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FORMULATION OF FORTIFIED MEDIA CONSISTING OF OIL PALM EMPTY FRUIT BUNCH BIOCOMPOST AND OIL PALM KERNEL SHELL BIOCHAR FOR THE ENHANCEMENT OF BIOACTIVE COMPOUNDS IN *Centella asiatica* (L.) URBAN

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ABSTRACT

Centella asiatica*, commonly known as Pegaga, is renowned in Malaysia for its abundant bioactive compound content, making it a highly valued herb. These bioactive compounds, including phenolics, antioxidants, and triterpenes, possess pharmacological activities that are beneficial for health. To optimise the properties of these bioactive compounds, Pegaga Kampung, a superior variety of* C. asiatica *in Malaysia, was cultivated in 14 different formulations of fortified media. The fortified media were formulated by incorporating various ratios of oil palm empty fruit bunch (OPEFB) biocompost, oil palm kernel shell (OPKS) biochar, organic fertiliser, inorganic fertiliser and soils. Our findings showed that fortified media with a high biocompost ratio of 25:75 (soil:biocompost) and enriched with inorganic fertiliser had a 33% enhanced total phenolic content (TPC). The combination of soil, biocompost and biochar at a ratio of 50:25:25, enriched with organic fertiliser, was found to increase the TPC by 30% and the antioxidant properties by 16% compared to cultivation in soil alone (control). This suggested that the use of fortified media containing OPEFB biocompost and OPKS biochar, along with organic or inorganic fertilisers, significantly improves the bioactive compound characteristics of* C. asiatica.

Keywords: bioactive compound, biochar Pegaga, biocompost, *Centella asiatica*.

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INTRODUCTION

Biocompost and biochar have been recognised for their beneficial effects on crop production, as they have been shown to improve the physical, chemical and biological properties of soils (Alfano *et al.*, 2009; Bastida *et al.*, 2010). For example, biocompost is commonly used as a fortification medium with soil. It works as the replenishment of nutrients in the soil and a nutrient source for plants. The amendment of soil with biocompost increases the water retention capacity (Zhou *et al.,* 2020). The application of biocompost in agriculture is gaining attention since chemical fertilisers and pesticides can significantly contribute to soil infertility

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(Jahangir *et al.,* 2021), environmental pollution (Singh & Prabha, 2017), and health hazards (Kalyabina *et al.,* 2021). Therefore, biocompost offers a sustainable and environmentally-friendly solution in the agricultural sector by reducing the reliance on chemical fertilisers and pesticides. It also provides an alternative approach to waste management, as it can be produced from waste materials, making it a greener option for improving soil health and crop productivity (Ozores-Hampton *et al.,* 1994). Hence, there is increasing demand worldwide for the application of biocompost from crop residues to croplands as a key countermeasure for enhancing soil carbon stock and reducing greenhouse gases emission from the agricultural sector (Jahangir *et al.,* 2021).

The ability of biochar to enhance the structure, porosity, particle size distribution and texture of soil is well known (Ding *et al.,* 2016). It has also been demonstrated to improve the chemistry of soil, including the pH, carbon content and cation exchange capacity (CEC) (Laghari *et al.,* 2016). The high surface area and porous nature of biochar contribute to its ability to retain water and nutrients (Li *et al.,* 2021; Wang *et al.,* 2019). Biochar has been recognised as a beneficial soil amendment that can enhance overall soil health and productivity. Research has shown that the application of biochar can increase the immobilisation of inorganic nitrogen and reduce ammonia volatilisation, indicating its potential for improving nitrogen management and minimising nitrogen losses in agricultural systems (Jindo *et al.,* 2020; Zhang & Guan, 2022). Although the impact of biochar on soil has been well documented, the knowledge of the soil environment due to the biochar implications is still in progress, especially on soil microbial communities and biogeochemical cycles. Research also showed that biochar helps in bioremediation (Patel *et al.,* 2022; Sharma *et al.,* 2020), carbon sequestration (Mona *et al.,* 2021) and nutrient adsorption (Gong *et al.,* 2019). Therefore, due to the beneficial effect of biocompost and biochar, this present study applied both biocompost and biochar produced from oil palm empty fruit bunch (OPEFB) and oil palm kernel shell (OPKS), respectively, to the soil media for the planting of *C. asiatica* and thus, find the optimum formulation that can enhance the production of the bioactive compound.

