DIVERSITY AND ANTIOXIDANT ACTIVITY OF
***Trametes* Fr. IN MALAYSIA**

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DISSERTATION SUBMITTED OF FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

FACULTY OF SCIENCE
UNIVERSITY MALAYA
KUALA LUMPUR

2010

UNIVERSITI MALAYA

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ABSTRACT

Research on antioxidant properties as a natural antioxidant, particularly in polypores, have been widely done since the past decade. It is known that antioxidant capacity is essential in protecting organisms and cells from damage brought on by oxidative which is believed to be the cause for ageing and degenerative diseases. In this study, biodiversity and antioxidant activity of Malaysian *Trametes* spp. which is from the group of polypores were investigated. Polypores were collected in Peninsular Malaysia forests from the year 2006 until 2007. The species were identified by taxonomic keys based on macromorphology and micromorphology analysis. *Trametes hirsuta, T. pocas*, *T. lactinea, T. menziezii* and *T. feei* were identified throughout the collection.

Methanolic and dichloromethane extracts of mycelia of *Trametes* spp. were analysed for antioxidant activity on 1,1-diphenyl-2-picrylhdrazyl (DPPH) radicals and reducing ability of CUPRAC assay. Furthermore, the total of phenolic content of methanol extracts of *Trametes* spp. was analysed and the relationship of the phenol contents with the antioxidant activity was observed. IC_{50} values of scavenging effect on DPPH radicals of methanolic extracts of *Trametes* spp. ranging from 7.296 to 27.324 mg/ml and dichloromethane extracts ranging from 36.2 to 66 mg/ml, respectively. The reducing ability of *Trametes* spp. ranging from 0.3457 to 0.5367 at 0.5 mg/ml. Total phenolic content of methanolic extracts of *Trametes* spp. ranging from 10.54 to 23.28 mg/g extract.

A good correlation was found between total phenolic content of *Trametes* spp. methanolic extracts on CUPRAC assay (r^2 = 0.9546) with antioxidant properties. However, a weak correlation was found between the scavenging effects on DPPH radicals of the methanolic extracts with the total phenolic content in *Trametes* spp. methanolic extracts (r^2 = 0.1332). These results show that both phenolic and non-

phenolic components were responsible for the total antioxidative activity of the *Trametes* spp. extracts. Therefore, *Trametes* spp. can be a natural source of antioxidants.

ABSTRAK

Kandungan antioksida dari kulat 'polypores' telah dikaji dengan meluasnya sejak sedekad yang lalu sebagai bahan antioksida semulajadi. Secara umum, kandungan antioksida diperlukan untuk melindungi organisma dan sel daripada rosak yang disebabkan oleh tindak balas oksidatif yang kemudiannya menyebabkan penuaan dan penyakit kanser. Dalam kajian ini, biodiversiti dan aktiviti antioksida spesis *Trametes* dari Malaysia yang mana adalah dari kumpulan kulat 'polypores' telah dijalankan. Kulat 'polypores' dikutip di hutan Semenanjung Malaysia dari tahun 2006 sehingga 2007. Pengecaman sepsis *Trametes* adalah berdasarkan analisis makromorfologi and mikromorfologi dengan menggunakan kekunci taksonomi. *Trametes hirsuta, T. pocas, T. lactinea, T. menziezii* and *T. feei* telah dikenalpasti melalui kutipan tersebut.

Ekstak metanol dan diklorometana dari miselia spesis *Trametes* dianalisa dari segi keupayaannya merencat radikal 1,1-diphenyl-2-picrylhdrazyl (DPPH) dan aktiviti penurunan menggunakan esei CUPRAC untuk menilai aktiviti antioksida. Seterusnya, jumlah kandungan fenol dalam ekstrak metanol dari spesis *Trametes* dianalisa dan hubungan di antara kandungan fenol dan aktiviti antioksida diperhatikan. Nilai IC_{50} aktiviti perencatan radikal DPPH dari ekstrak metanol spesis *Trametes* adalah dalam lingkungan 7.296 ke 27.324 mg/ml dan ekstrak diklorometana dari kadar 36.2 ke 66 mg/ml. Kadar aktiviti penurunan ekstrak methanol dari spesis *Trametes* adalah dari 0.3457 ke 0.5367 pada kepekatan 0.5 mg/ml. Jumlah kandungan fenol dalam ekstrak metanol spesis *Trametes* adalah dalam lingkungan 10.54 sehingga 23.28 mg/g ekstrak.

Korelasi yang bagus telah didapati di antara jumlah kandungan fenol dalam ekstrak metanol spesis *Trametes* dalam essei CUPRAC ($r^2 = 0.9546$) dengan aktiviti antioksida. Walaubagaimanapun, korelasi yang lemah didapati dalam kesan perencatan radikal DPPH ekstrak metanol spesis *Trametes* dengan jumlah kandungan fenol ($r^2 = 0.1332$). Keputusan ujian ini menunjukkan bahawa kedua-dua komponen fenol dan

bukan fenol terlibat dalam keseluruhan aktiviti antioksida spesis *Trametes*. Oleh itu, spesis *Trametes* berpotensi untuk dijadikan sumber antioksida semulajadi.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

ABTS	scavenging of the radical 2,2-azino-bis(3-ethylbenzo-thiazoline)-6 sulphuric acid
AO	reductant antioxidants
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
CAT	catalase
cm	centimeter
Cu^{2+}	copper ions
CuCl$_2$	cuprum chloride
Cu(II)-Nc	bis(neocuproine)copper(II) chloride
CUPRAC	Cupric ion reducing antioxidant capacity
DNA	Deoxyribonucleic acid
DPPH	1,1-Diphenyl-2-picrylhdrazyl
ET	electron transfer
Fig.	Figure
FRAP	Ferric reducing antioxidant properties
g	gram
GAE	gallic acid equivalents
GYMP	Glucose-yeast-malt-peptone
HAT	hydrogen atom transfer
IC$_{50}$	concentration providing 50% inhibition
KOH	Potassium hydroxide
L	liter
LDL	Low density lipoprotein
mg	milligram
ml	mill

mm	millimeter
Nc	Neocuproine
NH$_4$Ac	Ammonium acetate
nm	nanometer
OH˙	hydroxyl radical
ORAC	oxygen radicals absorbance capacity
PG	propyl gallate
PSK	polysaccharide-Krestin
PSP	polysaccharide-Peptide
ROS	reactive oxygen species
SOD	superoxide dismutase
TEAC	Trolox equivalent antioxidant capacity
TBHQ	tertiary butylhydroquinone
TPC	total phenolic contents
TRAP	total radical trapping antioxidant parameter
µg	microgram
µm	micrometer
β	beta
α	alpha
%	percentage

GLOSSARY OF MYCOLOGICAL TERMS

Aculei	having narrow spines.
Allantoid	slightly curved with rounded ends; sausage-like in form.
Agglutinated	fixed together as if with glue.
Applanate	flattened.
Basidiocarps	fruit body.
Basidiospores	a propagative cell.
Basidium	the cell or organ, diagnostic for basidiomycetes, from which, after karyogamy and meiosis, basidiospores (generally 4) are produced externally each on an extension (sterigma) of its wall.
Catahymenium	a hymenium in which hyphidia are the first-formed elements and the basidia embedded at various levels elongate to reach the surface and do not form a palisade.
Coralloid	much branched; like coral in form.
Coriaceous	like leather in texture.
Cystidia	a sterile body, frequently of distinctive shape, occurring at any surface of a basidioma, particularly the hymenium from which is frequently projects. Cystidia have been classified and name according to their origin, position, and form.
Decurrent	(of lamella), running down the stipe.
Dendrohyphida	irregularly strongly branched.
Dimidiate	shield-like; appearing to lack one half, without a stalk and semi-circular.
Effused-reflexed	stretched out over the substratum but turned up at the edge to make a pileus.
Ellipsoid	elliptical (oval) in optical section.
Endospore	the inner wall of a spore.
Flabelliform	like a fan, in the form of half-circle.
Glabrous	smooth, not hairy.
Globose	spherical or almost so.
Gregarious	in companies or groups but not joined together.

Hirsute	having long hairs.
Hispid	having hairs or bristles.
Hymenophore	a spore bearing structure.
Hymenium	a spore-bearing layer of a fruit body.
Hyphae (pl. hyphal)	one of the filaments of a mycelium.
Imbricate	partly covering one another like the tiles on a roof.
Lamellae	one of the characteristic hymenium-covered plates on the underside of the pileus; gill.
Lamellate	having lamellae.
Lunate	like a new moon; crescentic.
Perennial	living for a number of years.
Pileus	the hymenium-supporting part of the basidioma of non-resupinate.
Resupinate	flat on the substrate with the hymenium on the outer side.
Rhizomorph	a root-like aggregation of hyphae having a well-defined apical meristem (cf. mycelial cord) and frequently differentiated into a rind of small dark-coloured cells surrounding a central core of elongated coluorless cells.
Sessile	having no stem.
Stipe	a stalk.
Striate	marked with delicate lines, grooves or ridges.
Strigose	rough with sharp-pointed hairs; hispid.
Subglubose	not quite spherical.
Tomentose	having a covering of soft, matted hairs (a tomentum); downy.
Trama	the layer of hyphae in the central part of a lamella of an agaric, a spine of Hydnaceae, or the dissepiment between pores in a polypore.
Velutinate	thickly covered with delicate hairs, like velvet.
Ventricose	swelling out in the middle or at one side; inflated.

1.0   INTRODUCTION

Trametes is a genus from polypores group. Polypores are commonly known as conk or bracket fungi. They are a group of Basidiomycetes characterized by basidiocarps (fruit bodies) with hymenophore and basidiospores. Polypores are important members in the biodiversity of forest besides plants and animals. They usually occur as saprobes on logs, branches and other woody subsrata or pathogens on living trees. Myers (1980) reported on the alarming rate at which the tropical rainforest is disappearing. Based on the report mentioned, it is almost possible that fungi are extinct before they have been discovered and identified. Therefore, it is important to document the existence of microorganisms in the ecosystem before the habitat is lost forever.

Intensive studies on the systematics of Polypores have been published in Europe and North America (Gilbertson & Ryvarden, 1986, 1987; Ryvarden & Gilbertson, 1993, 1994), temperate and boreal East Asia (Li, 1991; Zhao & Zhang, 1992), in subtropical and tropical Asia Pacific (Corner, 1983; 1984; 1987; 1989a; 1989b; 1991; Chang, 2001) and recently in East Asia (Dai 2000; Núñez & Ryvarden, 2000, 2001). Cooke was the first mycologist who recorded various species from Peninsular Malaysia (Cooke 1883; 1884; 1885a, 1885b), followed by Chipp (1920), Thompson & Johnson (1953), Singh (1980) and Corner (1987, 1989a, 1989b, 1991). Recently, polypores in Malaysia were collected and catalogued by Salmiah (1997), Hattori (2000, 2001a, 2001b, 2003a, 2003b, 2005a); Hattori and Lee (2003); and Noraswati *et al.,* (2006). However, only a limited number of species were listed in Malaysia. This indicates that the taxonomy of the polypores is not widely known in Malaysia. The documentation of Malaysian fungal diversity was mostly studied by foreign mycologists, particularly as a part of the Commonwealth service (Jones *et al.,* 2007). Through the studies on the biodiversity of

mushrooms in the Malaysian forest, this will definitely expose young scientist to learn about the taxonomy and systematic of Polypores including *Trametes*.

Antioxidant compounds from mushroom have been widely used in natural products to replace the synthetic antioxidant due to their health risk and toxicity (Stone *et al*., 2003). Synthetic antioxidant such as BHA and BHT were found to have tumor-initiating as well as tumor-promoting action (Botterweck *et al.,* 2000). Antioxidant compound are able to reduce oxidative damage such as that caused by free radicals. Free radicals are produced in normal or pathological cell metabolism. However, the uncontrolled production of oxygen-derived free radicals can caused many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with ageing (Halliwell and Gutteridge, 2003).

Traditionally, mushrooms are collected from the forest by the indigenous communities and consumed as food (Lee *et al*., 2006) and medicines (Chang *et al.,* 2006). Edible mushrooms are alleged to exhibit significant antioxidant activity such as *Lentinula edodes* (Yang *et al.,* 2002), *Auricularia polytricha* (Chen *et al.,* 2003), *Pleurotus citrinopileatus* (Lee *et al.,* 2007), *Agaricus blezei* (Tsai *et al.,* 2006) and *A. biporus* (Elmastas *et al.,* 2007). Wild mushrooms which are widely found in the Peninsular Malaysia, namely *Marasmius berambutanus, M. ruforotula* and *M. guyanensis,* are proved to have antioxidant properties (Tan, 2007). Another species of wild mushroom that also exhibits antioxidant activity is *Lentinus squarrosulus* (Siti Fauziah, 2005).

Antioxidant properties of medicinal mushrooms such as *Trametes versicolar* (turkey tail) (Wasser (2002); *Ganoderma tsugae* (reishi) *G. lucidum, Dictyophora indusiata* (basket stinkhorn), *Grifola frondosa* (maitake), *Hericium erinaceus* (lion's mane) (Mau *et al*., (2002a,b & 2005); *Inonotus obliquus* (Cui, 2005); *Antrodia camphorate* (Chang chih) (Huang & Mau, 2007); and

Phellinus linteus (Nakamura *et al*., 2004) have been studied. These mushrooms were found to be medically active in several therapeutic effects such as antitumor, immunomodulating and chronic bronchitis (Wasser and Weis, 1999). The genus *Trametes* is a potential source of antioxidant properties as shown by a medicinal mushroom, *T. versicolor* (Yang *et al,* 1992; Kariya *et al.,* 1992 and Ng 1998).

The objectives of this study are:

   a. to study the taxonomy and biodiversity of *Trametes* in the Peninsular Malaysia.

   b. to screen the antioxidant activity of *Trametes* species collected.

2.0   LITERATURE REVIEW

2.1   Life cycle and ecology of *Trametes*

      Trametes are filamentous fungi composed of hyphae. The compatible strains are brought together by somatogamy (the fusion of vegetative cells which are not sexually differentiated) with the subsequent formation of dikaryotic hyphae (hyphae with 2 unfused, compatible nuclei in each cell). Fusion of the compatible nuclei (i.e., karyogamy) and a subsequent nuclear reductional division (i.e., meiosis) take place in a club-shaped cell called a basidium. Through the process of meiosis, the haploid nuclei will move out through short stalks (called sterigmata) on the basidium into the developing spores, which are known as basidiospores. The basidia are located on fertile hymenial layer which constitute a part of the fruiting body which also known as basidiocarp. The basidiocarp consists of many different forms. The classification within the *Trametes* species is based largely upon basidiocarp characteristic. Basidiospores can be discharged in the mid-region of the pore. Gravity will play its role when the basidiospores fall free from the basidiocarp. Then, it is disseminated widely through air. When the basidiospores have been disseminated, it has to germinate in order to fulfill its reproduction function.

Trametes are believed to be not host specific in the tropics (Hattori *et al.,* 2007). *Trametes* are usually found on logs, small branches and twigs. They are white rot species which degrade all of the major wood components (cellulose, hemicellulose, and lignin), leaving white residue after the degradation of wood (Wong and Wikes 1988). Most of the *Trametes* can only be found in an open area of secondary or primary forest which provided enough moisture, temperature, light, and aeration for growth. Corner (1989) has recorded a number of *Trametes* from the highland areas of Mount Kinabalu, Sabah but many of them can also be found in the lowland area of Peninsular Malaysia.

Ryvarden (1991) identified the genus *Trametes* as a cosmopolitan genus which is known from all main continents and all major climatic zones (i.e. boreal, temperate, subtropical and tropical). The growth and development of *Trametes* are influenced by the nutritional factors from woods and physical factors such as temperature, moisture, light, aeration, and gravity.

2.2 Morphology of Polypores

2.2.1 Macromorphology characters

Macromorphology characters can be seen via naked eye. This is an important character used for the classification of polypores.

a) *Type of basidiocarps*

The common types of basidiocarps are flabelliform, central stipitate, laterally stipitate and dimidiate shape as shown in Fig 2.0. However, there are some species that may develop from resupinate to effused-reflexed or distinctly pileate basidiocarps which may occur on the same log as in *Earliella scarbosa*.

b) *Attachment of basidiocarps*

A pileate basidiocarp broadly sessile or may taper at the base with a stipe. There may be transitions from sessile to stipitate or resupinate to effused-reflex in some species while others are consistent. The different types of attachment are imbricate, resupinate, sessile, and effused-reflex as shown in Fig. 2.1.

c) *Consistency*

Hyphal structure can be determined in the field based on the consistency of the fresh basidiocarps. Perennial basidiocarps with a dominance of skeletal hyphae are normally woody, while the trimitic ones will be tough and difficult to tear apart. Monomitic is soft and very sappy and it may shrink considerably during drying. This is often accompanied by a colour change.
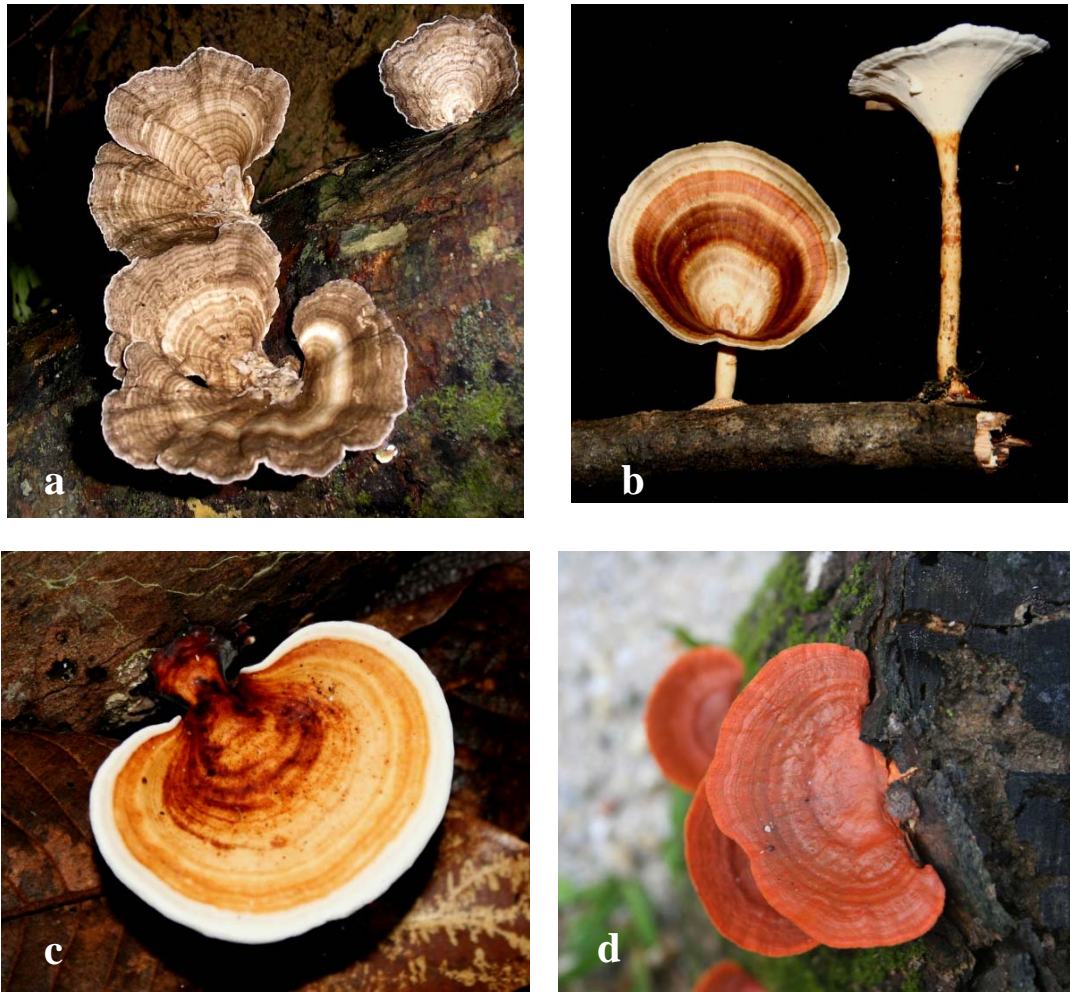
Fig. 2.0: Types of basidiocarp. a) Flabelliform shape in *Trametes menziezii* b) Central stipitate in *Microporus xanthopus* c) Laterally stipitate in *Microporus affinis* d) Dimidiate shape in *Pycnoporus sanguineus.*

d)   ***Construction of the basidiocarps***

     i)   ***Pilear surface***

In many species, the pileus has a distinct colour. The colour is apparently due to complex polyphenols which are very resistant to degradation by natural causes. In other species, the colour may fade because of the weather which has destroyed the pigments on the pilear surface. As an example, *P. sanguineus* is a species with brilliant reddish basidiocarp. White and light coloured species frequently become darker with age as the upper layer of hyphae collapse and become darker; ochraceous to brownish shades, resulting in a distinct zonation from the base to the margin where new hyphae are actively developing. This is typical in perennial basidiocarps. The pilear surface may

be glabrous or covered with hairs of different type. When glabrous the surface may be dull or shiny, in some cases with or without zonation, radial lines or raised ridges.

ii) *Margin*

In species with resupinate basidiocarps, the margin and its development might be an important character. Most species have more or less continuous margin with white or light colour. There are species which developed strands of hyphae or rhizomorphs. For other species, the basidiocarps often have a tendency to curl up and loosen along the margin on drying.

iii) *Stipe*

The consistency and colour of the stipe will normally be the pileus. However, in a few species, the stipe might be differently coloured. The tubes can be more or less decurrent on the stipes. For example, *Polyporus* species has the same width from the base to pileus. Meanwhile, in a few other species, more than one pileus may develop from common stipe or elongated base. There are also species which an applanate basidiocarp is a fan-shaped. However, for other species which are tapering towards the base, it may be difficult to decide whether a true lateral stipe is present or not. But if there is a distinct of non-poroid areas on the lower side of the base, close to the attachment, it is a good indication that a lateral stipe is present.

iv) *Pore surface*

The pore colour, shape and size are important characters to identify the species name. In some cases, the pore surface is bruised and becomes darker when touched. Pore shapes may be round or angular and sometimes the pores become irregular, sinuous to labyrinthine. It is important to measure the pores in terms of numbers per mm or cm. Depending on the species, the pores can almost be invisible to the naked eye or sometimes easy to see.

Fig. 2.1: Types of attachment of basidiocarp. a) Imbricate in *Rigidoporus microporus,* b) Resupinate in *Rigidoporus vinctus*, c) Sessile in *Coriolopsis retropicta*, d) Effused-reflex in *Earliella scarbosa*

v)   *Tubes and context*

In most species, the tubes are more or less concolorous with the pore surface but may become paler than the pore surface with age. In many species, there are no apparent differences either in colour or consistency between the tubes and the context. However, in other species, the tubes may have different colour and consistency. These differences may be an important taxonomic character for a particular species.

2.2.2   Micromorphology characters

Micromorphology characters are invisible to the naked eye. The microscopic characters of polypore are described comprehensively by Ryvarden (1991).

a)   Hyphae

The basidiocarp may consist of generative, skeletal and binding hyphae as shown in Fig. 2.2. Generative hyphae are the basic units of structure since they are always present in a basidiocarp. Vegetative or secondary hyphae may arise from the generative hyphae. The type of septation in the generative hyphae is a very important character in the classification of polypores. The septum between two hyphal cells can either be simple or a result from the formation of a clamp, but in rare cases, it can be from the formation of several clamps. Skeletal hyphae are normally unbranched and thick-walled to solid. Skeletal hyphae will have an even diameter throughout most of its length and no clamps or simple septa. Meanwhile, binding hyphae is branched, solid to very thick-walled and it is a non-septate hypae which often have more randomly oriented growth than skeletal hypae.

Hyphal System

The major microscopic characteristic separating the genera is the type of hyphal system, which can be monomitic, dimitic, or trimitic. Monomitic species have only septate generative hyphae (Fig. 2.2 a). Then, dimitic-skeletal species have both septate generative hyphae and a thick-walled of non-septate skeletal hyphae (Fig.2.2 a & b). These provide a hard structure found in many polypores. This can be seen in *Ganoderma tsugae*. Unlike dimitic-skeletal, dimitic-binding species have septate generative hyphae and branching binding hyphae (Fig. 2.2 a & c) which are responsible for holding the other hyphae together (e.g. *Laetiporus sulphureus*). Meanwhile, trimitic species have septate generative hyphae, skeletal hyphae and branching binding hyphae (Fig. 2.2 a, b & c). A good example of trimitic species is *Trametes* species.

b)   Basidiospores

Basidiospores are important for classification of the Basidiomycetes, and also for the polypores. The spore size of a few basidiospores may be larger or smaller than

the range given for a particular species in the taxonomy keys. Melzer's solution is used to observe the dextrinoid or amyloid reaction. When mounted in Melzer's solution, the basidiospores of some species change its colour from pale yellowish to strongly reddish brown (dextrinoid reaction) or grayish-bluish (amyloid reaction). For ornamented basidiospores, the measurements include the spines or aculei. The shape of the basidiospores are warted, oblong ellipsoid, globose, broadly ellipsoid, cylindric, lunate, and allantoid as shown in Fig. 2.3. Basidiospores of most polypores are smooth and thin-walled, but in a few genera they are ornamented with small warts, spines or longitudal striate. In the *Ganodermatacea*, all basidiospores have a thick yellowish to pale brown endospore, on which there is a regular pattern of warts or protuberances, and thus, making the basidiospores of this family very distinctive.

c)   **Cystidia and other sterile hymenial organs**

In many polypores, sterile organs occur among the basidia and it is important in delimiting genera and identifying specimens. Cystidia may be divided into two groups according to where they arise. Hymenial cystidia arise in the subhymenium whereas tramal cystidia arise in the trama (Fig. 2.4). Hymenial cystidia are usually of the same size or slightly larger than basidia. They can be thick to thin-walled. Sometimes, they are smooth while others have a crown of crystals or may even be encrusted for a considerable length. Several shapes of cystidia are thin-walled and apically encrusted; thick-walled and apically encrusted; smooth, tubular, ventricose and apically coarsely encrusted as shown in Fig. 2.5. Tramal cystidia arises as the name indicates, in the trama, and may or may not bend into the hymenium, often far beyond it. Usually they are the outer ends of skeletal hyphae with a widened strongly encrusted apex. Sometimes, such cystidia also occurs in the dissepiments or deeply embedded in the trama. They are often called skeletocystidia.
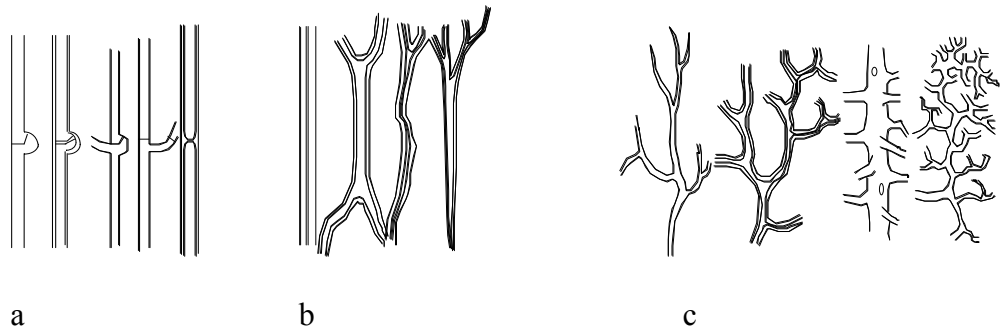
Fig 2.2: Types of hyphae. a) Generative hyphae, b) Skeletal hyphae, c) Binding hyphae. (Source: Hattori, unpubl.)
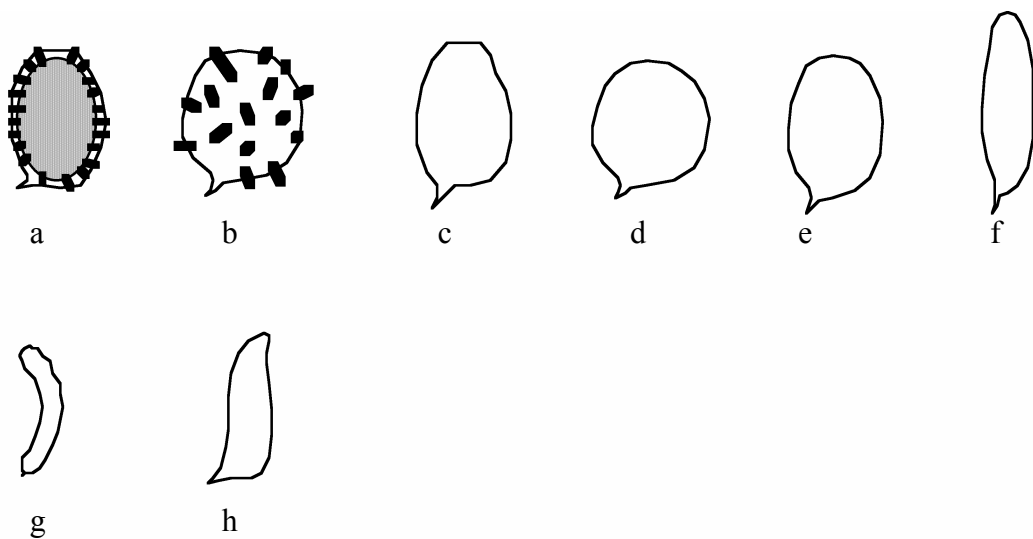
Fig 2.3: Types of basidiospores; a) With double wall (ganodermoid), b) Warted, c) Oblong ellipsoid, d) Globose, e) Broadly ellipsoid, f) Cylindric, g) Lunate, h) Allantoid. (Source: Hattori, unpubl.)

