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Characterisation of Andrographolide in Andrographis Paniculata under Different Cultivation Conditions

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The trend in the practical uses of herbal medicine has gained increasing momentum in recent years. One of the trends includes the use of the andrographolide-containing Andrographis paniculata, which is a herb that is traditionally used as medicine. There are not many studies have investigated the cultivation of this plant. The aim of this study is to develop a standard of procedure for cultivating Andrographis paniculata using the fertigation technique. A total of 6 treatments in 2 growth medium compositions were studied; treatments T1, T3, and T5 in Cocopeat-RHA (70:30) and treatments T2, T4, and T6 in 100 % cocopeat. 3 magnesium compositions of 10 ppm (T1 and T2), 50 ppm (T3 and T4), and 70 ppm (T5 and T6) in nutrient solution were also studied. Treatments T1, T3, and T5 showed the potential for better growth quality with a maximum plant height of 32.51 cm, 32.51 cm, and 31.75 cm. The andrographolide content was comparable with controls 1 and 2 (0.67-0.70 µg/mL). In higher magnesium compositions of the nutrient solution, the dry herb yield of T5 and T6 decreased from 2.4 g to 1.6 g and 2.4 g to 1.2 g. The T3 and T5 treatments exhibited a higher yield than T4 and T6 based on a comparison of dry herb yield. The incorporated fertigation technique showed an on-par quantity of andrographolide yield to that of conventional techniques (0.67-0.70 µg/mL) although it showed an advantage of overcoming the cleanliness problem faced in this study. Hence, the T3 standard of procedure was deemed the best, as it gave better plant growth quality with a maximum plant height of 31.75 cm, a maximum number of leaves (141 total count), a good dry herb yield of 2.2 g, and better andrographolide content 0.7 µg/mL. The fertigation technique incorporated with the combined Cocopeat-RHA medium and 50 ppm magnesium composition in the nutrient solution is suggested for the cultivation of Andrographis paniculata.

1. Introduction

Herbal medicine plays a significant role in maintaining human health and improving the quality of human life. A practice that has lasted throughout human history is the use of plants containing natural substances that can cure or lessen the symptoms of an illness. In recent years, the practical use of herbal medicines has gained momentum worldwide, reflected by an increasing number of scientific publications related to the therapeutic effect of these medicines, including randomised clinical trials (Wegener, 2017). This incident points towards the potential of commercially producing herbal medicine at a large scale for the global market.

One of the native plants acknowledged to have enormous commercial potential is *Andrographis paniculata.* Locally called *Hempedu Bumi* in Malaysia, this plant has an extremely bitter taste. *Andrographis paniculata* has been used traditionally to treat the common cold, inflammatory diseases, bowel constipation, high blood pressure, diabetes, and diarrhoea (Chao and Lin, 2010). Recently, the plant was used to treat HIV, hepatitis, cancer, kidney disorders, and swine flu (H1N1) (Seniya et al., 2014).

Andrographolide, a diterpene lactone, is the major bioactive compound in *Andrographis paniculata,* making up about 4 % of the dried whole plant, 0.8 %~1.2 % of the stem, and 0.5 %~6 % of the leaf extract (Cheung et al.,2001). The amount of andrographolide extracted from *Andrographis paniculata* is very low in individual

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plants, depending on different varieties and cultivation conditions. Increasing the biomass or productivity of *Andrographis paniculata* is one way of increasing its bioactive secondary metabolites on a large scale, i.e. by varying the cultivation method, optimising the nutrient distribution, and managing and standardising its nutrient formulation. In this study, *Andrographis paniculata* was cultivated using a soilless medium integrated with the fertigation technique to ensure better quantity and quality yield.

2. Methodology

2.1 Experimental and treatment designs

Experiments were carried out in a rain shelter situated at N29, Universiti Teknologi Malaysia (UTM), Johor, Malaysia. Saplings of *Andrographis paniculata* taken from Pusat Pertanian Sendayan, were transplanted into black polybags measuring 36 cm × 36 cm. Then, the plants enhanced with the nutrient solution were distributed using the fertigation technique. The site layout design was set up as in Figure 1. The treatments include two different medium compositions of cocopeat and RHA and three different nutrient formulations. The treatment details are given in Table 1.

