

## Antioxidative Responses of Tree Species in Ayer Hitam Forest, Selangor, Peninsular Malaysia

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### ABSTRAK

Tindakbalas oksidatif lapan spesies pokok, iaitu *Atrocarpus elasticus*, *Endospermum diadenum*, *Vitex pinnata*, *Pellacalyx axillaris*, *Garcinia atroviridis*, *Gironniera nervosa*, *Bouea oppositifolia* dan *Callerya atropurpurea* telah ditentukan bagi sampel yang diambil dari kedua-dua kawasan pendalaman dan tepian Hutan Simpan Ayer Hitam. Tindakbalas antioksidatif yang ditentukan termasuklah tahap penguraian oksidatif lipid pada membran sel dan juga kepekatan askorbat serta  $\alpha$ -tokoferol, dua antioksidan yang penting. Kesemua lapan spesies menunjukkan perbezaan yang bererti pada tahap penguraian oksidatif antara kawasan pendalaman dan tepian hutan di mana secara amnya, tahap penguraian oksidatif atau peroksidaan lipid pada membran adalah lebih tinggi pada sampel yang diambil dari kawasan tepian hutan kecuali *V.pinnata* dan *G.nervosa*. Kepekatan  $\alpha$ -tokoferol secara amnya juga didapati lebih tinggi di kawasan tepian hutan kecuali *V.pinnata* dan *G.nervosa*. Kepekatan askorbat walaubagaimanapun didapati lebih tinggi bagi semua sampel yang diambil dari kawasan tepian hutan. Ini menunjukkan bahawa terdapat tahap tegasan yang lebih tinggi dari segi tegasan oksidatif akibat gangguan yang lebih ketara di kawasan tepian hutan. *C.atropurpurea* mungkin merupakan satu spesies penunjuk yang baik dan sensitif dalam menentukan keadaan tegasan di dalam hutan sementara *V.pinnata* dan *G.nervosa* pula mungkin merupakan spesies pokok yang dapat mengurangkan tahap penguraian oksidatif dengan cekap dalam keadaan tegasan.

### ABSTRACT

Antioxidative responses of eight tree species namely *Atrocarpus elasticus*, *Endospermum diadenum*, *Vitex pinnata*, *Pellacalyx axillaris*, *Garcinia atroviridis*, *Gironniera nervosa*, *Bouea oppositifolia* and *Callerya atropurpurea* were determined from samples collected from both the interior and fringe forest regions of Ayer Hitam Forest Reserve. These antioxidative responses measured include the extent of oxidative deterioration of cellular membrane lipids as well as the concentrations of ascorbate and  $\alpha$ -tocopherol, two important endogenous antioxidants. All eight species showed significant differences in the extent of oxidative deterioration between the interior and fringe forest regions where generally higher levels of membrane lipid peroxidation or oxidative deterioration were observed in samples from the fringe forest regions except for *V.pinnata* and *G.nervosa*. Concentrations of  $\alpha$ -tocopherol were also found to be generally higher in the fringe forest regions except for *V.pinnata* and *G.nervosa*. Ascorbate concentrations were however found to be higher in all the tree species sampled from the fringe forest. This thus indicates higher levels of stress conditions with respect to oxidative stress manifested by higher levels of disturbance in the fringe forest regions. While *C.atropurpurea* may represent a good and sensitive indicator species in determining stress conditions in the forest, *V.pinnata* and *G.nervosa* may represent tree species that are efficient in minimising oxidative deterioration in stress conditions.