d)   Setae and setae hyphae

Setae and setae hyphae are confined to the Hymenochaetacae. They are dark brown, thick-walled, and these are important elements when they occur. They are hymenial and tramal setae. The setae are either subulate (i.e. they are the thickest at the base or taper evenly towards the top), or ventricose (i.e. with swollen part above the base and then more abruptly constricted at the top). In most species, the shape of the setae is fairly constant, while others, there may be a mixture of subulate and ventricose ones. Thus, a clear distinction might be difficult to draw. The shape of Setae hyphae is

often straight and run parallel to the tubes, but in some species, the apex is bent into and

beyond the hymenium. Setae hyphae may also present in the context. In some species,

branched setal hyphae may occur on the pilear surface.
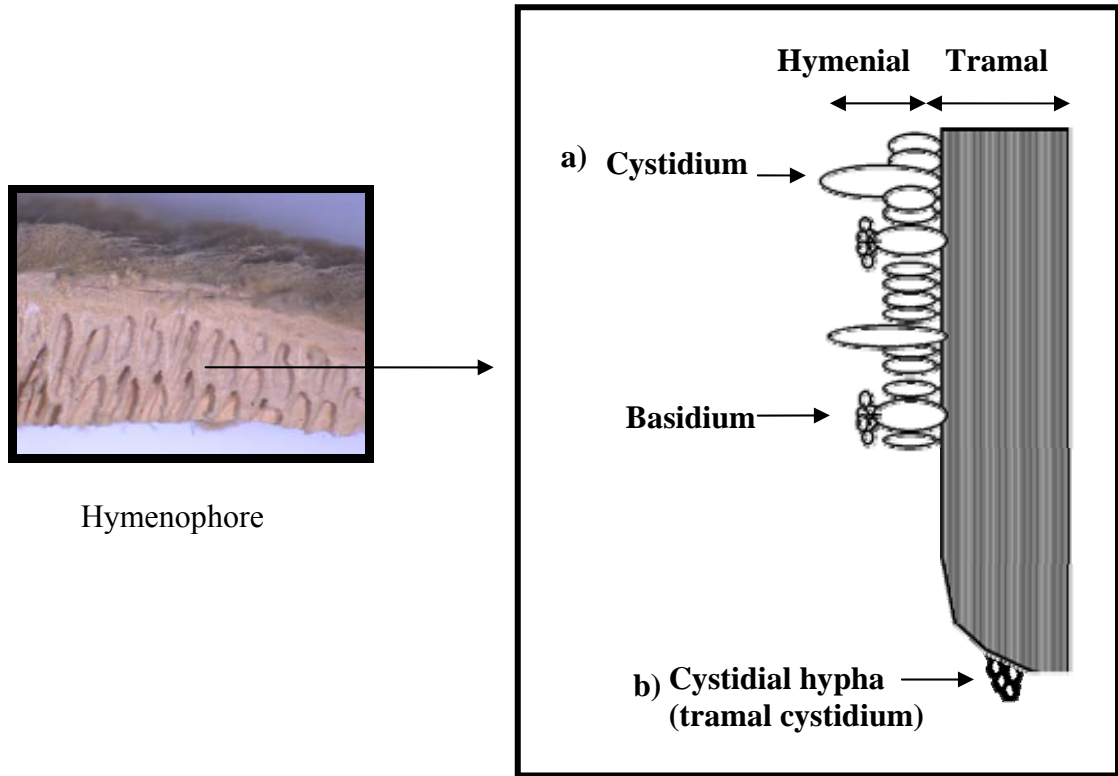
Hymenophore

Fig. 2.4: Positions of cystidia in Polypores a) Cystidia in hymenial and b) Cystidia in tramal of hymenophore. (Source: Hattori, unpubl.)
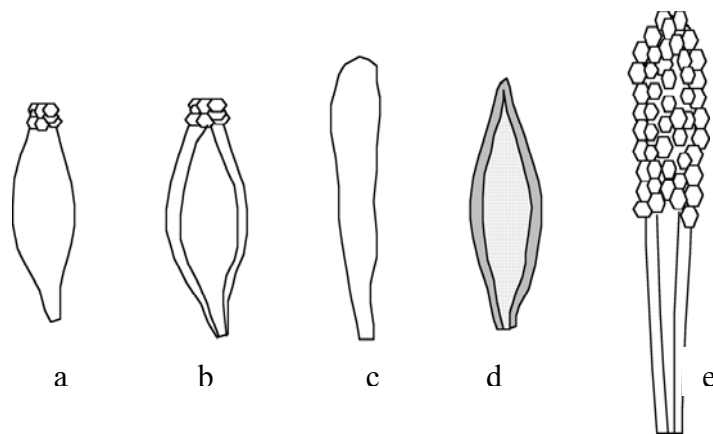
Fig 2.5: Types of cystidia; a) Thin-walled, apically encrusted,   b)Thick-walled, apically encrusted, c) Smooth, tubular, d) Ventricose, e) Apically coarsely encrusted. (Source: Hattori, unpubl.)

2.3   History of polypore nomenclature

Murill (1903) was the first to make a survey of the genera in the polyporaceae. Numerous discussion and debates on their typification and nomenclature had encouraged Cooke (1940, 1953 and 1959) to publish three successive lists of polypore genera. At the same time, Imazeki (1943) made an attempt to clarify the typification of the polypore genera represented in Japan and made the typification of some genera. A great contribution towards the stabilization of the nomenclature and typification was done by Donk (1960). However, Persoon (1801) and Fries (1821, 1828) had a wide generic concepts and used only a relatively restricted number of genera. In order to avoid too many changes, several generic names have been proposed for conservation by Cannon and Hawksworth (1983), Hawksworth (1984) and Ryvarden (1987).

2.4   Generic concepts

Fries used the hymenophore as the basic character. Based on their characteristics, all species with a poroid hymenophore were placed in *Polyporus* meanwhile those with a hydnoid in *Hydnum*. When the microscope was introduced as an aid in fungal classification, a new range of characters was put into use. Patouillard (1900) was the first to use these characters systematically. Microscopical information and together with chemistry, cytology and genetics were introduced both at the generic and specific levels. A survey of characters which have been used in classification of polypore genera was presented after a discussion of the generic concepts. Moreover, there are evolutions in Bondartzev-Singer-Imazeki era which was based on basidiocarp type, context, spores, and cystidia. Recently, Ryvarden-Gilbertson (1984) has established the modern generic classification based on hyphal systems, amyloidity-dextrinoidity, clamp connections, and decay type.

Ryvarden (1991) has completed the classification of all genera of polypores. The following families are accepted in this mycota:

HETEROBASIDIOMYCETES

Tramellaceae: *Elmerina, Protomerulius.*

HOMOBASIDIOMYCETES

Albatrellaceae: *Albatrellus.*

Agaricaceae: *Panellus, Porodisculus.*

Bondarzewiaceae: *Bondarzewia.*

Corticiaceae: *Byssoporia, Echinoporia, Gloeoporus, Grammothele, Poriodontia, Schizopora, Sistotrema, Trechispora, Theleporus.*

Cyphellaceae: *Stromatoscypha, Stigmatolemma.*

Echinodontiaceae: *Echinodontium.*

Fistulinaceae: *Fistulina.*

Ganodermataceae: *Amauroderma, Ganoderma.*

Hericiaceae: *Anomoporia, Heterobasidion, Wrightoporia.*

Hymenochaetaceae: *Coltricia, Coltriciella, Cyclomyces, Inonotus, Phellinus, Phylloporia, Pyrrhoderma.*

Polyporaceae: *Abortiporus, Amylocystis, Antrodia, Antrodiella, Auriporia, Bjerkandera, Ceriporia, Ceriporiopsis, Cerrena, Chaetoporellus, Climacocystis, Coriolopsis, Cryptoporus, Daedalea, Daedaleopsis, Datronia, Dichomitus, Diplomitoporus, Donkiporia, Earliella, Echinochaete, Flabellophora, Fomes, Fomitopsis, Gloeophyllum, Grifola, Hapalopilus, Haploporus, Heterobasidion, Hexagonia, Irpex, Ischnoderma, Jahnoporus, Junghuhnia, Laccocephalum, Laetiporus, Lenzites, Leptoporus, Leucophellinus, Lignosus, Megasporoporia, Melanoporia, Meripilus, Microporellus, Microporus, Nigrofomes, Nigroporus, Oligoporus, Oxyporus, Pachykytospora, Paratrichaptum, Parmastomyces, Perenniporia, Phaeolus,*

Physisporinus, Piloporia, Piptoporus, Polyporus, Pseudofavolus, Pycnoporellus, Pycnoporus, Pyrofomes, Rigidoporus, Skeletocutis, Spongipellis, Tinctoporellus, Trametes, Trichaptum, Tyromyces.

Thelephoraceae: *Boletopsis.*

2.5   The genus of *Trametes*

Cerrena, Coriolopsis, Cryptoporus, Daedaleopsis, Datronia, Earliella, Elmerina, Fomitella, Hexagonia, Lenzites, Megasporoporia, Microporus, Mollicarpus, Pycnoporus, Trametes and *Trichaptum* are put in synonym with *Trametes* by Corner (1989b).

Trametes group is characterized by a di-trimitic hyphal system, generative hyphae with clamps. It has binding hyphae which are usually of the tortuous type. The spores are hyaline, thin-walled, mostly cylindric, smooth and non-amyloid. The true hymenial cystidia are absent, tetrapolarity and they are growing almost exclusively on angiosperms. The species in this group produce mostly thin and pliable basidiocarps which are resistance from insects and desiccation for many weeks. This strategy and the apparent efficient spore dispersal may account for the widespread distribution of species in this group. Therefore, the genera are classified as cosmopolitan species. The descriptions of genera synonym with *Trametes* are as follow:

i)   *Cerrena* S.F.Gray

Corner (1989) placed *Cerrena* in synonymy with *Trametes* because of the similarity of the hyphal system. Gilbertson & Ryvarden (1986), Cunningham (1965) and Corner (1989) stated *Cerrena* is trimitic in its hyphal configuration. However, Westhuizen (1963) pointed out that Cerrena is bipolar, while *Trametes* is tetrapolar. The spores of *C. unicolor* are ellipsoid in contrast to the allantoid to cylindrical spores of most *Trametes* species. There are exceptions like in the tropical *T. cingulata* Berk.

which has exactly the same type of spores as in *C. unicolor*. *Cerrena* is a monotypic boreal genus. This genera is circumpolar in the northen boreal temperate zone.

ii)   *Coriolopsis* Murr.

The genus is closely related to *Trametes*. The basic character separating the two genera is the coloured hyphae of *Coriolopsis* which changes the basidiocarps from pale to deep brown colour. Their type of rot and nuclear behaviour are identical and Corner (1989) has placed the genus in synonym with *Trametes*. Ryvarden (1991) has accepted the pigmentation as a generic character. Therefore, *Coriolopsis* was accepted as a separate genus from *Trametes* but more likely and close relationship with *Trametes*.

iii)   *Cryptoporus* (Pk.) Shear.

Its trimitic hyphal system and cylindrical spores clearly suggest a close relationship to *Trametes*. The genus is unique by its volva covering, except for a small hole and the pore surface. *Cryptoporus* is an American-Asian genera.

iv)   *Daedaleopsis* Schroet.

Corner (1989) has placed the genus in synonym with *Trametes*. The genus is characterized by its pale coloured context, the long cylindrical spores and the dendrohyphida along the dissepiments. Its closest relative seems to be *Datronia* which has a very similar microstructure and rot, but has duplex basidiomes of a much darker colour. The genus is distributed at a temperate to boreal zone as circumpolar genera.

v)   *Datronia* Donk.

The genus is characterized by dark brown, usually duplex and rather thin basidiomes with a dimitic hyphal system composed of clamped generative hyphae and coloured skeletal hyphae. The spores are cylindrical and rather long (usually around 10um) and dendrohyphidia are present in the dissepiment in some species. The rot is white. Corner (1989) has placed the genus in synonym with *Trametes*. Therefore, *Datronia* is a cosmopolitan genera.

vi)   *Earliella* Murr.

The genus is related to *Trametes,* sharing the same type of hyphal system and spores. The basidiocarp, however, is deviating as it is frequently resupinate to effused-reflexed and develops a reddish cuticle on the pileus. Because of that, it is easily recognized in the field. Therefore, *Earliella* is pantropical genera.

vii)   *Elmerina* Bres.

The taxonomic position of the genus is uncertain as it resembles several genera in a number of characters. *Tyromyces* appears to be the closest as to hyphal structure, but representatives of this genus have normally thin dissepiments and smaller pores and without hyphal pegs. The hyphal pegs in the type of *Elmerina,* has the thick pore walls and the hymenophore which varies from large angular pores to lamellae point to a relationship with *Hexagonia* and *Trametes.* However, in these genera typical tortuous binding hyphae are present, a feature totally absent from all representatives of *Elmerina.* This genera   (has been widely spread) endemic in the tropical Asia as Asian genera.

viii)   *Fomitella* Murr.

Fomitella is a genus which is difficult to characterize. Through the hard and perennial basidiocarps, it closely resembles *Fomitopsis*, which however produces a brown rot, while the *Formitella* is white. Therefore, type of rot is very fundamental phylogenetically to keep the two genera separate. *Trametes* which has white rot, a similar hyphal system and spores as in *Fomitella,* is separated mainly by its annual, and mostly flexible to coriaceous basidiomes. In the field, the reddish cuticle which spreads from the base of the upper surface of the pileus, the woody consistency and the white rot, immediately reveal the generic identity. This genus is neotropical genera which is endemic to the tropical America.

ix)   *Hexagonia* Fr.

Hexagonia Fr. (1835) is a homonym of *Hexagonia* Poll. (1818). Thus, *Hexagonia* Fr. becomes illegitimate and *Hexagonia* Poll. disappear as a synonym of *Polyporus* Fr. Corner (1989) has placed the genus of *Hexagonia* Fr. in synonym with *Trametes*. The trimitic hyphal system with coloured skeletal hyphae and cylindrical spores suggest a close relationship to *Trametes*. There are difficulties in drawing a sharp borderline with some *Coriolopsis* species, but normally they are of a lighter colour and have shorter spores. *Hexagonia* is a pantropical genus.

x)   *Lenzites* Fr.

The genus is close to *Trametes* and as stated above basidiocarps of *L. betulina* and *T. hirsuta* are almost indistinguishable, and their microscopical characters are almost identical. However, the lamellate hymenophore and the distinct catahymenium which have pointed hyphal ends make *Lenzites* a distinct genus. There are some tropical species which have been transferred from the genus *Trametes* to the genus *Lenzites* because of their lamellate hymenophore.

xi)   *Megasporoporia* Fyv. & Wright

The genus is characterized by its large spores, resupinate basidiocarps and the dextrinoid skeletal hyphae. The closest relative is *Dichomitus* Reid which however has a non-dextrinoid binding hyphae. Corner (1989) has placed the genus of *Megasporoporia* in synonymy with *Trametes*. Thus, *Megasporoporia* is known as a pantropical genus.

xii)   *Microporus* Beauv.

The genus is highly characteristic by its stipitate basidiocarps, small cylindric spores and the crowded coralloid vegetative crystals. They may be interpreted as solid dendrohyphidia. Such organs are typical in genera of specialized habitats where long dry periods are the norm. *Microporus* is typically a genus of open sunny localities with

species like *Schizophyllum commune, Lentinus* sp. and *Coriolopsis polyzona* where they often occupy dry trunks. *Microporus* is closely related to *Polyporus* and it is seen as an evolutionary advanced staged adapted to dry areas. Corner (1989) has placed the genus in synonymy with *Trametes,* thus violating the principle of priority since *Microporus* is an older name than *Trametes.*

xiii)   *Mollicarpus* Ginns.

The genus related to *Coriolopsis* but is separated by the small subglubose spores and dextrinoid binding hyphae. As suggested by the name, the basidiocarps is of light weight and has a distinct duplex consistency. The latter is however of limited taxonomic weight as such duplex consistency and is found in many genera like *Trametes, Cerrena, Phellinus* and *Inonotus.*

xiv)   *Pycnoporus* Karst.

Pycnoporus is similar to *Trametes* in all characters except the bright reddish-orange colour. As colour is used, to a certain extent, as a pragmatic generic character, the genus is accepted. Corner (1987) merged it with *Trametes. Pycnoporus* was recognized as cosmopolitan genus.

xv)   *Trametes* Fr.

The generic concept is mainly based on the pileate basidiocarps, the trimitic hyphal system and the thin walled, IKI-spores. *Coriolus* is the thin basidiocarps with an indistinct black thin line between tomentum and the context of no taxonomic significance at the generic level. Duplex basidicarps may be found in many species, and in the genus there are all transitions between the thin and thick basidiocarps. *Lenzites* is very closely related. In principles, it is only separated by the distinct lamellate hymenophore. The pointed hyphal ends can be seen in *L. betulina,* the type species are also seen in *T. cubencis*, a species with small regular pores. As to the construction of

basidiocarps which inclusive of the pileus tomentum, spores and hyphal system, *L. betulina* comes close to *T. hirsuta.*

Coriolopsis Murr. is accepted as a separate genus, mainly based on its distinctly tinted skeletal hyphae which give the basidiocarps a general brown colour. This may be a character of a dubious value at the generic level, but is accepted as long as more detailed knowledge about genetics – cultural characters, sexuality and etc – are not known for most species including in the trimitic species complex. Corner (1989b) has a detailed discussion on all aspects of the genus and gives it a very wide circumscription neglecting characters such as the type of basidiocarp, type of rot and any reaction of hyphae in Melzer's reagent. All genera with trimitic hyphal system are placed in *Trametes*. This concept has some serious nomenclaturial consequences as three (*Coriolus, Lenzites* and *Coriolopsis*) of the genera which Corner places in synonymy of *Trametes,* have priority above that genus. In Corner's system, the valid and legitimate name of the trimitic group would be *Microporus* Beauv. 1805. However, Corner proposed to transfer *Microporus* to protect *Trametes* as the prior genus.

xvi)   *Trichaptum*

The genus is characterized by the purplish to violet pore surface in actively growing specimens, paling to buff or pale brown with age on drying. Microscopically the dimitic hyphal system, the cylindric spores and the cystidia are diagnostic. So far, only this feature has been demonstrated in polyporoid fungi in the Hymenochaetaceae and in some Heterobasidiomycetes. Its phylogenetic significance is unclear. Corner (1987) has a key to all species in the genus *Trichaptum* while Alexander *et al*., (1989) has discussed the genus in connection with a detailed study of *Trichaptum sector* (Ehrenb.:Fr) Kreisel. *Trichaptum* was classified as a cosmopolitan genus.

2.5.1 Classification of Trametes using taxonomy key

Key to families of Polyporaceae (Ryvarden & Johansen, 1980)

Fruitbodies white, cream, red, brown to black and generative hyphae are mostly with clamps. However, in some genera with simple septa, mostly appear without a black reaction with KOH. If monomitic without brown generative hyphae appears, setae absent.

Key to genera of Trametes (Ryvarden & Johansen, 1980) are as follows:

1. Spores & hyphae, non amyloid & non dextrinoid

2. Fruitbodies resupinate to pileate and then broadly attached or with a tapering lateral base

3. Spores smooth, fruitbodies resupinate to pileate

4. Generative hyphae with clamps

5. Spores thin-walled, globose to allantoid

6. Context white, cream to ochraceous

7. Hymenophore poroid

8. Cystidia absent

9. Hyphal system di-trimitic

10. Hyphal system trimitic, fruitbodies annual to perennial, pileate

11. Fruitbodies annual to biennial, crust absent or present

12. Fruitbodies broadly attached, dimidiate to conchate, usually no distinct mycelial disc at the attachment, coralloid element absent

13. Pileus smooth or tomentose to hirsute, tomentum less than 4 mm thick at the base, fruitbodies white, cream to pink

However, many of the species in the genera of *Trametes* proposed by Ryvarden & Johansen (1980) has undergone into other genera since the evolution name by generic concepts in a recent year. For the time being, the generic concepts and the key to the

genera of *Trametes* given by Núñez & Ryvarden (2001) are the most comprehensive and widely used as follows:

***Key to genera of Trametes* (Núñez & Ryvarden, 2001)**

Basidiocarps annual to biennial, pileate, sessile, dimidiate to flabelliform, single or imbricate, flexible to hard; pilear surface hispid to glabrous, often zonate; pore surface white, cream to pale grey; context white to isabelline, homogeneous or duplex, in some species with a dark line; hyphal system trimitic; generative hyphae with clamps and hyaline; skeletal hyphae thick-walled to solid, hyaline; in some species swelling in KOH; binding hyphae tortuous, solid, hyaline; in many species arboriform hyphae are present instead of skeletal and binding hyphae; cystidia lacking; basidiospores ellipsoid to allantoid, hyaline, thin-walled and negative in Melzer's reagent. This causes white rot in hardwoods, rarely on conifers. Based on the characteristics, it reflects the Cosmopolitan genus with common and widespread species.

2.5.2   Diversity of *Trametes* in Peninsular Malaysia

There are a number of references on Malaysian *Trametes* which date as early as 1921 by Chipp. The most detailed account of the *Trametes* in Peninsular Malaysia is that of the Corner in a series of articles and books (Corner, 1983-1991). Meanwhile, other significant references are Kuthubutheen (1981), Watling (1994), Lee *et. al.* (1995), Salmiah (1997) and Hattori (unpubl.). Table 2.0 is the lists of *Trametes* collected in Malaysia which includes the Corner's collection from Bukit Timah forest in Singapore.

Table 2.0: Diversity of *Trametes* in Malaysia.

No.	Fungus	New name	Location	References
1.	*Trametes (Microporus) affinis* Corner	*Microporus affinis* (Blume & T. Nees) Kuntze 1898	-Negeri Sembilan	Corner (1983-1991)
2.	*T. albobadia* (C.G. Lloyd) Corner		-Ketil R., Kelantan	Corner (1983-1991)

Table 2.0, continued

3.	*T. arcana* Corner		-Mt. Kinabalu, Sabah	Corner (1983-1991)
4.	*T. argenteiceps* Corner		-Slim River, Perak	Corner (1983-1991)
5.	*T. aurora* (Ces.) C.G. Lloyd	*Fomitopsis rhodophaea* (Lév.) Imazeki 1943	-P. Dayang Bunting, Langkawi -Perlis, Kangar -Sedili R., Johor	Corner (1983-1991)
6.	*T. badia* Berk.		-Kemaman, Terengganu -Tembeling, Pahang	Corner (1983-1991)
7.	*T. barbulata* Corner		-Mt. Kinabalu, Sabah	Corner (1983-1991)
8.	*T. benevestita* Corner		-Tembeling, Pahang	Corner (1983-1991)
9.	*T. biogilva* (C.G. Lloyd) Corner	*Polyporus rugulosus* Lév. 1844	-Langkawi, Kedah -K. Tekai, Pahang -Ketil, Kelantan	Corner (1983-1991)
10.	*T. borneoensis* C.G. Lloyd		-Johor -Mt. Kinabalu, Sabah	Corner (1983-1991)
11.	*T. brunneoleuca* (Berk.) Corner	*Coriolopsis brunneoleuca* (Berk.) Ryvarden 1972	-K. Pilah, Negeri Sembilan -Tembeling, Pahang - Ketil, Kelantan	Corner (1983-1991)
12.	*T. carnea* (Bl. Et Nees) Corner	*Fomitopsis rosea* (Alb. & Schwein.) P. Karst. 1881	-Negeri Sembilan	Corner (1983-1991)
13.	*T. carneo-nigra* (Berk.) Corner		-Kemaman, Terengganu -Ulu Kahang, Johor	Corner (1983-1991), Salmiah (1997)
14.	*T. castaneifumosa* Corner		-Fraser's Hill, Pahang	Corner (1983-1991)
15.	*T. corruga* Berk.	*Mollicarpus cognatus* (Berk.) Ginns 1984	-Pontian Kechil, Johor	Corner (1983-1991)
16.	*T. corrugate* (Pers.) Bres.	*Earliella scabrosa* (Pers.) Gilb. & Ryvarden 1985	-Pahang	Corner (1983-1991), Salmiah (1997)
17.	*T. deadaleoides* Corner		-K. Tekai, Pahang	Corner (1983-1991)
18.	*T. decorticans* Corner		-Gunung Panti, Johor -Tembeling, Pahang	Corner (1983-1991)

Table 2.0, continued

19.	*T. depauperata* Corner		-Gunung Panti & Ulu Tiram, Johor -Kemaman, Terengganu -Fraser's Hill, Pahang -Kuching, Sarawak	Corner (1983-1991)
20.	*T. dochmia* (Berk. Et. Br.) Corner	*Fomitopsis dochmia* (Berk. & Broome) Ryvarden [as '*dochmius*'] 1972	-Peninsular Malaysia -Mt. Kinabalu, Sabah -Santubong, Sarawak	Corner (1983-1991)
21.	*T. febris* Corner		-Sedili Kechil, Johor	Corner (1983-1991)
22.	*T. (fomitopsis) feei* (Fr.) Pat.	*Fomitopsis feei* (Fr.) Kreisel 1971	-Fraser's Hill, Pahang -Jeram Lenang, Kelantan	Corner (1983-1991), Watling (1994), Salmiah (1997)
23.	*T. flammula* Corner		-Cameron Highlands, Pahang	Corner (1983-1991)
24.	*T. flavidinigra* Corner		-Johor	Corner (1983-1991)
25.	*T. fulvirubida* Corner		-Johor	Corner (1983-1991)
26.	*T. granulifera* Corner		-Mt. Kinabalu, Sabah	Corner (1983-1991)
27.	*T. hirsuta* Corner		-Kuching, Sarawak	Corner (1983-1991)
28.	*T. hirsuta* (Wulf.) Pilat			Chipp (1921), Kuthubutheen (1981)
29.	*T. incana* Fr.		- Mt. Kinabalu, Sabah	Corner (1983-1991)
30.	*T. insularis*		-Jeram Lenang, Kelantan	Salmiah (1997)
31.	*T. internuntia* Corner	*Microporus internuntius* (Corner) T. Hatt. 2005	-Mt. Kinabalu, Sabah	Corner (1983-1991)
32.	*T. jejuna* Corner		-Tembeling, Pahang	Corner (1983-1991)
33.	*T. lactinea* (Berk.) Pat.		-Kuching, Sarawak	Corner (1983-1991)
34.	*T. linguiformis* Corner		-Fraser's Hill, Pahang -Mt. Kinabalu, Sabah	Corner (1983-1991)

Table 2.0, continued

35.	*T. lusor* Corner		-Gunung Panti, Johor	Corner (1983-1991)
36.	*T. malaysiana* Corner		-Gunung Panti, Johor -Mt. Kinabalu, Sabah	Corner (1983-1991)
37.	*T. menzeizii* (Berk.) Ryv.		-Kuching, Sarawak	Corner (1983-1991), Lee *et. al.,* (1995), Salmiah (1997)
38.	*T. meyenii* (Koltz.) C.G. Lloyd		-Fraser's Hill, Tekai & Jerantut, Pahang -Bkt Besi Hangit, Perlis -Mt. Kinabalu, Sabah	Corner (1983-1991)
39.	*T. microporoides* Corner		-Fraser's Hill & Tembeling, Pahang -Tapah, Perak	Corner (1983-1991)
40.	*T. modesta* (Fr.) Ryv.		-Angsi Forest, Negeri Sembilan	Corner (1983-1991), Hattori (unpubl.), Salmiah (1997)
41.	*T. molesta* Corner		-Tembeling, Pahang	Corner (1983-1991)
42.	*T. multiflabellata* Corner		-Angsi Forest, Negeri Sembilan -Fraser's Hill, Pahang -Mawai, Johor	Corner (1983-1991)
43.	*T. pallidilusor* Corner		-Padang Piol, Pahang -Kemaman, Terengganu	Corner (1983-1991)
44.	*T. paxillosa* Corner		- Tembeling & Tekai, Pahang	Corner (1983-1991)
45.	*T. perpallida* Corner		-Mt. Kinabalu, Sabah	Corner (1983-1991)
46.	*T. perstrata* Corner		-Tembeling, Pahang -Gunung Pulai, Johor	Corner (1983-1991)
47.	*T. pinsita* (Fr.) Fid. Et K. Fid.	*Trametes villosa* (Sw.) Kreisel 1971	-Tembeling & Cameron Highlands, Pahang	Corner (1983-1991)
48.	*T. polyblaster* Corner		-Endau Rompin, Johor	Corner (1983-1991)

