pertumbuhan kulat-kulat pereput kayu getah yang lain.

PRODUCTION OF ANTI-FUNGAL AGENT BY *SCHIZOPHYLLUM COMMUNE* AGAINST WOOD-DEGRADING FUNGI OF RUBBERWOOD

ABSTRACT

Wood-degrading fungi are serious threat to rubberwood. Chemical preservatives commonly used in rubberwood preserving industry become awareness as it give impact to health and environmental problem. Biological control may be used as an alternative method to solve this problem. In this study, twelve locally isolated wild strains of wood-degrading fungi were screened for their capability to produce antifungal agent. Results showed that methanol extract of *Schizophyllum commune* biomass provided the highest antifungal activity among the tested fungi with minimum inhibitory concentration (MIC) ranging from 0.1 to 5.0 μg/μl. Chemical compound presence in the biomass extract was analyzed via gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatograph (HPLC). It was found that 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP), a flavonoid fraction, was presence in the biomass extract. Optimization strategy based on one-factor-at-a-time (OFTA) method and statistical tool were employed to optimize the growth of *S. commune* in shake flask culture. Based on Plackett-Burman design (PBD), the variables such as yeast extract, glucose and $MgSO_4 \cdot 7H_2O$ significantly affected the fungus growth. The optimal values of these process variables were determined using Response Surface Methodology (RSM) coupled with Box-Behnken design (BBD). For the extraction of antifungal agent, the optimum conditions were 70.75% (v/v) methanol, 29 °C, and 145 rpm. All optimization studies were verified and the experimental data fitted well to the selected models with error percentage less than 1%. The extraction kinetics was also investigated using the Parabolic diffusion model, Power law model, Peleg's model,

and Elovich's model. All empirical models gave a good fit to the experimental data, in which the Power law model gave the highest R^2 and lowest RMSD values. To achieve higher production of biomass, total flavonoid (TF), and DDMP, the performance of a 2 l bubble column bioreactor was investigated. Results revealed that the highest production of biomass (35.11 g/l), TF (1.33 µg QE/mg sample), and DDMP (1.28 µg/mg sample) were achieved in culture grown at 30 ^{o}C. Furthermore, the oxygen transfer coefficient (k_La) of *S. commune* was also studied to investigate the oxygen transfer capabilities in the bioreactor considering the effect of aeration rate. It was found that the maximal biomass and antifungal agent production was achieved at 4 l/min with k_La value of 0.04 1/s. Several unstructured models for growth, product formation, substrate utilization, and substrate inhibition of the tested fungus were studied for glucose concentration ranged 20-100 g/l. Among various kinetic models tested, the Logistic model for microbial growth, the Logistic incorporated Zhang's Modified Luedeking-Piret model for product formation and substrate utilization, and the Mulchandani model for substrate inhibition provided accurate approximation of the fermentation kinetics with high R^2 and low RMSD values. The result also showed that antifungal agent production by *S. commune* using glucose as a substrate was mixed-growth product associated with α and $\beta \neq 0$. The produced methanol extract of the biomass was then applied on the rubberwood block to investigate its effectiveness. Results showed that the biomass extract containing antifungal agent, DDMP, was able to inhibit the growth of other tested wood-degrading fungi of rubberwood.

CHAPTER 1

INTRODUCTION

1.1 Research background

Rubberwood (*Hevea brasiliensis*), a native of the Amazon valley of South America, was introduced to India in the latter half of the 19^{th} century (Edwin and Muhamed Ashraf, 2006), and now is widely cultivated in mostly twenty countries of the world including Malaysia for natural rubber and wood panels production (Akhter, 2005).

Traditionally, rubberwood was a source of fuel, either in the form of firewood for the rural community or for the rubber sheet-curing and brick-making industries. It was also converted into charcoal for use in steel-making industry (Hoi, 1999; Hong, 1999; Hong and Sim, 1999; Hoi, 2002). Lew (1992) estimated that 67% of the total rubberwood consumed annually was used as fuelwood in Peninsular Malaysia before the development of the rubberwood industry. The processing of rubberwood in Malaysia began in the early 1970s (Ho and Roslan, 1999). It was firstly processed into block board cores and converted into chips for pulp and paper making (Mohd Nor, 1999). In the late 1970s, rubberwood was processed into sawn timber for export. As reported by Hong and Sim (1999), the export of rubberwood sawn timber in the year 1980–1995 increased more than tenfold in terms of volume and export values because rubberwood was used as a raw material in wood product manufacturing. The sawing process converted logs into sawn timber. In the process, undesirable defects such as knots, pith, and tapping marks are removed. At present, sawn rubberwood timber is widely used in the manufacture of furniture, doors, window frames, moldings, novelty items, and household utility items, whereas rubberwood logs are used to produce veneers, which are usually applied in the manufacture of plywood, hardboard, and solid moldings (Hong and Sim, 1999; Mohd Nor, 1999; Rahim, 1999; Hoi, 2002; Zhao, 2008).