Due to the extensive utilisation of wild resources and limited industrial-scale cultivation, *C. asiatica* has been categorised as an endangered plant species. The overexploitation of wild populations and inadequate cultivation practices have raised concerns about the sustainability and conservation of this herb (Heidargholinezhad *et al.,* 2023). In addition, there is no inconclusive determination of the best variety of *C. asiatica* in Malaysia (Bakar *et al.,* 2022; Hazirah *et al.,* 2017). Exploiting the best variety of *C. asiatica* for its pharmacological active ingredients is also challenging. This is because different environmental conditions (abiotic or biotic stress) of *C. asiatica* influence the concentration of those ingredients in the plant (Hazirah *et al.,* 2017; Seong *et al.,* 2023). Finding the ideal planting conditions, which include the soil formulation, is crucial in the efforts to preserve the *C. asiatica* species as well as to make it possible to grow this herb commercially. Hence, it is necessary to devise systematic cultivation of *C. asiatica* to ensure the sustainability and promising quality of the production of its biological compounds. This is because the cultivation conditions will influence the concentration of the bioactive compounds of *C. asiatica*. For example, the geographical area, environmental growth conditions and genetic material have been reported to significantly affect the bioactive compounds production in these herbs (Kunjumon *et al.,* 2022).

Balancing plant growth rate and bioactive compound yield is a laborious process. This is because, plant growth depends mostly on the primary metabolic pathways, utilising polysaccharides, fats, proteins and sugars that make up the plant's bulk. However, the bioactive compounds that contribute to medicinal benefits are derived from the secondary metabolic pathway. Since they are not essential for the survival of the plant, these compounds are produced at a much lower concentration compared to primary metabolites. To increase these metabolites, plant cell culture was explored as one of the techniques developed to increase metabolite concentration produced by plants. On a large scale, plant cell culture was able to produce an exiguous amount of compounds that can meet commercial demands. Some other approaches are the formulation of planting medium, such as by using biocompost and biochar (Ashokkumar *et al.,* 2022; De Morais *et al.,* 2022). Therefore, this study is aimed to enhance the bioactive compounds in *C. asiatica* by applying the OPEFB biocompost in combination with OPKS biochar in the fortified media formulation to grow *C. asiatica*.

MATERIALS AND METHODS

Production of Biocompost

OPEFB biocompost was produced based on Baharuddin *et al.* (2009). This biocompost was produced using the pressed and shredded OPEFB collected from Hulu Langat Palm Oil Mill, Dengkil, Selangor, Malaysia and palm oil mill effluent (POME) sludge collected from Felda Serting Palm Oil Mill, Negeri Sembilan, Malaysia. The OPEFB were mixed with POME sludge at the ratio of 1:1 (w/v) using a compost turner (Backhus, Germany) to form a compost windrow. The composting process was carried out for 20 days and the maturing for 10 days. Matured compost was stored in bags or drums prior to the formulation of fortified media for the planting of *C. asiatica*. The compost nutrient was analysed for quality control monitoring using inductively coupled plasma (ICP) analysis (Hammid *et al.*, 2022).

Production of Biochar

OPKS biochar was produced at Malaysian Palm Oil Board (MPOB) based on the method developed by Zainal *et al.* (2017). The microwave-assisted pre-carbonisation system with a maximum temperature of 300°C was used to produce OPKS biochar. The three magnetrons were set to shut down once the temperature of the system reached 250°C. The resulting heat from the combustion of OPKS was used to sustain the carbonisation process. The particle size of the biochar ranged from 6 to 15 mm. The biochar produced was sundried before being fortified in the media formulation.

Cultivation of *C. asiatica*

The study was conducted under greenhouse conditions. The mother plants of *C. asiatica* (Pegaga Kampung) were obtained from a local nursery in Sungai Buloh, Selangor, Malaysia. Small planting pots with drainage holes were used to grow the plant samples. Each pot was planted with five seedlings. The soil mixture was composed of topsoil, peat and sand at a ratio of 2:2:3 (v/v). All types of soil were supplied by Taman Pertanian Universiti, UPM and prepared accordingly. The formulation of fortified media was prepared as shown in *Table 1*. The water was provided twice daily to maintain the field's capacity.

TABLE 1. FORMULATION OF FORTIFIED MEDIA

Treatments	Compositions
T1	Soil 100% (Control)
T2	Soil enriched with IF
T3	Soil enriched with OF
T4	Soil: Biocompost [50:50]
T5	Soil: Biocompost [50:50] with IF
T6	Soil: Biocompost [75:25] with IF
T7	Soil: Biocompost [25:75] with IF
T8	Soil: Biocompost [50:50] with OF
T9	Soil: Biocompost [75:25] with OF
T10	Soil: Biocompost [25:75] with OF
T11	Soil: Biochar [50:50]
T12	Soil: Biocompost: Biochar [50:25:25]
T13	Soil: Biocompost: Biochar [50:25:25] with IF
T14	Soil: Biocompost: Biochar [50:25:25] with OF

Note: OF - organic fertiliser; IF - inorganic fertiliser.

