Effect of Chengal Wood (*Neobalanocarpus heimii*) on Physicochemical and Antimicrobial Properties of Coconut Sap (*Cocos nucifera*)

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Effect of Chengal Wood (*Neobalanocarpus heimii*) on Physicochemical and Antimicrobial Properties of Coconut Sap (*Cocos nucifera*)

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Abstract. Coconut sap from *Cocos nucifera* could be obtained from the coconut flower, and it is quite a popular fresh drink in Malaysia. Due to microbial activity, coconut sap may change to alcoholic and acidic by spontaneous fermentation. Traditionally, chengal wood chips (*Neobalanocarpus heimii*) have been added in coconut sap to slow down the fermentation process. This research aims to evaluate the physicochemical properties (pH, total acidity, total soluble solids, colour), volatile organic compounds (VOCs) and antimicrobial properties of coconut sap with and without the presence of chengal wood chips for ten weeks at 4 °C and 25 °C storage temperatures. The pH value of both samples was between 3.60 ± 0.01 to 6.66 ± 0.04. Total acidity ranged from 0.29 ± 0.11% to 4.26 ± 0.25%. Total soluble solids in both samples were around 8.3 ± 0.05 to 17.3 ± 0.05 °Brix. Colour analysis was also carried out based on the lightness value. Eighteen of VOCs were found in coconut sap with chengal wood chips versus 20 compounds without the chengal wood chips. Coconut sap with chengal wood chips kept at 4 °C had the lowest total plate count (1.19 x 104 - 1.78x106 CFU/mL) at week one until week seven, while total plate count of coconut sap without chengal wood chips was recorded as too numerous to count (TNTC) throughout the weeks. Chengal wood chip extracts inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans,* and *Aspergillus brasiliensis*, as shown by a clear zone of inhibition. In conclusion, the presence of chengal wood in coconut sap has helped to slow down the fermentation process and inhibit the growth of microorganisms. Hence it can help to prolong the shelf life of coconut sap.

1. Introduction

Coconut sap or 'nira' is a sweet natural traditional beverage obtained from the flower of the coconut tree (*Cocos nucifera*), which is a well-known member of the palm family *Arecaceae* [1]. This coconut sap is collected from cutting the unopened flower of coconut trees and which contains sugar (sucrose, glucose, and fructose) as well as other nutrients (minerals, vitamins, antioxidants, and other metabolites). Coconut sap is a traditional beverage that has been consumed by many peoples in Asian countries, including Malaysia. The drink is known as 'air tuak' in Kelantan, Terengganu, and Pahang. In

Indonesia, this sap is known as *neera* and in other countries, fresh coconut sap has its name such as *toddy* (alcoholic drink) in Sri Lanka, *maprau* in Thailand, and *tori* in India [2].

From the public point of view, coconut sap has many nutritional properties and medicinal uses. One of the benefits of this traditional beverage is to overcome constipation. The main cause of constipation is the lack of fibre in the daily food intake required by the body [3].

In the coconut sap, there are vitamins, including vitamin A, amino acids, fibre, and protein. Coconut sap contains properties that can enhance energy and refresh the body during the day. Besides that, the benefits of these beverages are to strengthen the muscles, the body's cells, help with eye care, and promote digestion, hence, increase the demand for this traditional beverages' product. Over-fermentation of this beverage may cause diarrhoea, hernia, and headache. Thus, it is crucial to control and slow down the development of the fermentation process. Previous studies have indicated that the researchers use chemical preservatives during sap tapping. As an example, the use of sodium metabisulphite or limestone in coconut sap. The effect of different preservatives is used to give a different flavour, texture, and colour of the coconut [4]. In Malaysia, coconut farmers added the chengal wood chips as natural preservatives during the harvesting of coconut sap. The previous study reported that chengal wood (*N. hemii)* have interesting biological activities due to the presence of several bioactive compounds, including phenolics and oligostilbenes [5], which have capabilities as antioxidants and antimicrobial agents. Hence, this study was performed to evaluate the effect of chengal wood chips as a natural antimicrobial agent to control or slow down the fermentation process to prolong the shelf life of coconut sap.