## INTRODUCTION

Plants, in their natural habitats, are often subjected to various stress conditions, which may be due to abiotic factors as well as biotic factors. Abiotic factors, which include drought (Price and Hendry 1991), salinity (Fadzillah *et al.* 1997), anoxia (Crawford 1993), herbicides (Westphal *et al.* 1992), ozone (Tausz *et al.* 1994), sulfur dioxide and other gas pollutants (Bowler *et al.* 1992) as well as biotic factors such as bacterial or fungal infections (Wojtaszek 1997) often lead to decreased growth and yield of the plants affected where in severe conditions, may even lead to death. In most stress conditions, the generation of reactive oxygen species (ROS), have been implicated, where accumulation of these ROS at higher than normal levels may cause various damages at the cellular and molecular levels (Scandalios and Wright 1990). An important measure of this oxidative damage is the extent of oxidative deterioration or peroxidation of tissue and cellular membrane lipids. Through evolutionary pressures however, plants have evolved an antioxidative mechanism comprising of enzymatic as well as non-enzymatic systems. Some plants are more efficient than others in regulating a satisfactory antioxidative defense against the stress conditions, and this is often influenced in part, by their endogenous antioxidant constituents.

The Ayer Hitam Forest Reserve, flanked by the Puchong Damansara Highway represents a forest ecosystem which can be divided into a more disturbed fringe forest region and a relatively less disturbed interior forest region. Disturbances arising mainly from human activities near the fringe forest which include atmospheric gas pollution may give rise to conditions of oxidative stress which can be measured from the concentration of malondialdehyde (MDA), representing the extent of oxidative deterioration of cellular membrane lipids. Indications of oxidative deterioration as well as status of endogenous antioxidant, namely ascorbate and  $\alpha$ -tocopherol, not only provide information on the level of stress and defense capacity of the plants, but may also provide information on the sensitivity of the plant species in detecting stress conditions. In this study, eight tree species were selected from both the interior and fringe forest regions of Ayer Hitam to determine differences between these two regions in terms of antioxidative responses manifested by different

levels of disturbances to these regions. Concentrations of MDA and two important endogenous antioxidants, namely ascorbate and  $\alpha$ -tocopherol, were determined in all the samples collected.

## MATERIALS AND METHODS

### *Plants Samples:*

Leaves of eight forest tree species namely *Atrocarpus elasticus* (terap), *Endospermum diadenum* (sesenduk), *Vitex pinnata* (leban), *Pellacalyx axillaris* (membuluh), *Garcinia atroviridis* (kandis), *Gironniera nervosa* (hampas tebu), *Bouea oppositifolia* (kundang), and *Callerya atropurpurea* (tulang daing) were collected from the fringe and interior regions of the Ayer Hitam Forest, Selangor. Leaf tissue of each plant collected was placed in sealed polythene bags, kept in crushed ice and quickly transported to the laboratory for immediate analyses.

### *Determination of Lipid Peroxidation :*

The level of lipid peroxidation in the leaf tissue, measured from concentration of MDA was determined by the thiobarbituric acid (TBA) reaction based on the method by Heath and Packer (1968) with slight modifications by Shaw (1995). Fresh samples (approximately 0.2 g) was homogenized in 1.5 ml 0.1 % (w/v) trichloroacetic acid and clean sand in a prechilled mortar and pestle at 0-4 °C. The homogenate was centrifuged at 10,000 xg (Universal 16R) for 5 minutes. 0.75 ml of the supernatant obtained was added into 2.25 ml of TBA reagent and the mixture was heated at 95 °C for 30 minutes and quickly cooled in an ice bath for 15 minutes. After centrifuging at 10,000 xg for 10 minutes, the absorbance of the supernatant obtained was measured at 532 nm with the value of non-specific absorption at 600 nm subtracted from the absorbance values. The concentrations of MDA were calculated using its extinction coefficient of 155 mM<sup>-1</sup>cm<sup>-1</sup> and expressed as nmol MDA/g fresh weight of sample. A total of five replicates were used for each plant species from each of the two (interior and fringe forest) locations.

