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Growth of *Caesalpinia sappan* L. by using Different Growing Media and Evaluation of Antioxidant Activity of Foliage

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Abstract-*Caesalpinia sappan* L. is a herb and dyeing plant which is found naturally in Thailand. Cultivation is an alternative way to conserve *C. sappan* in nature. Farmers in local areas are interested in planting *C. sappan* because of its agricultural by - products. This study investigated the effects on *C. sappan* L of rice husk, rice husk biochar, coconut coir dust and their combination as growing media on growth, TPC, TFC and antioxidant activity. The results showed that plant height, stem diameter, leaf length, leaf width, petiole length and leaf number increased from 1 to 8 months after transplanting to a field. All treatments produced greater plant height, stem diameter and leaf numbers after 8 months following transplanting, except for the coconut coir dust treatment (T4). In addition, there was a positive correlation between plant height and stem diameter. After three and a half years, stem diameter and the total flavonoid compound of *C. sappan* grown in rice husk (T2) were higher than control. At this period, there was no correlation between stem diameter and TPC, TFC or antioxidant activity. All the treatments of *C. sappan* produced diameters ranging from 49.2 to 69.9 mm. In conclusion, the growing media after composting had a greater effect on plant growth than non - composting. The identification of bioactive compounds and antioxidant activity from *C. sappan* for all treatments may play an important role in health benefits and for developing novel functional ingredients in animal feed.

Keywords: Antioxidant activity, rice husk, rice husk biochar, coconut coir dust, *Caesalpinia sappan*

1. Introduction

Rising trends in health care have resulted in an increasing demand for herbs. *Caesalpinia sappan* L. is known as sappanwood or faang in Thailand. *Caesalpinia sappan* is a small shrub tree which is used for traditional medicine in Thailand, India, Indonesia and the Philippines. *Caesalpinia sappan* is found in regions of India, Sri Lanka, Burma, Thailand, Malaysia and southern China (Zerrudo & Utomo, 2016). Heartwood is a main product of the *C. sappan* tree. It is used for both dyeing and traditional medicine. Its pharmacological properties are antibacterial, antiviral, anticoagulant, anti - inflammatory, antitumor and it can also be used as an immunostimulant (Badami *et al.*, 2004). *Caesalpinia sappan* tree produces phytochemicals in heartwood (Mathew *et al.*, 2007) which can be harvested after 7 - 8 years of growth or 6 - 9 m of height or 15 - 25 cm of stem diameter. It can be grown in hilly areas and in clayey soil with pH 5 - 7.5 and at a mean temperature 24 - 28 °C but it does not tolerate waterlogging (Zerrudo & Utomo, 2016). In Thailand, *C. sappan* tree is found in natural forest where some people harvest it. The economic cultivation of *C. sappan* can reduce this problem. The agricultural by - products of *C. sappan* grown commercially, such as rice husk, rice husk biochar, coconut coir dust, and sugarcane bagasse can be used for growing media. Rice husk can improve soil properties by decreasing soil bulk density and increasing organic carbon. Badar and Qureshi (2014) reported that the addition of rice husk to soil improved carbohydrate, crude protein, nitrogen and phosphorous content in sunflower plants. Thiyageshwari *et al.* (2018) found that composting of rice husk for 3 months was beneficial. Initially, pH was 7.1 but decreased to 6.1 during the compost process. At compost maturity, pH reached 8.6. After 3 months of composting, the total nitrogen, total phosphorus and total potassium increased from their initial values. Crop residues such as rice husk, wood material, nut shell and agricultural waste were burned at a low level of oxygen (pyrolysis process) to produce biochar. Rice husk biochar improved both chemical and physical soil properties (Abrishamkesh *et al.*, 2015). Biochar derived from rice husk affected plant growth by increasing stem size and leaf length of water spinach while biochar derived from wood increased root size and leaf width Milla *et al.* (2013). Coconut coir dust was used for moisture conservation and it improved organic matter in soil but had low nutritive value. However, coconut coir dust showed a high capacity of nutrient retention and biodegradable resistance (Arachchi and Somasiri, 1997). As economic cultivation of *C. sappan* takes many years, farmers will need additional income, for example, *C. sappan* leaves can be used as an animal feed additive. Widigdyo *et al.* (2017) indicated that flavonoid compound from heartwood extraction of *C. sappan* could decrease the amount of *Salmonella* in the intestine of quail. Harjit *et al.* (2016) reported antioxidant and anthelmintic properties of *C. sappan* leaves. The total phenol content of bitter gourd grown in rice husk ash (153.2 mg GAE/100 g leaf (dw) was highest followed by raw rice husk (150.3 mg GAE/100 g leaf (dw)) and control (135.5 mg GAE/100 g leaf (dw)) (Ratnayake *et al.*, 2018). Thus, the flavonoid and phenolic compound of *C. sappan* could be used for animal feed additive or supplementing it from a basal feed diet. The objective of this study is to encourage farmers in Thailand to cultivate

