Study on Heath Forest Species *Melaleuca cajuputi* as Potential Natural Herbicides Agent to Suppress Weed Growth in Landscape Management

Rashidi Othman*, Wan Masyitah Wan Daud¹, Razanah Ramya¹, Nursyafica Nadia Johari¹ and Zainul Mukrim Baharuddin¹

¹Department of Landscape Architecture, Kulliyyah of Architecture and Environmental Design (KAED), International Islamic University Malaysia, 53100 Kuala Lumpur

*Corresponding author: rashidi@iium.edu.my

Abstract. Weeds are diverse in habitats throughout the world. In landscape management, weed is of major concern because they compete for growth factors with landscape plant of interest. Modern management relies on the importance of synthetic chemicals to control weeds as unwanted plants to reduce the weed management cost. The use of herbicides is not an appropriate tool for controlling development of some weeds but can create negative effects to the environment. Managing the natural and landscape resources are not only designing for good views and environment merely, but need to sustain all of living environment through consideration. Therefore, this study was aimed to explore *Melaleuca cajuputi*, heath forest species as potential natural herbicides agent in sustainable landscape management. Three assessments were conducted to determine their effectiveness towards suppressing weeds without degrading the environmental quality index. Assessment of allelochemical compounds in *M. cajuputi* demonstrated that these species have allelopathic potential as a natural herbicide.

1. Introduction
In 1967, the Weed Science Society of America defined a weed as a plant growing where it is not desired or known as an unwanted and undesirable plant that utilize on land biodiversity and water resource and may affect human welfare. Although they account for not more than 1% of the total plant species on earth, they cause great problems nevertheless to humankind by interfering in food production, economic stability, health, and welfare [1]. Weed plants are one of the main problems in global agriculture. They are a setback for the management and harvest of the given culture, by decreasing productivity which leads to an annual loss of approximately US$95 billion. In landscape management, weeds are of concern because of their competition with other plants for growth factors. The discovery of synthetic natural herbicides for weed control in the late 1940s made reduced tillage practices more feasible because tillage was not the only method of weed control [2]. In Malaysia, dominant weeds are broad-leaved weeds in rice field [3]. The annual expenditure is approximately about US$4.10 million on herbicides for rice alone which almost 7% of the total cost of herbicides in Malaysia. Weeds constantly compete with crop plants to cause a considerable loss in their productivity, and have been documented as serious plant pests since ancient times [4]. The application
of allelochemicals as natural herbicides for weeds management tool has been reported [5], including their importance and interaction with the environment.

2. Materials and methods

2.1. Study site

The site selection is located within Jambu Bongkok Forest Reserve (370 ha) at Rantau Abang, Terengganu (4°55'N, 103°21'E) with altitude varying between 3-12 meters. Rantau Abang is a small village located 80 km from South of Kuala Terengganu. This site was chosen because it is one of the areas that is surrounded by the heath forest ecosystem and still have the traces of the forest. The heath forest ecosystem in this area is located near to the village and road systems. Even though there are wide area covered with heath forest, most of the ecosystem is already affected by human disturbance [6]. The sandy soil of the heath forest is often lacking in nutrients including nitrogen which impedes plant growth in these forests [7]. Over time, the sandy soil is covered by a thick layer of organic matter, allowing vegetation to develop. Heath forest is extremely fragile, once the organic layer is removed, no vegetation can regenerate because only sand remains. This heath forest was mainly dominated by Melaleuca cajuputi species. The study area is divided into three part of the forest: matured trees area, semi-matured trees and seedling areas as shown in figure 1.

![Figure 1. Category of heath forest species based on trunk diameter at Rantau Abang, Terengganu.](image-url)
2.2. **Plant maceration extraction**  
Sequential alkaline extraction method was used to extract free and bound phenolic compounds from sodium hydroxide (NaOH) for 12h in the oven at 60°C. The alkaline extract was treated with hydrochloric acid (HCl) to reach pH 2.0, centrifuged and the supernatant extract was collected then re-extracted with ethyl acetate [8]. The final concentration was resuspended with methanol for further analysis by HPLC.