The Malaysian rubber industry is well-known internationally for its developed and progressive R&D programs that enabled the country to establish itself as the world leader in rubber production, processing, and manufacturing technologies. Recently, the planting of rubber trees solely for wood extraction has been certainly a viable investment for investors when fully integrated with downstream rubberwood processing and product manufacturing (Hong and Sim, 1999). Kadir (1998) pointed out that so far, rubberwood was merely considered a residue of the rubber industry and commanded a poor price in the open market. The true value of the wood manifested only after it has been processed into semi-finished or final products. The rubber tree has an advantage over many other timber species because it can be exploited for both timber and latex. However, in the case of trees grown only for wood, the return on investment was improved if the wood is offered at a price competitive to other timber species (Hong and Sim, 1999). With this motivation, several plantation companies, such as Golden Hope Plantations and Guthrie, have moved into downstream processing of medium-density fiberboard (MDF) to take advantage of the lucrative value-added profits (Kadir, 1998).

Anthony (1998) reported that the Malaysian furniture industry was still considered to be at an early stage of development. In fact, the successful achievements in the processing and utilization of rubberwood through the R&D

efforts of the Forest Research Institute Malaysia (FRIM) have provided the growth impetus for the industry to scale greater heights in the future. On the other hand, the Malaysian furniture industry played an important role at the international level since it was able to turn cheap and plentiful timber into a value-added product at a competitive price (Hong, 1995). This generated several arguments on the trend of increasing rubberwood costs and questions on the long-term sustainability of the whole rubber and rubberwood-based furniture industry. Exports of the furniture industry grew by leaps and bounds from a cottage industry of RM120 million in 1986 to more than RM2.8 billion in 1997. With this, it has been estimated that the export of Malaysian furniture had continued to increase between 10% and 15% annually to reach an annual export turnover of RM4.1 billion in the future (Anthony, 1998). For example, the curved furniture components, such as chair backs and legs, were commonly made by laminating veneers, which raised the demand of rubberwood veneers and thus prompted the furniture industry to produce its own rubberwood veneers (Ho and Roslan, 1999). The main features of this industry that have emerged over the past decade are the remarkable upsurge in production output, the utilization of advanced technology, and the continuous upgrading of the sector. These developments indicated a prospectively vibrant sector of the industry. In 2008, export of furniture shot up to RM 8.7 billion, of which 80% was rubberwood-based furniture (Gan, 2009). On the other hand, the situation on the ground was complicated by several challenges ahead.

The biodegradation problem is one of the main reasons why rubberwood is less attractive for wood processing industries and was almost neglected in the past, although it was abundant in supply and easily available (Killmann, 2001; Hong, 1995). The high carbohydrate (sugar and starch) reserved deposited in the parenchyma is the major factors governing the high decay susceptibility of rubberwood (Salmiah, 1997). Zaidon *et al.* (2003) stated that these rubberwood products are generally less susceptible to biodeterioration agents than solid wood unless they are used in situations where exposure to moisture or risk of deterioration is likely.

Wood-degrading fungi that cause severe breakdown of wood are characterized either as brown rot and white rot (generally caused by Basidiomycetes) fungi or as soft rot (caused by Ascomycetes and Fungi Imperfecti) fungi (Hong *et al.*, 1999). Wong (1988) showed that the susceptibility of rubberwood to the three major types of wood rot fungi is in the following order of severity, in which the substrate mass loss due to soft rot (*Chaetomium globosum*) > white rot (*Pycnoporus sanguineus*) > brown rot (*Tyromyces palustris*). Salmiah (1997) stated that the capacity of both white rot and brown rot fungi to decompose rubberwood is reduced compared with the effect on temperate timbers (e.g., sweetgum and southern yellow pine). This might be because many tropical woods contain a higher proportion of tannins and phenolic compounds that have better fungistatic effects compared with temperate woods. As such, rubberwood appeared to be the most susceptible to soft rot decay compared with other nondurables such as punggai (*Coelostegia griffi thii*), jelutong (*Dyera costulata*), and kayu arang (*Diospyros* spp.). However, against the white rot fungus *Coriolus versicolor*, rubberwood has been found to be more susceptible than non-durable jelutong and ramin (*G. bancanus*) (Hong *et al.*, 1999). Therefore, it is necessary for rubberwood to be treated with appropriate preservatives for protection against the attacks of biodeteriorating organisms.

In Malaysia, there are several processes involved in treating rubberwood, either in the form of logs or sawn timber, such as dip treatment, dip-diffusion, pressure treatment, vacuum-pressure, oscillating pressure method (OPM), and double-vacuum process (Salamah *et al.*, 1993; Hafizoglu, 2005). For temporary protection from staining of cut ends of logs, 3% sodium pentachlorophenoxide or 2% captafol in a bituminous compound was applied (Gnanaharan and Mathew, 1982). Freshly felled logs can be kept under water in log ponds to protect them against splitting and attacks by insects and fungi (Mohd Dahlan *et al.*, 1999). For sawn timbers, it was necessary to treat them immediately after sawing to prevent the penetration of staining fungus. As an example, sawn rubberwood can be protected through the dipping process for a few seconds in a solution of 0.5–1% sodium pentachlorophenate and 2% borax in water before kiln seasoning, which is a drying process occurred in a large oven-like structure with controlling the air circulation, humidity, and temperature. Pressure impregnation for total protection gives satisfactory results for rubberwood due to its permeability (George, 1985; Mohd Dahlan *et al.*, 1999).