### *Determination of $\alpha$ -tocopherol :*

$\alpha$ -tocopherol was extracted from the leaves tissue based on the method by Hodges *et al.* (1996). Under dim light and over ice, 0.15g of fresh

sample was ground up with 1.5 ml acetone and clean sand in a mortar and pestle at 0-4°C. The mixture was extracted with 0.5 ml hexane followed by vortexing for about 30 seconds. The mixture was then centrifuged at 1000xg for 10 minutes. After the centrifugation, the top layer was removed and the hexane extraction was repeated twice. The assay mixture was prepared as described by Kanno and Yamauchi (1997). 0.5 ml of the hexane-extract was added into 0.4 ml 0.1% (w/v) PDT, (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine, prepared in ethanol) and 0.4 ml 0.1% (w/v) ferric chloride (prepared in ethanol). The volume was made up to 3.0 ml with absolute ethanol and the mixture was gently swirled and left for 4 minutes for colour development. Following this, 0.2 ml of 0.2 M orthophosphoric acid was added to the mixture and allowed to stand for 30 minutes at room temperature before absorbance of the mixture was measured at 554 nm. The blank was prepared in the same manner except that the absolute ethanol was used instead of the hexane-extract. A standard curve was prepared using  $\alpha$ -tocopherol (Sigma, type V) at various concentrations (0-1.4  $\mu\text{g/ml}$ ). 0.5 ml of  $\alpha$ -tocopherol was added into the solution as described above and amount of  $\alpha$ -tocopherol in the leaf sample was calculated based on the standard curve. A total of five replicates were used for each plant species from each of the two (interior and fringe forest) locations.

#### Determination of Ascorbate:

Ascorbate was extracted from the leaf tissue based on the method of John and Hughes (1983). 0.15 g of fresh sample was ground with pre-chilled mortar and pestle in 2.0 ml of 6% orthophosphoric acid in ice-cold conditions. The ground samples were then centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant obtained was obtained carefully titrated with DCPIP until a pink colouration was obtained. The volume of DCPIP used was compared against a standard curve to determine the amount of ascorbic acid in the samples. A total of five replicates were used from each of the two (interior and fringe forest) locations.

## RESULTS AND DISCUSSION

Oxidative deterioration of all the eight species sampled, as indicated by concentrations of MDA in their leaf tissues, were found to be significantly different between the interior forest and fringe

forest regions (Fig. 1). The MDA concentration was generally found to be higher in the leaf tissues sampled from the fringe forest which was relatively more disturbed compared to samples taken from the relatively undisturbed interior forest except for *Vitex pinnata* (Leban) and *Gironniera nervosa* (Hampas tebu) which had higher MDA concentration in the interior forest region. Higher levels of oxidative deterioration in the samples taken from the fringe forest indicate that the plants were subjected to higher levels of stress conditions which may partly be attributed by greater exposure to atmospheric pollution and inferior soil conditions (Bowler *et al.* 1992). The highest degree of difference in MDA concentrations between the interior and fringe forest with reference to the ratio of the fringe forest region MDA concentrations to interior forest region MDA concentrations was exhibited by *C. atropurpurea* followed by *B. oppositifolia*, *G. atroviridis*, *P. axillaris*, *A. elasticus*, *E. diadenum*, *V. pinnata* and *G. nervosa*.

Concentration of  $\alpha$ -tocopherol (Fig. 2), a lipid soluble antioxidant was also found to be generally higher in the fringe forest regions compared to the interior forest region except for *V. pinnata* and *G. nervosa* where significant differences were found in *E. diadenum*, *V. pinnata*, *C. atropurpurea*. The apparent ability of *V. pinnata* and *G. nervosa* to minimize oxidative deterioration in stress conditions as indicated by the lower levels of MDA concentrations in the fringe forest region may be attributed to their efficient ability in modulating and regulating the endogeneous  $\alpha$ -tocopherol to impede the chain reactions of oxidative deterioration. This may thus explain the lower levels of  $\alpha$ -tocopherol in both *V. pinnata* and *G. nervosa* sampled from the fringe forest regions. Concentration of ascorbate (Fig. 3), a water-soluble antioxidant on the other hand, was found to be higher for all species sampled from the fringe forest region compared to samples from the interior region where significant differences between these two forest regions were shown by *A. elasticus* (terap), *E. diadenum* (sesenduk), *V. pinnata* (leban), *B. oppositifolia* (kundang) and *C. atropurpurea* (tulang daing). Ascorbate, a reductant in the Halliwell-Asada pathway, may be more directly regulated by the environmental conditions compared to  $\alpha$ -tocopherol and thus more sensitive with respect to the antioxidative response shown by all the species samples.