2.3. **Determination of Total Phenolic Compound (TPC)**  
TPC determination was conducted by using the Folin-Ciocalteau assay as reported by [9] with slight modification. Quantification was performed with hydrolysed samples. Results were expressed as gallic acid equivalence (GAE) per gram dry weight sample using TECAN microplate reader.

2.4. **HPLC analysis of Phenolic Acids Content**  
HPLC analyses were proceed with Agilent 1200 series (Agilent Technologies, Palo Alto, CA, USA) equipped with the binary pump, an autosampler and a diode array detector (DAD). The HPLC column was a reverse-phase Zorbax SB-C18 (Eclipse 100 × 2.1 mm, 1.8 µm). The temperature of the column was set at 25°C. For the analysis, two mobile phases were used consist of 1% formic acid in water/ acetonitrile 90:10 v/v (phase A) and acetonitrile (phase B). the solvent gradient used developed as follows: 0-40% solvent B (0-20 min), 40-60% solvent B (20-25 min); 60-100% (25-25.1 min), 100% solvent B (25.1-35 min) and 100-0% solvent B (35-35.1 min) at a flow rate of 0.4 ml/min and with detection at 280 nm throughout the gradient [10]. The phenolic acid contents were identified through their retention times and their UV spectra as compared to standards.

2.5. **Determination of allelochemical effect through In Vitro model system**  
Seeds of the *Chloris barbata* (grass) were collected from a paddy field in Kepala Batas, Pulau Pinang. The tissue culture processed was modified based on [11]. The concentration of the water extractions was 100g/ L prepared for the experiment [12] from low to high concentration (0.5, 2.5 and 5.0 g/L). The experiment was observed during germination of weed species with three different stage of growth; Pre-emergent (Seed), Emergent (Seedling), Post-emergent (Individual plants). All stages of weed will be assessed with three different concentrations (high, medium and low concentration) at for four different periods of time (1st week – 4th week).

2.6. **Statistical analysis**  
Data were expressed as the mean ± standard deviation of triplicates solvents extraction for total phenolic compound (TPC) and phenolic acids content. One-way analysis of variance (ANOVA) with Tukey's test was conducted using XLSTAT-Pro (2014) statistical software (Addinsoft, Paris, France).
3. Result

3.1. Analysis of phenolic acid content and composition in Melaleuca cajuputi

Three different stages of M. cajuputi vegetative samples were analysed for total and individual phenolic content to investigate the stability of their phenolic acid composition profile. Analysis of variance on the data exhibited highly significant differences between the three stages and the individual phenolic acid content (figure 2). This further reinforced different stages of M. cajuputi tree can have a marked influence on the accumulation of allelochemicals. The changes in phenolic acid composition are complex because it changes with different period of tree age as indicated by the interaction components. The total phenolic content for young, semi-matured and matured M. cajuputi tree ranged from 892.97 to 1376.28 µg/g DW. The semi-matured tree (1376.28 µg/g DW) was detected to have the highest amount of total phenolic content while the matured tree (892.87 µg/g DW) was the lowest.

![Phenolic content diagram](image)

**Figure 2:** Comparison of phenolic acid content and composition of M. cajuputi between young, semi-matured and matured tree.

Comparison of phenolic acid profiles of the three different period of age showed variations in the profiles of individual phenolic acid compounds (figure 2). For example, young stage of tree with trunk diameter between 2 to 10 cm accumulated 6 major phenolic acid compounds mostly caffeic acid, vanillic acid, trans-p-coumaric acid, ferulic acid, 3-coumaric acid and 2-coumaric acid, whereas in semi-matured tree with trunk diameter between 25 to 50 cm accumulated only 4 types of phenolic acid compounds which are caffeic acid, vanillic acid, trans-p-coumaric acid and ferulic acid. For matured tree with trunk diameter between 80 to 130 cm, only three major phenolic acids were detected which are caffeic acid, vanillic acid and ferulic acid.
3.2. Analysis of allelopathic effect towards weed germination

All three stages of weed were assessed with three different concentrations (high, medium and low concentration) at four different periods of time (1\textsuperscript{st} week – 4\textsuperscript{th} week) (table 1).