An economical schedule for the industrial-scale treatment of rubberwood using boron compounds in the form of disodium octaborate tetrahydrate ($Na_2B_8O_{13}{\cdot}4H_2O$), disodium tetraborate decahydrate ($Na_2B_4O_7{\cdot}10H_2O$), and boric acid (H_3BO_3) has been developed, particularly for indoor applications, to protect from insects borers and fungi (Gnanaharan and Dhamodaran, 1993; Mohd Dahlan *et al.*, 1999). In addition, highly toxic, but safe once fixed, copper-chromium-arsenic (CCA) preservatives are widely used in many countries due to their efficacy and cost effectiveness (Gnanaharan and Dhamodaran, 1993). Mohd Dahlan *et al.* (1999) reported that CCA-treated rubberwood was rarely used in making furniture because of the unnatural color of the treated wood, but it can be used for construction or structural purposes to ensure resistance against termites and other biodeteriorating organisms.

Filamentous fungi are major source of bioactive secondary metabolites, and researchers has established the existence of biochemical pathways solely for the purpose of producing mycotoxins and other natural products in fungi through the study of ecological chemical interactions (Frisvad *et al.*, 2008). In this approach, fungi have been widely applied in agriculture as bio-control for pest management.

The first investigations on the potential of basidiomycetes as sources of antibiotics were performed by Anchel, Hervey, and Wilkins in 1941, whereby they succeeded in the isolation and identification of pleuromutilin, a diterpene that was useful in the treatment of mycoplasm infections in animals and served for the development of the first commercial antibiotic of basidiomycetes origin (Rosa *et al.*, 2003). As reviewed by Schuffler and Anke (2009), from 1940 until the early of 1950s, more than 2000 basidiomycetes fruiting bodies were screened for the production of antibiotics and resulted in the discovery of pleuromutilin, the lead compound for the semi-synthetic tiamulin used in veterinary practise and recently also in humans.

According to Reddy and Mathew (2001), there has been much interest in the possible use of wood-degrading fungi as biodegradation agent, particularly in the case of white rot fungi. Based on study done by Lara *et al.* (2003), wood-degrading fungi had unique capacity for degrading wood and its basic constituents, cellulose and lignin. These fungi used the cellulose fraction as a source of carbon and have the ability to completely degrade the lignin so as to access to the cellulose molecule. Additionally, Pointing (2001) stated that the white rot fungi belonging to the basidiomycetes group exhibited the most efficient and extensive lignin degradation. In fact, Song *et al.* (1998) previously reported that basidiomycetes divisions exhibited the anti-tumour properties by producing lentinan, schizophyllan, and meshima during secondary metabolite. These compounds can be used as the active ingredients of bioherbicides, bioinsecticides, and biofungicides products (Bennett *et al.*, 2001). Furthermore, with the great scientific and technical development of fungal isolation and taxonomy over a broad range of genera, more and more species of higher fungi within the Basidiomycota division showed antimicrobial properties and considered as new and tremendous source of potent bioactive natural resources (Rosa *et al.*, 2003; Rosecke and Konig, 2000).

Schizophyllum commune is a species of basidiomycetes belonging to the Schizophyllaceae of Agaricales (Hao *et al.*, 2010). It has been reported to be a filamentously growing fungus that produced exopolysaccharides (EPS), and secreted the β-glucans as a uniform, primary molecular structure. This EPS possessed a β-(1→3)-linked backbone with single β-(1→6)-linked glucose side chains, in which upon 100% oxidation would result in a large number of aldehyde groups that exhibited antibacterial properties (Hao *et al.*, 2010; Jayakumar *et al.*, 2010). The EPS has numerous potential applications that included emulsifiers, lubricants, stabilizer, and thickening agents (Kumari *et al.*, 2008; Jayakumar *et al.*, 2010).

Concerning pharmaceutical applications, the EPS has been used to treat a number of diseases, including AIDS, and also to enhance the effect of vaccines and anticancer therapies (Hao *et al.*, 2010). As reported by Lorenzen and Anke (1998) and Kumari *et al.* (2008), schizophyllan is a high molecular weight β-1,3-glucan (ranging from 6 to 12 x 10^6 g/mol) isolated from the mycelia of *S. commune* which shows antitumor properties. The mode of action seemed to involve the stimulation of the host animal's immune response rather than a direct inhibition of tumor cell growth. Additionally, Shittu *et al.* (2005) reported that a sizofiran, antitumour polysaccharide extracted from the culture broth of *S. commune* served as an effective immune-therapeutic agent for cervical carcinoma because it stimulated a rapid recovery of the immunological status impaired by radiotherapy. In addition, it was also reported that several different polysaccharide antitumour agents, such as hetero-β-glucans and their protein complexes, have been developed from the fruiting bodies, mycelia and medium of *S. commune*, particularly in China and Japan (Fagade and Oyelade, 2009).