C. sappan tree commercially by using its agricultural by - products and to also obtain income during the planting period or apply as a supplement with a concentrated feed from its bioactive compounds and antioxidant activity in foliage in order for animals.

2. Materials and Methods

2.1 Field Experiment

The field experiment was located at Burapha University, Sa Kaeo campus in Sa Kaeo province, Thailand. The soil is classified as Kabinburi series which belongs to a soil group of isohyperthemic Typic Kandiustults Ultisol (USDA taxonomy) (Land Development Department, 2019). The soil texture was clay loam. The mean annual air temperature was 27.9 °C (Climatological Center, 2019).

2.2 Plant Materials and Experimental Design

The experiment was conducted in Randomized Complete Block Design (RCBD) with 3 replications. There were 8 treatments of growing media including: control (T1), rice husk (T2), rice husk biochar (T3), coconut coir dust (T4), 1:1 v/v of rice husk : rice husk biochar (T5), 1:1 v/v of rice husk : coconut coir dust (T6), 1:1 v/v of rice husk biochar : coconut coir dust (T7) and 1:1:1 v/v of rice husk : rice husk biochar : coconut coir dust (T8). One year seedlings of *C. sappan* (30 - 40 cm) were transplanted to a field with 1.5m of row spacing and 2m of plant spacing. There were 2 rows per plot with 5 plants per row. All growing media were added to planting pits (50 x 50 x 50 cm) according to the experimental design before transplanting and then added again at 4 months after transplanting. N - P - K fertilizer 15 - 15 - 15 was applied to 60 g/ plant before transplanting and 4 months after transplanting.

2.3 Data Collections

The growth characteristics of *C. sappan* were measured every month for 8 months after transplanting. Data included plant height, stem diameter, number of leaves, leaf width, leaf length and petiole length. The stem diameter was measured after three and a half years of age after being planted in May 2020 which was half the harvesting period of 7 years.

2.4 Foliage Analysis

The fresh foliage (leaves and branches) of *C. sappan* samples were randomly collected at three and a half years of age after being planted in May 2020 (Figure 1) in trials in a community forest at Burapha University, Watthana Nakhon district, Sa Kaeo campus. An analysis of the total phenolic content (TPC), total flavonoid compounds (TFC) were conducted in three replications. An evaluation of the antioxidant activity of the *C. sappan* foliage extracts, such as DPPH radical scavenging assay and ferric reducing antioxidant power assay (FRAP) including commercial standard (Trolox) were also performed in triplicate. The *C. sappan* foliage samples were dried by using a hot air oven at 60 °C for 48 h (Figure 2) and mesh screen for analysis of TPC, TFC, DPPH, FRAP and Trolox at the Laboratory Equipment Center of Mahasarakham University in Thailand.

Figure 1. Cultivation of *Caesalpinia sappan* in the field (A), inflorescence (B), flower (C), stem (D) and pod (E) at three and a half years of age

Figure 2. The characteristics of fresh (A) and dried (B) appearance of *Caesalpinia sappan* foliage

2.5 Extraction and Determination of Total Phenolic Content

A 5 g dried *C. sappan* was extracted by using 80% methanol (V/V) on a shaking incubator set at 37°C for 12 h. The extract was filtrated and concentrated by using a rotary evaporator. After being re - filtered, the supernatant mixture was re - extracted by many iterations of the sample under identical conditions and then decanted into an amber vial where the total phenolic content and total flavonoid compounds were measured.