**Table 1.** Effect of allelochemical extracts of *M. cajuputi* on the *C. barbata* germination and growth at different period of time at low, medium and high concentration.

| WEEK 1 | Extract Concentration (g) | Shoot length (cm) | Shoot number | Radicle length (cm) | Plant height (cm) | Status |
|--------|---------------------------|-------------------|--------------|---------------------|------------------|--------|
| Low (0.5) | 0.4                      | 55                | 0.2          | 1.2                 | Rapid growth     |        |
| Medium (2.5) | 0.4                    | 5                 | 0.1          | 1.1                 | Slow growth      |        |
| High (5.0)  | 0.4                      | 5                 | 0.1          | 1.0                 | All radicle stunted |        |

| WEEK 2 | Extract Concentration (g) | Shoot length (cm) | Shoot number | Radicle length (cm) | Plant height (cm) | Status |
|--------|---------------------------|-------------------|--------------|---------------------|------------------|--------|
| Low (0.5) | 0.5                      | 110               | 0.3          | 1.4                 | Rapid growth     |        |
| Medium (2.5) | 0.4                    | 5                 | 0.1          | 1.1                 | 2 radicle stunted|        |
| High (5.0)  | 0.3                      | 5                 | 0.1          | 1.0                 | All radicle stunted |        |

| WEEK 3 | Extract Concentration (g) | Shoot length (cm) | Shoot number | Radicle length (cm) | Plant height (cm) | Status |
|--------|---------------------------|-------------------|--------------|---------------------|------------------|--------|
| Low (0.5) | 0.6                      | 157               | 0.4          | 1.6                 | Rapid growth.    |        |
| Medium (2.5) | 0.4                    | 5                 | 0.1          | 1.1                 | 3 radicle stunted|        |
| High (5.0)  | 0.3                      | 5                 | 0.1          | 1.0                 | All radicle stunted |        |

| WEEK 4 | Extract Concentration (g) | Shoot length (cm) | Shoot number | Radicle length (cm) | Plant height (cm) | Status |
|--------|---------------------------|-------------------|--------------|---------------------|------------------|--------|
| Low (0.5) | 0.7                      | 230               | 0.6          | 1.9                 | Rapid growth.    |        |
| Medium (2.5) | 0.4                    | 5                 | 0.1          | 1.1                 | All radicle stunted|        |
| High (5.0)  | 0.3                      | 5                 | 0.1          | 1.0                 | All radicle stunted |        |

According to the analysis of allelopathic effect towards weed germination (growth rates of *C. barbata*) in this in vitro model system, the seeds started germinating at day 2. Table 1 showed the effect of the allelochemical extracts from *M. cajuputi* on pre-emergent (seed), emergent (seedling), and post-emergent (individual plants) of *C. barbata* from week 1 until week 4. The result demonstrated that *C. barbata* seeds development showed a gradual inhibitory effect in germination rate through shoot number, radicle length, shoot length and plant height in all concentrations. The inhibition of seedling development increased from week 1 until week 2 as compared to low concentration of 0.5g/L. Concentration at 0.5 g/L was observed to have stimulatory effect on shoot length and number as well as radicle length and plant height. The number of seedlings of *C. barbata* showed a reduction of growth from 2.5 g/L until 5.0 g/L concentrations as compared to 0.5 g/L. In general, *M. cajuputi* allelochemical extract at 2.5 and 5.0 g/L showed constituents of allelopathic activity which influenced the inhibitory effect on pre-emergent, emergent and post-emergent of *C. barbata*.