On the other hand, *Pycnoporus sanguineus* is also very common on dead trees and plays an important ecological role in degrading woody forest litter (Djarwanto and Tachibana 2009; Hii, 2012). Meanwhile, the antimicrobial activity of *P. sanguineus* had been known since 1946, when poliporin was isolated against gram-positive and gram-negative bacteria and without toxicity to experimental animals (Rosa *et al.*, 2003). The basidiomes of *P. sanguineus* had been in use for a long time to prevent haemorrhages by Brazilian indigenous people and by America and African indigenous tribes for treatment of several ailments (Rosa *et al.*, 2003;

Smania *et al.*, 1995). Besides that, another wood-degrading fungus found in Malaysia, *Trametes versicolor,* is the most common species of the family Polyporaceae. 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 showed *Trametes* was among the most versatile of white-rotters with ongoing intensive research into bioremediation application.

Thus, exploration, conservation, and utilization of the fungi belonging to basidiomycetes can be expected to prove beneficial for human as well as the environment.

1.2 Problem statement

In Malaysia, rubberwood is now one of the most popular timbers used for the manufacture of quality furniture and other components. The attractive features of the rubberwood lay in its creamish colour and good woodworking properties. This had prompted many industries to use it as substitute for the highly priced remin timber. In fact, it has carved a niche for itself, and is the timber for many wood products (Hong and Sim, 1999).

In rubberwood, there is no distinction between the sapwood and heartwood (Killmann and Hong, 2000). It is considered that rubberwood contained only sapwood (Anonymous, 1982), and like the sapwoods of all timbers, is non-durable (Mohd Dahlan and Tam, 1987; Wong, 1988). Also, rubberwood is very prone to attack by fungi and wood borers in green and dry condition (Ho, 1999). Commonly, the wood-degrading fungi such as *Pycnoporus sanguineus*, *Lenzites palisotii* and *Ganoderma applanatum* could rapidly destroy the rubberwood. As reported by a few researchers, the high carbohydrate (sugar and starch) reserve deposited in the parenchyma is major factor governing the high decay susceptibility of rubberwood (Azizol and Rahim, 1989; Wong, 1993). In view of the high severity of the decay problem, there is a need for prompt preservative treatment against the attack of biodeteriorating organisms.

Boron and copper-chromium-arsenic (CCA) are reported to be important compounds in rubberwood preservation (Zaidon *et al.*, 2003; Hwang *et al.*, 2007). Boron is odourless and is relatively less toxic when compared to some of the other preservatives, e.g. lindane, which poses serious health hazard to the workers performing the treatment and the processing of treated timber. While CCA treated rubberwood is rarely used because of the unnatural colour of the treated wood. If the timber is to be used for construction or structural purpose it is best to treat with CCA to ensure resistance against termites and other biodeteriorating organisms (Mohd Dahlan, 1999). However, these compounds have become less popular nowadays due to their toxicity and hazardous effect to human beings (Zaidon *et al.*, 2008). Hence, there is a need to search for an alternative of rubberwood preservation especially from our natural resources.

In addition, biocontrol agents are promising alternatives to chemical control of molds and wood-degrading fungi (Tripathi and Dubey, 2004). As reported by Verma *et al.* (2007), fungal-based biological control agents have gained wide acceptance due to their broader spectrum in terms of disease control and production yield. Biofungicides are usually produced from secondary metabolites of fungi under an active culture cultivation process in which the fungi are not essential for vegetative growth in pure culture (Carlile *et al.*, 2001). *Trichoderma* biofungicides have been the modest biological control agents over the past 20 years (Ricard and Ricard, 1997; Verma *et al.*, 2007). For example, *Trichoderma harzianum* ATCC 20746 has been developed for the treatment of strawberries against gray mold *Botrytis cinerea* (Ricard and Ricard, 1997). There are also various fungal species that can be utilized as biological control agents, which may provide effective activity against various pathogenic microorganisms, such as *Ampelomyces quisqualis*, *A. niger*, *Candida oleophila*, *Chaetomium cupreum*, *C. globosum*, *Coniothyrium minitans*, *Cryptococcus albidus*, *Gliocladium virens*, *Gliocladium catenulatum*, *Fusarium oxysporum*, *Phlebiosis gigantea*, *Pythium oligandrum*, *Rhodotorula glutinis*, *T. harzianum*, and *Trichoderma polysporum* (Ricard and Ricard, 1997; Hofstein and Chapple, 1998; Carlile *et al.*, 2001; Soytong *et al.*, 2005; Kaewchai *et al.*, 2009). Based on the above observations, the biological control concept can be applied to the rubberwood industry for mold inhibition; however, at present, there are no reports on the usage of biological antifungal agent for rubberwood treatment.