2. Materials and Methods

2.1 Sample Collection and Laboratory Preparation of Coconut Sap

Coconut sap was collected by tapping the unopened inflorescence of the coconut trees at Salor, Pasir Mas, Kelantan. The tapping process has occurred during the daytime in July. The coconut sap was divided into two parts, namely coconut sap with and without the presence of chengal wood chips. Chengal wood was obtained from the coconut farmer and washed before being cut into small pieces and left until to dry under the sun (approximately two days). The sap was collected in the morning for the first collection and subsequently collected in the evening to avoid spontaneous fermentation. The end cut of the inflorescence had been tied with a plastic bottle.

The sterilised media bottle (100 mL) was used to store the coconut sap. The coconut sap with chengal wood chips had been divided into 100 mL sterilised media bottles with 100 mL filled into each bottle. The coconut sap with chengal wood chips was divided into two conditions and labelled with appropriate labelling, which involved 10 bottles for 4 °C and 25 °C, respectively. The media bottles were also marked with the week zero until week ten. The same procedures were used to store coconut sap without chengal wood chips. The samples were kept according to the selected conditions until further use.

2.2 Collection and Extraction Technique of chengal wood chips (Neobalanocarpus heimii)

The chengal wood chips had been used as preservatives in coconut sap throughout this study. The chengal wood was collected from coconut farmer at Salor, Pasir Mas, Kelantan, Malaysia. The chengal wood had been cut into a small piece of 40 mm x 25 mm 3 mm (height x width x thick) approximately. Ultrasonic extraction has been used for both ethanol and methanol solvents following the previous method [6] with some modifications. Media bottles containing 50 g of chengal wood chips and 500 mL of the solvents (ethanol and methanol) were extracted for 30 minutes at 60 °C. The filtration of the extract was proceeded using filter paper (Whatman no 4). After that, the resultant extract was concentrated in a rotary evaporator at a temperature of 40 °C. The percentage yield of crude was calculated as following [7]:

Percentage of yield (%) = [(Weight of extract) / (Weight of dried sample)] x 100

2.3 Physicochemical Analysis of Coconut Sap

2.3.1 pH Analysis.

The pH value of the coconut sap was measured using a digital pH meter following the previous method [10]. Before starting the analysis, the pH electrode was calibrated using a buffer solution at pH 7. The result of pH value was expressed as number range, which is acidic (0-6), neutral (7) and alkaline (8-14). All of the analysis was examined in triplicates (n=3). The average of the sample was calculated as the mean ± standard deviation.

2.3.2 Titratable Acidity.

The acidity of coconut sap was determined using titrable acidity test following the previous method [8]. The coconut sap (10 mL) was weighed into a beaker. Then, two drops of phenolphthalein indicator were added to each sample and titrated against 0.1N of sodium hydroxide (NaOH). The result shows changes in pink colour which indicated the endpoint of the titration. The determination of titratable acidity was expressed as the percentage (%) of lactic acid. All of the analysis was examined in triplicate (n=3). The average of the sample was calculated as the mean ± standard deviation. The following formula was expressed as below:

$$\% \text{ Total acidity} = (V1 \times N \times \text{Eq wt.} \times 100)/ (V2 \times 1000)$$

Where,
V1 is the volume of titrant used (mL)
N is the normality of titrant, NaOH (mEq/mL)
Eq. wt. is the equivalent weight of predominant acid (mg/mEq), Lactic acid is 90
V2 is the volume of sample used (mL)
1000 is a factor relating mg to grams (mg/g)

2.3.3 Total Soluble Solid.

The total soluble content of coconut sap was analysed according to the AOAC guideline [9]. It is used to determine the sugar content of coconut sap. The prism of the refractometer was cleaned up before used. The result was expressed as °Brix. All of the analysis was examined in triplicates (n=3). The average of the sample was calculated as the mean ± standard deviation.