Harvesting of Plants

The matured *C. asiatica* was harvested after 90 days of planting. Four plant samples from each of the treatments were selected randomly. The samples were used to determine the plant morphology, growth measurements, bioactive compounds, total phenolic content (TPC) and antioxidant activity. The leaves, petioles and roots were also oven-dried at 60°C for 48 hr and the dry weight was determined (Zin & Osman 2002).

Plant Morphology and Growth Measurements

All the plant samples were separated accordingly based on the plant parts, *i.e.*, leaves, petioles and roots for the measurement of quantitative traits. The measurement was based on the method explained by Pandey and Singh (2011). The fresh samples were characterised according to their number of leaves, leaf width and petiole length as an indicator of the plant growth measurement.

Plant Sample Preparation and Extraction

To prepare the samples for analysis, they were first washed with tap water to remove dirt and contaminants. The plant samples were then dried in a convection oven at 45°C for 48 hr until they reached a constant weight (Zin & Osman 2002). The dried samples were ground using a blender and sealed in polyethylene bags to prevent moisture exposure. The bioactive compounds, antioxidants, and TPC were extracted from 10 g of each powdered plant sample using 100 mL of ethanol as a solvent in an incubator shaker at 25 ± 2°C for 24 hr. The filtrates obtained through filtration with Whatman No. 1 filter paper were concentrated using a rotary evaporator at a temperature of 40°C for approximately 30 min. The dried extracts were weighed, dissolved in ethanol to a concentration of 1 mg/mL and stored at 4°C until use.

Analytical Procedures

Bioactive compounds. The presence of asiaticoside, madecacosside and asiatic acid in the extracted sample was analysed using high-performance liquid chromatography (HPLC) with a DAD detector. A Phenomenex Gemini 5 m NX-C18 LC column 250.0 × 4.6 mm was used as the stationary phase with a mobile phase of water-acetonitrile (70:30). The separation was performed with a flow rate of 1 mL/min and 0.01 mL volume of samples injected into the column at 25°C. Standards were prepared by dissolving 1.0 mg samples of asiaticoside (Sigma-Aldrich, Germany), madecacosside (Sigma-Aldrich, Germany) and asiatic acid (Sigma-Aldrich, Germany) in 1.0 mL of ethanol and analysed at 220 nm wavelength. This method was adopted by Siddiqui *et al.* (2011).

Total phenolic content. The determination of TPC of *C. asiatica* plant extracts was carried out based on Singleton *et al.* (1999). The Folin-Ciocalteau reagent (0.5 mL) was added to 0.5 mL of plant extract. The distilled water was used to adjust the volume of mixture to 8.5 mL of working volume. The mixtures were subjected to incubation for 10 min at room temperature and subsequently followed by the addition of 1.5 mL of 20% sodium carbonate (Na_2CO_3). The tubes were then incubated at 755 nm using the UV-spectrophotometer. The blank was prepared using distilled water. Analyses were performed in triplicates. The gallic acid standard curve was plotted against concentrations 50, 100, 150, 250 and 500 mg/L and calculated for the gallic acid equivalent (GAE)/100 g of the extracted sample.

Antioxidant activity. The antioxidant activity of *C. asiatica* plant extracts was determined based on their ability to scavenge free radicals as measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Akowuah *et al.*, 2004). The assay was conducted by adding 2 mL of a 0.1 mM DPPH methanolic solution to test tubes containing 200 µL of the plant extracts. The well-mixed solution was incubated in the dark for 1 hr. A control sample was prepared by replacing the plant extracts with 200 µL of methanol. After 1 hr, the absorbance was measured at 517 nm using a UV-spectrophotometer. All analyses were performed in triplicate. The percentage of DPPH scavenging activity was calculated using the following Equation (1):

$$\begin{array}{c}\text{DPPH}\\ \text{scavenging}\\ \text{activity (\%)}\end{array} = \frac{\text{Abs control-Abs sample}}{\text{Abs control}} \times 100 \qquad (1)$$

Statistical analysis. All analyses were performed in triplicates and the data reported in this study are mean with standard deviation. The linear correlation coefficient analysis was calculated using MS Office Excel 2010. The significant difference of $p<0.05$ between each treatment was analysed using Tukey's test performed in SAS software.