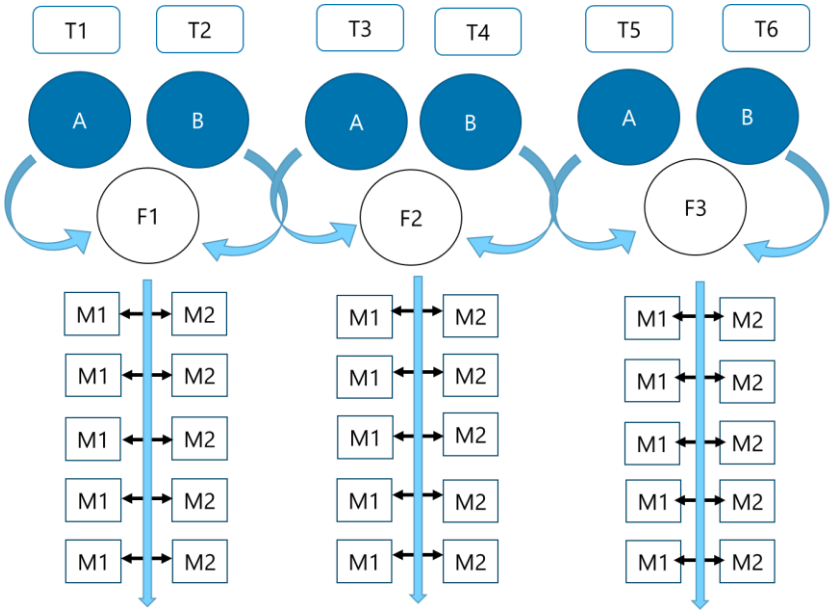

Figure 1: The experimental design of Andrographis paniculata using different media (M1 and M2) and nutrient solution compositions (F1, F2 and F3).

Table 1: The treatment details

Treatment	No. of Sets	Medium	Nutrient Solution
T1	5	70 % Cocopeat–30 % RHA (M1)	F1 – 10 ppm Mg
T2	5	100 % Cocopeat (M2)	F1 – 10 ppm Mg
T3	5	70 % Cocopeat–30 % RHA(M1)	F2 – 50 ppm Mg
T4	5	100 % Cocopeat (M2)	F2 – 50 ppm Mg
T5	5	70 % Cocopeat–30 % RHA (M1)	F3 – 70 ppm Mg
T6	5	100 % Cocopeat (M2)	F3 – 70 ppm Mg

The nutrient solution in this study was prepared based on the standard nutrient formulation of chilli (F2) provided by the Malaysian Agricultural Research and Development Institute (MARDI). This formulation was used to ration the magnesium composition according to 10 ppm Mg (F1) and 70 ppm Mg (F3) compositions. Magnesium was chosen as the nutrient in this study because a past study had shown the benefits of using Mg to increase the secondary metabolites in plants. In specific, Guo et al. (2015) reported that increasing Mg composition caused a positive response in the secondary metabolite (cardenolide) production of callus cultures of *Digitalis davisiana Heywood*, *D. lamarckii Ivanina*, *D. trojana Ivanina.* The nutrient solution for each

treatment in this study was prepared by mixing fertiliser A and fertiliser B. The electrical conductivity (EC) of the nutrient solution was set to 1.6 mS/cm^2 at pH 5.8.

2.2 Growth quality analysis

Periodic observations of the growth and quality of the plant were done, limited to the height, the number of branches and leaves, the fresh weight and the dry weight of the plant. The growth and yield attributes were recorded from 5 randomly selected plants taken from each treatment plot. All parameters were recorded as per the time of harvesting, which is just before maturity (flowering). Dry herbage samples were first dried in an oven at 45 °C until a constant dry weight was obtained.