2.6 Determination of Total Phenolic Content

TPC was determined using the Folin - Ciocalteu reagent as described by Kubola and Siriamornpun (2011) compared to the standard gallic acid. After the *C. sappan* extract reacted by being mixed with 10% of Folin - Ciocalteu reagent and 7.5% sodium carbonate (Na_2CO_3) solution, it was then incubated at room temperature for 90 min and the absorbance was measured at 725 nm using a spectrophotometer. The TPC of these extracts was calculated and expressed as gallic acid equivalents at a concentration of 1 to 1000 mg/L which is used as a standard. The quantitative results were expressed in grams of the dry weight sample (mg GAE/gDW) based on the gallic acid standard curve.

2.7 Determination of Total Flavonoid Compounds

Distilled water was mixed with 500 µl of the *C. sappan* extract followed by the addition of 5% $NaNO_2$ solution. A solution of 10% $AlCl_3.6H_2O$ was added and allowed to stand for another 5 min before 1 M NaOH was added and then mixed well by using a vortex. The absorbance was measured at 510 nm using a UV - vis spectrophotometer. The quantitative results were expressed in mg rutin equivalents in 1 g of dried sample (mg RE/g).

2.8 DPPH Free Radical Scavenging Assay

The scavenging activity of the *C. sappan* extracts was estimated by using 2, 2 - diphenyl - 1 - picrylhydrazyl (DPPH) free radicals which is a method adapted from Wanyo *et al.* (2016). The absorbance was measured spectrophotometrically at 517 nm. The free radical scavenging activity was calculated as: Radical scavenging effect (%) = $[(A_{DPPH} - A_{SAMPLE})/A_{DPPH})]$ x100 (A_{DPPH} = absorbance without extract; A_{SAMPLE} = absorbance with extract). A standard of Trolox was run ranging from

1 to 500 µg/mL. A standard curve was then produced by plotting the percentage of activity of Trolox against its concentrations. The results were expressed as mg Trolox equivalent antioxidant capacity in 1.0 g of dried sample (mg Trolox/g) as described by Chumroenphat *et al.* (2019).

2.9 Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant activity of the *C. sappan* extracts was determined from the FRAP assay which was adapted from Wanyo *et al.* (2016) and Kubola *et al.* (2011). The absorbance at 593 nm was measured, using the FRAP working solution as a blank. The antioxidant potential was determined from a standard curve plotted using the $FeSO_4 \cdot 7H_2O$ linear regression equation to determine the values of FRAP based on the FRAP assay. The concentration of Fe^{2+} - TPTZ (reducing capacity) was calculated by comparing the absorbance at 593 nm with the standard curve of the Fe (II) standard solutions ($FeSO_4 \cdot 7H_2O$ solution) at concentrations of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mM, respectively.

2.10 Data Analysis

All plant growth characteristics and chemical composition data were analyzed by using R statistics (R Core Team, 2019). The mean differences among the varieties were compared by using Duncan's new multiple range test (DMR).

3. Results and Discussion

Growth characteristics of *C. sappan* increased from 1 to 8 months. The different growth media showed significantly different effects on plant growth. Table 1 shows the plant height and stem diameter of *C. sappan.* Differences in plant height and stem diameter between treatments were found after the first month. While leaf characteristics showed differences among treatments at 5 to 8 months after transplanting (data not shown). The plant height showed significant differences between treatments at 1 ($p<0.05$), 7 ($p<0.001$) and 8 ($p<0.001$) months after transplanting. After 7 and 8 months, T1 (control) showed higher plant height than T4 (coconut coir dust) but it was not different from the other treatments. The stem diameter showed significant differences between treatments at 1 ($p<0.01$), 2 ($p<0.05$), 6 ($p<0.001$), 7 ($p<0.01$) and 8 ($p<0.001$) months after transplanting and at three and a half years of age. After the first two months, T2 (rice husk), T4 (coconut coir dust) and T8 (rice husk: rice husk biochar : coconut coir dust (1:1:1) treatments produced a greater stem diameter while T1 (control) showed the lowest value. After 7 to 8 months, the diameter of T4 (coconut coir dust) was low, compared to T1 (control). However, the diameter of T1 (control) was not different from that of the other treatments. T4 (coconut coir dust) had low plant height and a small diameter due to *C. sappan* not being able to tolerate waterlogging while coconut coir dust (T4) was able to conserve moisture (Mathew *et al.*, 2007). *C. sappan* tree showed a high value for the plant height and stem diameter of the control treatment. This indicated that *C. sappan* tree did not respond to the addition of growth media at 1 to 8 months after transplanting. After composting of the growth media at three and a half years of plant age, the stem diameter of *C. sappan* grown in rice husk