2.3.4 Colour Analysis.

The colour analysis was measured using a Konica Minolta Colour Reader CR 10 (Konica Minolta Optics, Inc., Japan). The colour measurement was observed using L*a*b* colour space with L* representing the lightness of colour, +a* is red while -a* is green, and +b* is yellow whereas -b* is blue. All of the analysis was taken in triplicate (n=3). The average of the sample was calculated as the mean ± standard deviation [10].

2.4 Volatile Organic Compounds

Volatile organic compounds (VOCs) were analysed using the previous method [11] with slight modification using GC-MS (Agilent Technologies, Diegem, Belgium) at Universiti Malaysia Kelantan. Coconut sap (50 mL) was gently shaken (10 min) with 1:1 (v/v) mixture of diethyl ether: n-pentane (20 mL) in a separating funnel and the aqueous phase was discarded. Then, the organic phase was collected and mixed with 1.0 g of anhydrous sodium sulphate and centrifuged at 9500 rpm for 20 min at 4 ºC. Gaseous nitrogen was used to evaporate the solvent and reduce the volume to 0.5 mL. The concentrated extract was kept under N_2 gas and stored at -20 °C in a vial (2.0 mL) until further analysis. The GC injector was set to the split mode at a carrier gas (He) with a flow rate of 1.0 mL min^{-1}. The oven temperature was programmed at 50 ºC with a final temperature of 260 ºC after 70 min. VOCs and non-VOCs were identified by comparing the peak with the library GCMS software.

2.5 Microbiological Analysis

2.5.1 Determination of Total Plate Count.

Coconut sap with chengal wood chips at 4 °C (1 mL) was transferred into 9 mL of sterile distilled water (SDW) (10^{-1}) from which, 1 mL was transferred to 9 mL of SDW (10^{-2}) and was then transferred to 9 mL of SDW (10^{-3}) and repeated with coconut sap with chengal wood chips at 25 °C, without chengal wood chips at 4 °C and 25 °C [12].

The coconut sap with chengal (100 μL) at 4 °C was pipetted from 10^{-1}, 10^{-2}, and 10^{-3} dilutions into separate, triplicate marked Petri dishes which contain De Man, Rogosa and Sharpe (MRS) Agar and Malt Extract Agar (MEA) Base. The lid of the Petri dish was half-opened to let the glass spreader in and moving the Petri dish while moving the spreader from top to bottom or side to side to spread the inoculum over the surface of the agar. The process was repeated with serial dilutions of coconut sap with chengal wood chips at 25 °C, without chengal wood chips at 4 °C and 25 °C. The Petri dishes were sealed with parafilm and incubated promptly (24 ± 1 hr) at 37 °C in an inverted position [12].

After the incubation period, colonies were grown in all Petri dishes. Normal plates contained 25 to 250 colonies. All colony forming units (CFU) were counted, including the pinpoint size, on selected plates. Dilutions were used, and the total number of colonies counted were recorded. Plate with the number of CFU more than 250 colonies was recorded as too numerous to count (TNTC). Meanwhile, plates with less than 25 colonies were recorded as too few to count (TFTC). The method was used based on the previous study with slight modification [12].

2.5.2 Preparation of Inoculum with Test and Control Culture.

A loopful of isolated colonies of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231 were transferred into 15 mL of nutrient broth for *S. aureus*, and *E. coli* and Sabouraud Dextrose Broth for *C. albicans*. Then, the broth cultures (*E. coli and S. aureus*) were incubated at 37 °C for 24 hours in a shaker to develop turbidity that exceeds or is equivalent to the 0.5 McFarland Standard. The broth culture was diluted using the nutrient broth to obtain the turbidity that is equivalent to McFarland. The broth cultures (*C. albicans*) were incubated at 25 °C for 24 hours in a shaker to develop turbidity [13]. The fungi for *Aspergillus brasiliensis* ATCC 16404 were inoculated on Sabouraud Dextrose Agar (SDA) in replicated plates. For *A. brasiliensis*, the fungi formed lawn and produce spores. In order to produce a sub-culture from the culture of *A. brasiliensis*, the small part of the lawn was cut by the surgical blade that had been sterilised with 70% ethanol and Bunsen burner flame, and it was then transferred into the new agar plate of SDA at the centre of the plate. When the spores had touched the agar surface, the agar was then incubated at 25 °C for two days to grow the culture.