2.3 Andrographolide extraction

The dried samples were ground into granular form. The andrographolide component was extracted using the method outlined in Karpakavalli et al. (2012) with slight modifications. First, 1 g of the sample was extracted with 25 mL of diluted ethanol (60:40) as the solvent, using a microwave set to 60 °C and 500 W intensity for 21 min. Then, the mixture was filtered and centrifuged. The supernatant was evaporated using a rotary evaporator until it concentrated at low temperature (40 °C to 50 °C) and a low rotational speed (40 rpm). The concentrated sample was further dried in an oven at a low temperature of 50 °C until a constant weight was achieved.

2.4 Andrographolide characterisation

A reverse-phase HPLC method was used to characterise the andrographolide as per the method outlined in Sajeeb et al. (2015) with slight modifications. First, a sample solution of 1,000 ppm or 1 mg/mL was prepared by dissolving the required quantity of amorphous residue in HPLC-grade methanol. The solution was then thoroughly shaken and sonicated until complete dissolution of the coarse and visible particles was achieved. Then, the solution was allowed to cool at room temperature and filtered through a 0.45-µm hydrophilic syringe filter. A total of 8 sample solutions of 1 mg/mL (including two control samples) concentrations were prepared and stored in HPLC 2 mL clean vials.

The andrographolide content in the amorphous residue was determined via reversed-phase HPLC with a C18 column. The elution was carried out with a binary solvent system of water and methanol (35:65) as the mobile phase at a flow rate of 0.7 mL/min maintained at ambient temperature. The sample injection volume was 20 µL, and the analyses were monitored with a UV-Vis detector at 223 nm.

The quantification of andrographolide in the sample was done based on the standard reference of Sajeeb et al. (2015). The standard solutions of different concentrations were analysed with the RP-HPLC method and peak areas were recorded. Referring to Figure 2, the retention time of the andrographolide was observed at 7.570 min.

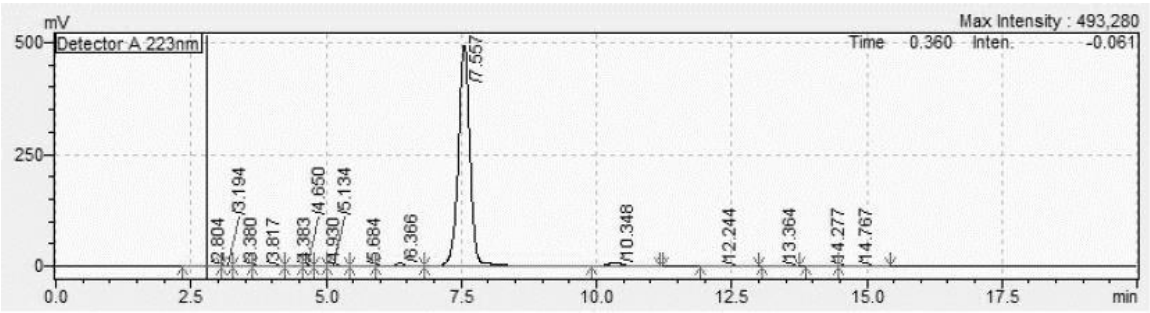

Figure 2: Chromatogram of the reference standard of andrographolide observed using RP-HPLC (Sajeeb et al*., 2015).*

Based on Figure 3, the linear regression equation of the calibration curve can be obtained, as per Eq(1).

$$Y = 57{,}250X + 37{,}719 \quad (1)$$

where X is the concentration and Y is the peak area, with a correlation coefficient (R^2) of 0.995. The sample solutions (1 mg/mL) were analysed and the peak areas recorded. Then, the concentration of andrographolide in the sample solutions was determined using Eq(1). The andrographolide content in this study was compared to that of the 2 controls: Control 1 represents conventional cultivation (Pusat Pertaninan Sendayan) prepared as per the above method whereas Control 2 was bought readily available from the market.