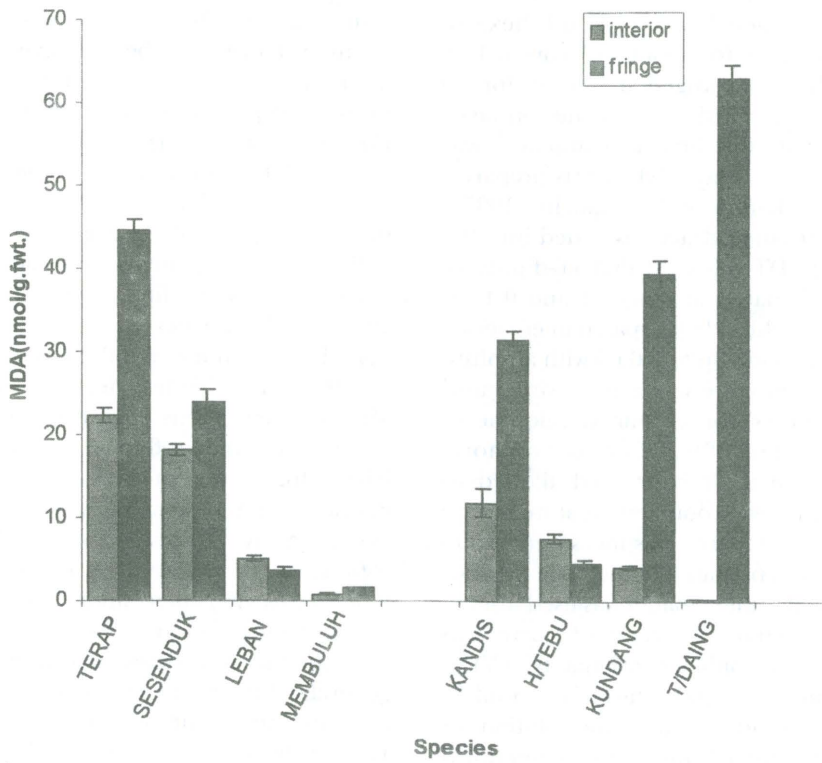


Fig. 1. Malondialdehyde concentration of eight tree species from the interior and fringe forest regions of Ayer Hitam Forest Reserve. Data are means  $\pm$  se (n=5 replicates)

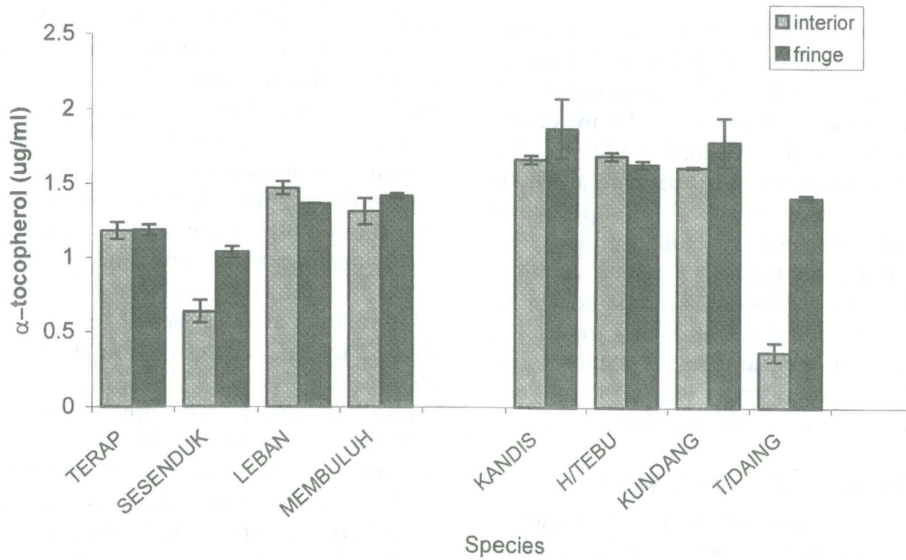


Fig. 2.  $\alpha$ -tocopherol concentration of eight tree species from the interior and fringe forest regions of Ayer Hitam Forest Reserve. Data are means  $\pm$  se (n=5 replicates)