(69.9 mm) was higher than control (47.9 mm), coconut coir dust (49.7 mm) and rice husk: coconut coir dust (1:1) (49.2 mm). This was also described by Thiyageshwari *et al.* (2018) who reported that total nitrogen, total phosphorus and total potassium increased after the decomposition of raw rice husk which improved soil nutrition. Moreover, there was a significant positive correlation ($p<0.01$) between plant height and stem diameter. Similarly, Mathew *et al.* (2007) reported that the height of *C. sappan* increased from 1 to 3 years with increasing diameter. In this study, the effect of rice husk biochar on the plant characteristics of *C. sappan* was not different from that of control. According to Borchard *et al.* (2014), biochar did not affect maize plant biomass or grain yield.

Characteristics of *C. sappan* leaves from 1 to 8 months after transplanting demonstrated in Figure 3. Leaf number increased corresponding to plant age in all treatments. Leaf width and leaf length increased from 1 to 6 months and were constant. The leaf length showed significant differences between treatments at 5 to 8 months after transplanting. The leaf lengths of T1, T5, T6, T7 and T8 were longest at these periods. The average leaf length at 8 months after transplanting was 48.8 cm. At 8 months after transplanting, T5 (rice husk: rice husk biochar (1:1)) had a greater leaf width than T1 and T6 (rice husk: coconut coir dust (1:1)) but it was not different from other treatments. The leaf number showed significant differences between treatments at 4 ($p<0.01$), 5 ($p<0.01$) and 6 ($p<0.05$) months after transplanting. In contrast, Ratnayake *et al.* (2018) showed that bitter gourd grown in raw rice husk and rice husk ash had higher leaf numbers than control. Milla *et al.* (2013) also reported that leaf number, leaf width, and leaf length of water spinach grown in rice husk biochar were higher than control. The petiole length showed significant differences between treatments at 4 ($p<0.05$) and 7 ($p<0.01$) months after transplanting. Moreover, there was a significant positive correlation ($p<0.01$) between stem diameter and leaf width.

Table 1. Plant height and stem diameter (mean±SD) of *Caesalpinia sappan* with different growth media at 1 to 8 months after transplanting.

Treatment	Month							
	1	2	3	4	5	6	7	8
	Plant height (cm)							
T1	28.0^{c}±8.0	31.4±8.5	51.5±17.7	77.9±30.7	116.7±38.0	149.2±38.0	194.1^{a}±38.7	238.5^{a}±42.6
T2	31.3^{abc}±8.8	33.8±8.9	48.7±8.0	67.7±17.0	103.5±40.2	129.2±49.3	162.8^{ab}±61.2	200.4^{ab}±62.9
T3	33.7^{a}±9.3	35.9±9.8	45.9±9.8	59.3±16.9	105.0±29.9	156.9±144.1	175.9^{ab}±40.6	215.0^{ab}±42.5
T4	28.0^{c}±6.8	30.5±8.6	47.6±12.9	64.9±19.1	96.2±32.3	119.7±34.7	148.2^{b}±40.6	187.8^{b}±47.5
T5	31.5^{abc}±7.8	33.7±8.1	49.4±9.9	69.7±23.0	109.1±35.9	139.0±40.1	176.9^{ab}±41.0	218.8^{ab}±44.8
T6	27.7^{c}±6.5	31.6±9.6	47.7±12.1	69.0±21.1	108.8±34.3	139.5±45.2	181.9^{ab}±36.5	218.5^{ab}±33.7
T7	32.7^{ab}±10.1	35.8±11.6	47.4±10.8	66.9±14.0	105.1±23.4	136.1±28.2	185.6^{ab}±33.6	229.0^{ab}±35.9
T8	28.2^{bc}±8.2	30.3±8.6	46.6±8.6	68.1±21.2	97.7±31.6	131.1±40.9	164.9^{ab}±45.1	209.8^{ab}±50.9
p - value	*	ns	ns	ns	ns	ns	***	***

Table 1. Plant height and stem diameter (mean±SD) of *Caesalpinia sappan* with different growth media at 1 to 8 months after transplanting. (cont.)