Table 2.0, continued

49.	*T. pocas*		-Mata Ayer, Perlis	Salmiah (1997)
50.	*T. polyporiformis* Corner		-North Borneo	Corner (1983-1991)
51.	*T. primulina* Corner		-Mt. Kinabalu, Sabah	Corner (1983-1991)
52.	*T. pseudodochmia* Corner		-Mt. Kinabalu, Sabah	Corner (1983-1991)
53.	*T. pubescens* (Schum.) Pilat		-Kuching, Sarawak	Corner (1983-1991)
54.	*T. rhodophaea* (Lev.) Corner	*Fomitopsis rhodophaea* (Lév.) Imazeki 1943	-Peninsular Malaysia	Corner (1983-1991)
55.	*T. rigidiceps* Corner		-Ketil, Kelantan -Jelebu & Angsi Forest, Negeri Sembilan -Tembeling & Cameron Highlands, Pahang	Corner (1983-1991)
56.	*T. rubida* (Berk.) Pat.		-Pahang -Johor	Corner (1983-1991)
57.	*T. rubrigrisea* Corner		-Reservoir Jungle, Singapore	Corner (1983-1991)
58.	*T. rufidochmia* Corner		-Bako National Park, Sarawak	Corner (1983-1991)
59.	*T. rugosituba* Corner		-Tembeling & Fraser's Hill, Pahang	Corner (1983-1991)
60.	*T. sacra* (Fr.) Corner	*Lignosus sacer* (Afzel. Ex Fr.) Ryvarden 1920	-Pattani, Kedah	Corner (1983-1991)
61.	*T. salina* Corner		-Pekan, Pahang -Port Swettenham, Selangor	Corner (1983-1991)
62.	*T. sanguinaria* (Kl.) Corner	*Polyporus rugulosus* Lév. 1844	-Mt. Kinabalu, Sabah	Corner (1983-1991)
63.	*T. sanguinea* (Fr.) Imaz.	*Pycnoporus sanguineus* (L.) Murrill 1904	-Malaysian regions	Corner (1983-1991)
64.	*T. (Earliella) scarbosa* (Pers.) G.H. Cunn.	Earliella scarbosa	-Cheka R., Pahang	Corner (1983-1991)
65.	*T. scopulosa* (Berk.) Bres.		-Bt. Caves, Selangor	Corner (1983-1991)
66.	*T. sediliensis* Corner		-Sedili R., Johor	Corner (1983-1991)
67.	*T. semitosa* (Berk.) Corner		-Sedili R., Johor -Cheka R., Pahang -Ketil R., Kelantan	Corner (1983-1991)

Table 2.0, continued

68.	*T. sepiicolor* Corner		-Cameron Highlands, Pahang -Mt. Kinabalu, Sabah	Corner (1983-1991)
69.	*T. suberosifusca* Corner		-Sedili R., Johor	Corner (1983-1991)
70.	*T. subincana* Corner		-Mt. Kinabalu, Sabah	Corner (1983-1991)
71.	*T. subligativa* Corner		-Tioman Island, Pahang	Corner (1983-1991)
72.	*T. sublutea* Corner		-Tembeling, Pahang	Corner (1983-1991)
73.	*T. socotrana*			Salmiah (1997)
74.	*T. telfarii* (Kl.) Corner	*Coriolopsis telfarii* (Klotzsch) Ryvarden 1972	-Gunung Raya, Langkawi, Kedah -Tembeling, Pahang -Bako National Park, Sarawak	Corner (1983-1991)
75.	*T. tenuis* (Hock.) Corner		-Common in Malaysia	Corner (1983-1991)
76.	*T. turpis* Corner		-Cheka R., & Tembeling Pahang	Corner (1983-1991)
77.	*T. umbrinopallens* Corner		-Tembeling, Pahang -Pasoh Forest, Negeri Sembilan	Corner (1983-1991)
78.	*T. verticallis* Corner		-Cheka R., Pahang	Corner (1983-1991)
79.	*T. villosa* (Fr.) Kreisel		Singapore	Corner (1983-1991), Salmiah (1997)
80.	*T. (Microporus) xanthopus* (Fr.) Corner	*Microporus xanthopus* (Fr.) Kuntze 1898	-Common in Malaysia	Corner (1983-1991)

Trametes spp. were frequently found in Pahang, Johor, Terengganu and Langkawi, all in the Peninsular Malaysia and also in Mount Kinabalu, Sabah and Kuching, Sarawak.

2.6   Free radicals and oxidative stress

Free radicals are highly reactive chemicals that attack molecules by capturing electrons and thus modifying chemical structures. Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of

oxidizing biomolecules, resulting in cell death and tissue damage (Halliwell and Gutteridge, 2003). However, the over-production of these reactive species due to oxidative stress can cause secondary to the diseases but in some instances they are causal, such as damage to biomolecules and cellular, atherosclerosis, diabetes, cancers and cirrhosis (Halliwell & Gutteridge, 1989). The current hypothesis favours the idea that lowering oxidative stress can contribute to the benefits of one's health.

2.7   Antioxidant

Antioxidants are of interest to the food industry because they can cause rancidity (Löliger, 1991). Antioxidants are also significant to biologists and clinicians because they could help to protect the human body against damage by reactive oxygen species (ROS). ROS is a collective term used to include oxygen radicals (O_2^-, $OH^{\cdot}$, $RO^{\cdot}$ etc) and several non-radical oxidizing agents, such as $HOCl$, H_2O_2, O_3 and $ONOO^-$. Reactive is a relative term (e.g. O_2^- is more reactive than O_2 but much less so than hydroxyl radical $OH^{\cdot}$ or $HOCl$).

The term antioxidants implicitly restricted to chain-breaking inhibitors of lipid peroxidations, such as α-tocopherol. However, free radicals generated *in vivo* damage many targets other than lipids, including proteins, DNA and small molecules. Hence, a broader definition of antioxidants is 'any substances that, when present at low concentrations   compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate' (Halliwell, 1990; Halliwell and Gutteridge 1989). The term oxidizable substrate includes almost everything (except H_2O) found in foods and in living tissues including proteins, lipids, carbohydrates and DNA. This definition emphasizes the importance of the target chosen and the source of oxidative damage in characterizing an antioxidant.

When ROS are generated in the living system, a wide variety of antioxidants comes into play (Aruoma, 1993; Diplock, 1985 and 1994; Fridovich, 1989; Sies. 1985).

Many reviews have covered the well-established physiological antioxidant roles of α-tocopherol, ascorbic acid and proteins such as superoxides dismutase (SOD), glutathione peroxidase, catalase and caeruloplasmin (Diplock, 1985; Fridovich, 1989; Halliwell, 1994a; Halliwell and Gutteridge, 1989; Kappus and Diplock, 1992; Liebler, 1993; Sies, 1985; Sies *et al.,* 1992). The utmost importance of these various antioxidants *in vivo* depends on which ROS is generated, how it is generated, where it is generated, and what target of damage is measured.

Hence, it is perfectly possible for an antioxidant to protect in one system but fail to protect, or even sometimes to cause damage in others. For examples, butylated hydroxyanisole (BHA) is a powerful inhibitor of lipid peroxidation, and yet a huge dietary dose of it can induce cancer of the rat forestomach and it has been suggested that oxidative DNA damage could be involved (Life Science Research Office, 1994; Schildermann *et al.,* 1995). Oxidative DNA and protein damage are the greatest importance in the cells of the human gastrointestinal tract and within the body. Oxidative DNA damage is a risk factor for cancer development, and the protein damage by reactive species is involved in cancer, cardiovascular, and neurodegenerative diseases (Feig and Loeb,1993; Davies and Dean, 1997; Hazen and Heinecke, 1997; and Halliwell and Gutteridge, 1999)

A wide range of compound has been suggested to act as antioxidants *in vivo* and in foods from β-carotene and metallothionein to histidine and also antioxidant compounds derived from plants, especially phenols such as quercetin, carnosol and thymol. How can such claims be evaluated? In evaluating the likelihood of direct antioxidant action *in vivo*, it is important to ask certain questions as in Table 2.1.

Table 2.1: Questions to ask when evaluating "antioxidants" *in vivo* (Halliwell, 1995)

No.	Questions
1.	What biomolecule is the antioxidant supposed to protect? Does enough antioxidant reach that target *in vivo*?
2.	How does it protect-by scavenging ROS, preventing their formation, or repairing damage?
3.	If the antioxidant acts by scavenging, can the resulting antioxidant-derived radicals themselves cause damage?
4.	Can the antioxidant cause damage in other biological systems?

2.7.1 Synthetic antioxidants

Antioxidants have been widely used as additives in fats and oils and in food processing to prevent or retard the oxidative deterioration of food. Some of the more popular synthetic antioxidants used are phenolic compounds such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ) and esters of gallic acid, e.g. propyl gallate (PG). Synthetic phenolic antioxidants are always substituted by alkyls to improve their solubility in fats and oils (Hudson, 1990). The four major synthetic antioxidants in used are subjected to a 'good manufacturing practice' limit of 0.02 % of the fat or oil content of the food (Simic,1981). The most suitable antioxidant for vegetable oils is TBHQ. BHA and BHT are fairly stable to heat and are often used for stabilization of fats in baked and fried products. BHA is also synergistic with PG (Angelo, 1996).

Although the synthetic antioxidants are powerful in protecting the product quality in food distribution, excessive antioxidants added to food might produce toxicities or mutagenicities, and thus endanger the health of people (Williams 1993, 1994). Recently, BHA and BHT were found to have tumor-initiating as well as tumor-promoting action (Botterweck *et al.*, 2000). Therefore, numerous efforts have been put in to search and identify compounds that can act as suitable antioxidants to replace synthetic ones (Löliger, 1991). Natural products have been investigated as healthier and safer than synthetic antioxidants (Valenzuela & Nieto, 1996).

2.7.2   Natural antioxidants

Natural antioxidants are being extensively studied for their capacity to protect organism and cells from damage brought by oxidative stress. Natural antioxidants are found in almost all plants, microorganism, fungi and even in animal tissues (Pokorny, 1999). Almost all organisms are well protected against free radical damaged by oxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT), or chemical compounds such as α-tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione (Niki *et al.,* 1994; Mau, Lin, & Song, 2002;).

There are over 600 carotenoids that occur naturally in plants. Most of them have been identified in human serum being; α- and β-carotene, the xanthins (β-cryptoxanthin and zeaxanthin), lycopene and leutein (Krinsky 1993; Gerster 1993). Most carotenoids are pigments that give fruits and flowers their colour and provide protection from the photochemical reactants which was formed during photosynthesis. The carotenoids have a characteristic structure consisting of conjugated double bonds, of which many possess vitamin A activity which is able to act as pro-vitamin A while others, including lycopene, are devoid of any vitamin A activity.

Carotenoids are antioxidants or radical scavengers with broad range of potencies (Doba *et al.* 1985; Brakely *et al.* 1988; Carbonneau *et al.* 1989). This includes the inhibition of lipid peroxidation (Halliwell *et al.* 1995), protection against phototoxicity (Davison et al. 1993; Krinsky 1989b; Black and Mathews-Roth 1991), which may protect against skin cancer, protection against neoplastic transformation (β-carotene, α-carotene, canthaxanthin, α-tocopherol and lycopen) (Bertram *et al.* 1991), and inhibition of the early steps in carcinogenesis (Goswami *et al.* 1989).

Beta-carotene is found in many common vegetables including carrots, spinach, kale, broccoli and in mango and papaya fruits. Beta-carotene is involved with cell communication, apoptosis and gene regulation, as well as acting as a direct

antioxidant (Krinsky 1993). β -carotene is a powerful quencher of singlet oxygen and a free radical scavenger (Foote 1968; Foote *et al.* 1970a,b), and it provides a protection against cancer (Gester 1993). Antioxidant capacity of β –carotene varies with oxygen tension, and its activity is the greatest at a low oxygen tension found under physiological conditions (Burton and Ingold 1984; Kinsky 1989a). Experimental evidence has shown that β –carotene inhibits micronuclei formation (Yager *et al.* 1990), which is the characteristic of a number of genotoxic carcinogens. Micronucleic are formed either due to DNA breakage that results in a broken chromosome or by damaging the microtubules. Epidemiological evidence suggests that β –carotene has a role in the prevention of both cardiovascular disease and cancer (Pappalardo *et al.* 1996)

Flavonoids are polyphenolic compounds found in plants. It gives the food its colour, texture and taste (Harbourne 1986). Experimental data shows that flavanoids are associated with the inhibition of tumorigenesis and tumour growth *in vivo* (Huang *et al.* 1992; Chung 1992). The flavonoids are split into major groups including the anthocyanins, flavonols, flavones, cathechins and flavanones. In addition to their biological roles in humans, it can be seen that most important compounds are involved in the maintenance of capillary walls (Havsteen 1983). The flavonoids possess a range of antioxidant defence mechanism. These include the scavenging of radicals (Husain *et al.* 1987), and superoxide anions (Robak and Gryglewski 1988), together with the sequestration of metal ions (Takahama 1985); although some can act as pro-oxidants in the presence of copper ions (Cu^{2+}).

Vitamin E is an essential lipid-soluble vitamin in the human diet, and it is found in cell membranes and plasma lipoproteins. Severe deprivation of vitamin E is known to lead to the neurological damage (Muller and Goss-Sampson 1990). As an antioxidant which is active in lipid-soluble compartments of the cell, vitamin E contains a number of tocopherols. Alpha-tocopherol is one of the most important free radical scavengers

and inhibits peroxidation in cell membranes by scavenging peroxyl radicals. Vitamin E was found to inhibit hydroperoxide which can damage the DNA (Yang and Schaich 1996).

Vitamin C (ascorbate) is an essential micronutrient required for normal metabolic functioning of the body (Jaffe, 1984). Humans and other primates have lost their ability to synthesize vitamin C due to a mutation in the gene coding for L-gulono-γ-lactone oxidase, an enzyme required for the biosynthesis of vitamin C via the flucuronic acid pathway (Woodall and Ames, 1997). As a result, humans have to obtain vitamin C through diet. A lack of vitamin C in the diet causes the deficiency disease of scurvy (Levine 1986). Vitamin C is a co-substrate for various biosynthetic enzymes, including the hydroxylases and oxygenases involved in the synthesis of collagen, carnitine, and catecholamines (Burri and Jacob 1997; Tsao 1997).

The function of vitamin C is to reduce the active cancer metal ion of these enzymes (Burri and Jacob 1997; Tsao 1997). Additionally, the ability to maintain metal ions in the reduced state is related to the redox potential of vitamin C. The advantages and disadvantages of synthetic and natural antioxidants are summarized in Table 2.2 (Valenzuela & Nieto, 1996).

Table 2.2: Advantages and disadvantages of natural and synthetic antioxidations

Synthetic antioxidants	Natural antioxidants
Inexpensive	Expensive
Widely applied	Use restricted to some products
Medium to high antioxidant activity	Wide ranging antioxidant activity
Increasing safety concern	Perceived as innocuous substances
Use banned for some of them	Increasing use and expanding applications
Low water-solubility	Broad range of solubilities
Decreasing interest	Increasing interest

2.7.3 Antioxidant from mushrooms

Mushrooms have been used for traditional foods and medicines in Asia (Chang, 1996). In Thailand, *A. polytricha* was found to reduce weight, decreasing sticky

blood, decreasing blood sugar of diabetics and repairing skin due to their antioxidant activity of mycelial extract and extracellular polysaccharide production (Chen *et al.*, 2003). In China, Tsai *et al.,* (2006) have reported that ethanolic and hot water extract from *Agaricus blezei, Agrocybe cylindracea* and *Boletus edulis* were effective antioxidant properties. These mushrooms have been used as food and food flavoring materials in soups and sauces. *Pleurotus citrinopileatus* Sing. (Lentinaceae) is also an edible mushroom popular in China, India and Japan. It was found to have antioxidant properties. Ethanolics extracts from fruit bodies of *P. citrinopileatus* were more effective in antioxidant properties than those from mycelial and filtrate extracts (Lee *et al.,* 2007).

Elmastas *et al.,* (2007) have analysed the antioxidant activity of seven wild edible mushroom species (*Agaricus bisporus, Polyporus squamosus, Pleurotus ostreatus, Lepista nuda, Russula delica, Boletus badius, Verpa conica*) from northern Turkey. According to the result of the study, methanolic extract of the mushroom species has significant antioxidant activity against various antioxidant systems *in vitro*. Phenolic compound seems to be the main components responsible for the antioxidant activity of all mushroom species extracts. Some common edible mushrooms consumed in Asian culture have also possess antioxidant activity, which is well correlated with their total phenolic content (Yen & Hung, 2000; Yang *et al.,* 2002; Cheung *et al.,* 2003; Mau *et al.,* 2002,2004; Cheung & Cheung, 2005; Lo & Cheung, 2005).

Mushrooms contain various polyphenolic compounds recognized as an excellent antioxidant due to their ability to scavenge free radicals by single-electron transfer (Hirano *et al.,* 2001). Phenolic compounds were found to have antioxidant activity in the inhibition of LDL (Low density lipoprotein) oxidation (Teissedre and Landrault, 2000). The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals (Deckers, 1997). Phenolics are

one of the major groups of nonessential dietary components that have been associated with the inhibition of atherosclerosis and cancer (Williams & Iatropoulos, 1997).

Besides the wild edible mushrooms and cultivated mushrooms, several studies have been done on the antioxidant properties of medicinal mushrooms. *Cordyceps sinensis,* an entomogenous fungus, is the best known traditional medicines. Recent studies have demonstrated aqueous extracts and methanol extracts from this mushroom could scavenge hydroxyl radicals (Cai *et al.,* 2004; Zhang *et al.,* 2003). Methanolic and water extracts of *Ganoderma lucidum*, *G. tsugae* and *G. atrum* are high in antioxidant abilities (Yen & Wu, 1999; Yang *et al.,* 2002; Mau *et al.,* 2002; Mau & Tsai 2005 and Gong *et al.*, 2006). In addition, *Auricularia, Flammulina, Ganoderma, Grifola, Lentinus, Trametes* (*Coriolus*) and *Tremella* have been demonstrated to possess significant medicinal properties (Wasser, 2002). This is followed by *T. versicolor* which has antimicrobial, antiviral and anti-tumor properties (Jong and Birmingham, 1993; Ulrike *et al,* 2005). Nowadays *T. versicolor* is mainly used as an adjuvant in the treatment of cancer (Tsang *et al,* 2003; Hattori *et al,* 2004). It has been demonstrated that extracts obtained from this mushroom are likely to show stimulatory effects on the immune system and to inhibit the growth of cancer cells.

The best known commercial polysaccharopeptide preparations of *T. versicolor* are polysaccharide-Krestin (PSK) and polysaccharide-peptide (PSP). Both products are obtained from the extraction of *T. versicolor* mycelia. PSK is a Japanese product, while PSP is Chinese product which was first isolated in 1986 (Yang and Van, 1986). Both products have similar physiological activities but are structurally different. PSK is produced from CM-101 strain of *T. versicolor* whereby the extraction is done by salting out with ammonium sulfate from the hot water extract. On the other hand, PSP is produced from Cov-1 strain of *T. versicolor* whereby the extraction is done by using alcoholic precipitation from the hot water extract. PSK may act as an antioxidant by

enhancing superoxide dismutase (SOD) (Kariya *et al.,* 1992) and glutathione peroxidase activities to protect tissue damage from harmful effects of free radicals (Kobayashi *et al.,* 1993 and Mau *et al.,* 2002).

Inonotus and *Phellinus* have been used as traditional medicines for the treatment of gastrointestinal cancer, cardiovascular disease, tuberculosis, liver or heart diseases, fester, stomachache, bloody gonorrhoea, stomach ailments and diabetes (Nakamura *et al.,* 2004 and Sutton *et al.,* 2005). It has been reported that these mushrooms produce a bundle of yellow antioxidant pigments that are composed from hispin derivatives and polyphenols (Mo *et al.,* 2004; Lee *et al.,* 2006a and Lee *et al.,* 2006b).

2.7.4   Antioxidant assay

In recent years, various methods were used to evaluate antioxidant activity of foods, serum and other biological fluids. Antioxidant capacity assays may be broadly classified as electron transfer (ET)- and hydrogen atom transfer (HAT)-based assays (Huang *et al.,* 2005 and Prior *et al.,* 2005). The majority of HAT-based assays involve a reaction scheme in which antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds. These assays included low-density lipoprotein autoxidation, oxygen radicals absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), and crocin bleaching assays (Huang *et al.,* 2005 and Prior *et al.,* 2005). Most of these assays are kinetic-based, meaning that they are more concerned with the rate rather than thermodynamic conversion efficiency of the radical reaction with the antioxidant. An exceptional assay is ORAC (Cao *et al.,* 1995) which deals with both kinetic and thermodynamic aspects of the reaction and reported results based on the net area under curve of the fluorescence decay/time curve of the probe in the presence and absence of antioxidants.

On the other hand, ET-based assays measure the capacity of an antioxidant in the reduction of an oxidant, which changes colour when reduced (Huang *et al.,* 2005 and Prior *et al.,* 2005). The degree of colour change is correlated to the concentration of antioxidant in the sample. These assays generally set a fixed time for the concerned redox reaction, and measure thermodynamic conversion during that period. ET-based assays include ABTS/TEAC (Miller *et al.,* 1993), Folin-Ciocalteu (Folin and Ciocalteu, 1927; and Singleton *et al.*, 1999), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Blois, 1958), Feric reducing ability of plasma (FRAP) (Benzie and Strain, 1996) and cupric ion reducing antioxidant capacity (CUPRAC) (Apak *et al.*, 2004 and Apak *et al.,* 2005), using different chromogenic redox reagents with different standard potentials. Although the reducing capacity of a sample is not directly related to its radical scavenging capability, it is a very important parameter of antioxidants.
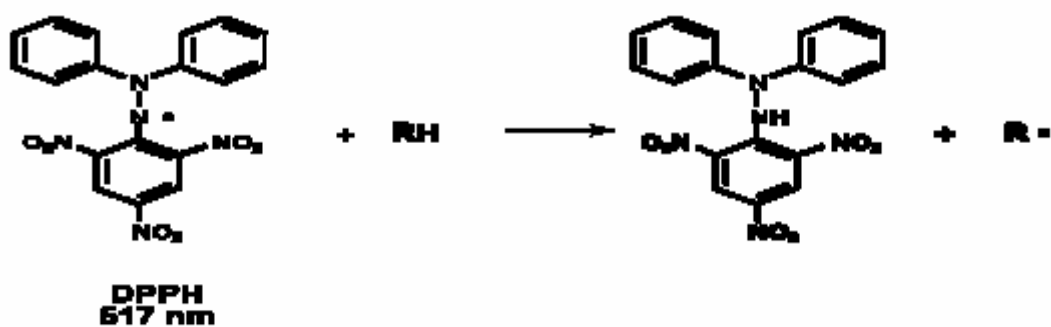
Fig. 2.6: Structure of DPPH and its reduction by an antioxidant. (Source: Prakash, 2001)

DPPH radical scavenging assay is the most simple and inexpensive method to measure antioxidant capacity. The DPPH method can be used for solid or liquid samples and is not specific to any particular component, but applies to the overall antioxidant capacity of the sample. The mechanisms of DPPH methods are absorption spectra of the stable, free radical changes when the molecule is reduced by an antioxidant species. The structure of DPPH and its reduction by an antioxidant are shown in Fig. 2.6. The odd electron in the DPPH free radical gives a strong absorption

maximum at 517 nm and appears in purple. The colour turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The result of decolourization is stoichiometric, with respect to the number of electrons captured.

CUPRAC (cupric ion reducing antioxidant capacity) method is another simple, versatile, and low-cost antioxidant capacity assay compared to DPPH method. The chromogenic redox reagent used for CUPRAC assay was bis(neocuproine) copper(II) chelate (Apak *et al*., 2004; Apak *et al.,* 2005). This reagent was useful at pH 7, and the absorbance of the Cu(I)-chelate formed as a result of redox reaction with reducing polyphenols was measured at 450 nm. The colour was due to the formation of Cu(I)-Nc chelate. The reaction conditions such as reagent concentration, pH, and oxidations time at room and elevated temperatures were optimized (Apak *et al*., 2004 and Apak *et al.,* 2005).

The chromogenic oxidizing reagent of the developed CUPRAC method, i.e., bis(neocuproine)copper(II) chloride (Cu(II)-Nc), reacts with n-electron reductant antioxidants (AO) in the following manner:

$$nCu(Nc)_2^{2+} + \text{n-electron reductant (AO)} \rightarrow nCu(Nc)_2^+ + \text{n-electron oxidized product} + nH^+$$
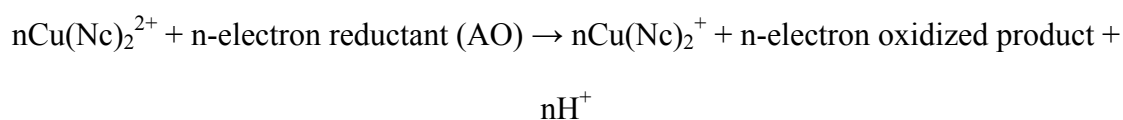
In this reaction, the reactive Ar-OH groups of polyphenolic antioxidants are oxidized to the corresponding quinines (ascorbic acid is oxidized to dehydro-ascorbic acid) and Cu(II)-Nc is reduced to the highly coloured (Cu(I)-Nc chelate showing maximum absorption at 450 nm. Although the concentration of Cu^{2+} ions was in stoichiometric excess of that of neocuproine in the CUPRAC reagent for driving the

above redox equilibrium reaction to the right, the actual oxidant was the $Cu(Nc)_2^{2+}$ species and not the sole Cu^{2+}, because the standard redox potential of the Cu(II/I)-neocuproine was 0.6V, much higher than Cu^{2+}/Cu^{+} couple (0.17V) (Apak *et al.,* 2005). As a result, polyphenols were oxidized much more rapidly and efficiently with Cu(II)-Nc than with Cu^{2+}, and the chromogen (i.e., Cu(II)-Nc. The liberated protons are buffered in NH_4Ac medium. In the normal (N) CUPRAC method, the oxidation reactions were essentially completed within 30 minutes. The linear calibration curves of the tested antioxidants as absorbance *vs* concentration with respect to the CUPRAC method generally gave correlation coefficients close to 1, i.e., r≥ 0.999, within the absorbance range of 0.1-1.2.

There are several advantages of the CUPRAC method (Apak *et al.,* 2004; Apak *et al.,* 2005). The CUPRAC reagent is fast enough to oxidize thiol-type antioxidant (Apak *et al.,* 2005; Tütem & Apak, 1991). Besides, the reagent is more stable and accessible than other chromegenic reagents (e.g., ABTS, DPPH). The cupric reducing ability measured for a biological sample may indirectly but efficiently reflect the total antioxidant power of the sample even though no radical species are involved. The redox reaction has changed to a coloured chelate of Cu(I)-Nc which is relatively insensitive to a number of parameters adversely affecting certain reagents such as DPPH, air, sunlight, humidity and pH to a certain extent. Thus, the CUPRAC method is easily and diversely applicable in conventional laboratories using standard colorimeters rather than necessitating sophisticated equipment and qualified operators.