2.5.3 Inoculation.

Within 15 minutes, the sterile cotton swab was dipped into the adjusted inoculum. After that, it was rotated a few times and pressed firmly against the upper inside wall of the tube to express excess fluid. Then, the sterile cotton swab was streaked on the entire agar plate surface three times. The plate has been turned at 60 °C to obtain even inoculation [13].

The tested organism (*A. brasiliensis)* was performed by measuring 10 mL of sterile distilled water. The sterile distilled water was then poured on the lawn and scratch with the surgical blade until the spores mix with the sterile distilled water. After that, the inoculum was pipetted (100 μL) and transferred to another SDA plate. The glass spreader was sterilised with 70% ethanol and Bunsen burner flame. After that, the lid of the Petri dish was half-opened to let the glass spreader to enter. The Petri dish was rotated while moving the spreader from top to bottom or side to side to spread the inoculum over the surface of the agar. The agar surface was let to be fully covered with inoculum [13].

2.5.4 Disc Diffusion Method.
Filter paper discs were prepared by cutting paper discs of 6.0 mm in diameter from Whatman No. 1 filter paper using a punch. About 6.0 mm diameter of sterile blank disc was used to inseminate two different solvent extracts (ethanol and methanol). The test was performed by the disc diffusion method. The discs contained 10 μL of ethanol and methanol extracts with the concentration of 100 mg/mL, respectively, which were placed on the agar that contains microorganisms (*E. coli* and *S. aureus*) with the sterile distilled water (SDW) as a negative control, 10 μL of trimethoprim (Sigma-Aldrich) with the concentration of 50 mg/mL, and 10 μL of flucunazole (Sigma-Aldrich) with the concentration of 150 mg/mL as a positive control to test the presence of *C. albicans* and 10 μL cycloheximide (Sigma-Aldrich) with the concentration of 50 mg/mL as a positive control to test *A. brasiliensis*. The test performed in triplicates (n=3) for each microorganism and all the Petri plates were allowed to rest for 10 min at room temperature for the diffusion of the extract and incubated at 37 °C for 24 hours for microorganism (*E. coli* and *S. aureus*) growth and 25 °C for 24 hours for *A. brasiliensis* and *C. albicans* to grow. The antibacterial activities were then measured using the ruler in the unit millimetre (mm) based on the clear zones of inhibition [13].

3. Results and Discussion

3.1 The Yield of Ethanol and Methanol Extracts
The percentage yield of the chengal wood crude extracts obtained using two types of solvents is shown in Table 1. The percentage yield of chengal wood obtained for ethanolic extract (13.06%) is lower than methanolic extract (17.06%). This result is in a good agreement with previous research [14], where it was reported that different extraction techniques that used different solvents gave different amounts of extract yield. It is also in line with the previous study, which stated the solvent polarity and the method of extraction affects the percentage of yield [15,16]. Thus, in this study, methanol shows the best solvent for extracting chengal wood extract using the ultrasonic water bath.