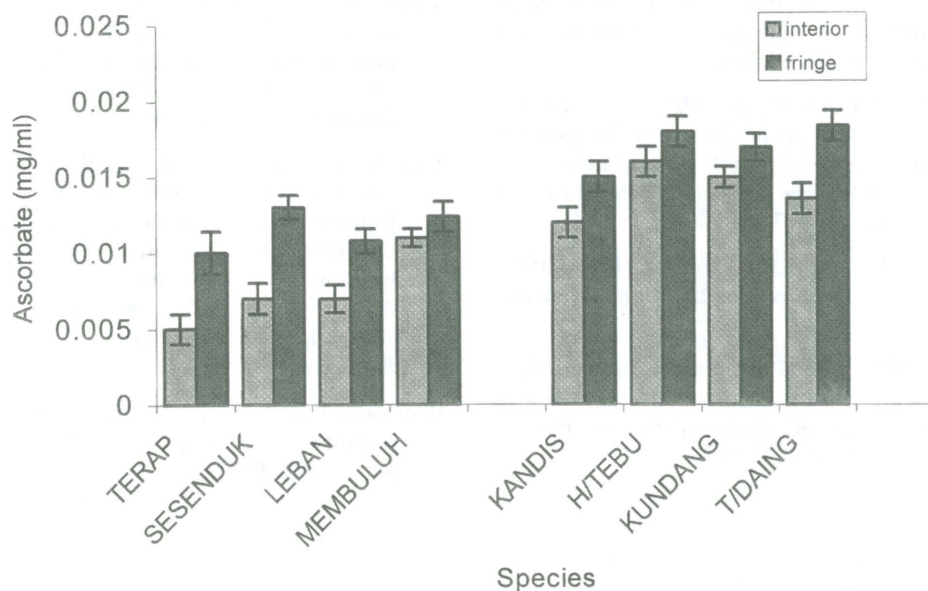


Fig. 3. Ascorbate concentration of eight tree species from the interior and fringe forest regions of Ayer Hitam Forest Reserve. Data are means  $\pm$  se ( $n=5$  replicates).

## CONCLUSION

In agreement with a previous study (Fadzillah and Faridah Hanum 1999), oxidative deterioration has been shown to be a significant antioxidative response and may be a suitable indicator of forest disturbance. In addition, *C. atropurpurea* may be a good and sensitive indicator species in determining stress conditions in the forest regions while *V. pinnata* and *G. nervosa* represent tree species that are efficient in modulating their endogenous  $\alpha$ -tocopherol content to minimize oxidative deterioration in stress conditions.

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## Economic Valuation of Forest Goods and Services of Ayer Hitam Forest, Puchong, Selangor

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### ABSTRAK

*Penukaran tanah hutan kepada kegunaan lain telah menyebabkan kehilangan kepada kepelbagaian biologi dan lain-lain nilai ekonomi kepada komuniti. Bagaimanapun, potensi nilai ekonomi sumber-sumber hutan tidak diambilkira sewajarnya oleh pembuat polisi dan perancang pengguna tanah. Satu kajian penilaian ekonomi sumber hutan dijalankan di Hutan Ayer Hitam (AHFR), Puchong Selangor untuk menentukan nilai ekonominya dan kesannya ke atas perubahan guna tanah. Kajian ini memberi tumpuan kepada penilaian sumber kayu, rekreasi, peranan ke atas komuniti dan pemuliharaan hidupan liar. Keputusan menunjukkan bahawa nilai ekonomi Hutan Simpan Ayer Hitam adalah tinggi dan jika kita tidak mengambilkira nilai tersebut dalam pembangunan guna tanah boleh menunjukkan petanda yang salah kepada pembuat polisi. Perancangan penggunaan tanah pada masa depan hendaklah mengambilkira bukan sahaja pulangan berasaskan kepada pasaran tetapi juga lain-lain faedah alam sekitar.*