3.0   MATERIALS AND METHODS

3.1   Collection of samples

Trametes spp. were collected from Langkawi, Kelantan, Terengganu, Pahang, Selangor and Johore in Peninsular Malaysia (Fig. 3.0). Several field trips to collect the polypores were carried out from February 2006 to November 2007 in Peninsular Malaysia.
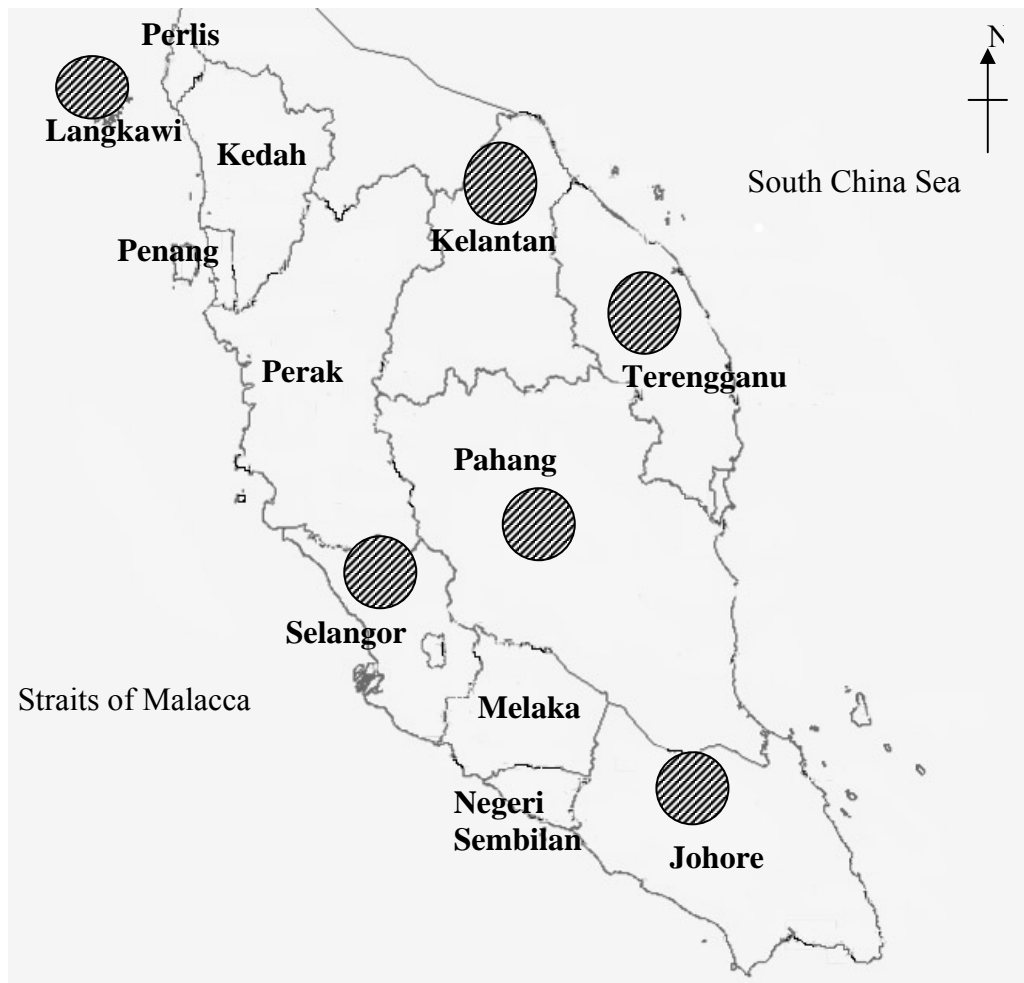
Fig. 3.0: Map of Peninsular Malaysia showing the collection sites of *Trametes*.

Two field trips were held at Endau-Rompin, Johore National Park which situated at the southern part of Peninsular Malaysia on 15th -18th February 2006 and 5th -6th Jun 2007. Endau-Rompin is known as an important conservation area. Therefore,

the highest collection is expected in that area since the forest is not for inland uses and it protects many species of flora and fauna.

Then, a field trip was conducted at the Orang asli village, Pos Senderut, Kuala Lipis, Pahang, which is situated near the back bone of Peninsular Malaysia on $6^{th} - 9^{th}$ of March 2006. Orang Asli is an indigenous community in Peninsular Malaysia. Most of the Orang Asli villages are located near the river and forest reserved.

Next, a field trip was conducted in Langkawi on the 29th May – 3rd of June 2006. Langkawi is situated in the northern part of Peninsular Malaysia. The collections were done in several places that are located at Kg. Buku, Teluk Datai, Pasir Tengkorak and Gunung Raya in Langkawi. These locations were recognized as a recreation area and classified as the forest beach hill.

A field trip was conducted at the east coast of Peninsular Malaysia in Kelantan and Terengganu. The collection in Kelantan was located at the Forest Recreation Park Jeram Lenang and Bukit Bakar on the $22^{nd} - 25^{th}$ of February 2007. In Terengganu, polypores were collected at the Forest Recreation Park on the $19^{th} - 21^{st}$ of November 2007. Almost all parts of Terengganu were covered and this includes areas located in Sekayu, Cemerung, Rantau Abang, Lata Belantan, Lata Tembakah and Bukit Raja. Both Forest Recreation Park in Kelantan and Terengganu consist of dipterocarp forest. Therefore, polypores are expected to be found abundantly in those areas as woods decay fungi.

Field trips were made to collect the polypores throughout the year from 2006 until 2007 at the Forest Research Institute Malaysia, Kepong, Selangor. It is situated in the west coast of Peninsular Malaysia. These field trips were carried out when ever possible and quite frequent in order to ensure the consistency of the data collected.

Polypores are wood-rotting fungi. Therefore, polypores are most probably found on dead wood, standing or fallen trees, stumps, fallen branches or logging slash. Some

polypores can be found on standing living trees. Essential items were used to collect polypores in this study which are: sharp knife to remove the basidiomes from the substratum; paper bags for storing specimens; hand lens to observe the macroscopic features; pen and data sheets for recording and making field notes. In addition, digital camera was used to record the fresh specimens in the natural habitat during the field trip. At the time of collection, the following information had been recorded on the field label:

- Date of collection

- Locality

- Name of collector and field code

- Additional information was recorded such as notes on colour and colour changes, consistency, and other characters that are not discernible from dried specimens.

The specimens were carefully collected from the substrate to prevent damage. Pieces of wood with basidiocarps attached were chopped carefully. Each collection was placed in a different paper bag of appropriate size. Photographs with code label were made before specimens are dried. The specimens were placed in the dryer at 40°C with the field label. Then, polypores were stored into the bag with labels and had been placed at the University of Malaya herbarium (KLU-M), Rimba Ilmu Kuala Lumpur and the duplicate specimens were sent to the Wood Mycology Lab (WML), Forest Research Institute Malaysia (FRIM).

3.2 Morphology of *Trametes*:

3.2.1 Macromorphology analysis

Basidiocarps were described based on its shape and attachment. The features on the pileus and stipe were also described. Based on the colour, shape and size, an observation of the pore surface has been recorded. Essentially, the macromorphology analysis was done on fresh specimens.

3.2.2 Micromorphology analysis

Radial section of the pileus was made in order to take a piece of the context tissue. The tissue was placed on a microscope slide with potassium hydroxide (KOH, 5%) and followed by phloxine (1%). Phloxine was used to stain the generative hyphae. Then, the cover slip was placed on the specimen. The slide was observed under phase-contrast compound microscope. Based on the observation, features and the images of the specimen were recorded. The same method was also used for cotton blue reagent to stain the hyphae wall. From the observation, all types of hyphae were recorded.

This is followed by another observation whereby the spores of the polypores were collected and placed on the microscope slide with Melzer's reagent. The reaction in Melzer's reagent is important in identifying its species. Based on the reaction in Melzer's reagent, the spores can be identified as amyloid or dextrinoid. If the spores colour changed from yellow to blue, it is an amyloid. However, if the spores colour remained in yellow, then it is a dextrinoid. From the results, the identification was made based on taxonomy key by Núñez & Ryvarden (2001).

3.3 Preparation of pure cultures

Pure cultures of *Trametes* spp. were prepared by transferring a piece of sterile tissue taken from the context of basidiocarp. Then, it is placed centrally on the malt

extract media (MEA) culture under sterile condition at the field in five replicates. The mycelia growing out from the tissue after 3 days it were sub-cultured for two or three times in order to obtain the pure culture. Pure cultures of *Trametes* specimens were stored and maintained in the slant MEA media at 28°C (Appendix A1).

3.4 Growth of mycelial biomass in liquid culture for the extraction of antioxidants.

Glucose-yeast-malt-peptone (GYMP) agar media were prepared as in Appendix A2. GYMP liquid media (100 ml) were prepared in 250 ml Erlenmeyer flask and autoclaved at 121°C for 20 minutes. Ten discs of inocula (7 mm diameter) were cut from the edge of a 7 day-old colony grown on the GYMP agar media. Then, the discs were transferred into sterile GYMP liquid media (100 ml) in twenty replicate flasks per species. Mycelial biomass was harvested after 14 days incubation at 25°C and it was freeze dried using Labconco freeze dryer at -180°C under vaccum. After following all the process, only then the dry weight of mycelia biomass were recorded.

3.5 Extraction of bioactive compounds

Dried mycelial biomass was soaked in methanol in 250 ml Erlenmeyer flasks and covered with aluminum foil. It was left overnight in a chamber at a room temperature, 28°C.

The mycelium was separated by filtration using Whatman No. 1 filter paper. The supernatant extraction was transferred into round bottom flasks and dried using rotary evaporator. The crude extract was then transferred into the pre-weighed glass vial and rotary evaporated till dryness to yield crude extract. The weight of all crude extracts obtained were measured and stored at -20°C before antioxidant analysis. Dichloromethane extracts were prepared similar to the methanolic extracts.

3.6   1,1-Diphenyl-2-picrylhdrazyl (DPPH) radical scavenging assay

Free radical-scavenging activity of *Trametes* extracts against stable 1,1-diphenyl-2-picrylhdrazyl (DPPH) were determined spectrophotometrically. Experiments were performed according to Blois (1958). The hydrogen atom or electron donation abilities of the corresponding extracts were measured from the bleaching of the purple-coloured methanol solution of DPPH.

The solution of DPPH in methanol (0.06 mM) was prepared daily, before UV measurement (Appendix A3). Methanol was used as blank. Acid ascorbic was used as positive control. Stock extract solution (50mg/ml) were prepared by dissolving 0.05 g of methanol extract in 1 ml of methanol. The stock was further diluted with methanol to give a range for 1 to 5 mg/ml. One ml of various concentrations (1 to 5 mg/ml) of the *Trametes* spp. methanolic extracts on was added to 4ml of 0.06 mM methanolic solution of DPPH in a disposable cuvette. Absorbance was read against a blank at 517 nm after a 0', 1', 2' and every 15 minutes intervals until the reaction reached a plateau at room temperature in the dark. Each concentration was tests in triplicate. The absorbance of the DPPH methanolic solution without additional of extract was used as control. Several concentrations of *Trametes* methanolic extracts ranges from 4 to 50 mg/ml were repeated using the same procedure after incubation period based on the plateau reaction before.

Inhibition of free radical by DPPH in percent (I %) was calculated as follows:

$$(\text{Inhibition, I \%}) = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

When A_{blank} is the absorbance of the control reaction (containing all reagent except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of

inhibition (%) against extract concentration. The same procedure was repeated for *Trametes* dichloromethane extracts.

3.7   Cupric ion reducing antioxidant capacity (CUPRAC) assay

Cuprum (II) chloride ($CuCl_2$),   ammonium acetate (NH_4Ac) and neocuproine (Nc) were prepared as in Appendix A4. All of the solutions were prepared according to the Apak *et al*. (2008) method.

One ml of $CuCl_2$ solution, 1 ml of Nc and 1 ml of NH_4Ac solutions was added with 1 ml extract at different concentrations (0.05 to 1 mg/ml) in cuvette. Each concentration was tested in triplicate. The mixture was allowed to stand for 30 minutes for stabilization of the blue colour formed. The absorbance was measured at 450nm.

3.8   Determination of total phenolic contents (TPC)

Total phenolic content in the *Trametes* extracts were determined using Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) and gallic acid was used as a standard phenolic compound. One ml aliquots of 2, 4, 6, 8, and 10 mg/ml gallic acid were oxidized with 250µl of 10% Folin-Ciocalteu reagent to establish a standard calibration curve. After three minutes, 500µl of Na_2CO_3 (10%) was added, then the mixture was allowed to stand at room temperature for one hour to neutralize the reaction. Based on the results, the absorbance of the resulting blue colour was measured at 750 nm and the calibration curve was drawn. All the determinations were performed in triplicates. One ml of 0.5 mg/ml concentrations of the *Trametes* extracts were mixed with the same reagents as described above. One hour later, the absorption was measured to determine the total of phenolis in the extracts. The total of phenolic content of *Trametes* extract was expressed as µg gallic acid equivalents (GAE)/ mg extract.

4.0   RESULTS AND DISCUSSION

4.1   Biodiversity of *Trametes* in Peninsular Malaysia

In this study, specimens were collected in once or twice times per collecting site for a period of two years, randomly. Only 23 out of 306 specimens were identified as *Trametes* species. They were *Trametes feei* (8 specimens), *T. hirsuta* (1 specimen), *T. lactinea* (2 specimens), *T. menziezii* (9 specimens) and *T. pocas* (3 specimens). Figure 4.0 showed the distribution of these *Trametes* species in Peninsular Malaysia. *Trametes feei*, *T. menziezii* and *T. pocas* were commonly found in the Peninsular Malaysia while *T. hirsuta* and *T. lactinea* were rarely found. These results show that *Trametes* species can be found in all parts of Peninsular Malaysia. *Trametes* is a cosmopolitan species, in East Asia extending to subtropical to warm temperate China, Japan, Taiwan and Far East Russia (Núñez and Ryvarden, (2001). However, foray must be done frequently to obtain more species of *Trametes* at different forest type and altitude in Peninsular Malaysia.

Referring to the Fig. 4.0, the highest specimens (eight specimens) were collected in Endau-Rompin, National Park. Two visits were done at Endau-rompin, Johore National Park which is situated at the southern part of Peninsular Malaysia on the 15th to18[th] of February 2006 and 5th to 6th of June 2007. This place was recognized as an old National Forest Reserve and is protected under Wildlife Department. Even though the highest specimen was occurred in this place, but only two species of *Trametes* were identified as   *T. feei* and *T. menziezii.* Lower specimens (seven specimens) were collected in Selangor when compared to Endau-Rompin, National Park. However, more species of *Trametes* were found in that area. They were *T. feei, T. menziezii, T. lactinea* and *T. pocas.* More species were found due to the frequent foray in Forest Research Institute Malaysia (FRIM), Kepong, Selangor which is dominated by trees from

Dipterocarpaceae family and it is a well-managed forest. This does not include the *T. lactinea* which was collected twice on the stump at the roadside. The composition of canopy and sub-canopy trees (heights, crown architecture and density) can influence the amount and quality of light transmitted to the forest floor (Rich *et al.,* 1993; Clearwater *et al.,* 1999 and Leaky *et al.,* 2005). Light are important factor for growth of mushroom. Therefore, most of the specimens were collected at an open area such as roadside and along the canopy trail.

Intermediate specimens (four specimens) were collected in Terengganu with three species of *Trametes* which were *T. feei, T. menziezii* and *T. pocas.* Only one trip was done in the Forest Recreation Park located at Sekayu, Cemerung, Rantau Abang, Lata Belantan, Lata Tembakah and Bukit Raja on the 19th to 21st of November 2007. The lowest specimens (one specimen) of *Trametes* were collected in Kelantan and Langkawi. Even though the forest types in Kelantan are similar with forest in Terengganu, which contained Dipterocarp trees but only one specimen of *T. feei* was found in Kelantan. The lowest specimens in Kelantan might be due to the limited places during the field trip on the 22nd to25[th] of February 2007. The specimens were collected only at Jeram Linang and Bukit Bakar Forest Recreation Park. This situation is different in Terengganu whereby more collections were made just in one trip. Moving on, the forest type in Langkawi which is a beach hill forest, may affect the collection of *Trametes* species as only one *T. menziezii* was found in Langkawi which is situated in the northern part of Peninsular Malaysia on the 29th of May to 3rd of June 2006. The results were not expected as Corner (1989b) found *T. aurora T. biogilva* and *T. (Coriolopsis) telfarii* in Langkawi.

In this study, *T. hirsuta* was only found in Kuala Lipis, Pahang. This rare species was found on a log in logging area. The presence of large logs allows the occurrence of threatened and rare plolypore species (Bader *et al.,* 1995; Sippola *et al.,* 2001; Stokland

& Kauserud, 2004; Heilmann-Clausen and Christensen, 2005). However, Corner (1989b) has reported that this species was found in Kuching, Sarawak. The common species of *Trametes* which is *T. feei* was also collected in Kuala Lipis, Pahang but not in the logging area. It was found on the stump at the trail to the indigenous people village on the 6th to 9th of March 2006. Orang Asli is the indigenous communities in the Peninsular Malaysia. Most of their villages are located near the river and the forest reserve. However, Corner (1989b) has reported that more than ten species of *Trametes* were found in Pahang.

The climate of Peninsular Malaysia is very much influenced by the monsoons. Throughout the year, it experiences two rainy seasons associated with the Southwest monsoon from May to August and the Northeast monsoon from November to February. Heavy rainfalls are expected during the inter-monsoon months (March–April and September–October) (Suhaila and Jemain, 2007). Therefore, it was recommended that the collection of basidiomycetes was done after rainy season since the fruit body of mushrooms will be induced after stimulated by dried and followed by the rainfall in the forest.

The total numbers of *Trametes* species in this study were relatively low when compared with the previous collection in 1921 until 1997 (Chipp, 1921; Kuthubutheen, 1981; Corner, 1983-1991; Watling, 1994; Lee *et. al.,* 1995; Salmiah, 1997; and Hattori, unpubl.). This was mainly caused by the impact of the global climate change. White *et al.,* 1999 has reported a decreased in net ecosystem productivity (NEP) occurring after about 1.5°C of warming. The decreased in NEP were associated with the decline or death of tropical or temperate forest. Fungi also have been report to have both low and high congruence with the diversity of plants in the forest ecosystems (Saetersdal *et al.,* 2004; Humprey *et al.,* 2000). Therefore, forest is seen as an important habitat of polypores.
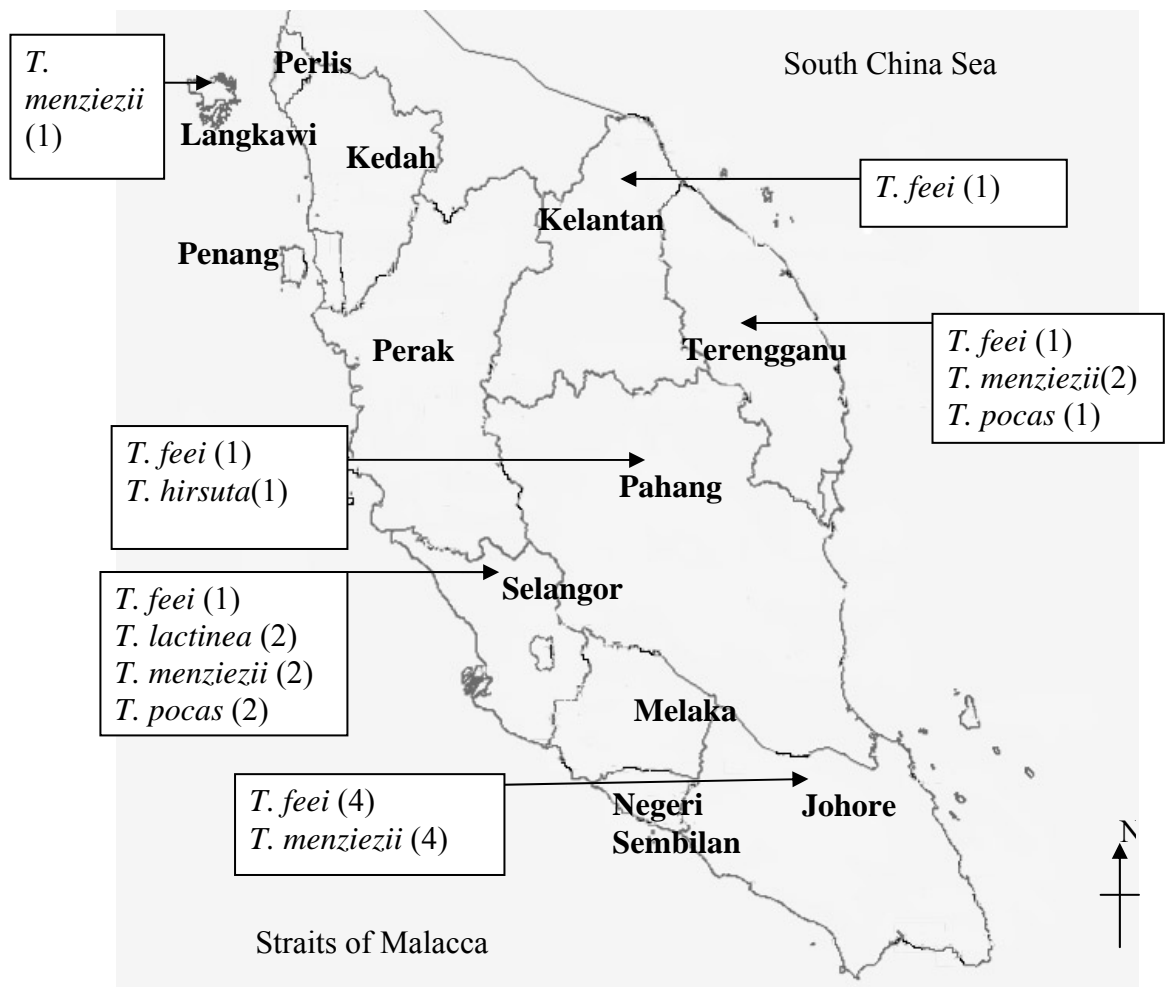
Figure 4.0: Distributions of *Trametes* spp. in Peninsular Malaysia. Number of specimen indicated in bracket.

Robledo and Rension (2009) have reported that the early and late forest plots in their study had the lowest population of polypores. This is followed by the young and mixed forest. Meanwhile, the highest populations of polypores were found in the matured forest. The highest polypores species in the matured forest is typical in most studies (Hattori & Lee, 2003; Hattori, 2005b; Brown *et al.,* 2006; Lonsdale *et al.,* 2008). Based on the studies, it is suggested that matured forest should be protected and young forest must be well managed in order to promote higher proportion of matured forest.

More species of *Trametes* in Malaysia may disappear slowly unless serious attempts are made to increase their population. Most importantly, the conservation of fungi in the forest can be followed by two tracks based on: 1) protection of sites with a diverse range of polypores; or 2) nature conservation measures in the

forest subject to management. In reality, a combination of both approaches is needed (Dahlberg *et al.,* 2009).

4.2   Description of *Trametes*

Trametes is a polyphyletic genus. Corner (1989b) has classified *Cerrena, Coriolopsis, Cryptoporus, Daedaleopsis, Datronia, Earliella, Elmerina, Fomitella, Hexagonia, Lenzites, Megasporoporia, Microporus, Mollicarpus, Pycnoporus, Trametes* and *Trichaptum* in the same category with *Trametes*. They are similar due to the type of basidiocarps which is annual to biennial, pileate, dimidiate to flabelliform, single or imbricate, flexible to hard; pilear surface hispid to glabrous, often zonate; hyphal system trimitic; basidiospores ellipsoid to allantoid, hyaline, thin-walled and negative in Melzer's reagent.

However, only *Coriolopsis, Daedaleopsis, Datronia, Earliella, Hexagonia, Lenzites, Microporus, Pycnoporus, Trametes* and *Trichaptum* can be found in Malaysia (Jones *et al.,* 2007). *Coriolopsis, Datronia, Earliella, Hexagonia, Lenzites, Microporus,* and *Pycnoporus* are almost similar with *Trametes*. However, Ryvarden (1991) has found the special characters in each genus to differentiate them from *Trametes.* Lamellate hymenophore and distinct catahymenium in *Lenzites* is the special feature which cannot be found in *Trametes.* Unlike *Trametes, Microporus* has stipitate basidiocarps and small cylindric spores. *Earliella* has a resupinate to effused-reflexed basidiocarps and develops a reddish cuticle on the pileus while *Trametes* has sessile basidiocarps and ellipsoid spores. The context of *Coriolopsis, Datronia,* and *Hexagonia* are based on the colour that is pale to deep brown meanwhile *Trametes* has whitish to isabelline. *Pycnoporus* has special basidiomes in bright reddish-orange which is different with *Trametes.*

Trametes was identified based on basidiocarps annual to biennial, pileate, sessile, dimidiate to flabelliform, single or imbricate, flexible to hard; pilear surface hispid to glabrous, often zonate; pore surface white, cream to pale grey; context white to isabelline, homogeneous or duplex, in some species with a dark line; hyphal system trimitic; generative hyphae with clamps and hyaline; skeletal hyphae thick-walled to solid, hyaline; in some species swelling in KOH; basidiospores ellipsoid to allantoid, hyaline, thin-walled and negative in Melzer's reagent.

In this study, polypores were collected during the field trips from various sites as in Figure 4.1. Each specimen, or several, if they were clearly of the same species, were gathered and labeled. Normally, specimens were sorted into their main group or genus as described by Ryvarden (1991). Any features and colour of the fruit body were noted on a data sheet. Then, sketches and photograph were made before the specimens were dried. The specimens were dried at 40°C and stored in boxes with insect repellent such as mothballs because of the possible insects' colonization. Micromorphology characters such as basidiospore and hyphae of each specimen were observed under phase contrast microscope and the features were sketched under lucida lense. Then, digital photo was recorded using camera attached with the microscope. Macromorphology and micromorphology of each specimen were analysed using the taxonomy keys (Núñez & Ryvarden, 2001). *Trametes feei, T. hirsuta, T. lactinea, T. menziezii* and *T. pocas* were identified and described as in Figures 4.1-4.19.

1.   *Trametes feei* (Fr.) Pat. (Núñez & Ryvarden, 2001)

Basidiocarps annual, solitary to imbricate, dimidiate, semicircular, mostly applanate, 1-5 mm thick at the base, coriaceous soft when fresh and tough when dry (Fig. 4.1).

Pilear surface Pink or pale brick red when fresh, fades when it dries, first velutinate and soft to touch, then it becomes agglutinated and glabrous, strongly zoned in old specimens, from the base are dotted, warts and more outgrowths (Fig. 4.1).

Pore surface whitish-pink when fresh, pore round and entire, 6-8 per mm, tubes concolorous with the pore surface; 3 mm thick (Fig. 4.2).

Hyphal system trimitic, generative hyphae with clamps, thin-walled; skeletal hypahe thick-walled, hyaline; binding hyphae thin-walled, branched (Fig. 4.3).

Cystidia absent.

Basidiospores ellipsoid, 3-5 x 2-3μm (Fig. 4.3 & 4.4).

Habitat/Substrata. On hardwoods. Kepong, Selangor.
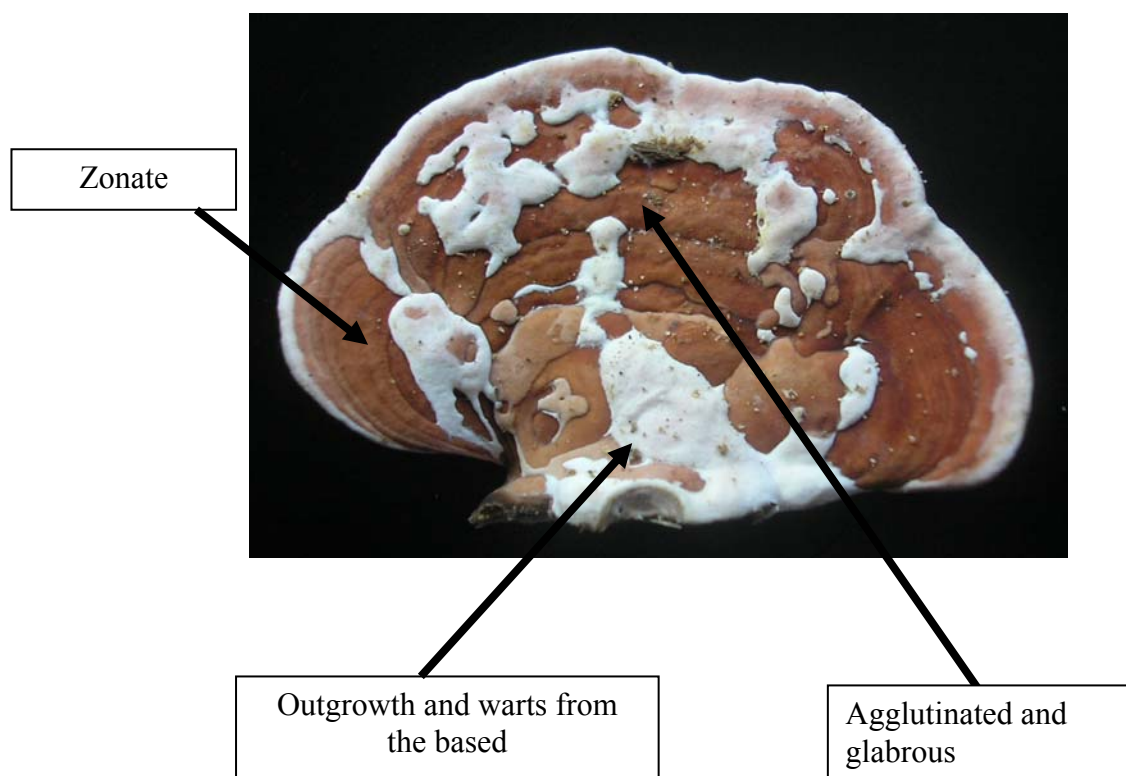
Fig. 4.1: Basidiocarp of *T. feei.*

Fig. 4.2: Pore surface of *T. feei.*

Material examined Malaysia, Selangor, Kepong, Forest Research Institute Malaysia (FRIM), Engkabang Trail, 12 Jan. 2006, Noraswati Mohd Nor Rashid, KUM 70015; Malaysia, Johor, Endau Rompin National Park, Lubuk Tapah, 16 Feb. 2006, Noraswati Mohd Nor Rashid, KUM 70046; Malaysia, Pahang, Kuala Lipis, Pos Senderut, 8 Mac 2006, Noraswati Mohd Nor Rashid, KUM 70087; Malaysia, Kelantan, Jeram Lenang, 22 Feb. 2007, Noraswati Mohd Nor Rashid, WML 07/1.