4. Discussion

Both species studied at the cultivar levels was reported to change in different environments. [13, 14]. The bioavailability of phenolic acid compounds is a complex issue and depends on many factors such as location, times of year, cultivar and their relationship of all factors [15]. In this study, looking at the influences of different age of tree and total phenolic acid content interactions, variations in both the total phenolic content and the individual phenolic acid compounds exhibit strong relationships between *M. cajuputi* stage or period of maturity. Therefore, major factors influencing allelochemicals production are location, age and their interactions (figure 2). Genotype x environment interactions on
biochemical composition has been previously reported for phenolics accumulation in potatoes. [16] reported that potatoes cultivated on loam soils in warm dry regions with low altitudes over a three-year period contained a lower amount of total phenolics than those cultivated in cooler and more humid regions on sandy loam soil. In a similar study they found that organically grown potatoes contained higher levels of phenolics than did the same varieties grown in a conventional manner. It is hypothesized that the chemically untreated plants defend themselves against unfavourable extrinsic factors with higher levels of polyphenols. Furthermore, it is known that plants that are exposed to abiotic and biotic stresses increase their production of phenolics as a defence mechanism [16, 17].

5. Conclusion
This research will establish a new body of knowledge on the application of allelopathic species of Melaleuca cajuputi as potential natural herbicides in landscape towards sustainable landscape management. Creation of bio herbicides based on allelochemicals generates the opportunity to exploit natural compounds in plant protection and shows the possibility to cope with evolved weed resistance to herbicides and may be based on the structure of compounds occurring in nature, which could be used without any risks as selective and eco-friendly herbicides. Eco-friendly trends in weed management drives scientists to reach for innovative sources and tools. A natural control strategy may be warranted because of concerns about environmental effects of chemical herbicides or mechanical control, lack of efficacy of other methods, or simple economics. Therefore, a natural control in weeds management today introduces a new finding towards bio herbicide products which are safer to be used as well as by using reactions between plants.

6. References
[1] Qasem J R and Foy C L 2001 J. Crop. Prod. 4 43.
[2] Voltarelli V M, Pedro J, Ribeiro N and Lima M I S 2012 Acta. Bot. Bras. 26 779.
[3] Karim S M R, Azmi M and Ismail B S 2004 Weed. Biol. Manag. 4 177.
[4] Jabran K, Mahajan G, Sardana V and Chauhan B S 2015 Crop. Prot. 72 57.
[5] Nornasuha Y and Ismail B S 2017 Malays. Appl. Biol. 46 1.
[6] Syurhani A W, Hakeem K R, Faridah-Hanum I, Alias M S and Ozturk M 2014 Emir. J. Food Agric. 26 1114.
[7] Rashidi O and Ruzaimi M R 2013 Hutan heath: Khazanah landskap dan warisan ekologi Negara. (Kuala Lumpur: Yamani Angle Sdn. Bhd.)
[8] Aarabi A, Mizani M, Honarvar M, Faghihian H and Gerami A 2016 J. Food. Meas. Charact. 10 42.
[9] Baba S A and Malik S A 2015 J. Taibah. Univ. Sci. 9 449.
[10] Zhao H, Sun J, Fan M, Fan L, Zhou L, Li Z and Guo D 2008 J. Chromatogr. A 1190 157.
[11] Othman R 2009 Biochemistry and genetics of carotenoid composition in potato tubers (Ph.D. thesis. Lincoln University).
[12] Ismail B S, Syamimi H, Wan Juliana W A and Nornasuha Y 2016 Sains. Malay. 45 517.
[13] Dencic S, Kastori R, Kobiljski B and Duggan B 2000 Euphytica. 113 43.
[14] Banziger M and Cooper M 2001 Euphytica. 122 503.
[15] Fraser P D and Bramley P M 2004 Prog. Lipid Res. 43 228.
[16] Hamouz K, Lachman J, Vokal B and Pivec V 1999 Rost. Vyroba. 45 293.
[17] Lewis C E, Walker J R L, Lancaster J E and Conner A J 1998 Aust. J. Plant. Physiol. 25 915.

Acknowledgements
The research was supported by the Ministry of Higher Education Malaysia (MOHE) and International Islamic University Malaysia (IIUM) under research grant PRIGS18-001-0001.