### ABSTRACT

*The conversion of forestland to other land uses has resulted in substantial loss of biodiversity and other potential economic values to the community. However, the potential economic values of forest resources have been largely ignored by policy makers and land use planners. An economic valuation of forest resources of Ayer Hitam Forest (AHFR), Puchong, Selangor was carried out to determine its economic value and its impact on land use changes. The study focused on valuation of timber resources, recreation, community roles and wildlife conservation. The results show that the economic value of AHFR is substantial and ignoring this value in land use development would provide a wrong signal to policy makers. Future land use planning should consider not only market-based economic returns but also its non-market and other environmental benefits.*

### INTRODUCTION

Malaysian tropical forest is well known for providing valuable timber resources to the state governments and the community in terms of direct and indirect monetary and non-monetary benefits. Forests also provide a source of food and genetic resources of many agricultural crops, materials used in medicine, eco-tourism and recreation opportunities, and help in maintaining favourable environmental conditions

as well as 'research labs.' In the past, however, the forest has been viewed mainly as a source of timber to feed the wood-based industries, which produce a variety of products for domestic and export consumption. The other equally important components of the forest ecosystem such as environmental services, however, have not attracted much attention until very recently. This is indeed an unfortunate situation knowing the fact that tropical forests are very rich in flora and fauna.

The natural products that come from the forests include latex, steroids, edible oils, rattans, bamboo, spices, pesticides, and dyestuffs while some of the consumer goods made from forest products are coffee, lubricants, glue for postage stamps, golf balls, chewing gums, toothpaste, shampoo, mascara and lipstick. The market in these industrial products is worth billions of dollars per year.

The full potential of the biologically diverse tropical forests has never been completely quantified in economic or monetary terms. While it is relatively simple to determine the economic value of timber because of its readily available market price, it is not as simple to calculate the economic value of recreation, wildlife conservation, medicinal plants species or biological diversity. This could be an important factor for the past neglect on the non-timber components of the forest ecosystem in the decision to convert forest to non-forest uses. The economic potential of these resources has not been very much appreciated. Since the economic value of these resources is difficult to determine, their real potential as income generators has not been fully explored. There is a strong need for studies to be carried out to quantify to the fullest extent the economic value of all forest goods and services. Only then we would have a complete view on the costs and benefits of comparing alternative forestland use options.

This paper discusses the economic value of Ayer Hitam Forest, Puchong, Selangor with emphasis on timber resources, recreation, benefits to community and wildlife conservation. The implications of the study on land use options are discussed in the final section of the paper.

#### **VALUATION OF FOREST GOODS AND SERVICES: THE NEED AND APPROACH**

Economists generally depend on market prices to indicate the value of goods and services. For goods and services exchanged in a well-defined market, information on prices and quantities are readily available. This information can be used to estimate the value of certain goods and services by constructing a demand curve. Unfortunately, not all forest goods and services have market prices. This is particularly true for most

of the non-timber forest products or services such as water, recreation, wildlife, wild fruits and genes. One characteristic of such goods or services is the occurrence of 'free riders', in which case consumers refuse to express their true willingness-to-pay (wtp), but could obtain utility from the good or service. As such, prices might be distorted leading to inappropriate estimation of the true economic value of the resources. The major role of valuation is, therefore to assign the value to goods and services with distorted or non-existent market prices or to value them in terms of their opportunity cost.

Typically, the benefits derived from forest resources are to be measured in terms of market price or willingness-to-pay of users or consumers for using and experiencing the goods and services. An approximation of users' wtp for certain recreational opportunities, for instance, can be developed from a demand curve, which indicates the quantity of use that users in a market would be willing and able to purchase at each price. Other estimates could be in terms of the expenditures on preventive measures taken by consumers or users to avoid a future loss. Thus, conservation of forest resources could be seen as a form of wtp for current, as well as, future benefits.