Notes The pinkish colour throughout the fruit body and the small pores are the characteristics for this species.
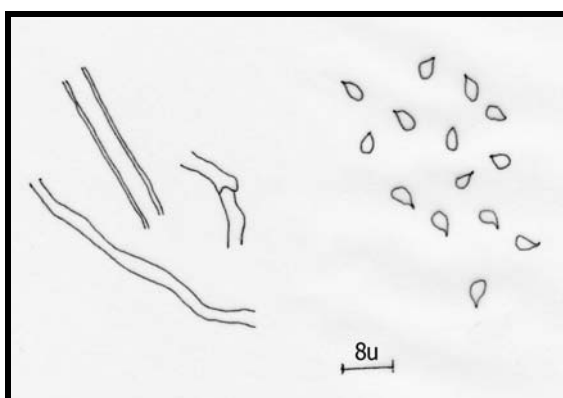
Fig. 4.3: Hyphae and basidiospores of *T. feei* (KUM 70015=KUM 70046=   KUM 70087)
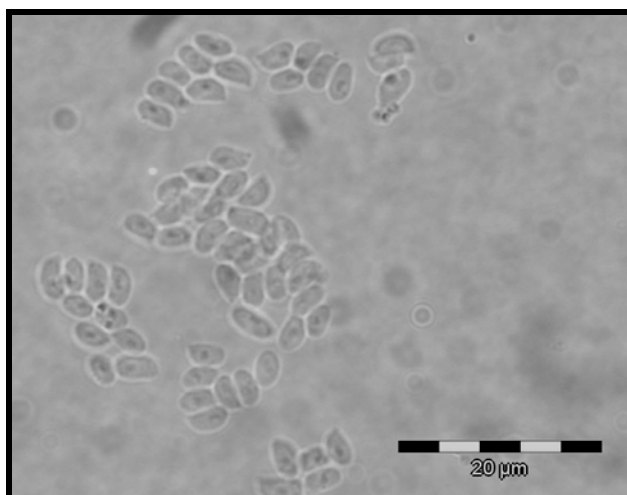
Fig. 4.4: Basidiospores of *T. feei.*

2.   ***Trametes hirsuta*** (Fr.) Pilát (Núñez & Ryvarden, 2001)

Basidiocarps annual, pileate, coriaceous when fresh, dimidiate, applanate (Fig. 4.5).

Pilear surface whitish to grey, often covered with algae when old, hirsute, concentrically zonate (Fig. 4.5).

Pore surface white to tan, becoming grayish when old, pores circular, 3-4 per mm, tubes concolorous with the lower context, 6 mm long; context duplex, the upper layer grey, soft-fibrous, up to 3 mm thick, at least at the base separated by a thin black line from the lower part, the latter ivory white, corcky, up to 1.5 cm thick (Fig. 4.6).

Hyphal system trimitic, generative hyphae with clamps, thin walled, skeletal hyphae thick-walled, hyaline, with few branches, binding hyphae thin-walled and brunched (Fig. 4.7).

Cystidia absent.

Basidiospores ellipsoid, 6-8 x 3-4 μm (Fig. 4.7 & 4.8).

Habitat/Substrata. On hardwoods. Pos Senderut, Kuala Lipis, Malaysia.

Material examined: Malaysia, Pahang, Kuala Lipis, Pos Senderut, 8 Mac 2006, Noraswati Mohd Nor Rashid, KUM 70093.

Notes: The grey, hirsute pilear surface and the grayish pore surface are characteristic of *T. hirsuta*.

Concentrically zonate

Hirsute

Covered with algae
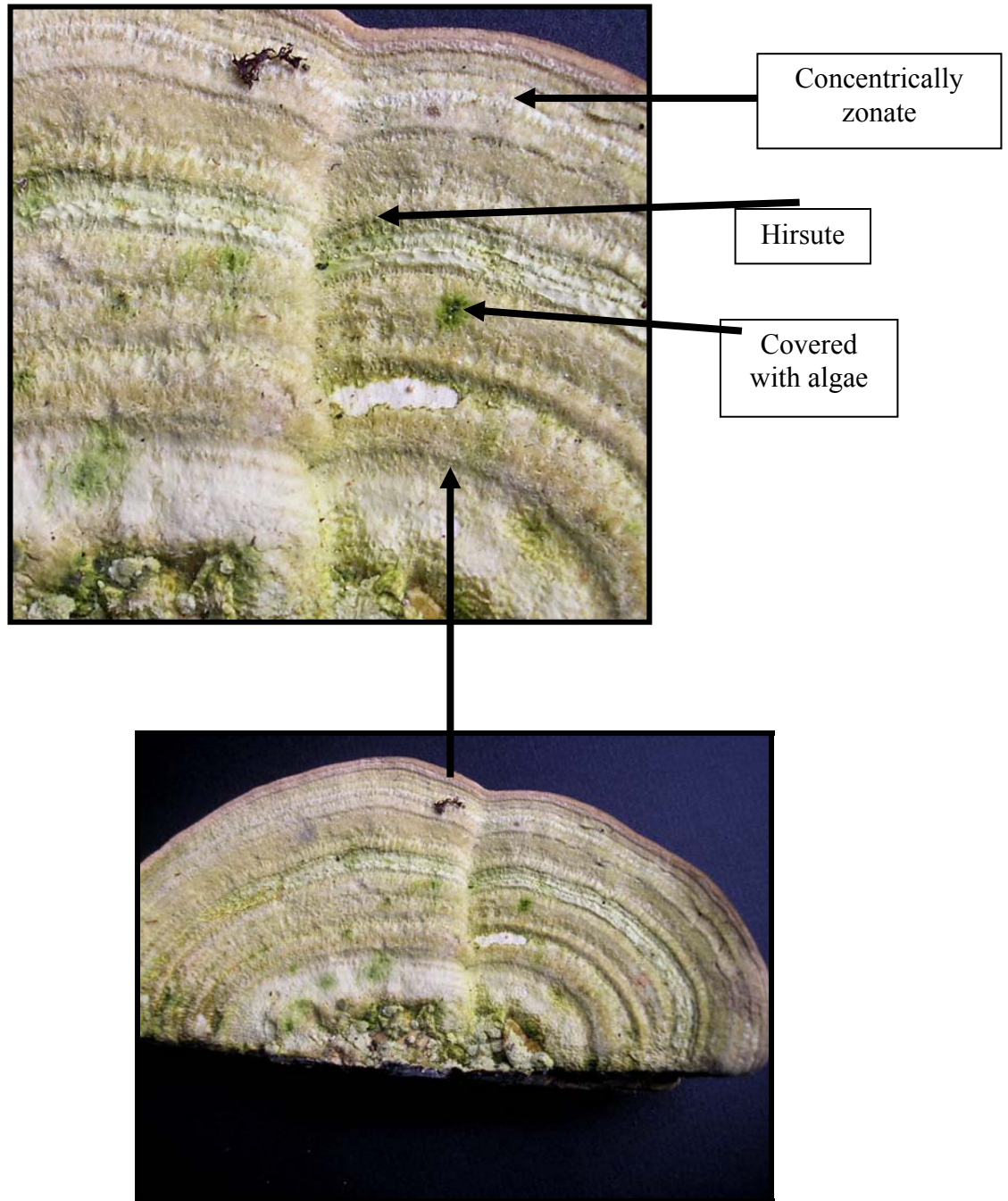
Fig. 4.5: Basidiocarp of *T. hirsuta*

Fig. 4.6: Pore surface of *T. hirsuta.*
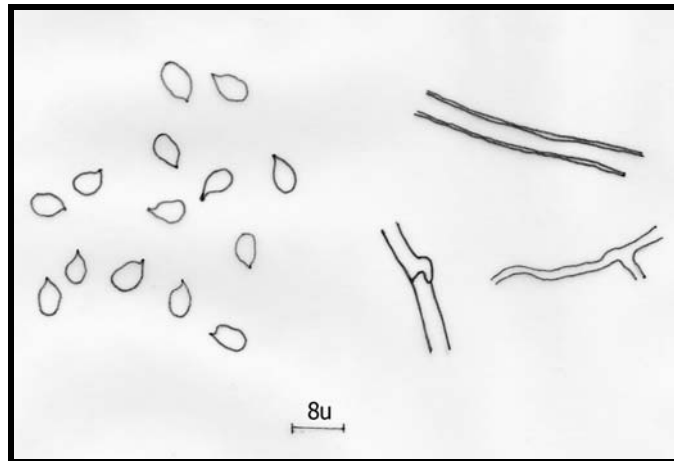
Fig. 4.7: Basidiospores and hyphae of *T. hirsuta* (KUM 70093).
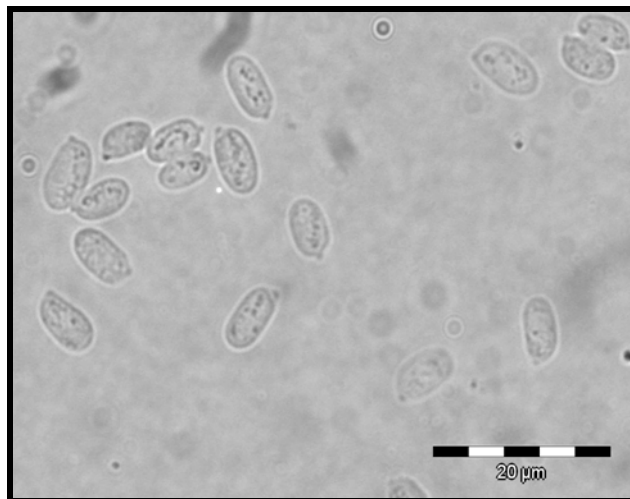
Fig. 4.8: Basidiospores of *T. hirsuta* (KUM 70093).

3. *Trametes lactinea* (Berk.) Pat. (Núñez & Ryvarden, 2001)

Basidiocarps annual to biannual, pileate, solitary, dimidiate to semicircular, applanate, 10 cm broad and wide, 1.1 cm thick, consistency leathery in fresh and become corky to woody hard when dry (Fig. 4.9).

Pilear surface dull, first white to cream, becoming ochraceous to grey or tan, soft and velvety to touch, with age becoming warted or with irregular outgrowth especially near the base, azonate, radially striate, margin entire to weakly lobed, obtuse and thick, concolorous or paler than the rest of pilear surface (Fig. 4.9).

Pore surface cream, ochraceous to pale fulvous, pores round, 1.5-2.0 per mm. (Fig. 4.10).

Hyphal system trimitic; generative hyphae with clamps, hyaline and thin-walled, collapsed and not easy to find in dried specimens; skeletal hyphae abundant, hyaline, thick-walled; binding hyphae abundant, hyaline and thin-walled (Fig. 4.11).

Basidiospores ellipsoid, 6-8 x 2-3.5 μm (Fig. 4.11 & 4.12).

Habitat/Substrata. On several hardwood. Open area. Selangor, Malaysia.

Material examined: Malaysia, Selangor, Kepong, Desa Jaya, 20 Mac 2006, Noraswati Mohd Nor Rashid, KUM 70117; Malaysia, Selangor, Kepong, Desa Aman Puri, 12 July 2006, Noraswati Mohd Nor Rashid, KUM 70150.

Notes. The species is recognized by the velvety, azonate pileus, thick basidiocarps and large pores.

Warted

Azonate

Margin entire to
weakly lobed
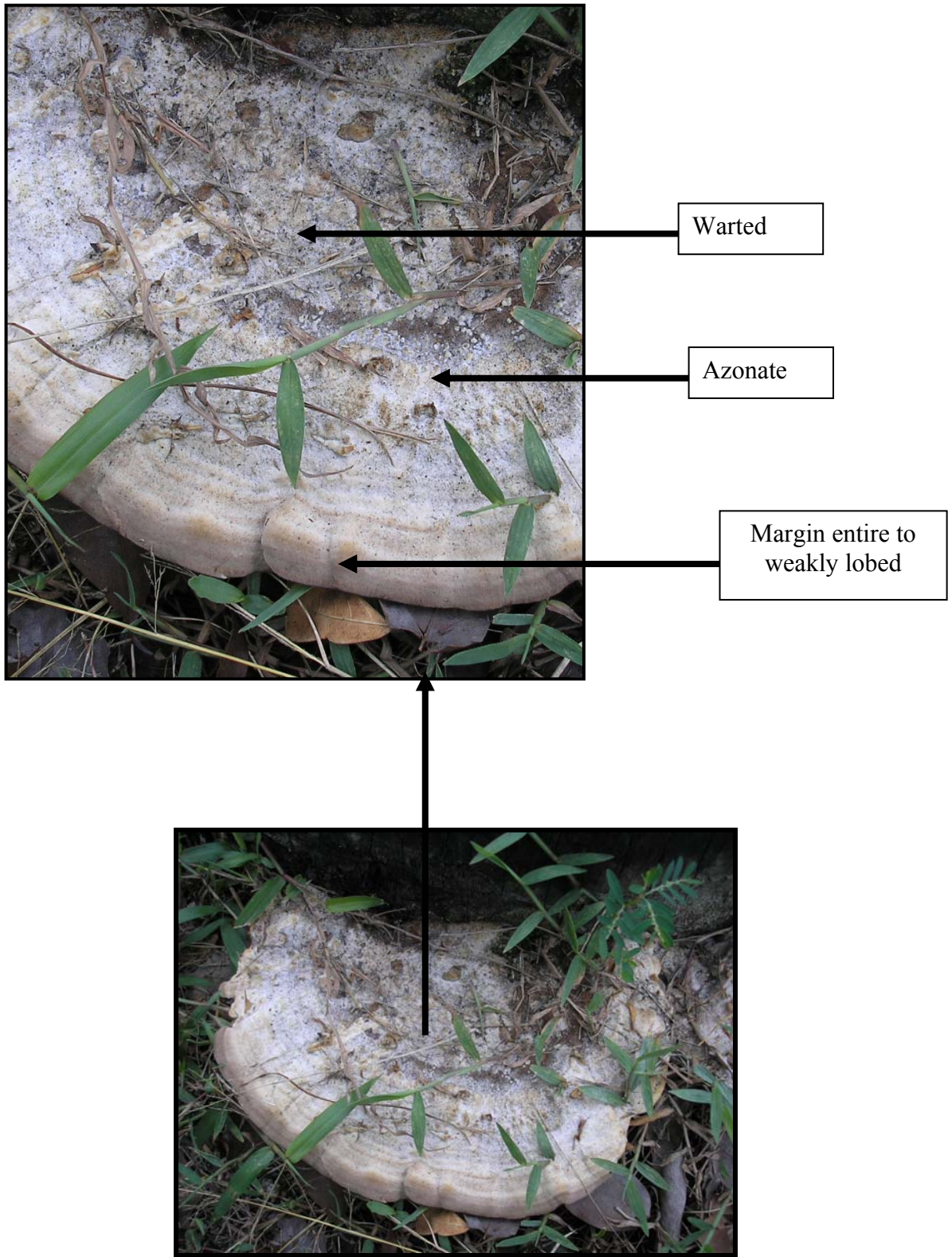
Fig. 4.9: Basidiocarp of *T. lactinea*

Fig. 4.10: Pore surface of *T. lactinea*
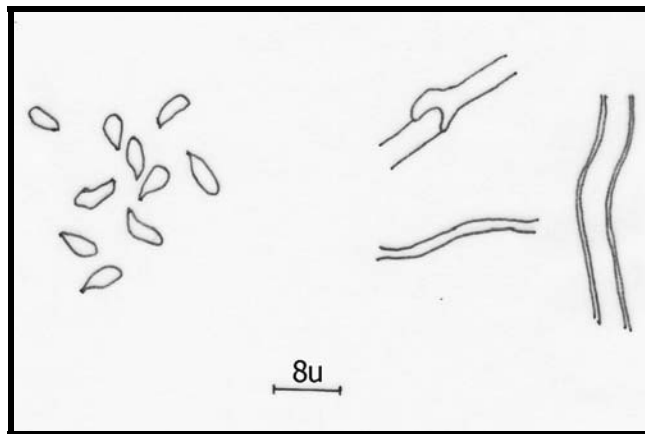
Fig. 4.11: Basidiospores and hyphae of *T. lactinea* (KUM 70117=KUM 70150)
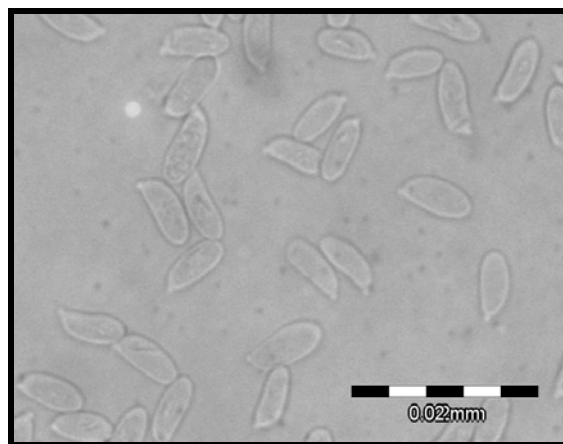
Fig. 4.12: Basidiospores of *T. lactinea* (KUM 70117=KUM 70150)

4.   *Trametes menziezii* (Berk.) Ryvarden (Núñez & Ryvarden, 2001).

Basidicarps annual, variable in size, pileate, single or imbricate to gregarious, applanate, substipitate, dimidiate to flabelliform, almost circular when the lobes behind partly meet over the point of attachment, up to 15 cm wide and long, 3-8 mm thick, flexible and tough when fresh, becomes hard when kept longer in thicker specimens, margin thin and acute, strongly lobed and usually deflexed in dry specimens (Fig. 4.13).

Pilear surface first white to ochraceous, in most cases, it becomes grayish in different shades, darker towards the base, first appreassed velvety, usually it soon becomes glabrous, 1-3 mm wide concentric zones, smooth or slightly sulcate, wrinkled radially when dry, in some specimens with an outgrowth from the base and normally paler than the pileus (Fig. 4.13).

Stipe or contracted base often distinct with a 2-1 cm long sterile area between the pore layer and the substrate, white to deep grey.

Pore surface cream coloured in fresh specimens, become pale tan when dry, in old and wrinkled specimens more deep ochraceous, pores variable, partly entire, round and small, 6-7 per mm, but usually larger, round to angular and from 2-6 per mm and tubes concolorous with the pore surface (Fig. 4.13).

Hyphal system trimitic; generative hypae with clamps, hyaline, thin walled; skeletal hypae hyaline, thick-walled; binding hypae abundant, thin-walled (Fig. 4.14).

Basidiospores ellipsoid to cylindrical, 3.5-5 x 1-2 μm, rarely found in dry specimens (Fig. 4.14 & 4.15).

Habitat/Substrata.On dead hardwoods, often in open and dry localities. Johore, Malaysia.

Material examined Malaysia, Johore, Endau Rompin National Park, Takah Tujuh, 16 Feb. 2006, Noraswati Mohd Nor Rashid, KUM 70044; same location, 17 Feb. 2008, Noraswati Mohd Nor Rashid, KUM 70074; Malaysia, Kedah, Langkawi, Kg. Buku,

2 Jun 2006, Noraswati Mohd Nor Rashid, KUM 70143; Malaysia, Terengganu, Sekayu, 20 Apr. 2007, Noraswati Mohd Nor Rashid, WML 07/140.

Notes The species is variable especially the size of the pore. The basidiocarps always substipitate with grayish zonate pileus.

Concentric zones

Margin thin acute

Outgrowth from the base

Lobes behind meet over

Pore surface cream coloured
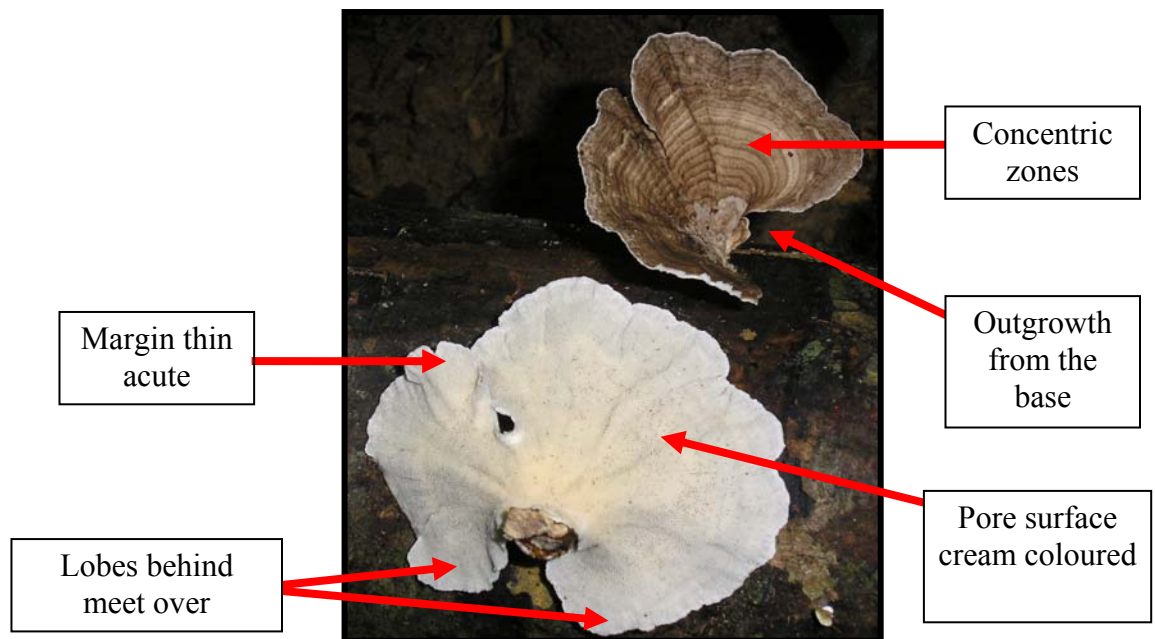
Fig. 4.13: Basidiocarp of *T. menziezii*

8u
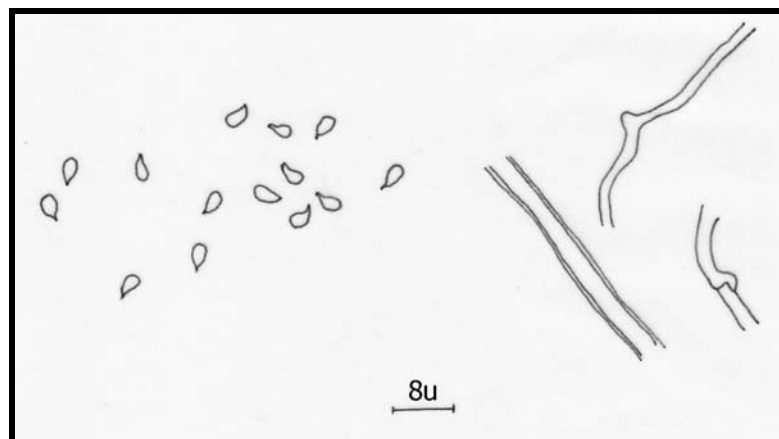
Fig. 4.14: Basidiospores and hyphae of *T. menziezii* (KUM 70044=KUM 70074=KUM 70143)
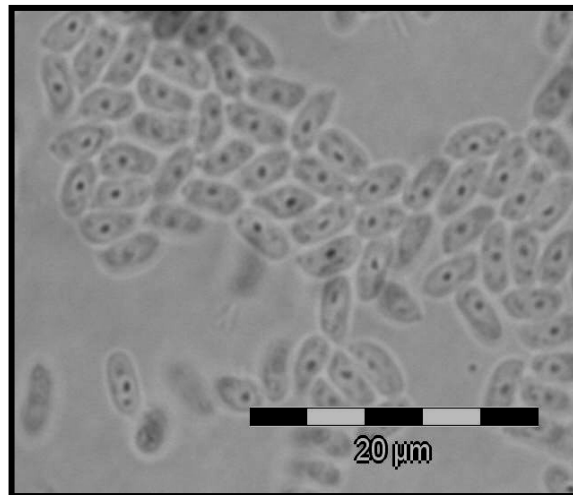
Fig. 4.15: Basidiospores and hyphae of *T. menziezii* (KUM 70044=KUM 70074=KUM 70143)

5. *Trametes pocas* (Berk.) Ryvarden (Núñez & Ryvarden, 2001).

Basidiocarps annual to biennial, pileate, dimidiate to flabelliform, more rarely effused-reflexed, often fused laterally to form compound basidiocarps, flexible, up to 5 cm and long,up to 1 mm thick at the base, margin thin, undulated to lobed in fresh specimens; curled in dry specimens (Fig. 4.16).

Pilear surface white, yellow-grey to unevenly pale to dirty brown, frequently greenish because of algae, strigose to hirsute, coarsely towards the base, distinctly zonate with persistent tomentum (Fig. 4.16).

Pore surface white to cream, pores angular, 1-3 per mm, tubes shallow, up to 1 mm long; context white and thin (Fig. 4.17).

Hyphal system trimitic; generative hyphae with clamps, hyaline, thin-walled; skeletal hyphae hyaline, thick- walled to solid; binding hypae solid, hyaline (Fig. 4.18).

Cystidia absent.

Basidiospores broadly ellipsoid, 4-5 x 2-3 μm (Fig. 4.18 & 4.19).

Habitat/ Substrata. On hardwords. Selangor, Malaysia
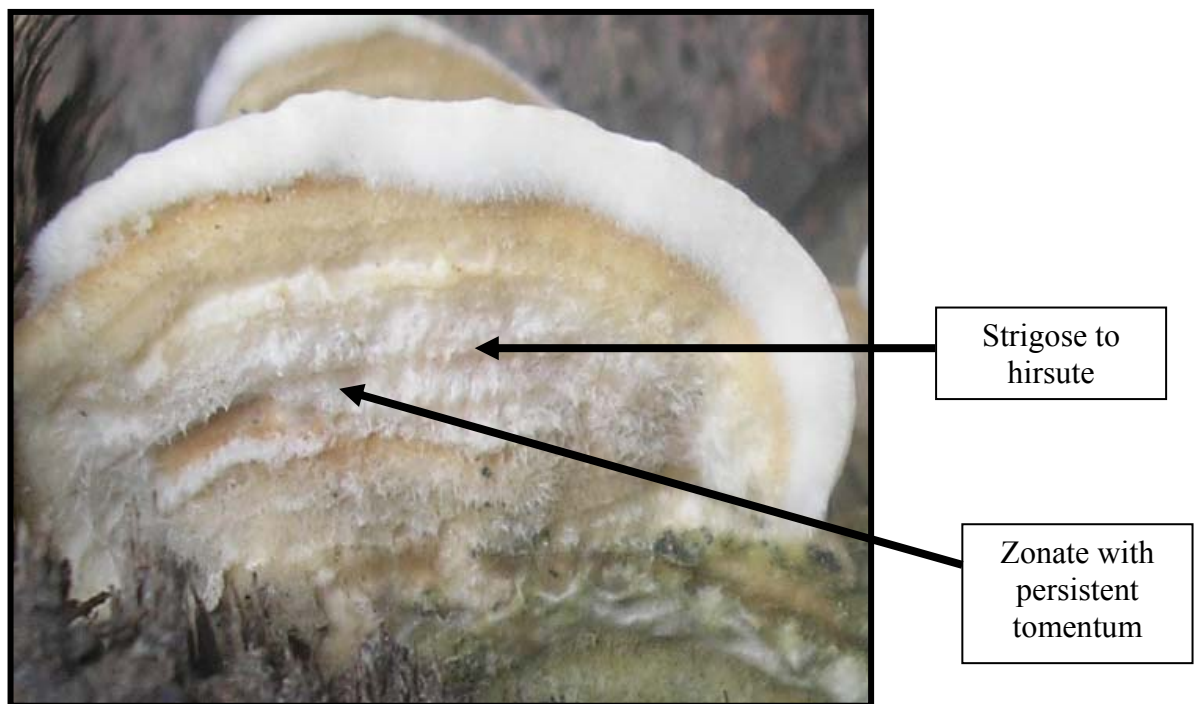
Fig. 4.16: Basidiocarp of *T. pocas.*

Fig. 4.17: Pore surface of *T. pocas.*

Material examined Malaysia, Kuala Lumpur, University Malaya, 20 Sept. 2006, Noraswati Mohd Nor Rashid, KUM 70160; Malaysia, Selangor, Kepong, Forest Research Institute Malaysia (FRIM), Jalan Bukit Bujang, 12 Jan. 2006, Noraswati Mohd Nor Rashid, KUM 70161.