RESULTS AND DISCUSSION

Effect of Different Formulations on the Growth of *C. asiatica*

Plant growth can be boosted by enhancing the physicochemical properties and nutrient content of the soil, which will be affecting the nutritional effects and secondary metabolite compounds of the plants. It has been recognised that the biocompost and biochar from plant organic matter act as a substitute for agrochemicals (Bernal-Vicente *et al.,* 2008). This study investigated the plant growth performance using 14 formulated fortified media. Results on the growth measurements of *C. asiatica* on different treatments are provided in *Table 2*. It can be observed that the addition of biocompost in the fortified media increased the width of the leaf as compared to soil only or soil with biochar only. The number of leaves showed that the highest treatment bearing a value of 32.75 ± 12.28 was seen in T2 followed by T14 with a value of 32.00 ± 5.41 whereas the lowest number of leaves were seen in T9 and T1 (control) with a value of 17.75 ± 1.50 and 18.75 ± 8.65 number of leaves, respectively. T2, which is soil with inorganic fertiliser is the planting medium applied by most nurseries in Malaysia. Therefore, T2 has been observed to exhibit better growth compared to other treatments. Among the treatments, T8 showed the highest petiole length, while T11 showed the lowest. It is possible that climatic differences or variations in plant response to the formulated fortified media may lead to conflicting outcomes. However, it is important to note that the statistical analysis of treatment effects, conducted with Tukey's test (HSD), showed that the number of leaves and petiole length were not statistically significant. This study also showed the effect of the formulated fortified medium on the leaves width. It can be observed that the T1-T2 generates the smallest leaves width between 3.88-3.58 cm. There is no significant difference for treatment T3-T14, except T7 which has the highest average leaves width of 4.48 ± 0.36 cm. The small variation of the leaf width among the treatment might be due to different amounts of nutrients being adsorbed by the plants, attributed to the similar factors discussed for number of leaves.

Effect of Different Formulations on the Total Phenolic Content of *C. asiatica*

Diverse environmental stresses influence a plant's TPC production, concentration and accumulation levels (Samaniego *et al.,* 2020). The results of TPC in *C. asiatica* on different treatments are shown in *Table 3*. It should be highlighted that when compared to T1, the treatments designated T2, T7, T8 and T14 exhibited an improvement in TPC in leaves (control). According to Duncan's Multiple Range tests, the TPC value in treatments T2, T7, T8 and T14 differs significantly from T1.

Several factors contribute to the enhancement or decrement of phenolic content. The concentration of different secondary plant products is heavily dependent on growing conditions and affects the metabolic pathways responsible for the accumulation of associated natural products

TABLE 2. GROWTH MEASUREMENTS OF *Centella asiatica* ON DIFFERENT TREATMENTS

Treatments	Compositions	No. of leaves	Leaf width (cm)	Petiole length (cm)
T1	Soil 100% (control)	18.75 ± 8.65^{a}	3.38 ± 0.23^{b}	5.58 ± 1.74^{a}
T2	Soil enriched with IF	32.75 ± 12.28^{a}	3.58 ± 0.34^{b}	8.95 ± 2.14^{a}
T3	Soil enriched with OF	21.50 ± 8.88^{a}	3.82 ± 0.23^{ab}	6.08 ± 1.81^{a}
T4	Soil: Biocompost [50:50]	21.30 ± 11.20^{a}	3.55 ± 0.34^{b}	6.83 ± 2.94^{a}
T5	Soil: Biocompost [50:50] with IF	25.50 ± 7.04^{a}	3.83 ± 0.30^{ab}	7.20 ± 1.85^{a}
T6	Soil: Biocompost [75:25] with IF	20.30 ± 5.90^{a}	3.55 ± 0.20^{ab}	6.78 ± 0.80^{a}
T7	Soil: Biocompost [25:75] with IF	23.80 ± 9.21^{a}	4.48 ± 0.36^{a}	7.23 ± 2.94^{a}
T8	Soil: Biocompost [50:50] with OF	25.50 ± 13.96^{a}	3.95 ± 0.42^{ab}	10.23 ± 2.97^{a}
T9	Soil: Biocompost [75:25] with OF	17.75 ± 1.50^{a}	4.00 ± 0.35^{ab}	8.18 ± 1.55^{a}
T10	Soil: Biocompost [25:75] with OF	26.30 ± 15.41^{a}	4.18 ± 0.45^{ab}	7.32 ± 2.16^{a}
T11	Soil: Biochar [50:50]	20.00 ± 12.02^{a}	3.70 ± 0.29^{ab}	5.23 ± 2.99^{a}
T12	Soil: Biocompost: Biochar [50:25:25]	24.00 ± 5.88^{a}	3.55 ± 0.47^{ab}	5.50 ± 1.04^{a}
T13	Soil: Biocompost: Biochar [50:25:25] with IF	28.50 ± 7.76^{a}	3.88 ± 0.25^{ab}	6.75 ± 1.72^{a}
T14	Soil: Biocompost: Biochar [50:25:25] with OF	32.00 ± 5.41^{a}	4.00 ± 0.78^{ab}	7.30 ± 2.22^{a}

Note: *Mean of growth measurements, values in row followed by the same letter indicates no significant difference of growth measurements at $p<0.05$ level by Tukey's tests. OF - organic fertiliser; IF - inorganic fertiliser.