Treatment	Month							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
mean	30.1	32.9	48.1	67.9	105.3	137.6	173.8	214.7
	Stem diameter (mm)							
T1	6.5^{b}±1.5	6.9^{b}±1.8	10.0±2.3	13.3±2.9	17.7±4.5	21.8^{ab}±4.7	27.0^{a}±7.1	33.9^{a}±7.4
T2	7.5^{a}±1.1	8.9^{a}±6.0	10.5±1.7	12.5±2.0	16.6±5.0	19.0^{ab}±4.7	23.3^{ab}±6.4	27.8^{ab}±8.2
T3	7.0^{ab}±1.0	7.3^{b}±1.2	10.1±1.2	12.5±1.7	16.6±2.9	19.1^{ab}±3.0	23.6^{ab}±4.2	29.6^{ab}±6.5
T4	7.5^{a}±0.8	7.7^{ab}±1.2	10.3±1.7	12.3±1.9	15.6±3.1	17.8^{b}±3.2	21.5^{b}±3.9	26.6^{b}±4.6
T5	7.1^{ab}±1.4	7.2^{b}±1.3	10.2±1.5	12.7±2.2	16.3±3.2	20.4^{ab}±4.8	25.2^{ab}±7.6	30.4^{ab}±9.5
T6	6.9^{ab}±0.9	7.0^{b}±1.2	9.8±1.6	12.5±2.1	16.7±3.2	20.9^{ab}±4.0	26.2^{ab}±5.3	32.1^{ab}±7.0
T7	7.0^{ab}±1.2	7.0^{b}±1.3	10.1±1.5	13.4±2.6	17.9±3.9	22.8^{a}±5.4	27.1^{a}±5.6	32.4^{ab}±6.1
T8	7.7^{a}±1.1	7.9^{ab}±1.4	10.0±1.9	12.0±2.6	16.5±4.4	20.4^{ab}±5.5	24.8^{ab}±6.6	29.4^{ab}±7.2
p - value	**	*	ns	ns	ns	***	**	***
mean	7.1	7.5	10.1	12.6	16.7	20.3	24.8	30.3

T1 = Control, T2 = rice husk, T3 = rice husk biochar, T4 = coconut coir dust, T5 = rice husk : rice husk biochar (1:1), T6 = rice husk : coconut coir dust (1:1), T7 = rice husk biochar : coconut coir dust (1:1), T8 = rice husk : rice husk biochar : coconut coir dust (1:1:1), * significance at $p<0.05$, ** significance at $p<0.01$, *** significance at $p<0.001$. Different letters in the same column represent significant differences

Table 2. Correlation coefficient among diameter, total phenolic content, total flavonoid compounds, DPPH free radical scavenging assay, ferric reducing antioxidant power (FRAP) assay and Trolox of *C. sappan* foliage.

Item	Diameter	TPC	TFC	FRAP	DPPH	Trolox
Diameter	1					
TPC	- 0.07	1				
TFC	0.27	- 0.63	1			
FRAP	- 0.63	- 0.41	0.45	1		
DPPH	- 0.60	- 0.44	0.52	0.99	1	
Trolox	- 0.60	- 0.44	0.52	0.99	1	1

TPC = total phenolic content, TFC = total flavonoid compounds.