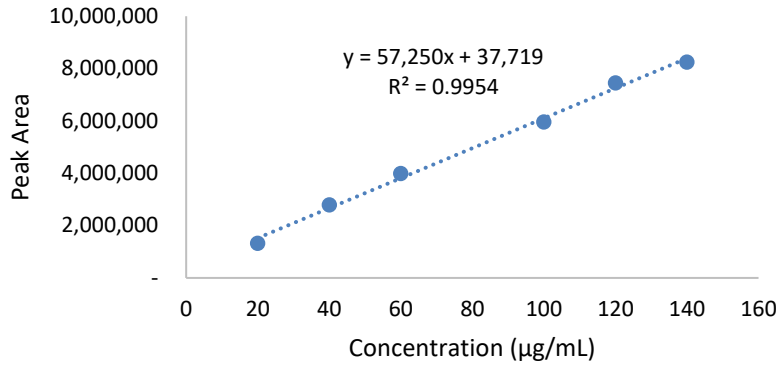

Figure 3: Calibration curve of the reference standard of andrographolide (reproduced from Sajeeb et al*., 2015).*

3. Result and discussion

3.1 Plant vegetative components

The results of this study are shown in Table 2 where the values constitute the average of 5 measurements of randomly selected plants per treatment with a respective confidence interval at a 95 % confidence level and a t-value of 2.776. The data were recorded during harvesting at maturity. The highest mean plant height was observed for T1 and T3 (32.51 cm) followed by T5 (31.75 cm). The result shows that the plants treated in mixed RHA and cocopeat as the growth media had slightly higher plant height compared to the 100 % cocopeat. This is because the plant growth is slightly superior in combined soilless media (SM) (T1, T3 and T5), rather than the 100% cocopeat medium (T2, T4 and T6). A study showed that substituting Rice Husk Ash with 50 % of recommended K could enhance maize growth and other growth parameters (Saranya et al., 2018). RHA was also found to increase the growth and yield of Cucumis sativus (Rusli et al., 2018) and tomatoes (Truong et al., 2017). The current findings vary from a similar study that investigated different media compositions of cocopeat and Empty Fruit Bunch compost for the cultivation of *Andrographis paniculata.* The result of the study showed that a medium combination according to a 7:3 ratio gave the best results (Shara et al., 2017). Noorhanin et al. (2013) reported that the highest *Andrographis paniculata* plant growth was observed when grown in 100 % cocopeat fed with inorganic fertiliser.

Similar observations were noted for the number of branches per plant in this study. There were no significant differences between T1, T2, T3, T4, and T5 with the maximum number of branches noted for T5 (23.40 per plant) and T6 showing the lowest number of branches (17). As reported earlier, the number of branches is slightly superior with the combined soilless media rather than the 100 % cocopeat. This result shows the potential of RHA in enhancing plant growth (Shara et al., 2017).

T3 gave a significant maximum leaf count per plant of 141 followed by T1 with 105.40. Other treatments showed no significant differences in the number of leaves, with T4 yielding the lowest (69). The leaves of *Andrographis paniculata* contain the highest percentage of andrographolide (4 %) compared to its other parts and contribute to the andrographolide yield per plant (Chao and Lin, 2010). This study showed that the increased plant growth in T3 was probably due to the perfect combination and balance between nutrient availability in the nutrient solution and the suitable pH of the combined medium (Shara et al., 2017).

Table 2: Plant vegetative attributes for the different treatments

Treatment	Plant height (cm)	Number of branches per plant	Number of leaves per plant	Dry Weight (g)
T1	32.51±2.39	19.60±3.12	105.40±55.99	2.4±0.68
T2	30.73±6.35	19.60±4.17	98.60±28.56	2.4±0.68
T3	32.51±3.96	22.00±3.51	141.00±28.90	2.2±3.51
T4	31.14±8.66	19.20±4.15	69.00±16.70	1.2±0.55
T5	31.75±10.10	23.40±6.43	93.00±41.22	1.6±0.68
T6	24.64±5.75	17.00±2.48	76.40±20.33	1.2±0.55

The dry weight (g) of the plant indicates its biomass production. Table 2 shows that the dry weight decreased as the magnesium content in the nutrient solution was increased. Another observed trend was that at high concentrations of magnesium, the combined RHA-cocopeat media yielded a significantly better dry weight than the 100 % cocopeat medium. The maximum dry weight obtained was 2.4 g for T1 and T2, followed by T3 with an insignificantly different dry weight of 2.2 g. This study proves that, with abundant magnesium or nutrient content, the biomass production will be lower, but the combined media gave better nutrient uptake efficiency (Shara et al., 2017).