(Akula & Ravishankar, 2011). In this study, different components and amounts of fortified media were used. The nutrients present in the fortified media could be one of the factors influencing the phenolic content of the samples. Further investigation is needed to elucidate the specific role of fortified media in the observed increase in phenolic concentration. When *C. asiatica* is being planted using the nursery's treatment (T2), it showed that this herb had a great TPC with a value of 12.44 g/mg. However, when the soil is fortified with biocompost at a ratio of 25:75 and then enriched with inorganic fertiliser biweekly, it shows a better result with a value of 13.06 g/mg. Since both treatments were enriched with inorganic fertiliser biweekly, the enhancement of phenolic levels could probably be due to the plant being supplied with adequate nutrients as the characteristics of the inorganic fertiliser itself. Inorganic fertiliser is known as a readily formed nutrient where it releases its nutrients rapidly once applied (Belay *et al.*, 2002).

On the other hand, the treatments which are enriched with organic fertilisers also showed a positive effect in the enhancement of TPC in *C. asiatica*. However, it requires other materials such as biocompost and biochar to be fortified with soil to achieve this significant difference. Treatment labelled T8, which is fortified with soil and biocompost with a ratio of 50:50 showed a better amount of TPC when compared with T1. This observation was also supported by a study conducted by Siddiqui *et al.* (2011) where the composition of 50% of compost tea and 50% of NPK had the highest value for plant growth and yield. T8 had a value of 12.13 mg/g of TPC. However, when biochar was added to the composition of fortified media with a ratio of (50:25:25) and supplemented with organic fertiliser, the TPC was recorded at 12.73 mg/g, which is higher than T2 and T8. This result is in agreement with Schulz *et al.* (2013), whereby, the beneficial effects of biochar and compost on plant growth might be because of the rising levels of total organic carbon and total nitrogen, both of which are essential for plant growth. Although the value of phenolic content in T14 is much lower compared to T7, it should be emphasised that these treatments were in the same group (a) when statistically analysed, with no significant difference between treatments labelled T2, T7, T8 and T14.

The usage of inorganic and organic fertilisers has been debated for many centuries in the agricultural world. Both have their benefits and circumstances. It has been documented that organic fertiliser had lower performance compared to inorganic fertiliser (Pan *et al.*, 2020). Meanwhile, organic fertilisers are a sustainable choice for nourishing plants, as they release their nutrients slowly, in response to the plant's specific needs and environmental conditions. This gradual nutrient release promotes a healthier growing environment by avoiding soil exhaustion, as noted by Oad *et al.* (2004). However, it is important to note that organic fertilisers may require more time and investment due to their lower growth performance, which can be a concern for farmers. Nevertheless, the long-term benefits of using organic fertilisers outweigh the initial challenges, as they contribute to sustainable agriculture practices and promote

TABLE 3. TOTAL PHENOLIC CONTENT OF *Centella asiatica* IN DIFFERENT TREATMENTS

Treatments	Compositions	Leaves (mg/g)	Petiole (mg/g)	Roots (mg/g)
T1	Soil 100% (control)	9.83 ± 0.20^{cd}	1.89 ± 0.07^{cd}	1.55 ± 0.03^{a}
T2	Soil enriched with IF	12.44 ± 0.47^{a}	1.89 ± 0.02^{cd}	1.39 ± 0.09^{ab}
T3	Soil enriched with OF	9.23 ± 0.25^{de}	1.07 ± 0.05^{ab}	0.99 ± 0.23^{c}
T4	Soil: Biocompost [50:50]	5.36 ± 0.11^{h}	1.57 ± 0.08^{h}	1.18 ± 0.08^{bc}
T5	Soil: Biocompost [50:50] with IF	4.50 ± 0.48^{h}	2.89 ± 0.21^{ef}	1.53 ± 0.19^{a}
T6	Soil: Biocompost [75:25] with IF	5.15 ± 0.30^{h}	1.76 ± 0.07^{a}	0.99 ± 0.03^{c}
T7	Soil: Biocompost [25:75] with IF	13.06 ± 0.15^{a}	2.12 ± 0.05^{de}	1.16 ± 0.02^{bc}
T8	Soil: Biocompost [50:50] with OF	12.13 ± 0.55^{a}	1.37 ± 0.08^{bc}	1.50 ± 0.10^{a}
T9	Soil: Biocompost [75:25] with OF	11.02 ± 0.42^{b}	1.71 ± 0.09^{de}	1.40 ± 0.07^{ab}
T10	Soil: Biocompost [25:75] with OF	8.02 ± 0.33^{f}	2.30 ± 0.16^{b}	1.06 ± 0.07^{c}
T11	Soil: Biochar [50:50]	6.71 ± 0.31^{g}	1.24 ± 0.10^{hg}	1.00 ± 0.02^{c}
T12	Soil: Biocompost: Biochar [50:25:25]	10.37 ± 0.26^{bc}	$1.36 \pm 0.07f^{g}$	1.20 ± 0.02^{bc}
T13	Soil: Biocompost: Biochar [50:25:25] with IF	8.59 ± 0.44^{bc}	2.18 ± 0.17^{fg}	1.00 ± 0.12^{bc}
T14	Soil: Biocompost: Biochar [50:25:25] with OF	12.73 ± 1.07^{a}	1.63 ± 0.06^{e}	1.20 ± 0.05^{bc}