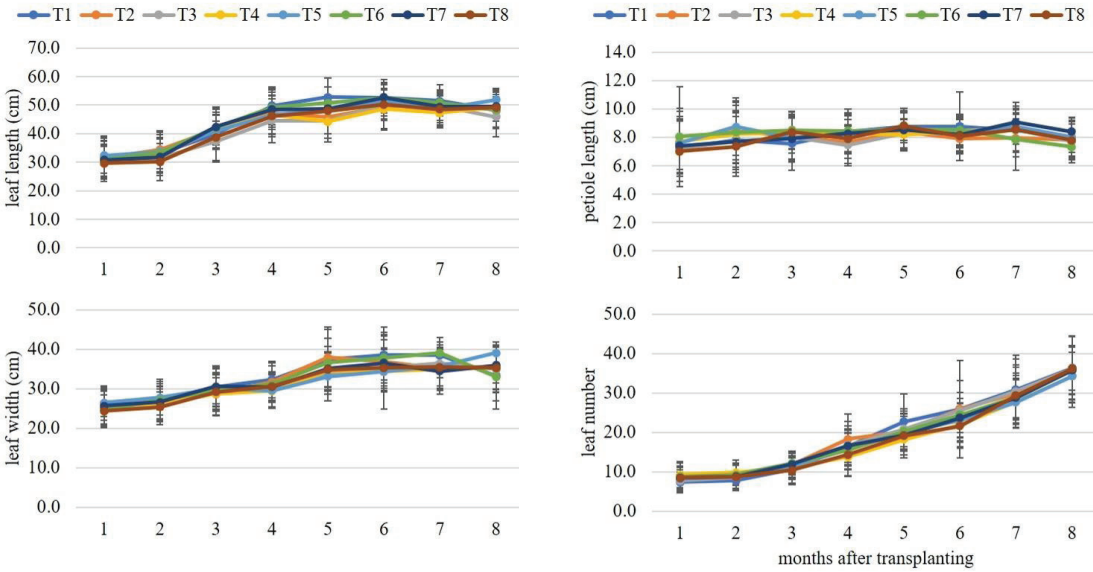

Figure 3. Leaf characteristics of *C. sappan* with different growth media at 1 to 8 months after transplanting (SD bar)

Table 3. Effects of the different growing media on *C. sappan* diameter, total phenolic content, total flavonoid compounds, DPPH free radical scavenging assay, ferric reducing antioxidant power (FRAP) assay and Trolox (mean±SD).

Treatment	Diameter	TPC	TFC	FRAP	DPPH	Trolox
T1	47.9^{b}±9.3	26.7^{ef}±0.6	49.7^{b}±1.7	63.0^{a}±1.5	63.5^{a}±2.3	21.1^{a}±0.8
T2	69.9^{a}±8.1	26.8^{ef}±0.9	59.7^{a}±0.4	43.6^{d}±1.7	46.5^{c}±1.1	15.4^{c}±0.4
T3	59.5^{ab}±7.2	31.3^{bc}±1.0	29.2^{f}±1.9	33.8^{e}±2.0	37.0^{d}±1.6	12.1^{d}±0.5
T4	49.7^{b}±4.7	33.6^{ab}±0.7	39.6^{d}±1.5	52.1^{c}±2.3	52.9^{b}±3.0	17.5^{b}±1.0
T5	54.8^{ab}±9.1	28.8^{de}±1.9	47.5^{b}±1.3	64.2^{a}±1.2	61.7^{a}±2.5	20.5^{a}±0.9
T6	49.2^{b}±13.2	25.3^{f}±0.3	43.4^{c}±1.4	57.3^{b}±2.1	55.5^{b}±2.2	18.4^{b}±0.7
T7	58.5^{ab}±11.3	29.2^{cd}±0.4	35.3^{e}±0.6	46.9^{d}±1.1	46.7^{c}±1.9	15.4^{c}±0.6
T8	54.4^{ab}±12.4	34.2^{a}±1.7	35.4^{e}±0.6	46.5^{d}±1.5	46.1^{c}±1.2	15.2^{c}±0.4
p - value	*	***	***	***	***	***
mean	55.5	29.5	42.5	50.9	51.3	17.0

T1 = Control, T2 = rice husk, T3 = rice husk biochar, T4 = coconut coir dust, T5 = rice husk : rice husk biochar (1:1), T6 = rice husk : coconut coir dust (1:1), T7 = rice husk biochar : coconut coir dust (1:1), T8 = rice husk : rice husk biochar : coconut coir dust (1:1:1), * significance at $p<0.05$, *** significance at $p<0.001$. Different letters in the same row represent significant differences, TPC = total phenolic content (TPC was determined in comparison with standard gallic acid and the results expressed in terms of mg GAE/g), TFC = total flavonoid compounds (TFC was determined in comparison with standard rutin and the results expressed in terms of mg RE/g)

The stem diameter of *C. sappan* planted in different growing media had no correlation to TPC, TFC or antioxidant activity (Table 2). However, the diameter of the stem had a close relationship with the height of the stem. The results show that the height and size of the stem diameter did not affect the value of TPC, TFC or free radicals in *C. sappan* foliage. The use of different growing media for *C. sappan* resulted in statistically significant differences in the diameter, TPC, TFC and antioxidant activity. To our knowledge, the bioactive compounds and antioxidant activity of *C. sappan* at three and a half years of age which was half the harvesting period of 7 years. are reported for the first time in this paper for publication.