Notes Easy to recognize because of the thin, pliable basidiocarps with a hirsute pileus and broadly ellipsoid basidiospores.
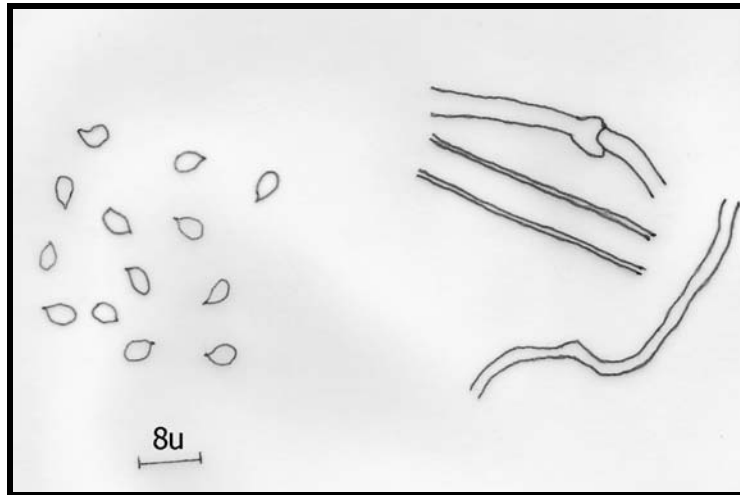
Fig. 4.18: Basidiospores and hyphae of *Trametes pocas* (KUM 70160=KUM 70161).
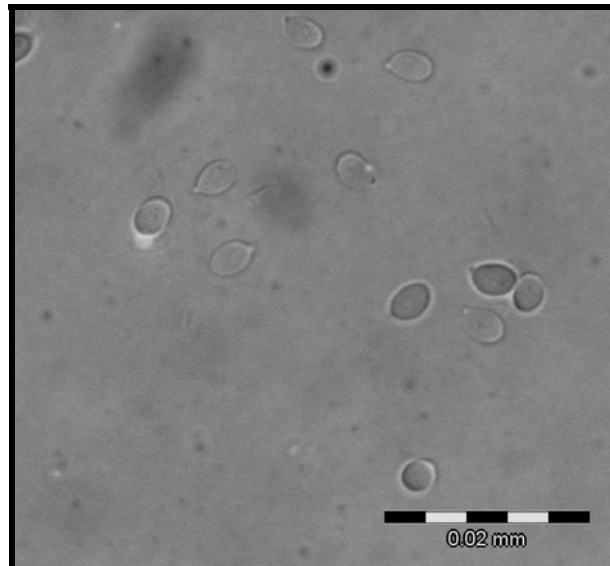
Fig. 4.19: Basidiospores of *T. pocas* (KUM 70160=KUM 70161).

Trametes feei is also known as *Fomitopsis feei*. The special character for this species is the pinkish and flexible basidiocarps. *Trametes hirsuta* is easily recognized through the greyish hirsute structure or hairy development at the upper side of the pileus meanwhile *T. lactinea* has a thick basidiocarps and a warty structure on the pileus. This is followed by *T. menziezii* that has a zonate, thin and fan shape or flabelliform pileus. *Trametes pocas* is usually recognised by its effused-reflex, small and thin basidiocarps; with hairy structure on the pileus. *Trametes lactinea* (6-8 x 2-3.5 μm) and *T. hirsuta*

(6-8 x 3-4 μm) have ellipsoid basidiospores which are bigger than *T. feei* (3-5 x 2-3μm) and *T. menziezii* (3.5-5 x 1-2 μm). The smallest basidiospores is *T. pocas* (4-5 x 2-3 μm) which has a broad ellipsoid. All of the five *Trametes* species have the same trimitic system hyphae; basidiospores ellipsoid, hyaline and negative reactions in Melzer's reagent; and flexible basidiocarps.

4.3   Production of antioxidants from *Trametes* spp.

4.3.1   Yield of extracts from mycelia of *Trametes* spp.

Mycelial biomasses of *Trametes* spp. were obtained by fermentation in 2L GYMP liquid media under static condition for 14 days at 28°C. The mycelium and the liquid media was freeze dried to obtain the dried mycelial biomass. The yields of dried mycelial biomass of *Trametes* spp. were summarized in Table 4.1, ranging from 27-43.5 g/L. The highest yield of mycelial biomass was *T. lactinea* (43.5 g/L), followed by *T. menziezii* (39.6 g/L), *T. hirsuta* (29.8 g/L) and *T. pocas* (28.0 g/L), respectively. The lowest yield of mycelial biomass was *T. feei* (27.0 g/L). Growth rate of fungi were measured by colony radius or mycelial dry weight (Long and Harsh, 1918). The highest yield of mycelial biomass of *T. lactinea* might be due to the rapid growth of the mycelia compared with other *Trametes* species. The texture of the mycelial mat may affect the yield of mycelial biomass of *Trametes* spp. The texture of the mycelial mat was described as cottony: rather long, single mycelial hyphae spreading in all directions; wooly: fairly long interwoven hyphae or groups of hyphae, somewhat matted, resembling woollen cloth; felty: cottony or woolly mycelium, which has become matted or packed; or lacunose: mycelial surface depressed or indented (Long and Harsh, 1918; Nobles, 1948, 1965).

Methanol was used to extract the polar compounds and dichloromethane to extract non polar compounds from the mycelial biomass of *Trametes* spp. The yields of

methanol extract of *Trametes* spp. ranging from 5.03%-12.63% were higher than dichloromethane extracts ranging from 0.03%-0.15% as shown in Table 4.2. The higher yield was mainly due to the fact that filtrate may contain large amount of polar compounds using methanol solvent than non-polar compounds in dichloromethane solvents.

Table 4.1: Dry weight of mycelial biomass of *Trametes* spp.

No.	Code of extract	Scientific name	Weight (g)/L
1	KUM 70160	*T. pocas*	28.0
2	KUM 70155	*T. menziezii*	39.6
3	KUM 70150	*T. lactinea*	43.5
4	KUM 70093	*T. hirsuta*	29.8
5	KUM 70015	*T. feei*	27.0

Mau *et al.*, (2005) has reported that the yield of methanolic mycelial extract of *Ganoderma tsugae* at 40.64% was higher than yield of methanolic extracts from fruiting bodies at 8.46%. Apparently, yield of methanol extracts of *Trametes* spp. seemed to be in the range of higher yields compared with yield of methanolic extracts from the fruiting bodies of *G. tsugae*.

Table 4.2: Yields of methanolic and dichloromethane extracts from *Trametes* spp.

Sample	Extraction yield (%)	
	Methanol (MeOH)	Dichloromethane (DCM)
T. pocas	6.39	0.07
T. menziezii	10.83	0.08
T. lactinea	2.80	0.07
T. hirsute	5.03	0.03
T. feei	12.63	0.15

4.3.2   Radical-scavenging activity of *Trametes* extracts

The methanol and dichloromethane extracts of *Trametes* spp. were tested for their scavenging effect on DPPH. The data and graphs for the determination of the time (minutes) require for the methanolic and dichloromethane extracts of *Trametes* spp. and

acid ascorbic to react with DPPH radicals and reach the steady state are given (refer to Appendix B1). The amount of time taken for methanolic extracts of *Trametes* spp. to reach a steady state is 30 minutes after thereaction while dichloromethane extracts of *Trametes* spp. requires 45 minutes. The positive control used was ascorbic acid when it reacted immediately after DPPH was added and shows a rapid kinetic behaviour. The methanolic and dichloromethane extracts of *Trametes* spp. exhibited moderate kinetics as shown in Figs. 4.20 & 4.21 whereby the methanol extracts exhibited faster reaction kinetics than dichloromethane extracts.
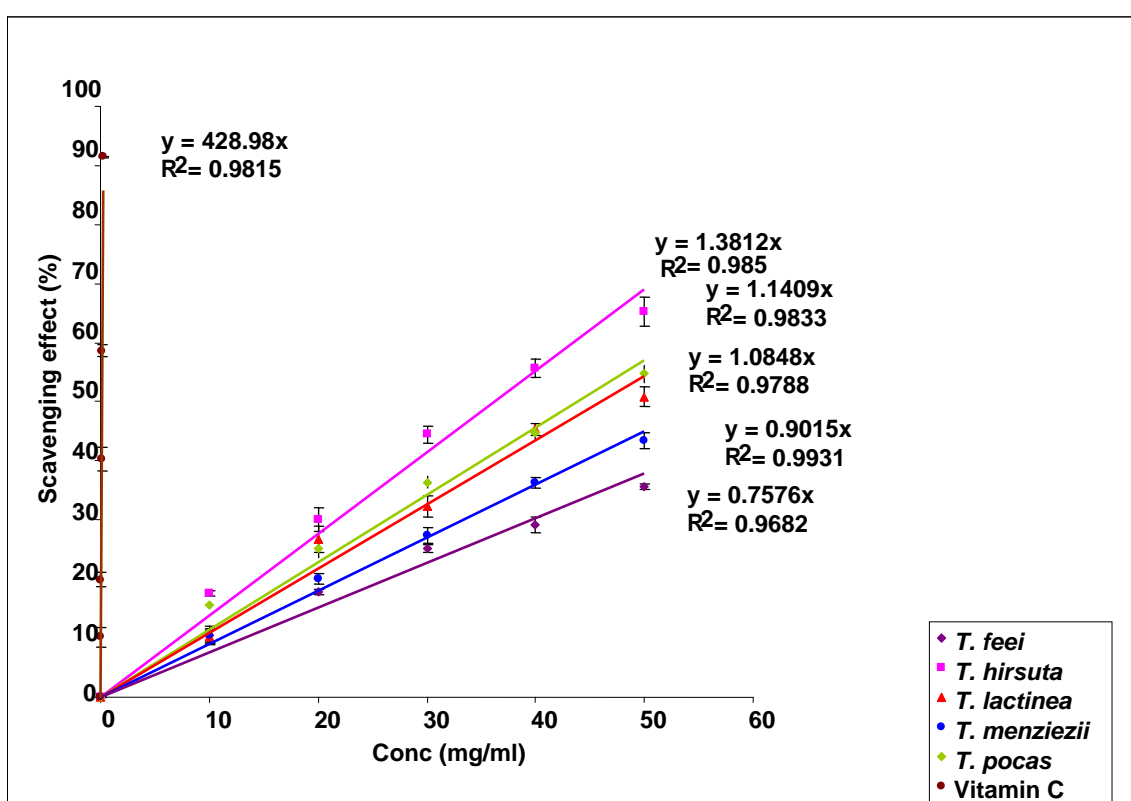
Fig. 4.20: Scavenging effect (%) of *Trametes* spp. dichloromethane extracts on DPPH radicals at 30 minutes. Each value is expressed as mean ± standard deviation (*n* = 3).

It can be seen that DPPH radical scavenging effect of methanolic and dichloromethane extracts of *Trametes* spp. increased as concentration was increased. Antioxidant can deactivate (scavenge or quench) free radicals by two major mechanism: by reduction via electron transfer or by hydrogen atom transfer that may also occur in parallel (Huang *et al.,* 2005). The end results are the same, regardless of the mechanism,

but the kinetics are differ (Prior *et al.,* 2005). DPPH scavenging is considered to be mainly based on electron transfer whilst hydrogen atom transfer is a marginal reaction pathway (Foti *et al.,* 2004). The contribution of a particular pathway depends on the species involved. The initial step includes the reactions of electron and/or H atom transfer from an antioxidant to the free radical. Therefore, different kinetics model have been shown in methanolic and dichloromethane extracts of *Trametes* spp.
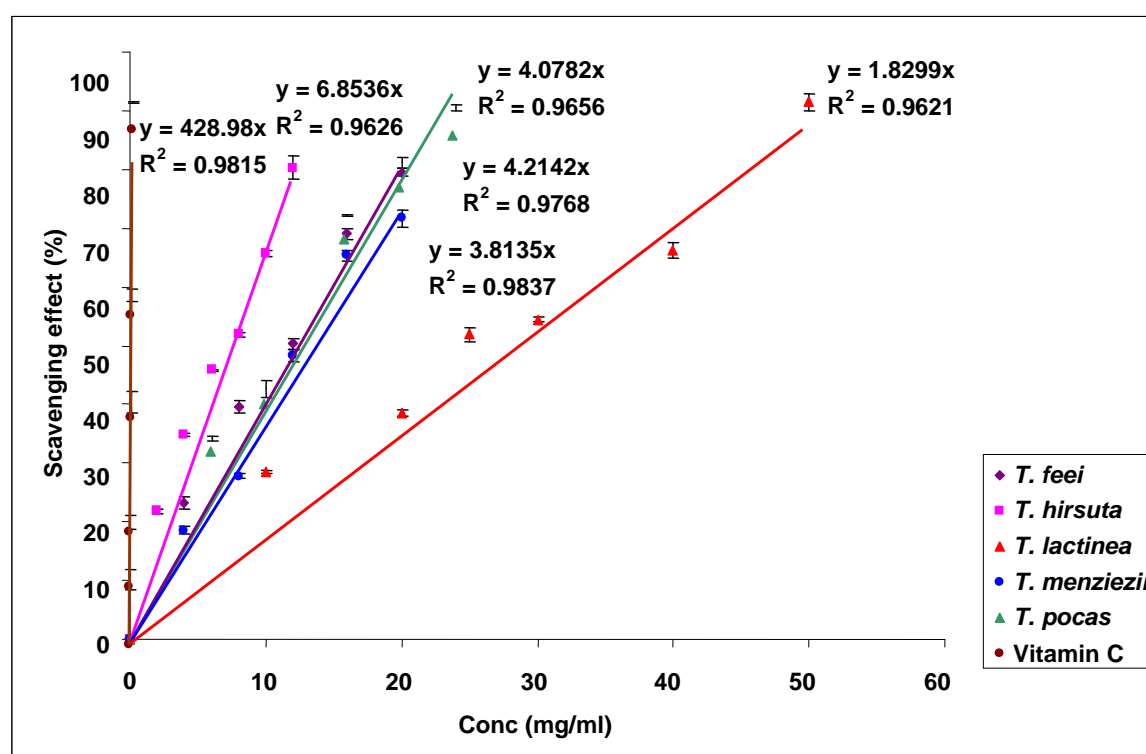
Fig. 4.21: Scavenging effect (%)of *Trametes* spp. methanolic extracts on DPPH radicals at 45 minutes. Each value is expressed as mean ± standard deviation (*n* = 3).

The IC_{50} values were calculated from the graphs of DPPH radical scavenging ability versus concentrations of crude extracts at steady state. The lower the IC_{50} values, the more efficient the antioxidant activity of the extracts. IC_{50} values of methanolic extracts of *Trametes* spp. ranging from 7.296 to 27.324 mg/ml and dichloromethane extracts ranging from 36.2 to 66 mg/ml was displayed in Fig. 4.22.

Generally, methanol extracts exhibited lower IC_{50} values than dichoromethane extracts. Among the methanol extracts, *T. hirsuta* showed the most efficient antioxidant activity with IC_{50} value of 7.296 mg/ml. This was followed by *T. feei, T. pocas* and

T. menziezii with IC_{50} of 11.87 mg/ml, 12.26 mg/ml and 13.111 mg/ml. The lowest IC_{50} value in methanol extracts was showed by *T. lactinea* at 27.324 mg/ml.

The dichloromethane extracts of *T. hirsuta* exhibited the highest DPPH scavenging activity with IC_{50} value of 36.2 mg/ml, followed by *T. pocas*, *T. lactinea* and *T. menziezii* with IC_{50} value of 43.825 mg/ml, 46.09 mg/ml and 55.463 mg/ml. The lowest antioxidant activity was exhibited by *T. feei* with IC_{50} value of 66 mg/ml. Ascorbic acid (Vitamin C) was used as a positive control for antioxidant activity in this study. The IC_{50} value of ascorbic acid obtained was much lower, which was 0.117 mg/ml compared to the methanol and dichloromethane extracts of *Trametes* spp.

Antioxidant activity of vegetables was varianced due to the type of extraction solvent and antioxidant assay used (Manzocco *et al.,* 2001). In this study, polar compounds of methanol extract from mycelia of *Trametes* spp. exhibited a strong antioxidant activity than non-polar compounds. Therefore, only methanol extracts of *Trametes* spp. showed better scavenging activity. Hence, the study has further analysed the reducing ability and quantification of total phenolic content.

Mau *et al.,* (2004b and 2005) found that methanol extracts from mycelial of *Antrodia camphorata* and *Ganoderma tsugae* exhibited antioxidant properties with more than 50% scavenging activity at concentration of 10 mg/ml while methanol extracts from mycelial of *Trametes* spp. in this study was showed less than 50% scavenging activity at 10 mg/ml. Scavenging effect on DPPH radicals of methanol extracts from fruit bodies of *Coriolus(Trametes) versicolor, G. lucidum* and *G. tsugae* were 24.6 %, 67.6% and 73.5 % at 0.64 mg/ml (Mau *et al.,* 2002a). In this study, methanol extracts from mycelial of *Trametes* spp. exhibited only 4.39-1.17% of scavenging effect on DPPH radicals at same concentration, 0.64 mg/ml. These results indicated that the antioxidant activity in *T. feei, T. hirsuta, T. lactinea, T. menziezii* and

T. pocas were lower than *Antrodia camphorata, Coriolus(Trametes) versicolor, G. lucidum* and *Ganoderma tsugae.*

The various research groups used different protocols which differed in the concentration of DPPH (22.5-250 µM), incubation time (5 min-1h), reaction solvent and pH of the reaction mixture. High concentration of DPPH in the reaction mixture gives an absorbance beyond the accuracy of spectrophotometric measurements (Ayres, 1949; Slone and William, 1977). As a result of these differences in the reaction conditions, the IC_{50} values for even the standard antioxidants like ascorbic acid and BHT varies significantly between researchers. Therefore, it is not possible to compare the results of different laboratories (Kano *et al.,* 2005; Ricci *et al.,* 2005).
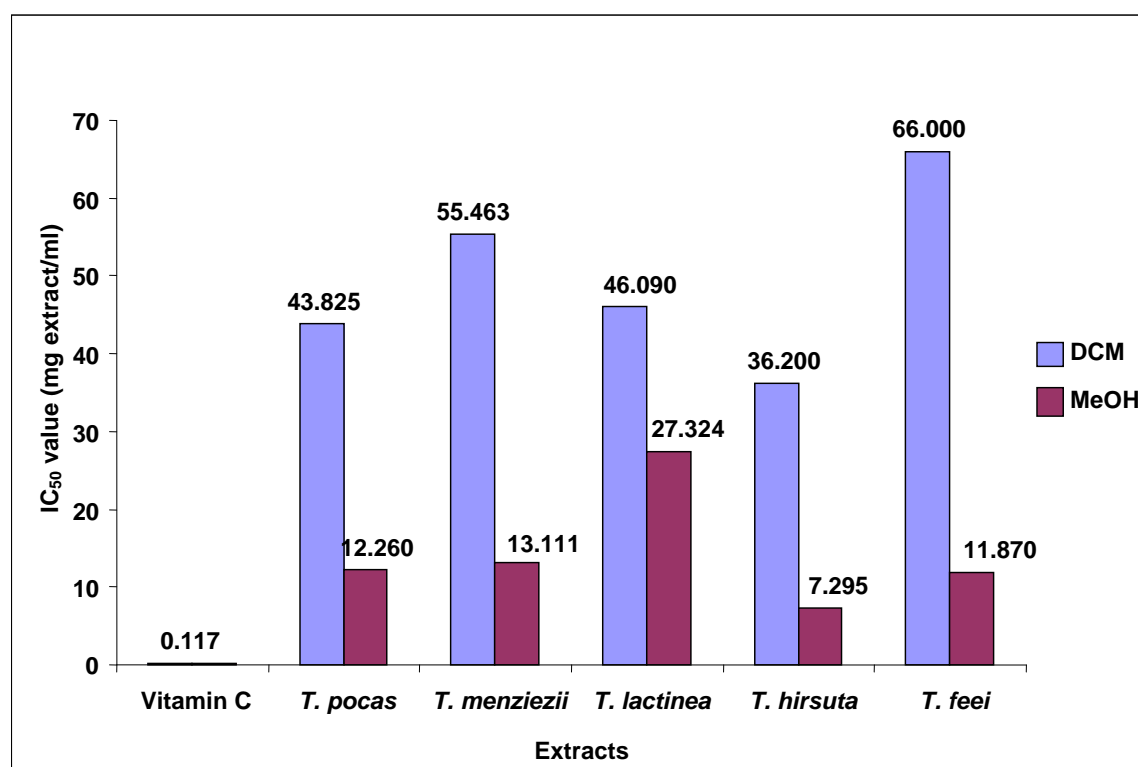
Fig. 4.22: IC_{50} values of methanolic and dichloromethane extracts of *Trametes.*

4.3.3   CUPRAC assay of *Trametes* extracts

The methanol extracts of *Trametes* spp. were tested for their reducing ability in the CUPRAC assay. The data for the determination of the absorbance with various concentrations in oxidation reaction with the CUPRAC reagent are given in Appendix B. The graph of the methanol extracts of *Trametes* spp. as absorbance versus concentration with respect to the CUPRAC method was displayed in Fig. 4.23, which generally gave correlation coefficients close to 1, i.e., r ≥0.999, within the absorbance range of 0.1-1.07. Reducing ability of methanol extracts of *Trametes* spp. were excellent and increased steadily with the increased concentration. The highest reducing ability at 0.5 mg/ml was exhibited by *T. feei* was 0.5367, followed by *T. pocas*, *T. hirsuta* and *T. menziezii* were 0.4681, 0.4279 and 0.3965, respectively. The lowest reducing ability at 0.5 mg/ml was exhibited by *Trametes lactinea* was 0.3457. Ascorbic acid (Vitamin C) was used as a positive control for antioxidant activity in this study. The reducing ability of ascorbic acid obtained was much better than *Trametes* spp., which was achieved 0.59 at concentration lower than 0.5 mg/ml.

Mau *et al.* (2002a) has found reducing powers of methanolic extracts from fruit bodies of *G. tsugae*, and *G. lucidum* were 1.26 and 0.99 at 2 mg/ml while methanolic extracts from mycelia of *Trametes* spp. were exhibited better at 2 mg/ml which were 1.38-2.15. Mau *et al.* (2002a) has also observed reducing power of *T. versicolor* was 0.79 at 4.0 mg/ml while in this study, *Trametes* spp. was exhibited 2.77-4.29 at 4.0 mg/ml. Mau *et al.*, (2003) has found that reducing powers of methanolic extracts from submerged mycelia of *A. camphorata* were >0.64 at 2.5-10 mg/ml compared to methanol extracts of *Trametes* spp. in this study were >1.73 at 2.5-10 mg/ml.

In this study, CUPRAC assay was used based on reduction of Cu(II) to Cu(I) by antioxidants while the results from the previous studies were obtained by FRAP assay. FRAP has two major flaws: (1) FRAP assay is conducted at acidic pH (3.6) to maintain

iron solubility; (2) FRAP assay does not measure thiol antioxidants, such as glutathione (Prior *et al.,* 2005). Thus, FRAP may not give comparable relative values in physiological conditions. Furthermore, the CUPRAC reagent is fast enough to oxidize thiol-type antioxidant (Apak *et al.,* 2005; Tütem & Apak, 1991). Therefore, *Trametes* spp. has showed much better antioxidant activity compared with other mushroom species in the previous studies.
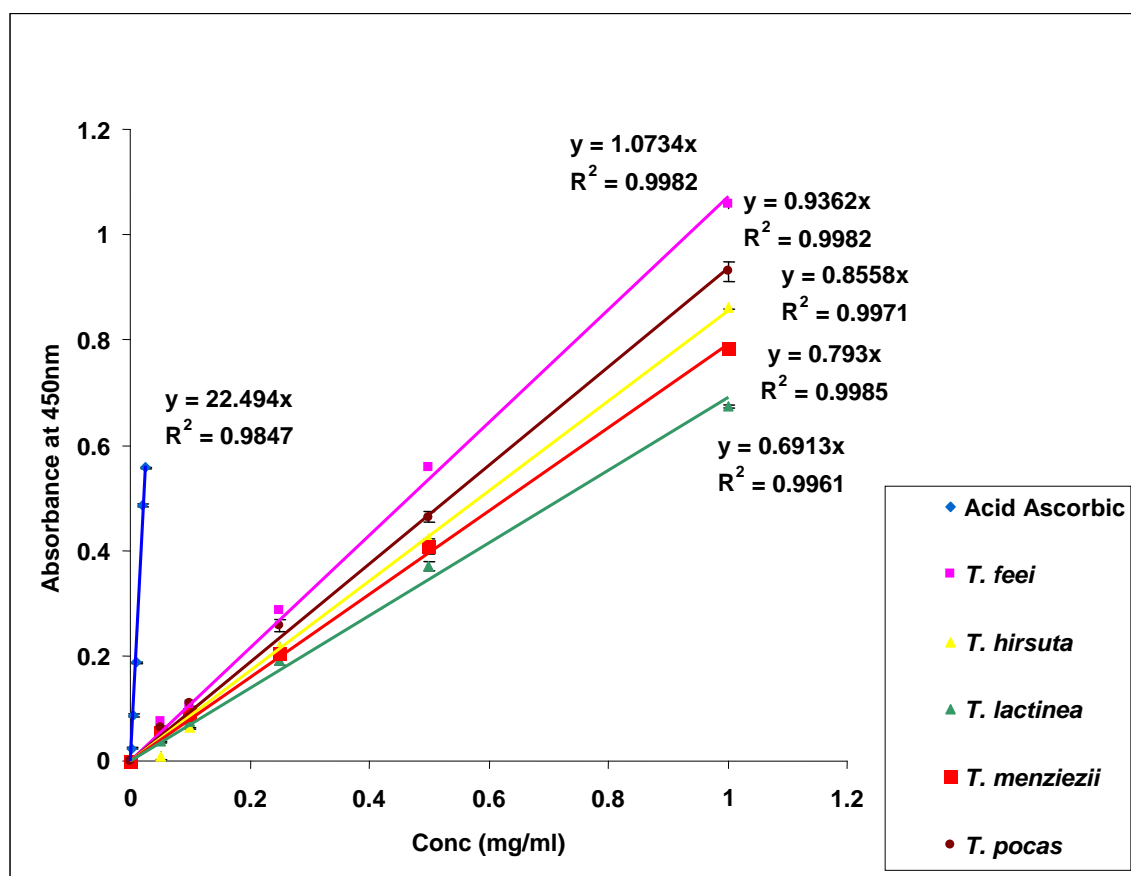
Fig. 4.23: CUPRAC assay of *Trametes* extracts. Each value is expressed as mean ± standard deviation (n = 3).

The reducing power of methanol extracts of *Trametes* spp. mycelia might be due to its hydrogen-donating ability, as described by Shimada *et al.,* (1992). As a result, *Trametes* spp. mycelia might contain some reductones, which could react with free radicals to stabilize and terminate radical chain reactions.

4.3.4 Total phenolic content of *Trametes* extracts

The Folin-Ciocalteu method is an electron transfer based assay and gives reducing capacity, which has normally been expressed as phenolic contents (Prior *et al.,* 2005). The amount of phenolics compounds was calculated as gallic acid equivalents as in Appendix B3. Total phenolic content of methanolic extracts of *Trametes* spp. ranging from 10.54 to 23.28 mg/g extract was showed in Fig. 4.24. The highest total phenolic content was found in *T. feei* (23.28 mg/g) followed by *T. pocas* (17.4 mg/g), *T. hirsuta* (15.54 mg/g) and *T. menziezii* (15.2 mg/g). The lowest total phenolic content was in *T. lactinea* (10.54 mg/g).

Total phenolic content in methanolic extracts of fruit bodies commercial mushrooms were 6.27-10.24 mg/g (Yang *et al.,* 2002). This is followed by *G. tsugae* with 35.6 mg/g (Mau *et al.,* 2005), *Agaricus blazei* with 5.67 mg/g (Tsai *et al.,* 2007), *G. lucidum*, 47.25 mg/g and *T. versicolor,* 23.28mg/g (Mau *et al.,* 2002a). Elmastas *et al.,* (2007) has found the total phenolic compounds in fruit bodies of methanolic extract of *Polyporus squamosus* which was 13.9 mg/g and *Pleurotus ostreatus* with 12.1 mg/g. It seems that the total phenolic compound in methanolic extracts from mycelia of *Trametes* spp. in this study was better than fruit bodies commercial mushrooms, *A. blazei, P. squamosus* and *P. ostreatus*. However, it was surprised that *T.feei* showed the similar total phenolic compound with *T. versicolor* which was 23.28 mg/g. Lee *et al*., (2007) has found antioxidant component, including ascorbic acid, β-carotene, tocopherols and total phenols were found in three extracts of fruit bodies, mycelia and filtrate from *Pleurotus citrinopileatus.* Therefore, it is revealed that some other components has also existed and has contributed in the antioxidant properties of *Trametes* spp.