Note: *Mean of growth measurements, values in row followed by the same letter indicates no significant difference of growth measurements at $p<0.05$ level by Tukey's tests. OF - organic fertiliser; IF - inorganic fertiliser.

soil health. Biochar, on the other hand, can play a vital role in enhancing the total plant count (TPC) due to its remarkable absorption and adsorption capabilities (Rawat *et al.*, 2019). This study found that the high concentration of phenolic compounds in T14 might be due to a greater uptake of nutrients from the fertiliser. Therefore, it can be concluded that the combination of this fortified media (T14) is beneficial since it aids in the release of nutrients when the plant most needs them, resulting in probable less leaching and nutrient waste. The potential of biochar to act as a soil conditioner and improve the chemical composition of soil could make it healthier and better suited for growing plants, herbs, or crops. Biochar is also known to absorb heavy metals from soils (Mustafa *et al.*, 2018). Different types and amounts of fertiliser had different effects on the TPC of *C. asiatica* which was also supported by other studies (Nguyen *et al.*, 2010).

Effect of Different Formulations on the Antioxidant Activity of *C. asiatica*

Enhancement of antioxidant activity was also determined based on several treatments formulated in this study. *C. asiatica* is one of the herbs that are well-known to have high antioxidant activity. Previous researchers have documented the great benefits of this herb, especially in its antioxidant activity since this herb can scavenge radical components thus providing consumers with a nourished and youthful appearance. Biologically, consuming foods containing high antioxidant activity could also help maintain health as the radicals, if not being scavenged could cause detrimental effects to the cells such as cancers. In this study, the results obtained from different formulations of fortified media showed different values in antioxidant activity as shown in *Table 4*.

When compared to the control (T1), the treatments T8 and T14 are among those that are able to increase the antioxidant activity in *C. asiatica*. The highest antioxidant activity was obtained from T14 with a value of 56.13% in its leaf extracts. This treatment was also in line to show an enhancement in the TPC of *C. asiatica*. This could probably be due to the phenolic compounds being a combination of various compounds (phenolic acid and alcohols, flavonoids, stilbenes, tocotrienols and tocopherols), which are a good source of antioxidants (Zheng *et al.*, 2011). Several studies reported that high TPC also resulted in high antioxidant activity (Cai *et al.*, 2004; Shan *et al.*, 2005; Wong *et al.*, 2006). The combination of soil, biocompost at 50:50 and soil, biocompost and biochar at (50:25:25) with the enrichment of organic compounds might be suitable for the enhancement of both antioxidant activity and TPC.

However, in comparison to other treatments, apart from T14 and T8, the control group (T1) exhibited a higher value (48%). This observation could potentially be attributed to the plant experiencing stress, which could have resulted in a contrasting effect. It is well noted that T1 is not being supplied with other nutrients besides the soil only, however, this treatment still had a high antioxidant activity value. Plant exposure to stress conditions, for example, stress from drought, salt or heavy metal could induce reactive oxygen species