On the other hand, filamentous fungus fermentation is a complex process as compared to bacterial and yeast fermentation (Xu and Yang, 2007). It is reported that a complex medium composition is needed for fungal fermentation during secondary metabolites (Zhang *et al.*, 2011). Meanwhile, Marwick *et al.* (1999) stated that if the harvesting of secondary metabolites be the main purpose, then the specific nutrient (e.g. carbon or nitrogen source) supply to sustain growth sometimes had to be limited to keep the cell in stationary phase, while the substrate needed for the formation of the desired product has to be present in excess. For example, K_2HPO_4 serves as phosphorous source and mainly controlling the production of secondary metabolites rather than the mycelia growth rate (Xu and Yang, 2007). In addition, physical condition during fermentation and extraction process plays a crucial role in controlling the quantity and quality of the desired product. Thus, the optimization study on different parameters for fungal fermentation and product formation should be investigated.

Bubble column bioreactor is a promising device for gas-liquid mass transfer and is being considered in biochemical application, especially for fermentation process (Kantarci *et al.*, 2005). It is an elongated non-mechanically stirred bioreactor with an aspect ratio of height/diameter through which is a unidirectional flow of gases (Nanou *et al.*, 2012). Additionally, bubble column bioreactor provides several advantages as compared to other types of bioreactors. First and foremost, it has an excellent heat and mass transfer characteristic, followed by the low operating and maintenance cost required due to lack of moving parts and compactness (Kantarci *et al.*, 2005; Nanou *et al.*, 2012). Furthermore, bubble column bioreactor with a bubble-free aeration through membrane provides suitable alternative for transferring gas without inducing cell damage through shear stress. It had been reported that batch bioreactor study was convenient systems for determination of the suitable conditions for maximum productivity (Chattopadhyay *et al.*, 2002). The knowledge gained in kinetics and production rate makes possible the application of principles and practices in understanding the critical parameters influencing mycelia growth and product yield in batch system.

1.3 Research objectives

In view of the above observations, this study was carried out with the following objectives:

1. To screen for the presence of antifungal agent from twelve locally isolated wild strains of wood-degrading fungi.
2. To optimize the chemical and physical parameters for enhancement of growth of selected fungus in shake flask culture using a statistical method.
3. To optimize the antifungal agent extraction parameters using a statistical tool, select and validate various kinetic models for batch solvent extraction process.
4. To investigate the fungal growth and antifungal agent production by the selected fungus in a 2 l bubble column bioreactor.
5. To select and validate kinetic models of growth, antifungal agent production, glucose utilization, and substrate inhibition in a bubble column bioreactor.
6. To determine the effectiveness of antifungal agent produced on the rubberwood block panel.

1.4 Scope of study

The preamble of this study was to investigate the ability of selected filamentous fungi to inhibit the growth of wood-degrading fungi of rubberwood. The antifungal activity was examined using minimum inhibitory concentration (MIC) assay coupled with broth dilution method using 96-well microtitre plate.

Firstly, process optimization of various variables using one-factor-at-a-time (OFAT) method on the production of biomass including media composition (e.g., yeast extract, malt extract, glucose, peptone, KH_2PO_4, K_2HPO_4, $Na_2HPO_4 \cdot 12H_2O$, $MgSO_4 \cdot 7H_2O$, $(NH_4)_2SO_4$, and KCl) and fermentation conditions (e.g., pH, incubation temperature, and agitation rate) were studied in order to obtain the optimum conditions for fungus growth under shake flask cultivation. Process optimizations using a statistical approach with selected independent parameters during fermentation process was also discussed. Response surface methodology (RSM) coupled with Box-Behken Design (BBD) were used in this study.

Secondly, the effect of extraction parameters (e.g., solvent concentration, extraction temperature, and mixing rate) on the production of antifungal agent was also carried out in order to obtain the optimum conditions for antifungal agent produced. Process optimizations using a statistical approach with selected independent parameters during extraction process was also discussed. Response surface methodology (RSM) coupled with Box-Behken Design (BBD) were used in this study. In order to describe the kinetics and mechanism of extraction process, several empirical models was simulated with the experimental data, such as parabolic diffusion model, power law model, Peleg's model, and Elovich's equation. The validity of each model was elucidated by the linear correlation coefficient (R^2), root mean square deviation (RMSD), and mean relative percentage deviation (P) value.

Thirdly, the production of antifungal agent was also studied in a bubble column bioreactor by employing one-factor-at-a-time (OFAT) method. Batch cultivations were characterized by constantly changing environmental conditions, such as aeration rate, temperature, and glucose concentration. Next, the kinetic studies of cell growth, product formation, substrate utilization, and substrate inhibition using bubble column bioreactor were carried out to evaluate the fermentation characteristics. The models were then tested and the results obtained for each parameter was compared. The validity of each model was clarified by the linear correlation coefficient (R^2), root mean square deviation (RMSD), and variance (σ) value.

Finally, the antifungal agent produced was applied onto the rubberwood block panel to study its effectiveness against selected wood-degrading fungi.

1.5 Organization of the thesis

There are five chapters in this thesis and each chapter describes the sequence of this research.