Mushrooms contain phenolic compounds which are recognized as an excellent antioxidant due to their ability to scavenge free radicals by single-electron transfer

(Hirano *et al.,* 2001). Phenolic compounds were found to have antioxidant activity in the inhibition of LDL oxidation (Teissedre and Landrault, 2000). The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals (Deckers, 1997).
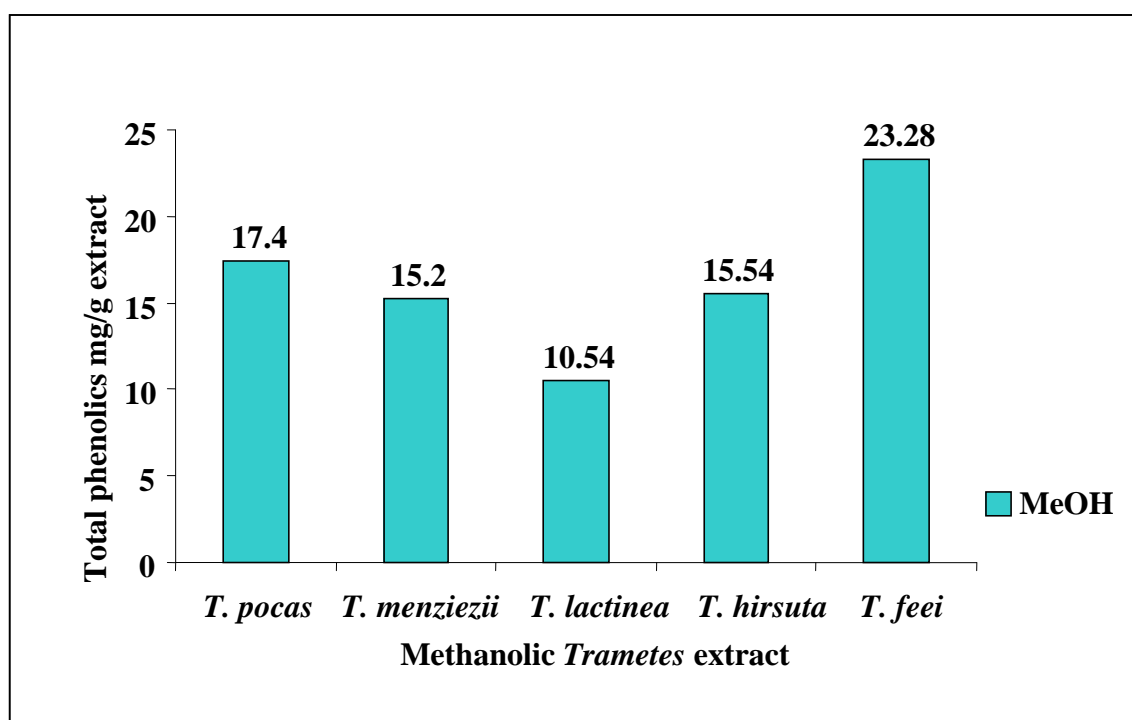
Fig. 4.24: Total phenolic content of *Trametes* methanolic extracts.

Numerous studies have conclusively showed that consumption of food high in phenolic content can reduce the risk of heart disease by slowing the progression of atherosclerosis, because they act as antioxidants (Halliwell and Gutteridge, 2003; Kaur and Kapoor, 2002; Kahkonen *et al.,* 1999).

4.3.5 Correlation of total phenolic content with DPPH and CUPRAC assay

Correlation of total phenolic content and scavenging effects on DPPH radicals of all *Trametes* extracts and also the correlation with CUPRAC reducing ability are

shown in (Fig. 4.25a and b). A weak correlation was found between the scavenging effects on DPPH radicals of the methanolic extracts with the total phenolic content in *Trametes* spp. ($r^2 = 0.1332$). This result might explain that the antioxidant of the extract was attributed to other non-phenolic compounds. Mushrooms have been proven as source of polyprenols (Kukina *et al.,* 2005) and carotenoids (Mui *et al.,* 1998), which are non-polar groups that posesses high antioxidant activity. On the other hand, a strong correlations was found in *Trametes* methanolic extracts between its reducing ability and total phenolic contents ($r^2 = 0.9546$). This phenolic compound may contribute directly to antioxidative action (Duh *et al.,* 1999). It was reported that the antioxidant activity of phenolics was mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators and single oxygen quencher (Rice-Evans *et al.,* 1995).

Overall, it could be reasonable to suggest that both phenolic and non-phenolic components were responsible for the total antioxidative activity of the *Trametes* spp. extracts. Elmastas *et al.,* (2007) has found α-tocopherol, β-carotene and total phenolic compounds as the natural antioxidant components in methanolic extracts from wild edible mushrooms. Some common edible mushrooms also possess antioxidant activity, which is well correlated with their total phenolic content. Possitive correlation between the scavenging activity of the methanolic extract with total phenolic content in *L. edodes* ($r^2 = 0.99$) and *Volvariella volvacea* ($r^2 = 0.93$) have found by Cheung *et al.,* (2003). Correlations of total phenol content of *Pleurotus citrinopileatus* were moderately to highly associated ($r = 0.425$-0.948) with antioxidant properties has reported by Lee *et al., (*2007).
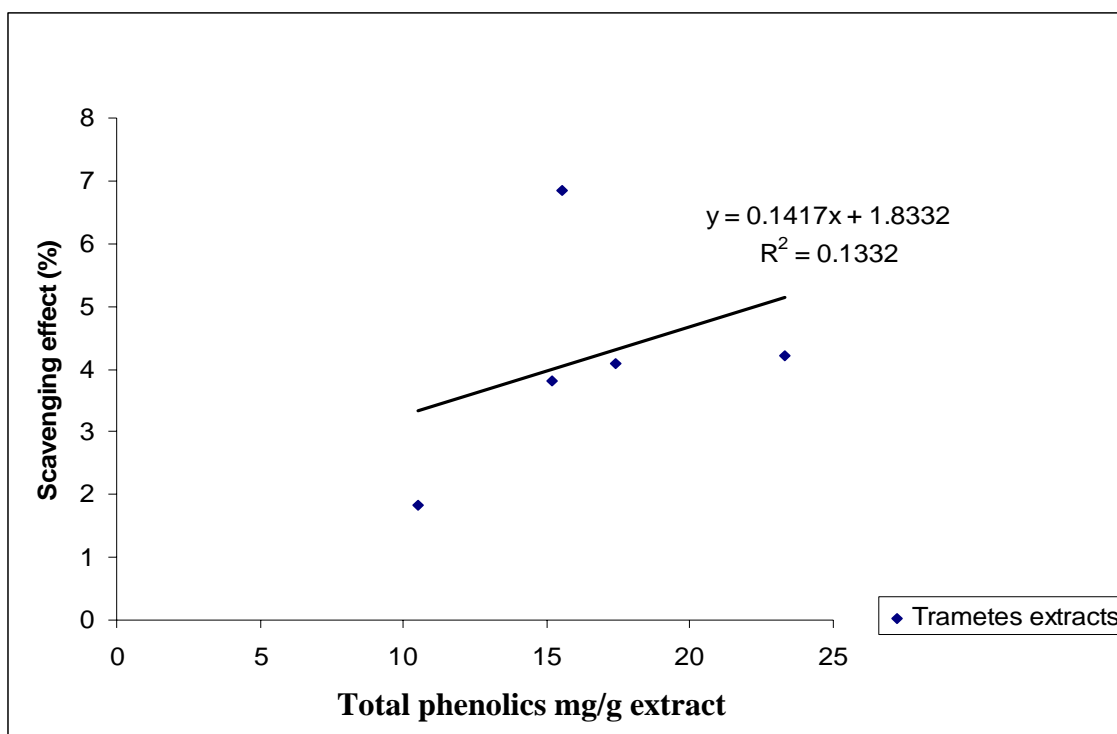
Fig. 4.25a: Correlation between total phenolic content of *Trametes* extracts and scavenging effect on DPPH radicals.
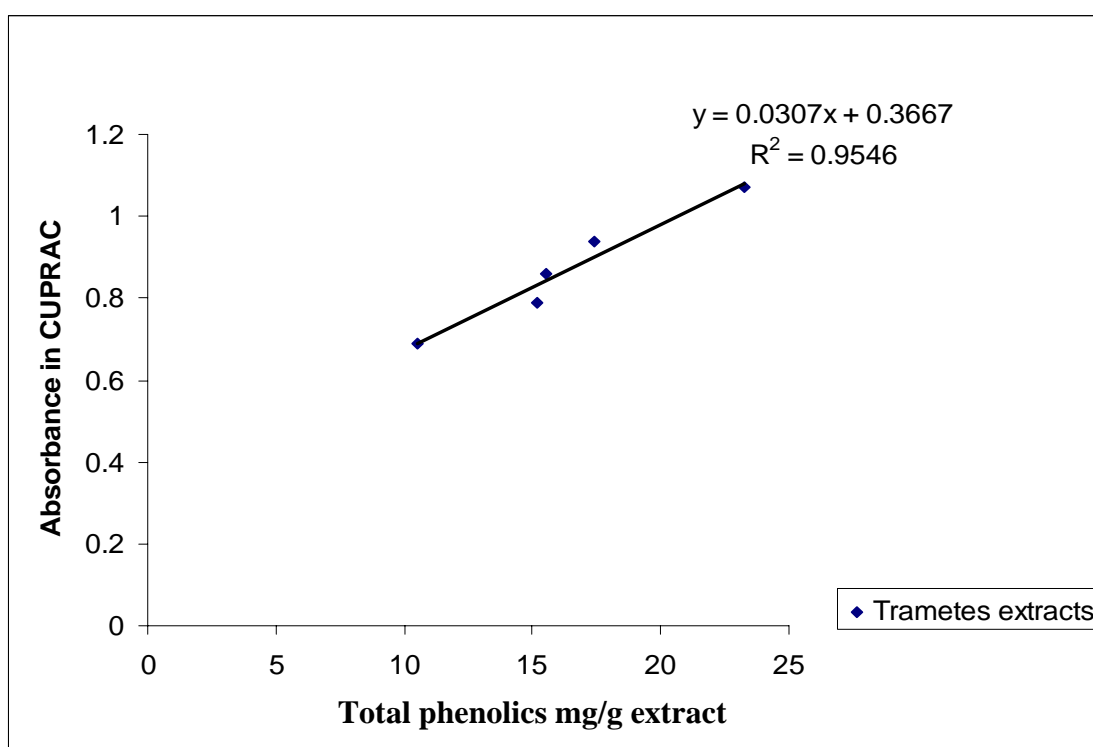
Fig. 4.25b: Correlation between total phenolic content of *Trametes* extracts and CUPRAC reducing ability.

5.0 CONCLUSION

Trametes feei, T. hirsuta, T. lactinea, T. menziezii and *T. pocas* were collected in Peninsular Malaysia from 15th of February 2006 to 25th of November 2007. All of the *Trametes* spp. specimens were succesfully documented in this study. From the data collection, it can be seen that *T. feei, T. menziezii* and *T. pocas* were commonly found in the Peninsular Malaysia while *T. hirsuta* and *T. lactinea* were rarely found. *Trametes* spp. was found mostly in the area of Selangor where frequent collections were made.

In DPPH assay, methanol extracts of *Trametes* spp. were more effective in antioxidant activity than dichloromethane extracts. This result indicates the good antioxidant properties were affected by polar compounds in *Trametes* spp. extracts. *Trametes hirsuta* has showed the best DPPH radical scavenging effect among the *Trametes* spp. with IC_{50} value at 7.295 mg/ml in methanolic extracts and 36.2 mg/ml in dichloromethane extract. On the other hand, *T. feei* showed excellent reducing ability of 0.5367 at 0.5 mg/ml and have the highest phenolic contents of 23.28 mg/g extract. Antioxidant properties of *Trametes* spp. are highly dependent on total phenolic content on CUPRAC assay ($r^2 = 0.9546$). Therefore, high contents of total phenols in the *Trametes* spp. methanolic extracts were responsible for their antioxidant properties. However, a weak correlation was found between the scavenging effects on DPPH radicals of the methanolic extracts with the total phenolic content in *Trametes* spp. ($r^2 = 0.1332$) which contributed by non-phenolic components such as carotenoids and tocopherols. Based on the results obtained, methanolic extracts of *Trametes* spp. more effective in reducing power than in antioxidant activity using the DPPH. Antioxidant compound in *Trametes* spp. extracts may reduce oxidative damage which caused by free radicals from cell and tissues. Free radicals can caused many diseases such as

cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with ageing.

The mycelial biomass of *Trametes* species can be produced uniformly and in large amounts by liquid fermentation for the extraction of natural antioxidant. Searching wild sources may bring new natural products into the food industry with safer and better antioxidants that provide good protection against the oxidative damage, which occurs both in body and our daily foods. Therefore, *Trametes* spp. could be introduced for this purpose, as natural source of antioxidants such as food supplement. In addition, these compounds may have many industrial uses as preservatives in food and cosmetics. With the established antioxidant activity of the *Tramete*s spp. methanolic extracts, the chemical characteristics of the antioxidative components in the extracts should be fuher investigated.

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Preparation of chemicals and media

1) Malt extract agar (MEA)

Malt extract	20g
Bacteriological agar	20 g
Distilled water	1000 ml

Autoclave at 121°C for 20 min.

2) Glucose-yeast-malt-peptone (GYMP)

$MgSO_4.7H_20$	0.4 g
KH_2PO_4	0.4 g
K_2HPO_4	0.4 g
NH_4Cl	0.4 g
Glucose	6.0 g
Peptone	3.2 g
Yeast extracts	3.2 g
Malt extract	3.2 g
Agar	3.2 g
Distilled water	400 ml

Autoclave at 121°C for 20 min.

3) DPPH solutions

0.0078 g of powder DPPH (Sigma) was dissolved in 20 ml methanol. From this stock of DPPH, 3 ml was taken out and dissolved with 47 ml of methanol. This 50 ml of DPPH solution was used for the test. It should be freshly prepared for every day.

4) CUPRAC solutions

Cuprum(II) chloride ($CuCl_2$) solution, 1.0×10^{-2} M, was prepared by dissolving 0.4262 g $CuCl_2 \cdot 2H_2O$ in 250ml distilled water. Ammonium acetate (NH_4Ac) buffer at pH = 7.0, 1.0 M, was prepared by dissolving 19.27 g NH_4Ac in 250 ml distilled water. Neocuproine (Nc) solution, 7.5×10^{-3} M, was prepared by dissolving 0.078g Nc in 50 ml of 96% ethanol. All of the solutions were prepared daily according to the method Apak *et al*., 2008.

RAW DATA

1) Antioxidant activity of methanol and dichloromethane extracts of *Trametes* spp. on DPPH radicals.

a) Absorbance at 517 nm in triplicate of single concentration of methanolic extracts to reach plateau reaction; with calculate mean and standard deviation for 0, 1, 2 and every 25 minutes interval.

	Conc(mg/ml)	Time	R1	R2	R3	Raverage	STDEV
T. pocas (KUM 70160)	1	0'	0.326	0.309	0.327	0.321	0.010
		1'	0.305	0.289	0.305	0.300	0.009
		2'	0.299	0.28	0.296	0.292	0.010
		15'	0.233	0.216	0.231	0.227	0.009
		30'	0.199	0.183	0.197	0.193	0.009
		45'	0.171	0.156	0.169	0.165	0.008
		60'	0.163	0.146	0.164	0.158	0.010
T. menziezii (KM 70155)	5	0'	0.227	0.237	0.260	0.241	0.017
		1'	0.202	0.212	0.236	0.217	0.017
		2'	0.191	0.202	0.226	0.206	0.018
		15'	0.078	0.088	0.114	0.093	0.019
		30'	0.034	0.042	0.044	0.040	0.005
		45'	0.036	0.038	0.049	0.041	0.007
		60'	0.033	0.029	0.031	0.031	0.002
		75'	0.034	0.029	0.028	0.030	0.003
T. lactinea (KUM 70150)	5	0'	0.294	0.282	0.31	0.295	0.014
		1'	0.256	0.237	0.267	0.253	0.015
		2'	0.235	0.214	0.245	0.231	0.016
		15'	0.09	0.068	0.096	0.085	0.015
		30'	0.045	0.024	0.048	0.039	0.013
		45'	0.041	0.026	0.044	0.037	0.010
		60'	0.039	0.024	0.041	0.035	0.009
T. hirsuta (KUM 70093)	5	0'	0.112	0.104	0.099	0.105	0.007
		1'	0.103	0.084	0.089	0.092	0.010
		2'	0.087	0.082	0.074	0.081	0.007
		15'	0.051	0.079	0.047	0.059	0.017
		30'	0.051	0.078	0.047	0.059	0.017
		45'	0.053	0.079	0.058	0.063	0.014
		60'	0.053	0.081	0.050	0.061	0.017

Continued

T. feei (KUM 70015)	3.2	0'	0.265	0.256	0.256	0.259	0.005
		1'	0.247	0.237	0.238	0.241	0.006
		2'	0.222	0.213	0.213	0.216	0.005
		15'	0.082	0.074	0.077	0.078	0.004
		30'	0.064	0.060	0.065	0.063	0.003
		45'	0.063	0.059	0.065	0.062	0.003
		60'	0.061	0.058	0.063	0.061	0.003

b)   Graph of the mean of absorbance at 517 nm of the methanol extracts of *Trametes* spp. at 0, 1, 2 and every 15 minutes interval.
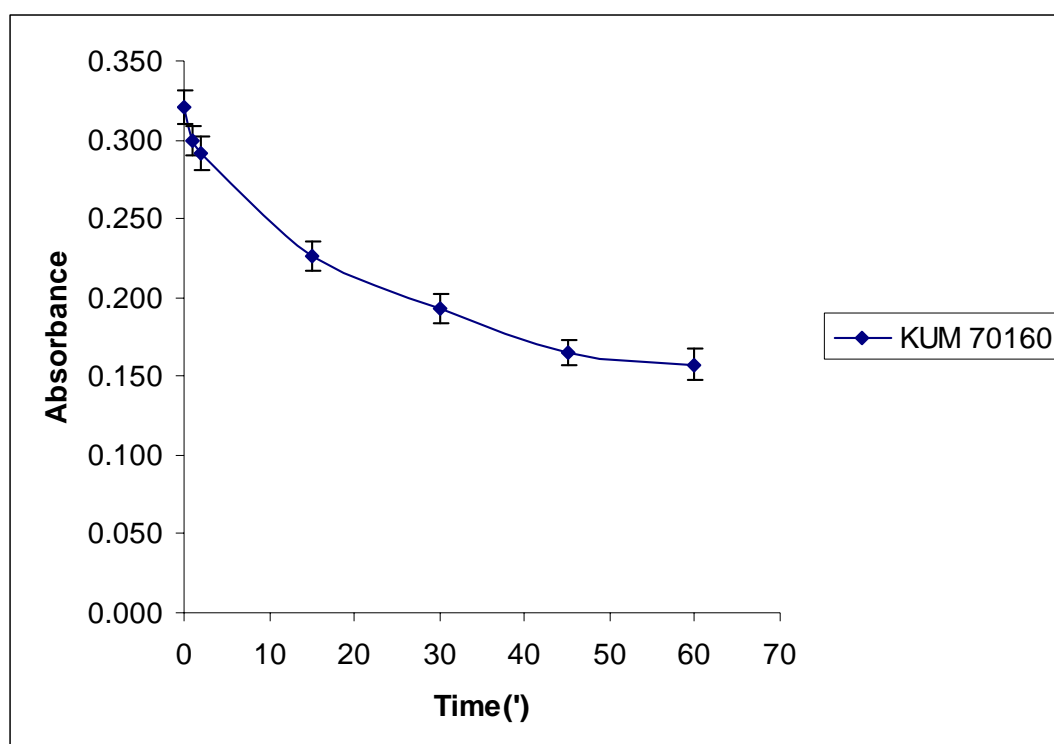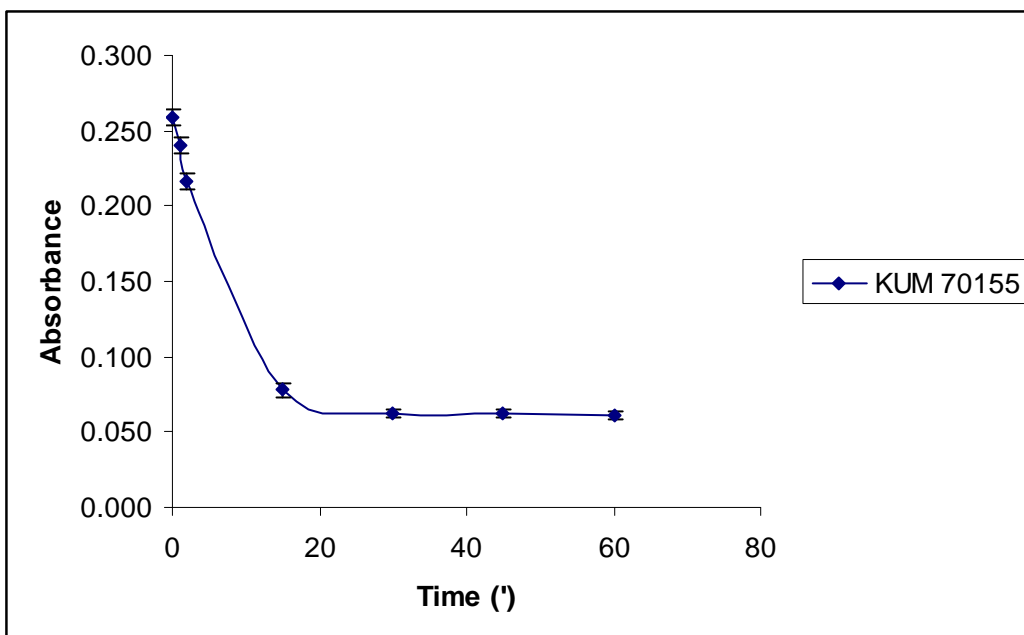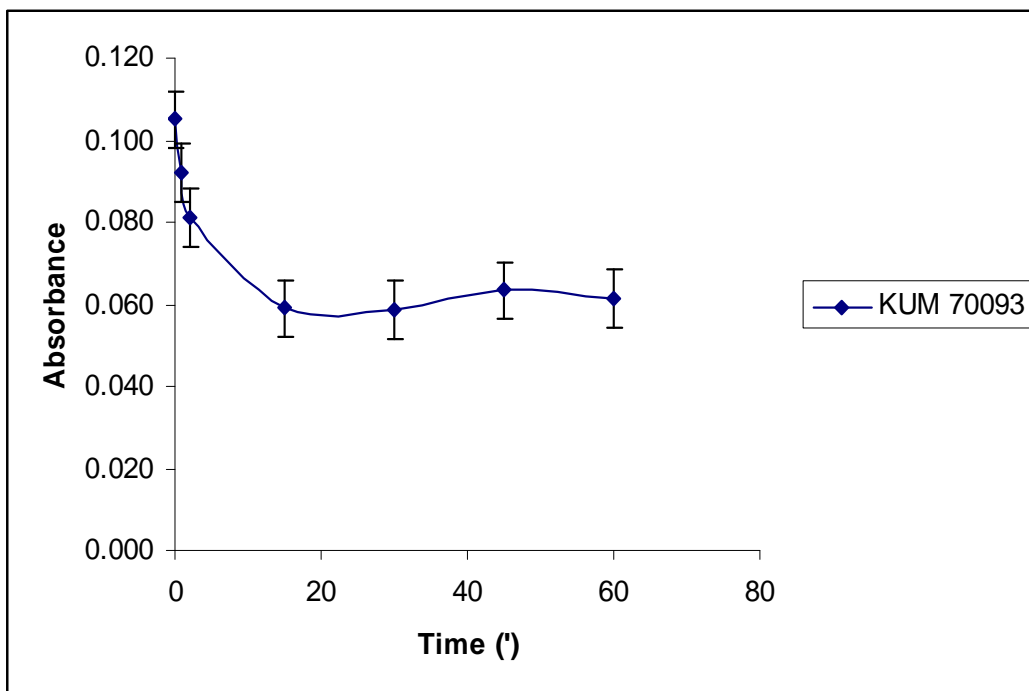
c)   Absorbance at 517 nm in triplicate of single concentration of dichloromethane extracts to reach plateau reaction; with calculate mean and standard deviation for 0, 1, 2 and every 25 minutes interval.
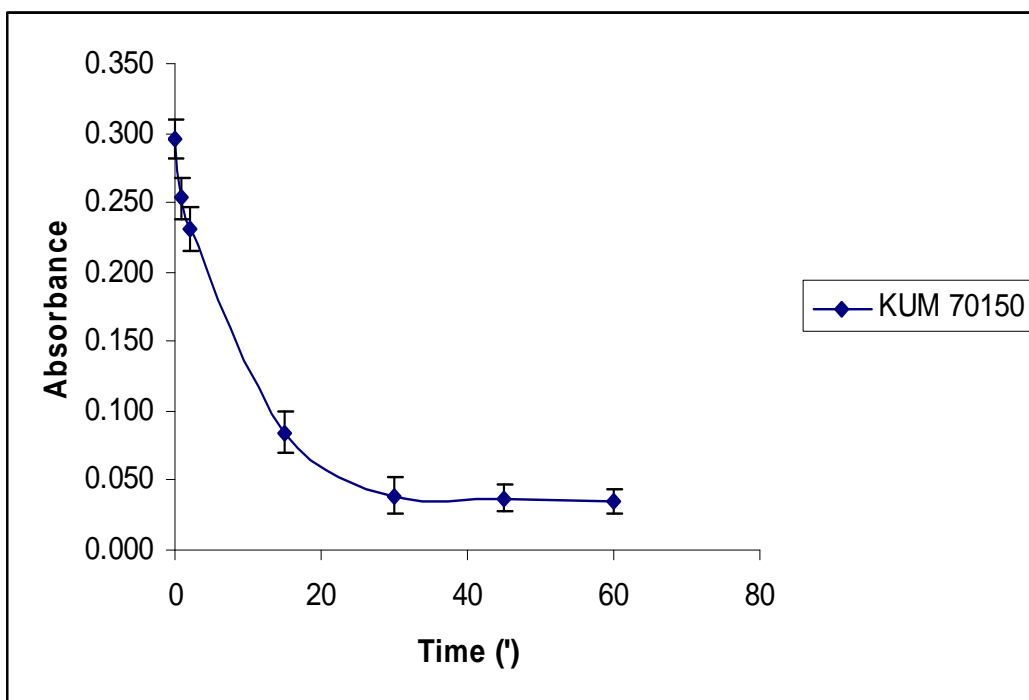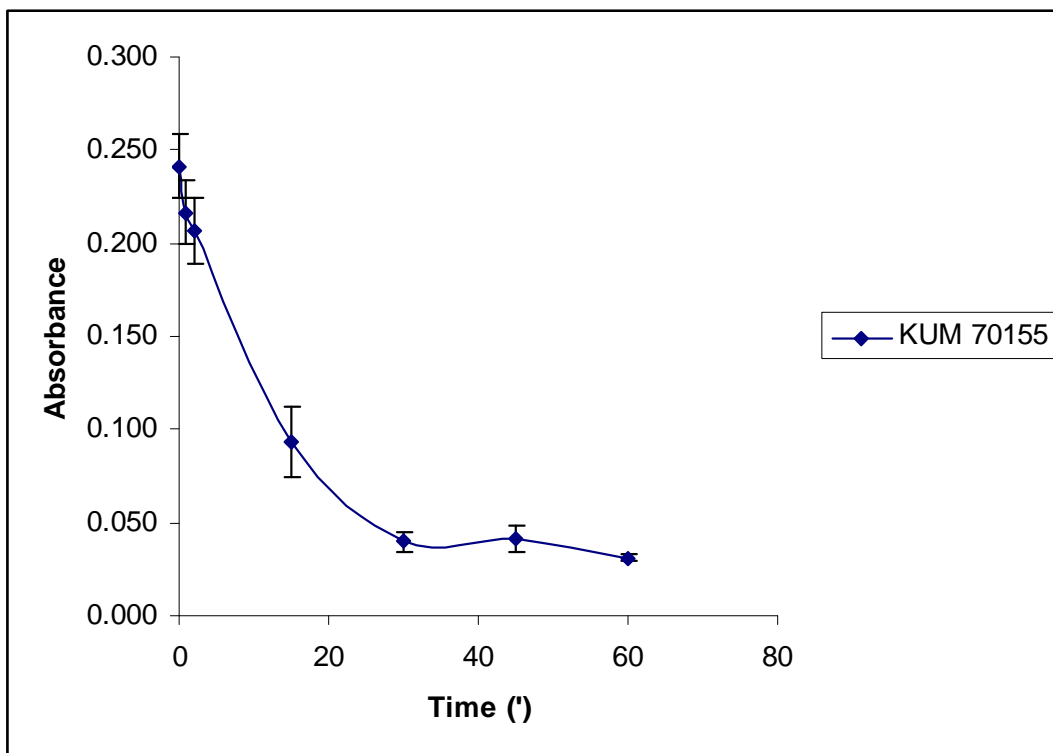
Extract	DPPH	conc (mg/ml)	Time	R1	R2	R3	AVG	STDEV
T. pocas (KUM 70160)	0.497	1.0	0'	0.458	0.465	0.459	0.461	0.004
			1'	0.457	0.465	0.459	0.460	0.004
			2'	0.458	0.464	0.458	0.460	0.003
			15'	0.456	0.463	0.457	0.459	0.004
			30'	0.456	0.461	0.457	0.458	0.003
			45'	0.455	0.461	0.457	0.458	0.003
			60'	0.455	0.461	0.457	0.458	0.003
T. menziezii (KUM 70155)	0.497	1.5	0'	0.456	0.461	0.450	0.456	0.006
			1'	0.452	0.457	0.447	0.452	0.005
			2'	0.450	0.453	0.444	0.449	0.005
			15'	0.426	0.426	0.424	0.425	0.001
			30'	0.418	0.414	0.417	0.416	0.002
			45'	0.412	0.408	0.420	0.413	0.006
			60'	0.409	0.404	0.420	0.411	0.008
T. lactinea (KUM 70150)	0.497	1.5	0'	0.444	0.446	0.439	0.443	0.004
			1'	0.442	0.443	0.437	0.441	0.003
			2'	0.440	0.442	0.435	0.439	0.004
			15'	0.436	0.433	0.425	0.431	0.006
			30'	0.435	0.432	0.421	0.429	0.007
			45'	0.434	0.431	0.418	0.428	0.009
			60'	0.434	0.433	0.416	0.428	0.010
T. hirsuta (KUM 70093)	0.497	0.5	0'	0.361	0.364	0.356	0.360	0.004
			1'	0.350	0.357	0.346	0.351	0.006
			2'	0.344	0.352	0.340	0.345	0.006
			15'	0.302	0.309	0.304	0.305	0.004
			30'	0.283	0.284	0.289	0.285	0.003
			45'	0.267	0.264	0.276	0.269	0.006
			60'	0.263	0.264	0.276	0.268	0.007
T. feei (KUM 70015)	0.492	2.0	0'	0.483	0.481	0.489	0.484	0.004
			1'	0.483	0.481	0.482	0.482	0.001
			2'	0.470	0.474	0.474	0.473	0.002
			15'	0.466	0.464	0.467	0.466	0.002
			30'	0.465	0.464	0.466	0.465	0.001
			45'	0.465	0.461	0.465	0.464	0.002
			60'	0.465	0.460	0.463	0.463	0.003