The average values of diameter, TPC, TFC and antioxidant activities of *C. sappan* are shown in Table 3. There were significant differences in diameter, TPC, TFC and antioxidant activities between treatments. Using rice husk (T2) as a *C. sappan* growing media resulted in the largest stem diameter, which was different from the control treatments (T1), T4 (coconut coir dust) and T6 (rice husk : coconut coir dust (1:1)), but not different from the other treatments. Badar and Qureshi (2014) showed that adding rice husk into soil improved growth, total chlorophyll, carbohydrate, crude protein, nitrogen and phosphorus content in sunflower plants. This was due to rice husk increasing soil organic matter and fertility, especially after composting. Muscolo *et al.* (2019) reported that plant bioactive compounds (total antioxidant capacity, total chlorophyll, carotenoids and ascorbic acid) were higher in leaves of *Brassica rupestris* grown in soil with high nitrogen, C/N ratio and organic matter than those grown in soil with low nitrogen, C/N ratio and organic matter. On the other hand, *B. rupestris* grown in soil with low nitrogen, C/N ratio and organic matter had a high amount of total phenols. Soil organic matter had a positive correlation with microbial biomass carbon and total antioxidant capacity. This shows that microorganisms were involved in soil organic matter and soil fertility.

The contents of TPC and TFC of *C. sappan* planted in different materials were in the range of 25.3 to 34.2 mg GAE/g DW, 29.2 to 59.7 mg RE/g DW, respectively. These are well known safe natural compounds of a medicinal plant of the Leguminosae family in Ayurveda with a long - established record for the development of novel ingredients, whether for human food or animal feed. Although the heartwood of *C. sappan* is often used for medicinal purposes rather than other parts of this plant due to its contents of water soluble flavonoids, namely, brazilin, protosappanin and haematoxylin of which Brazilin is one of the most important bioactive natural compounds, Badami *et al.* (2004) reported that the *C. sappan* leaves are used as an ingredient of Jamu which is a traditional medicine from Indonesia. A previous study by Harjit *et al.* (2016) found that all the TPC of *C. sappan* leaves from various extracts, such as dichloromethane, ethylacetate and methanol of tannic acid equivalents per mg of dried extract (TAE µg/mg extract) had 10.09, 6.32 and 14.05, respectively, which are lower than that obtained in our research. In this study, the TFC of *C. sappan* extracts was also higher than the various extracts of *C. sappan* leaves, such as petroleum ether, dichloromethane, ethylacetate and methanol (Harjit *et al.*, 2016). The TPC in the legumes varies depending on the types of legumes or growth

period, such as ungerminated legumes, germinated legumes or beans that have been fermented or processed through dry heat in accordance with the procedure of Dajanta and Rongkom (2017).

The *C. sappan* with the control treatment (T1) and T5 (rice husk: rice husk biochar (1:1)) provided the highest FRAP, DPPH and Trolox as shown in Table 3. Previous research showed that application of rice husk biochar to soil increased stem size and leaf length of water spinach (Milla *et al.*, 2013). The addition of rice husk biochar increased soil pH, EC, calcium, silica, magnesium and some trace elements (Fe, Al, Na, Mn, Zn) while the addition of fresh rice husk increased potassium (Milla *et al.*, 2013). Therefore, growing media treatment of rice husk and rice husk biochar (T5) might provide more nutrients to *C. sappan* leaves than other treatments. However, this is the first report to find that the lack of growing media for *C. sappan* (the control treatment) leads to a high increase in antioxidant activities, especially in DPPH and Trolox which had the highest antioxidant activities. Harjit *et al.* (2016) evaluated the antioxidant activity of *C. sappan* leaves which were procured from forest (Dhanlakshami Agro Plantations and Consultancy, Tamilnadu). This research assumed that *C. sappan* is a forest plant that possibly does not adjust to planting and does not respond to the growing media.

4. Conclusion

At 1 to 8 months after transplanting, all the growing media, except for coconut coir dust, used for the growth of *C. Sappan* resulted in the same plant height, stem diameter and leaf characteristics as the control treatment. After three and a half years the stem diameter and TFC of *C. sappan* grown in rice husk were higher than control. This demonstrates that growing media after composting is more effective than after non - composting. The TPC, TFC of *C. sappan* after being planted for three and a half years at Burapha University, Sa Kaeo campus, indicated that *C. sappan* showed it can be a source of antioxidants. The anti - inflammatory activity of *C. sappan* crude extract and other compounds from its different edible parts will be further studied.

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6. References

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