Chapter 1 presents the expansion of rubberwood in Malaysia and the existing preservation methods during industrial processing. The use of higher fungi as a source of antifungal agent is also described. This chapter also presents the problem statement, research objectives, scope of research and thesis organization.

Chapter 2 covers an overview of related knowledge of Malaysia's rubberwood industry and its limitation. This chapter also reports about the potential ability of filamentous fungi, the extraction method used for bioactive compound production and the bioreactor system for fungal fermentation.

Chapter 3 presents the materials and methods describing the experimental procedure in the research for antifungal agent production. This chapter also contains the analytical and characterization methods of sample.

Chapter 4 presents the results and discussion covering the experimental data and results obtained for screening of antifungal agent in shake flask culture. The optimization data with different parameters on mycelia growth and product formation are reported from the Design of Experiment. The empirical model on the extraction kinetics under optimized condition also been presented. This chapter also discusses the factors that influence the fungal growth and product formation in a bubble column bioreactor. The kinetic and modelling for growth, product formation, substrate utilization, and substrate inhibition are also highlighted. This chapter also covers the effectiveness study for the antifungal agent produced.

Chapter 5 presents the overall conclusions that were based on the findings obtained in this study (Chapter 4). Recommendations for future research are also given in this chapter.

CHAPTER 2

LITERATURE REVIEW

2.1 Development of rubberwood in Malaysia

2.1.1 Rubber plantation revenue

The rubber tree (*Hevea brasiliensis*), which belongs to the family Euphorbiaceae, is indigenous to the Amazon forests of Brazil and represents the major source of natural rubber in the world (De Vis *et al.*, 2006). In 1877, nine seedlings of rubber were planted behind the house of the British resident Sir Hugh Low in Kuala Kangsar, Perak, Peninsular Malaysia, and these are believed to be the oldest rubber trees in Malaysia (Kiam, 2002). Then, systematic breeding and selection works of rubber clones to improve productivity has been on-going process in the Malaysian Rubber Board for almost nine decades. Since it embarked on the process, six series of clones with a total of 185 clones had been developed and recommended to the industry (Malaysian Rubber Board, 2011). In 1980s, the Rubber Research Institute of Malaysia (RRIM) sent a delegation to Brazil to source for more materials to widen its clonal stock for breeding purposes. More than 20 clones of rubber trees were planted in Malaysia (Hong, 1999).

Initially, rubber trees were extensively grown for the production of natural rubber. Latex could be collected economically from a rubber tree for 25–30 years, with its production decreasing gradually; a considerable quantity of rubberwood is obtained during replantation (Akhter, 2005). A mature rubber tree (>25-years old) in Malaysia is about 30 m tall with an average branch-free bole of 3 m at breast height. The trees in Malaysian rubber plantations are much smaller in size and have been bred for the production of latex without considering the volume of wood produced (Hong, 1999). In 1991, the total area planted with rubber trees in Malaysia was reported to be 1,820,000 ha (Ismariah and Norini, 1999). However, the area of rubber trees under cultivation by estates and small holdings in Malaysia decreased from 1,389,000 ha in 2001 to 1,021,000 ha in the year 2009 (Table 2.1), a 26.4% decreased in the total area of rubber cultivation in Malaysia (FAO, 2010; Malaysian Rubber Board, 2011). This might have resulted from some estates converting to more profitable commodities such as oil palm. However, the future demand for rubberwood is expected to grow, particularly with the scarcity of indigenous timber species (Ismariah and Norini, 1999). Thus the criteria for breeding of rubber trees would in the future include those for production of wood in addition to latex (Hong, 1995; Killmann and Hong, 2000). Another factor that promotes rubber tree replanting is the market price of rubberwood. Smallholders of rubber plantations will demand the highest price possible, as rubber trees are worth RM1000–4000/m^3, depending on the quality and quantity of rubberwood as well as the locality of the holdings (Yahaya, 1998). Taking the economic life of rubber trees as 25 years, Yahaya (1998) estimated that rubberwood production could be up to 3,207,000 m^3 in 2012, of which 581,000 m^3 (18.12%) would be from estates and 2,626,000 m^3 (81.88%) would be from smallholdings.

There was a slight difference in the rubber tree plantation areas among the different parts of Malaysia (Table 2.2). From 2007 to 2009, the total planted area in Peninsular Malaysia decreased by 22.25%, while in Sabah and Sarawak the total planted area remained stable. This might be due to the availability of large tracts of land which are suitable for commercial agriculture in East Malaysia (Sabah and

Sarawak). In fact, to overcome the declining areas of plantation, management of sustainable forest plantation was also practiced. In tandem with the development, additional new planting areas of 0.25 billion m^2 in Sabah and 0.05 billion m^2 in Sarawak, established by government agencies under the Ninth Malaysia Plan, were reported to enable additional production of rubber and rubberwood (The Star, 2009). This stabilized the plantation areas from 2007 to 2010.