TABLE 4. ANTIOXIDANT ACTIVITY OF *Centella asiatica* IN DIFFERENT TREATMENTS

Treatments	Compositions	Leaves (%)	Petiole (%)	Roots (%)
T1	Soil 100% (control)	48.39 ± 2.33^{ab}	5.13 ± 1.88^{bcd}	2.56 ± 1.10^{bc}
T2	Soil enriched with IF	38.30 ± 1.06^{cd}	6.85 ± 0.41^{abcd}	5.78 ± 0.58^{abc}
T3	Soil enriched with OF	31.47 ± 4.39^{d}	3.85 ± 3.25^{cd}	2.43 ± 1.12^{c}
T4	Soil: Biocompost [50:50]	8.80 ± 7.26^{e}	2.78 ± 4.29^{d}	5.80 ± 2.38^{abc}
T5	Soil: Biocompost [50:50] with IF	3.80 ± 3.11^{e}	2.75 ± 1.58^{d}	6.37 ± 1.19^{abc}
T6	Soil: Biocompost [75:25] with IF	4.17 ± 3.02^{e}	5.46 ± 1.02^{bcd}	7.76 ± 0.71^{abc}
T7	Soil: Biocompost [25:75] with IF	41.35 ± 0.01^{bc}	8.06 ± 0.04^{abcd}	5.54 ± 0.01^{abc}
T8	Soil: Biocompost [50:50] with OF	49.33 ± 0.03^{ab}	6.53 ± 0.01^{abcd}	6.18 ± 0.00^{abc}
T9	Soil: Biocompost [75:25] with OF	41.89 ± 0.02^{bc}	7.25 ± 0.01^{abcd}	8.62 ± 0.01^{a}
T10	Soil: Biocompost [25:75] with OF	43.15 ± 0.01^{bc}	9.48 ± 0.06^{ab}	6.77 ± 0.01^{abc}
T11	Soil: Biochar [50:50]	30.25 ± 0.01^{d}	7.92 ± 0.01^{abcd}	8.94 ± 0.04^{a}
T12	Soil: Biocompost: Biochar [50:25:25]	47.91 ± 0.01^{b}	8.83 ± 0.01^{abc}	8.70 ± 0.02^{a}
T13	Soil: Biocompost: Biochar [50:25:25] with IF	46.31 ± 0.01^{bc}	11.16 ± 0.00^{a}	7.82 ± 0.02^{ab}
T14	Soil: Biocompost: Biochar [50:25:25] with OF	56.13 ± 0.04^{a}	11.62 ± 0.01^{a}	10.76 ± 0.03^{a}

Note: *Mean of growth measurements, values in a row followed by the same letter indicates no significant difference of growth measurements at p<0.05 level by Tukey's tests. OF - organic fertiliser; IF - inorganic fertiliser.

(ROS) to mass-produce (Moradi & Ismail, 2007; Murata *et al.,* 2007; Takahashi & Murata, 2008). The mass-produce of this ROS could disrupt the stability of the cell membrane and thus accelerate protein deformation and nucleic acid damage resulting from the inhibition of plant growth (Choudhury *et al.,* 2013; 2016). This phenomenon can be reflected as shown in *Table 2*, where almost all T1's growth measurements are seen to be lower compared to other treatments. Plants connected by stolons or rhizomes are more prone to encountering heterogeneous availability of resources. This variability in resource availability can pose challenges for these plants in capturing essential resources, which may affect their growth and development (Klimesova & Bello, 2011; Ye *et al.,* 2013; You *et al.,* 2016). Therefore, treatments that are enriched with organic fertilisers showed enhancement in the value of the antioxidant activity. The usage of organic fertiliser is advisable as it provides a suitable and healthier growing environment, thus, giving benefits not only to the plant but also to the soil properties, including functionality, structure, porosity as well as water holding capacity (Assefa & Tadesse, 2019).

Effect of Different Formulations on the Triterpene Composition of *C. asiatica*

Madecacosside, asiaticoside and asiatic acid are secondary metabolites that fall under the category of triterpene compounds. These three triterpenoids serve as the main biomarkers of *C. asiatica*, contributing to the distinctive properties and uniqueness of this herb. Results on the occurrence of triterpenes compounds of *C. asiatica* on different treatments are tabulated in *Table 5*. It can be observed that madecacosside was only present in T1-T5 and T14, where the highest value was observed in T2. On the other hand, it was observed that the presence of asiaticoside compound, which is known for its potential effects on collagen synthesis, anti-wrinkle properties, brain tonic properties, and wound healing effects, was not detected in any of the treatments. The preliminary sample showed the highest presence of asiaticoside compound with a value of 1.30 mg/g, while the market sample had a lower value of 0.24 mg/g. On the other hand, asiatic acid was found to be highest in T10 as compared to other treatments.

Asiatic acid was enhanced in T10, with a value of 32.55 mg/g. This increase in asiatic acid production can be attributed to the synergistic effect of the combination of soil, biocompost and biochar enriched with either inorganic or organic fertilisers (Siddiqui *et al.,* 2011). However, this compound was observed only in treatments T6 to T13. It should be noted that asiaticoside is a derivative of asiatic acid, and asiatic acid is a derivative of asiaticoside due to the synthesis and hydrolysis processes involved in their formation. Therefore, although asiaticoside was not detected in all treatments, it is possible that asiatic acid, which is a precursor of asiaticoside, could be present. This suggests that the functions and benefits of asiaticoside in *C. asiatica* may still be available in the form of asiatic acid. Both asiatic acid and asiaticoside are known to have anti-aging, wound-healing and collagen synthesis properties.