3.2 Andrographolide content

Andrographolide is the major bioactive compound in *Andrographis paniculata (AP)*. Table 3 shows the estimation of andrographolide content for each treatment and control. Control 1 shows the estimation of andrographolide content for conventional cultivation (AP systems) and Control 2 shows the estimation of andrographolide content from AP (bought readily available from the market). Both Controls had 0.68 µg/mL of andrographolide concentration.

This study showed that the systems of fertigation incorporated with soilless media gave the highest andrographolide content for treatment T3 (0.7 µg/mL) and the lowest for T5 (0.67 µg/mL). Overall, there was no significant difference in the andrographolide content of all treatments and controls. This result contradicts the findings of Behera et al. (2014), who concluded that the integration of drip fertigation increased the herbage and oil yield of mint rather than the conventional foliar method. This result could be different because of the un-optimised nutrient availability in the past study, and the constant electrical conductivity (EC) of the nutrient solution in this study (1.6 mS/cm). Studies have shown that salinity (EC) could also affect the AP system (Rajpar et al., 2019).

Table 3: Estimation of andrographolide in each treatment.

Treatment	Peak Area of HPLC	Concentration of Andrographolide (µg/mL)
Control 1	1,208.39	0.68
Control 2	1,016.07	0.68
T1	1,106.84	0.68
T2	1,753.36	0.69
T3	2,145.10	0.70
T4	1,880.21	0.69
T5	878.18	0.67
T6	1,503.56	0.69

There was no significant trend in the effect of magnesium composition and medium composition on the andrographolide yield. Therefore, it can be concluded that magnesium and media compositions do not affect the bioactive compound of *Andrographis paniculata*. However, although the chemical fertiliser, NPK, affected herb yield, no significant difference in the andrographolide yield was observed, as compared to using no fertiliser treatments (Verma et al., 2015).

In the context of soilless medium composition, different soilless medium compositions were found to affect the bioactive compounds of dill and parsley leafy aromatic plants (Saleh et al., 2019). However, the same trend was not observed in this study because the composition of RHA in the total medium was kept constant. That is, the pH value between each treatment was also constant. Hence, the ratio of 30% RHA composition should be reconsidered in future studies.

Overall, the integrated fertigation system and soilless medium cultivation showed on-par quality and quantity of andrographolide as compared to both controls, which used conventional cultivation techniques. Therefore, this study established a Standard Operating Procedure (SOP) to integrate an unconventional cultivation technique for the commercial cultivation of *Andrographis paniculata*.

3.3 Standard of procedure

The Standard Operating Procedure (SOP) of T3 was decided as the best, as it gave better growth quality with a maximum plant height of 31.75 cm, the maximum number of leaves (141 total count), good dry biomass yield of 2.2 g, and better andrographolide content of 0.7 µg/mL. Hence, the fertigation technique incorporated with combined RHA-cocopeat medium and 50 ppm magnesium composition in the nutrient solution could be used to cultivate *Andrographis paniculata.*

4. Conclusions

This study developed a standard of procedure to cultivate *Andrographis paniculata* in soilless media integrated with the fertigation technique. The combined soilless media (RHA and cocopeat) showed more superior growth and dry yield compared to the 100 % cocopeat medium. Also, higher magnesium content decreased the dry yield of the plant. Furthermore, there was no significant difference in the andrographolide content of each treatment and the controls. The T3 treatment was chosen as the SOP, as it provided better growth quality with a maximum plant height of 31.75 cm, the maximum number of leaves (141 total count), a good dry herb yield of 2.2 g, and better andrographolide content of 0.7 µg/mL. Thus, the fertigation technique incorporated with combined RHA-Cocopeat and 50 ppm magnesium composition in the nutrient solution can be used to cultivate *Andrographis paniculata.*

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