Table 2.1. Total rubber trees planted in Malaysia from 2001 to 2010 (FAO, 2010; Malaysian Rubber Board, 2011)

Year	Estates (billion m^2)	Small Holdings (billion m^2)	Grand Total (billion m^2)
2001	0.96	12.94	13.89
2002	0.85	12.64	13.49
2003	0.78	12.47	13.26
2004	0.64	12.14	12.79
2005	0.57	12.14	12.71
2006	0.54	12.09	12.64
2007	0.53	11.95	12.48
2008	0.61	11.86	12.47
2009	0.61	9.60	10.21
2010	0.62	9.67	10.29

Table 2.2. Planted area of rubber trees in Peninsular Malaysia, Sabah, and Sarawak from 2001 to 2010 (Malaysian Rubber Board, 2011)

Year	Peninsular Malaysia (billion m^2)	Sabah (billion m^2)	Sarawak (billion m^2)
2001	11.52	0.87	1.50
2002	11.39	0.63	1.47
2003	11.05	0.64	1.57
2004	10.57	0.65	1.57
2005	10.49	0.65	1.57
2006	10.43	0.65	1.57
2007	10.20	0.71	1.57
2008	10.19	0.71	1.57
2009	7.93	0.71	1.57
2010	7.75	0.94	1.59

2.1.2 Potential characteristics of rubberwood

Rubberwood (*H. brasiliensis*), like any other wood, is a lignocellulosic material, non-homogeneous in nature and orthotopic in structure. Its density is not uniform and its mechanical properties varied longitudinally, radially, and tangentially (Mohd Shukari, 1999). After 25 years, rubber trees normally had clear boles 3 to 10 m in height and a diameter of up to 50 cm at breast height (Lim and Ani, 1999). The structural elements within rubberwood consist of 61.5% fibers, 9.5% vessels, and 29.0% parenchyma cells. The fiber length varies from 1.10 to 1.78 mm, the fiber width is from 26 to 30 µm, and the cell wall thickness ranges from 5.1 to 7.0 µm. The wood is fine, straight-grained, and light yellowish to white in colour, similar to the civit (*Swintonia floribunda*) or champa (*Michelia champaca*), with an approximate specific gravity of 0.56 (Mohd Nor, 1999).

The bending properties, compressive and shear strength, and hardness of the rubberwood, as shown in Table 2.3, indicated that it had good overall woodworking and machining qualities for sawing, boring, turning, nailing, and glaring. In addition, its strength and mechanical properties are also suitable for use in furniture making (Mohd Shukari, 1999; Hong, 1995). According to Hong (1995) and Killmann (2001), timber with an air-dry density of 560–650 kg/m^3 is classified as medium-dense timber. As can be seen in Table 2.3, rubberwood (under air-dry seasoning conditions) has a density of 640 kg/m^3, which falls into the medium-dense timber category. Air-dried rubberwood, with a moisture content of 17.2%, has higher modulus of rupture (MOR) and modulus of elasticity (MOE) values at 66 N/mm^2 and 9240 N/mm^2, respectively, than green wood (Mohd Shukari, 1999). In addition, this medium-dense timber is suitable for wide application as it can easily be steam bent or stained to resemble any other timber (Gnanaharan and Dhamodaran, 1993; Nganthavee, 2002).

Table 2.3. Physical and mechanical properties of rubberwood (Mohd Shukari, 1999)

Properties	Seasoning Condition	
	Green	Air-dry
Moisture content (%)	52.0	17.2
Specific gravity (based on oven-dried weight and volume at test)	0.53	0.55
Density (kg/m^3)	800	640
Static bending		
Modulus of rupture, MOR (N/mm^2)	58	66
Modulus of elasticity, MOE (N/mm^2)	8800	9240
Comparison parallel to grain Maximum crushing strength (N/mm^2)	25.3	32.3
Comparison perpendicular to grain Stress at limit of proportionality (N/mm^2)	3.65	4.69
Side hardness		
Load to embed a 11.28mm diameter steel sphere to one half its diameter (N)	3030	4320
Shear parallel to grain Maximum shearing strength (N/mm^2)	9.0	11.0

Table 2.4 shows the comparative mean strength properties of rubberwood at different ages for the clone PB 260. The specific gravity increased with increasing tree age. This is in agreement with studies carried out by previous researchers (Lim, 1996; Lim and Ani, 1999), who stated that the specific gravity of the same clone (PB 260) tends to increase slightly with age. On the other hand, the MOR, MOE and the compression parallel to grain for this clone are not significantly different for different age groups, whereas the hardness, shear parallel to the grain, and the cleavage are significantly different at the 95% confidence limit between the different age groups. Nevertheless, the overall strength properties are higher in the older than in the younger trees for rubberwood clone PB 260, thus indicating that the old trees are hardier.