TABLE 5. TRITERPENE COMPOUNDS OF *Centella asiatica* IN DIFFERENT TREATMENTS

Treatments	Compositions	Madecacosside (mg/g)	Asiatic acid (mg/g)
T1	Soil 100% (control)	0.8 ± 12.3^a	ND
T2	Soil enriched with IF	0.9 ± 8.9^a	ND
T3	Soil enriched with OF	0.9 ± 11.2^a	ND
T4	Soil: Biocompost [50:50]	0.8 ± 7.0^a	ND
T5	Soil: Biocompost [50:50] with IF	0.8 ± 5.9^a	ND
T6	Soil: Biocompost [75:25] with IF	ND	31.1 ± 2.9^a
T7	Soil: Biocompost [25:75] with IF	ND	29.0 ± 3.0^a
T8	Soil: Biocompost [50:50] with OF	ND	28.4 ± 1.6^a
T9	Soil: Biocompost [75:25] with OF	ND	28.0 ± 2.2^a
T10	Soil: Biocompost [25:75] with OF	ND	32.6 ± 3.0^a
T11	Soil: Biochar [50:50]	ND	26.4 ± 1.0^a
T12	Soil: Biocompost: Biochar [50:25:25]	ND	26.9 ± 1.7^a
T13	Soil: Biocompost: Biochar [50:25:25] with IF	ND	27.8 ± 1.7^a
T14	Soil: Biocompost: Biochar [50:25:25] with OF	0.8 ± 5.4^a	ND

Note: *Mean of growth measurements, values in row followed by the same letter indicates no significant difference of growth measurements at $p<0.05$ level by Tukey's tests. ND - Not detected; OF - organic fertiliser; IF – inorganic fertiliser.

However, it was reported that asiatic acid is less bioavailable due to the rapid metabolism measured (Meeran *et al.*, 2018; Rush *et al.*, 1993). This is because, derivatives of asiatic acid by the modifications at C-11 and C-28 positions are more potent and had higher bioavailability to exhibit its beneficial effect (Meeran *et al.*, 2018).

The variation in the values of triterpene compounds could be attributed to factors such as harvesting time and geographical location, which may vary across different months or seasons. These factors can influence the growth and development of the plant, as well as the accumulation of secondary metabolites, including triterpenes. Environmental conditions, such as temperature, humidity, sunlight and soil composition, can affect the synthesis and accumulation of triterpenes in plants (Meeran *et al.*, 2018). Studies conducted by Alqahtani *et al.* (2015) have suggested that *C. asiatica*, which originates in Australia, exhibits higher amounts of triterpenoids during the summer season, indicating an optimum time for harvesting. Similarly, research by Puttarak and Panichayupakaranant (2012) has documented that different provenance in Thailand and cultivation during different months also greatly influence the yield of bioactive compounds in *C. asiatica*. This highlights the potential impact of geographical location and harvesting time on the triterpenoid content of *C. asiatica*. For example, *C. asiatica* harvested in Thailand during March provided higher triterpenoids with a value of 37.2 mg/g dry powder whereas *C. asiatica* from Songkhla had the highest amount of triterpenes compounds (37.4 mg/g dry powder) when it was harvested in December. However, *C. asiatica* collected from Nakornsrithammarat and Ratchaburi (different provinces in Thailand) produces the lowest content of triterpene compounds across all harvesting periods.

CONCLUSION

A suitable and appropriate formulation of fortified media as a planting medium of *C. asiatica* determined is treatment T14. This treatment is a mixture of soil, biocompost and biochar with a ratio of 50:25:25 enriched with organic fertiliser. This treatment resulted in an enhancement in the TPC and antioxidant activity of *C. asiatica*. The formulated fortified media could be a suitable alternative to organic farming in the agriculture world as most of these materials are a mixture of recycled waste products of oil palm biomass, which is utilised as a value-added product. The best treatment for the specific chemical properties depends greatly on the needs of the plants and preferences in terms of cost and environmental impact. If one plans on producing a plant using organic materials without having to think of the cost, it is advisable to use the organic method such as treatment T14, which specifically focuses on using organic materials such as biocompost, biochar and organic fertilisers. However, if one is concerned about the cost and time and neglects the environmental pollution, it can be advisable to use treatment T2 and treatment T6, where treatment focuses on using inorganic fertiliser which may cause detrimental to the environment after a long period of usage. Treatment T6 on the other hand gives an option of organic and inorganic materials, which comprises biocompost enriched with inorganic fertiliser.

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