d)   Graph of the mean of absorbance at 517 nm of the dichloromethane extracts of *Trametes* spp. at 0, 1, 2 and every 15 minutes interval.
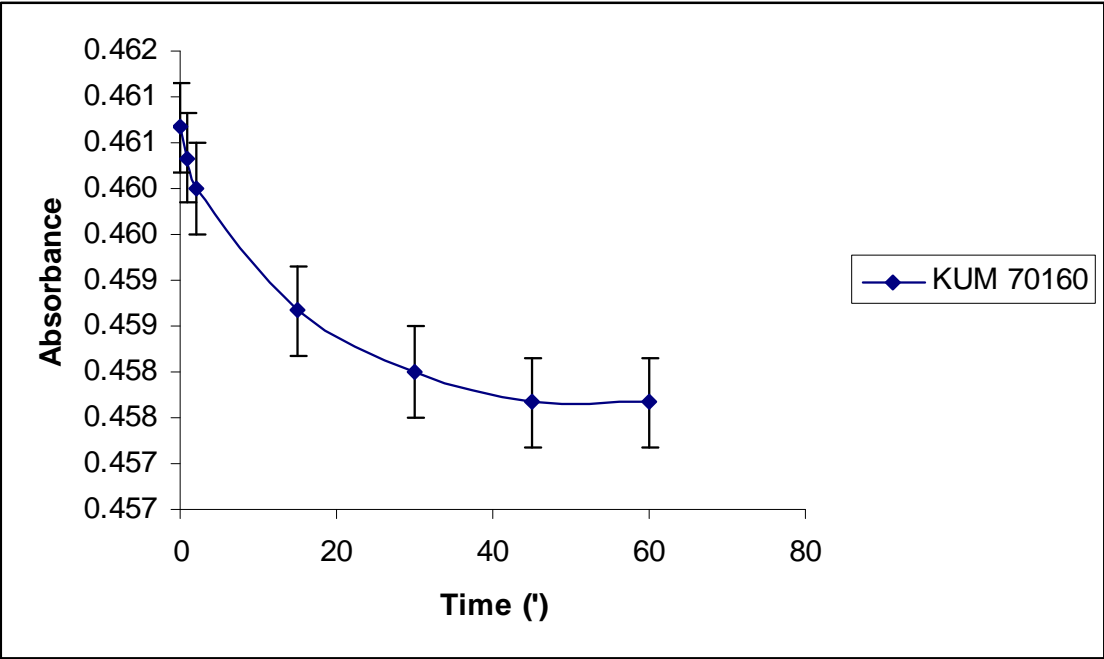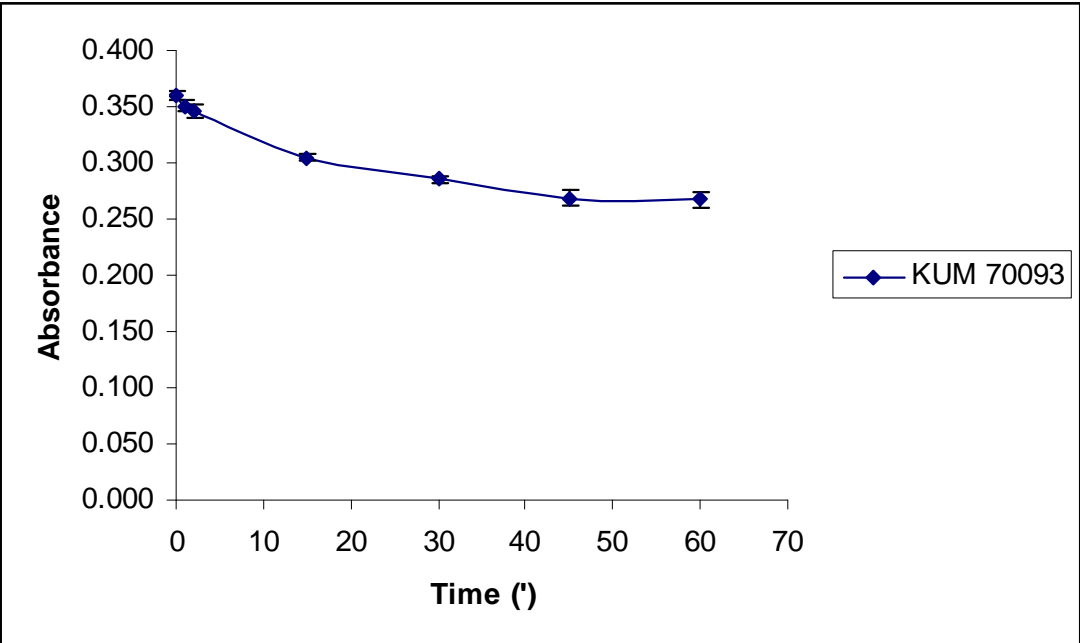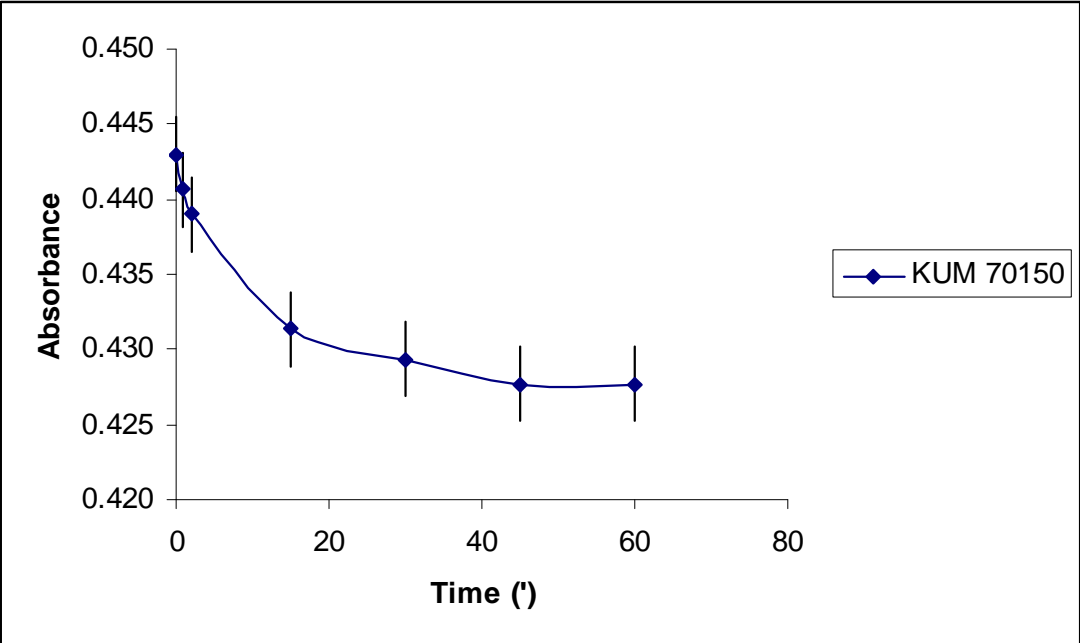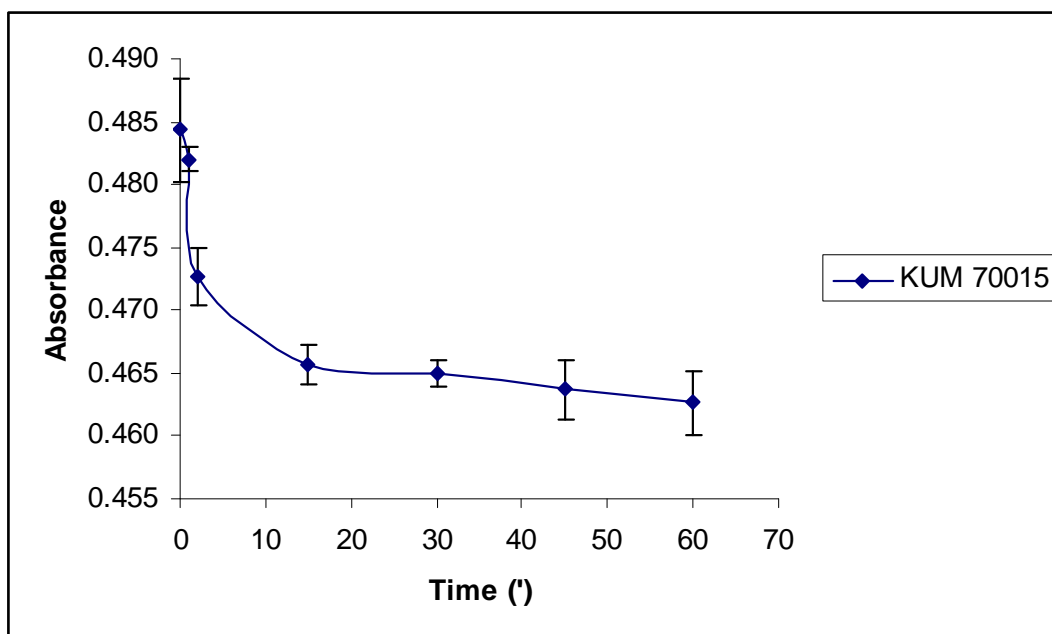
e)   Absorbance at 517 nm on methanol and dichloromethane extracts of *Trametes* spp. in triplicate of five to six different concentrations of stock extracts and positive control, with calculated mean and standard deviation at steady state

   i)   *Methanolic extracts*

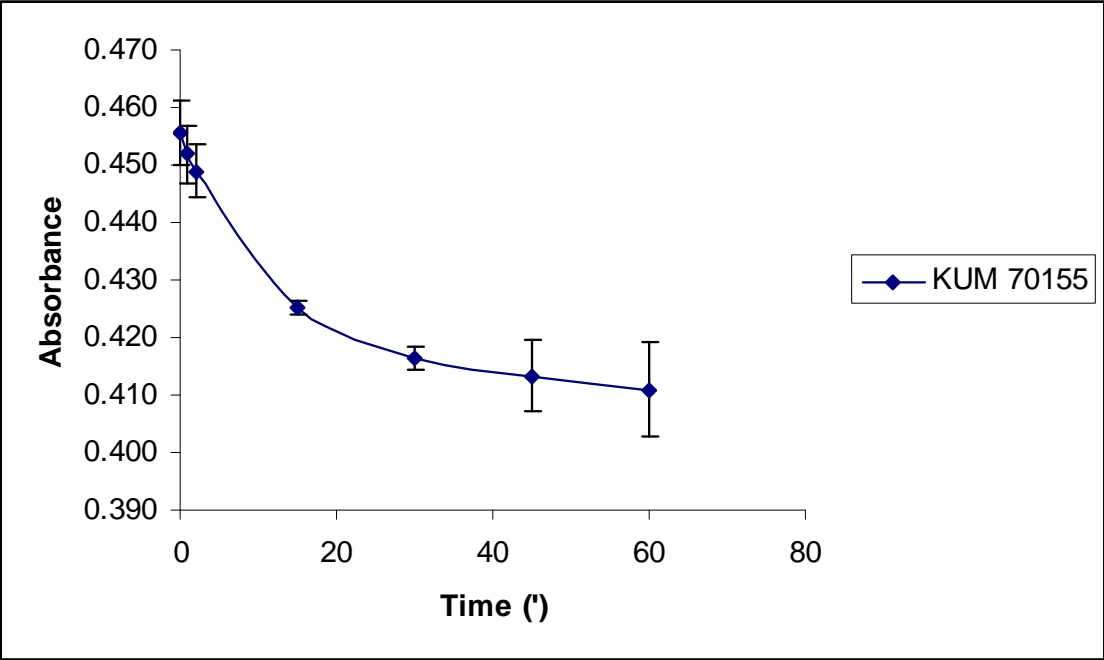| Code	Conc (mg/ml)	R1	R2	R3	Raverage	STDEV	DPPH
T. menziezii	0	0	0	0	0	0	0
	4	0.403	0.399	0.405	0.402	0.0031	0.494
	8	0.356	0.359	0.355	0.357	0.0021	0.494
	12	0.25	0.256	0.26	0.255	0.005	0.494
	16	0.176	0.169	0.169	0.171	0.004	0.494
	20	0.148	0.138	0.134	0.14	0.0072	0.495
T. pocas	0	0	0	0	0	0	0
	6	0.322	0.326	0.325	0.324	0.0021	0.493
	10	0.287	0.295	0.28	0.287	0.0075	0.501
	16	0.137	0.138	0.138	0.138	0.0006	0.493
	20	0.089	0.098	0.091	0.093	0.0047	0.493
	24	0.047	0.049	0.044	0.047	0.0025	0.487

Continued

T. lactinea	0	0	0	0	0	0	0
	10	0.355	0.353	0.345	0.351	0.0053	0.495
	20	0.302	0.307	0.305	0.288	0.0025	0.495
	25	0.243	0.232	0.24	0.245	0.0057	0.495
	30	0.225	0.228	0.224	0.229	0.0021	0.495
	40	0.171	0.159	0.17	0.167	0.0067	0.495
	50	0.045	0.034	0.048	0.039	0.0074	0.495
T. hirsute	0	0	0	0	0	0	0
	2	0.38	0.379	0.376	0.378	0.002	0.484
	4	0.316	0.314	0.316	0.315	0.001	0.484
	6	0.263	0.262	0.262	0.262	0.001	0.484
	8	0.235	0.232	0.231	0.233	0.002	0.484
	10	0.168	0.166	0.163	0.166	0.003	0.484
	12	0.083	0.097	0.1	0.093	0.009	0.474
T. feei	0	0	0	0	0	0	0
	4	0.384	0.381	0.373	0.38	0.006	0.494
	8	0.304	0.293	0.297	0.3	0.006	0.493
	12	0.242	0.242	0.25	0.24	0.005	0.493
	16	0.146	0.151	0.142	0.146	0.005	0.473
	20	0.1	0.094	0.097	0.097	0.003	0.473
Vit C	0	0	0	0	0	0	0
	0.01	0.449	0.433	0.438	0.440	0.0082	0.490
	0.05	0.389	0.389	0.399	0.392	0.0058	0.490
	0.10	0.302	0.283	0.292	0.292	0.0095	0.490
	0.15	0.207	0.199	0.198	0.201	0.0049	0.487
	0.20	0.043	0.042	0.041	0.042	0.0010	0.490

ii)   *Dichloromethane extracts*

Code	Conc (mg/ml)	R1	R2	R3	Raverage	STDEV	DPPH
T. pocas	0	0	0	0	0	0	0
	10	0.409	0.408	0.409	0.409	5.5377	0.484
	20	0.364	0.369	0.354	0.362	11.3354	0.484
	30	0.307	0.313	0.304	0.308	17.1415	0.484
	40	0.268	0.274	0.261	0.268	22.9375	0.484
	50	0.216	0.214	0.229	0.22	28.7434	0.484

Continued

T. menziezii	0	0	0	0	0	0	0
	10	0.438	0.436	0.425	0.433	0.007	0.484
	20	0.383	0.392	0.386	0.387	0.0046	0.484
	30	0.344	0.357	0.352	0.351	0.0066	0.484
	40	0.304	0.308	0.313	0.308	0.0045	0.484
	50	0.278	0.276	0.266	0.273	0.0064	0.484
T.lactinea	0	0	0	0	0	0	0
	10	0.443	0.435	0.432	0.437	0.0057	0.486
	20	0.366	0.358	0.344	0.356	0.0111	0.486
	30	0.326	0.322	0.339	0.329	0.0089	0.486
	40	0.263	0.262	0.271	0.265	0.0049	0.486
	50	0.23	0.241	0.245	0.239	0.0078	0.486
T. hirsute	0	0	0	0	0	0	0
	10	0.399	0.4	0.403	0.401	0.0021	0.486
	20	0.34	0.349	0.33	0.34	0.0095	0.486
	30	0.267	0.265	0.278	0.27	0.007	0.486
	40	0.21	0.212	0.224	0.215	0.0076	0.486
	50	0.161	0.164	0.183	0.169	0.0119	0.486
T. feei	0	0	0	0	0	0	0
	10	0.398	0.4	0.402	0.4	0.002	0.487
	20	0.387	0.382	0.391	0.387	0.0045	0.487
	30	0.368	0.362	0.364	0.365	0.0031	0.487
	40	0.34	0.342	0.352	0.345	0.0064	0.487
	50	0.313	0.311	0.316	0.313	0.0025	0.487

f)   Percentage   of   DPPH   scavenging   effect   by   methanol   and dichloromethane extracts of Trametes spp. at steady state of three replicates at five to six different concentrations with calculated mean and standard deviation.

i)   methanol extracts

Code	Conc (mg/ml)	R1	R2	R3	Raverage	STDEV
T. pocas	0	0	0	0	0	0
	10	0.409	0.408	0.409	0.409	5.5377
	20	0.364	0.369	0.354	0.362	11.3354
	30	0.307	0.313	0.304	0.308	17.1415
	40	0.268	0.274	0.261	0.268	22.9375
	50	0.216	0.214	0.229	0.22	28.7434

Continued

T. menziezii	0	0	0	0	0	0
	10	0.438	0.436	0.425	0.433	0.007
	20	0.383	0.392	0.386	0.387	0.0046
	30	0.344	0.357	0.352	0.351	0.0066
	40	0.304	0.308	0.313	0.308	0.0045
	50	0.278	0.276	0.266	0.273	0.0064
T. lactinea	0	0	0	0	0	0
	10	0.443	0.435	0.432	0.437	0.0057
	20	0.366	0.358	0.344	0.356	0.0111
	30	0.326	0.322	0.339	0.329	0.0089
	40	0.263	0.262	0.271	0.265	0.0049
	50	0.23	0.241	0.245	0.239	0.0078
T. hirsuta	0	0	0	0	0	0
	10	0.399	0.4	0.403	0.401	0.0021
	20	0.34	0.349	0.33	0.34	0.0095
	30	0.267	0.265	0.278	0.27	0.007
	40	0.21	0.212	0.224	0.215	0.0076
	50	0.161	0.164	0.183	0.169	0.0119
T. feei	0	0	0	0	0	0
	10	0.398	0.4	0.402	0.4	0.002
	20	0.387	0.382	0.391	0.387	0.0045
	30	0.368	0.362	0.364	0.365	0.0031
	40	0.34	0.342	0.352	0.345	0.0064
	50	0.313	0.311	0.316	0.313	0.0025
Vit C	0	0	0	0	0	0
	0.01	8.37	11.36	10.61	10.20	1.6676
	0.05	20.61	20.61	18.57	19.930	1.1778
	0.10	38.37	42.24	40.41	40.340	1.9359
	0.15	57.49	59.14	59.34	58.657	1.0153
	0.2	91.22	91.43	91.63	91.427	0.2050

ii) *Dichloromethane extracts*

Code	Conc(mg/ml)	R1	R2	R3	Raverage	STDEV
T. pocas	0	0	0	0	0	0
	10	15.5	15.7	15.5	15.57	0.1155
	20	24.79	23.76	26.86	25.14	1.5788
	30	36.57	35.33	37.19	36.36	0.9471
	40	44.63	43.39	46.07	44.7	1.3412
	50	55.37	55.79	53.1	54.75	1.4471

Continued

T. menziezii	0	0	0	0	0	0
	10	9.5	9.91	12.19	10.53	1.4493
	20	20.87	19	20.25	20.04	0.9525
	30	28.93	26.24	27.27	27.48	1.3572
	40	37.19	36.36	35.33	36.29	0.9318
	50	42.56	42.97	45.04	43.52	1.3294
T. lactinea	0	0	0	0	0	0
	10	8.85	10.49	11.11	10.15	1.1677
	20	24.69	26.34	29.22	26.75	2.2927
	30	32.92	33.74	30.25	32.3	1.8249
	40	45.88	46.09	44.24	45.4	1.0129
	50	52.67	50.41	49.59	50.89	1.5951
T. hirsuta	0	0	0	0	0	0
	10	17.9	17.7	17.08	17.56	0.4276
	20	30.04	28.19	32.1	30.11	1.9559
	30	45.06	45.47	42.8	44.44	1.4379
	40	56.79	56.38	53.91	55.69	1.558
	50	66.87	66.26	62.35	65.16	2.4526
T. feei	0	0	0	0	0	0
	10	9.24	10.47	11.5	10.4	1.1315
	20	18.28	17.86	17.43	17.86	0.425
	30	24.44	25.68	25.26	25.13	0.6307
	40	30.18	29.77	27.72	29.22	1.318
	50	35.73	36.14	35.11	35.66	0.5186

2) Antioxidant activity on CUPRAC assay.

   a) Absorbance at 450 nm of five different concentrations of methanolic extracts and control, with calculate mean and standard deviation.

Extract	Conc (mg/ml)	R1	R2	R3	AVG	STD
Ascorbic acid	0.0025	0.023	0.023	0.026	0.024	0.0017
	0.005	0.087	0.084	0.089	0.087	0.0025
	0.01	0.186	0.185	0.189	0.187	0.0021
	0.02	0.481	0.489	0.486	0.485	0.0040
	0.025	0.558	0.556	0.559	0.558	0.0015

Continued

T. feei	0.05	0.078	0.07	0.08	0.076	0.0053
	0.1	0.105	0.109	0.102	0.105	0.0035
	0.25	0.287	0.281	0.287	0.285	0.0035
	0.5	0.553	0.556	0.565	0.558	0.0062
	1	1.05	1.068	1.055	1.058	0.0093
T. hirsuta	0.05	0.017	0.007	0.004	0.009	0.0068
	0.1	0.062	0.068	0.065	0.065	0.0030
	0.25	0.214	0.226	0.212	0.217	0.0076
	0.5	0.422	0.418	0.428	0.423	0.0050
	1	0.863	0.862	0.859	0.861	0.0021
T. lactinea	0.05	0.038	0.034	0.038	0.037	0.0023
	0.1	0.076	0.062	0.084	0.074	0.0111
	0.25	0.201	0.19	0.184	0.192	0.0086
	0.5	0.361	0.372	0.378	0.370	0.0086
	1	0.67	0.673	0.678	0.674	0.0040
T. menziezii	0.05	0.051	0.064	0.054	0.056	0.0068
	0.1	0.09	0.097	0.079	0.089	0.0091
	0.25	0.211	0.205	0.203	0.206	0.0042
	0.5	0.415	0.391	0.419	0.408	0.0151
	1	0.783	0.782	0.785	0.783	0.0015
T. pocas	0.05	0.063	0.06	0.065	0.063	0.0025
	0.1	0.113	0.111	0.102	0.109	0.0059
	0.25	0.26	0.246	0.268	0.258	0.0111
	0.5	0.454	0.464	0.474	0.464	0.0100
	1	0.919	0.952	0.919	0.930	0.0191

3)   Total phenolic content of methanoli extract of *Trametes* spp.

a) Gallic acid equilibrium (GAE) by total phenolic content of five different concentrations, in triplicate with calculate mean and standard deviation:

Conc	R1	R2	R3	Raverage	STDEV
0	0	0	0	0	0
2	0.064	0.065	0.064	0.064	0.0006
4	0.125	0.126	0.137	0.129	0.0067
6	0.200	0.180	0.188	0.189	0.0101
8	0.230	0.244	0.246	0.240	0.0087
10	0.264	0.274	0.279	0.272	0.0076

b) Graph of Gallic acid equilibrium (GAE) by total phenolic content

$y = 0.0292x$

$R^2 = 0.9852$
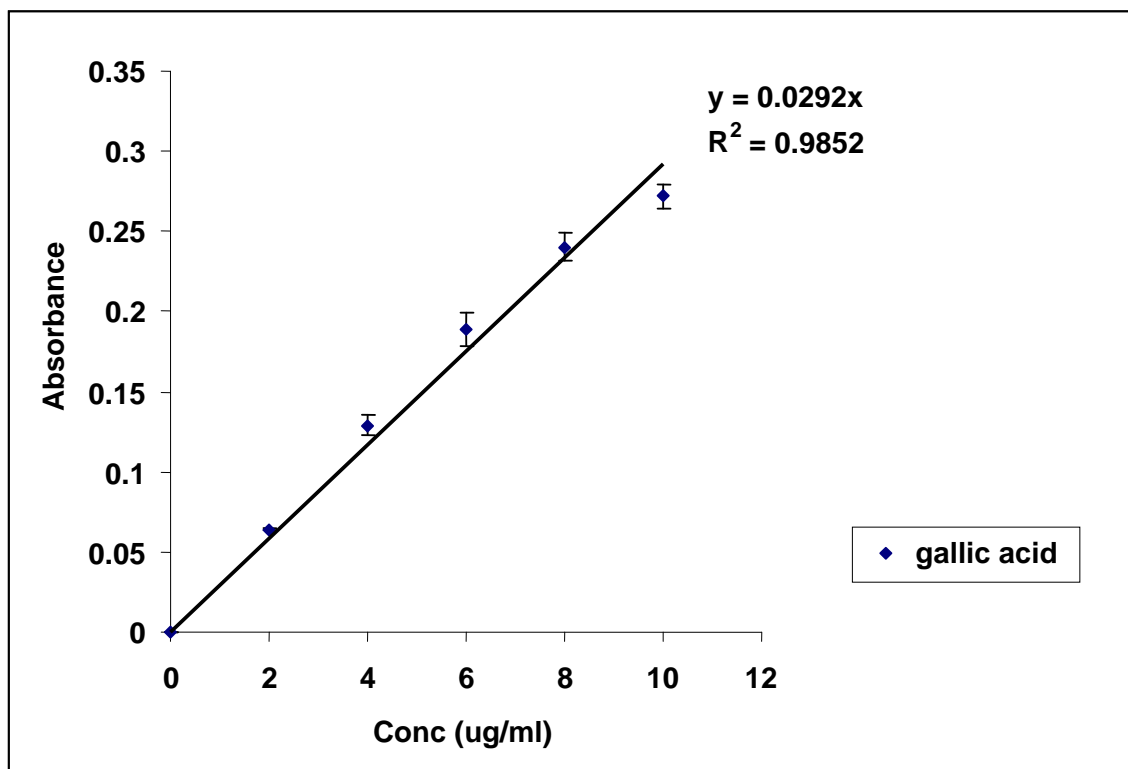
b) Total phenolic contents of methanolic extracts of *Trametes* spp. per mg GAE in triplicate with calculate mean and standard deviation:

Code	R1	R2	R3	AVG	STDEV	Total phenolics ug GAE/0.5mg extract	Total phenolics GAE mg/g extract
T. pocas	0.256	0.252	0.253	0.2537	0.0021	8.7	17.4
T. menziezii	0.225	0.218	0.224	0.2223	0.0038	7.6	15.2
T. lactinea	0.158	0.154	0.151	0.1543	0.0035	5.27	10.54
T. hirsuta	0.231	0.221	0.228	0.2267	0.0051	7.77	15.54
T. feei	0.341	0.356	0.322	0.3397	0.0170	11.64	23.28