Table 2.4. Compressive strength properties of rubberwood clone PB 260 at different ages (Mohd Shukari, 1999)

Properties	Age group			Scheffe' Test
	3 yrs	8 yrs	14 yrs	
Moisture content (%)	13.63±1.18	15.30±0.66	14.58±1.67	n.s.
Specify gravity	0.56±0.02	0.57±0.03	0.58±0.02	n.s.
Modulus of rupture, MOR (N/mm^2)	81.01±5.00	84.74±9.65	81.28±5.70	s
Modulus of elasticity, MOE (N/mm^2)	370±810	8534±872	8564±1337	n.s.
Compression parallel to grain (N/mm^2)	33.04±2.13	33.19±4.21	33.55±2.67	n.s.
Hardness (N)	3849±258	4265±505	4187±226	s
Shear parallel to grain (N/mm^2)	11.46±0.62	13.19±1.41	12.48±0.93	s
Cleavage (N/mm width)	12.57±1.29	14.16±2.11	14.47±1.45	s

Note: ± refers to the corresponding standard deviations; s – significantly different at 95% confidence limit by Scheffe' Test; n.s. – not significant.

Fresh, sawn rubberwood is white to creamy in colour, sometimes having a pinkish tinge and weathering to a light straw or light brown colour (Lim and Ani, 1999; Killmann and Hong, 2000). The natural colour of rubberwood is one of the principal reasons why it is popular in Japan. All colours are distributed in a sphere known as the solid colour. Whitish wood is often preferred in many applications because it gives a clean and fresh impression, and the wood can easily be stained using dye or pigment (Minemura, 1999). Thus, rubberwood was increasingly used to replace more traditional timber (e.g., *Fagus* spp. and *Quercus* spp.) in a wide variety of applications. As a result, rubberwood became a good substitute for ramin (*Gonystylus bancanus* Baill) due to its favourable qualities and light colour (Killmann and Hong, 2000). Another added advantage of rubberwood is its good dimensional stability; its shrinkage or swelling rate is lower compared with that of other tropical species (Anthony, 1998). Extensive research and aggressive marketing have contributed to making rubberwood one of the most important export timbers and a substitute for light tropical hardwoods in the production of furniture and indoor building components.

Fresh rubberwood contains 1.0–2.3% free sugars and 7.5–10.2% starch, making it easily attacked by fungi and insects (Anthony, 1998). The free carbohydrate content has also created other problems in terms of the setting of cement in cement-bonded panels manufactured in Malaysia (Akhter *et al.*, 1994). However, this problem may be resolved by open-air storage of the chips, which reduces sugar and starch levels to 0.2% and 1.0%, respectively (Killmann and Hong, 2000).

2.1.3 Rubberwood-based industry

In the past 25 years, rubber trees have been planted for latex and timber purposes, particularly in Peninsular Malaysia, and the finished products of rubberwood have captured a lucrative export market. Currently, the Malaysian rubber industry produces a broad range of products from natural rubber to rubberwood-based products. For example, rubberwood has been established as a major wood product in several countries, particularly for the production of furniture, furniture components, and wood panel products, as well as for construction and decorative use (Balsiger *et al.*, 2000). In fact, a strong demand for rubberwood is based on sawn timber, which was reflected in the increase in exports from 95,700 m^3 in 1984, valued at RM29 million, to about 221,000 m^3, valued at RM98.7 million, in 1989, which is an increase of approximately 98% by value (Hong, 1995). As reported by Chan *et al.* (2005), in 2004 the rubber products sector contributed RM19.6 billion to the country’s export earnings, of which rubberwood products comprised RM6.5 billion. Table 2.5 showed the export value contributed by the Malaysian rubberwood sector from 2005 to 2009.

Table 2.5. Export value contribution of the Malaysian rubberwood subsector from 2005 to 2009 (Malaysian Rubber Board, 2011)

Product	Export Value Contribution of Rubberwood (RM million)					
	2005	2006	2007	2008	2009	2010*
Sawn timber	386.2	69.8	55.2	27.1	34.3	16.8
Furniture	4,665.3	5,127.4	5,331.9	5,536.9	4,998.6	1291.5
Mouldings	698.1	796.3	915.3	744.1	686.4	170.5
MDF	1,106.7	1,144.9	1,180.9	1,156.1	1,033.4	289.2
Chipboard	266.7	266.9	364.9	391.7	250.9	66.5
Builders' carpentry & joinery	116.1	102.7	101.8	100.5	98.8	22.4
Wooden frame	12.7	12.2	13.2	12.4	16.2	2.9
Total	7,251.8	7,520.2	7,963.2	7,968.8	7,117.8	1859.8

Note: *January to June

Due to this demand, in addition to new mills established solely for rubberwood processing, a number of traditional sawmills have converted to sawing exclusively rubberwood to maintain production capacity and minimize running cost. In 1993, there were 116 stationary and 26 mobile sawmills that processed only rubberwood (Killmann and Hong, 2000; Killmann, 2001). However, in 1994, there were more than 150 sawmills that processed only rubberwood (Hong, 1995). Meanwhile, there were a number of mobile mills that operated in plantations and smallholdings. Rubberwood became popular for use in several wood panel products. In 1999, Malaysia had medium-density fiberboard (MDF) mills with 13 production lines using primarily rubberwood (Killmann, 2001). For the production of MDF, rubberwood is usually the sole raw material, whereas chipboard usually uses either rubberwood or material from mixed-species groups (Norini, 2002).