Resource economists have yet to agree on a taxonomy of economic values. There are many classifications of values and benefits given in the literature (Barbier 1992, Munasinghe 1993, Pearce 1993). In general, the following category of economic values are used:

- (i) Direct use values refer to the productive or consumptive values of ecosystem components or functions. Direct uses may be marketed or non-marketed, with some of the latter activities often being important for the subsistence needs of local communities. An example of a marketed direct use is timber resources, which can be harvested and sold to consumers. The use of medicinal herbs collected from the forest resources by local communities is an example of non-marketed direct use. Marketed uses may be important for both domestic and international markets. In general, the value of marketed goods and services is easier to measure than the value of non-marketed and subsistence direct uses.



- (ii) Indirect use values refer to the value of environmental functions that support or protect an economic activity. For instance, a tropical forest protects watersheds and store carbon dioxide. Tropical forests also include many plant species, which in turn may have ecological functions. The values of environmental functions can be derived from the supporting or protecting economic activities that have directly measurable values.
- (iii) Option values relate to the amount that an individual or society would be willing to pay to conserve an ecosystem for future uses. For example, preservation of biological diversity can preserve wild genes for future uses such as improvement of a fruit species. Wild fruit and fish may prove to be extremely valuable genetic stocks in the future, because many of these wild plants and fish have genes that can help resist some kind of diseases.
- (iv) Existence values refer to society's willingness-to-pay to conserve biological resources for their own sake, regardless of their current or optional uses. For instance, many people reveal their wtp for the existence of biological resources such as wildlife and landscape without participating in the direct use of the wildlife and landscape through recreation.

The method employed to determine each value mentioned above depends on the nature of forest goods and service in question. For the direct use value, the methods available include market-based technique, changes in productivity approach, relocation cost, and damage cost avoided. The contingent valuation approach can be used to value the indirect use, option and existence values. This method requires good understanding of forest goods and services production system. It is not the intention of this paper to discuss each method used in valuing a good or service. A good literature on the methods used can be found in IIED (1994) and Mitchell and Carson (1989).

## MATERIALS AND METHODS

### *Location of the Study Area*

The study area is the Ayer Hitam Forest (AHFR), Puchong, Selangor, which is located in a strategic

place in a rapidly developing urban community. Some of the development projects that have been completed in the vicinity include an agriculture project, world class sports complex, a multi-million dollar housing project, incineration plant and waste disposal area, and an equestrian park. The forest reserve has also been excised for the highway linking Seri Serdang and Damansara Puchong Highway. The new administration city, Putrajaya, is just a short drive away and so is the capital city of Kuala Lumpur. The forest area, therefore, could provide excellent recreation and eco-tourism opportunities for urban dwellers.

The forest belongs to the Lowland Dipterocarp forest type. It is classified as a secondary disturbed forest because it has been logged and treated several times since 1930's. Currently, the forest comprises six compartments, namely, compartments 1, 2, 12, 13, 14 and 15. These compartments make up a total area of 1,248 ha. According to the Forestry Department record, the area of AHFR has decreased substantially from the original forest area of about 4,267 ha in 1965. The extent of forest area and the percentage of area loss as compared to the original area are shown in Table 1.

The AHFR is the only remaining lowland forest reserves left in the Klang Valley. It is an excellent demonstration area for students to learn about various aspects of forestry. In addition, the forest area offers research opportunities for scientists interested in the working of a tropical lowland forest ecosystem. It also serves as an important 'green lung' for the urban city of Kuala Lumpur.

Considering the factors mentioned above, a general function of AHFR is to promote the protection of a lowland forest ecosystem that would serve the needs for education, research, and recreation not only for UPM community but also the urban areas (Petaling Jaya, Subang Jaya, Kelang, Kuala Lumpur) and dwellers surrounding the forest reserve (Seri Serdang, Seri Kembangan, Puchong, Kajang and Bangi). Thus, the management objectives of AHFR are as follows:

- to promote systematic and coordinated research into the working of a lowland rainforest ecosystem;
- to provide training areas in forest biology, forest production, forest management,