Table 1. Yield of ethanol and methanol extract of chengal wood using an ultrasonicator water bath

Extraction solvent	Weight dried sample (g)	Weight of Extract (g)	Yield % (w/w)
Ethanol	50	6.80	13.60
Methanol	50	8.53	17.06

3.2 Physicochemical Analysis of Coconut Sap

3.2.1 pH Analysis.
Based on Table 2, the pH results obtained from coconut sap with the presence of chengal wood chips and without chengal wood chips on week zero were 6.00 ± 0.01 and 4.00 ± 0.01, respectively. However, the result slightly increased in the sample with chengal wood chips at 25 °C and 4 °C storage conditions for week one and two but continued to decrease gradually on week three until week ten. Furthermore, the coconut sap without chengal wood chips on 25 °C and 4 °C storage conditions also increases at week one but continued to decrease on week two until the end of the week. According to a previous study by Naknean et al. [17], the decrease in pH value is caused by the fermentation of yeast. The yeast can convert sucrose to glucose and fructose by invertase and finally transform into alcohol. The addition of preservative in coconut sap was to inhibit the conversion of sucrose. The previous study by Lasekan et al. [18] reported that palm sap has shown a neutral pH value of approximately 7. Hence, the low pH value indicates that the fermentation process has occurred.

Table 2. pH level of coconut sap

Week	With Chengal wood chips		Without Chengal wood chips	
	25 °C	4 °C	25 °C	4 °C
0	6.00 ± 0.01	6.00 ± 0.01	4.00 ± 0.01	4.00 ± 0.01
1	6.61 ± 0.25	6.66 ± 0.04	5.38 ± 0.08	5.59 ± 0.29
2	6.37 ± 0.03	6.46 ± 0.13	4.62 ± 0.05	5.15 ± 0.02
3	4.83 ± 0.01	6.22 ± 0.06	4.67 ± 0.01	4.91 ± 0.02
4	4.78 ± 0.01	6.25 ± 0.01	4.11 ± 0.02	4.86 ± 0.01
5	4.70 ± 0.04	6.18 ± 0.02	4.01 ± 0.01	4.80 ± 0.02
6	4.44 ± 0.02	6.13 ± 0.02	4.02 ± 0.01	4.80 ± 0.01
7	4.44 ± 0.03	5.96 ± 0.03	3.93 ± 0.01	4.73 ± 0.02
8	4.41 ± 0.02	5.83 ± 0.03	3.80 ± 0.01	4.76 ± 0.01
9	4.40 ± 0.01	5.20 ± 0.01	3.75 ± 0.01	4.70 ± 0.01
10	4.18 ± 0.01	5.16 ± 0.03	3.60 ± 0.01	4.51 ± 0.01

3.2.2 Titratable Acidity.
Based on Table 3, the result for titratable acidity obtained in coconut sap with and without chengal wood chips on week zero was 0.29 ± 0.12 and 0.36 ± 0.05 %, respectively. The total acidity for both samples with and without chengal wood chips increased gradually without any fluctuated value. In week ten, the highest percentage of total acidity in the sample without chengal wood chips at 25 °C is 4.26 ± 0.11 % while the least percentage of total acidity in the sample with chengal wood chips at 4 °C is 0.54 ± 0.04 %. According to this result, it indicates that the chengal wood in the sample has helped to reduce the acidity percentage by slowing down the formation of lactic acid bacteria (LAB). The previous study by Marius et al. [19] reported that lactic acid produced by LAB was responsible for the drop of pH value to lead yeast to fasten fermentation processes. As the pH drop, the acidity of coconut sap increases due to the activity from the LAB.

Table 3. Titratable acidity of coconut sap

Week	With Chengal wood chips		Without Chengal wood chips	
	25 °C (%)	4 °C (%)	25 °C (%)	4 °C (%)
0	0.29 ± 0.12	0.29 ± 0.11	0.36 ± 0.05	0.36 ± 0.05
1	0.54 ± 0.10	0.29 ± 0.03	0.83 ± 0.09	0.63 ± 0.12
2	0.59 ± 0.10	0.38 ± 0.07	1.14 ± 0.10	0.72 ± 0.04
3	0.62 ± 0.15	0.38 ± 0.03	2.21 ± 0.08	0.78 ± 0.07
4	0.68 ± 0.01	0.35 ± 0.09	2.25 ± 0.09	0.80 ± 0.11
5	0.72 ± 0.10	0.42 ± 0.05	2.78 ± 0.03	0.92 ± 0.09
6	0.78 ± 0.12	0.39 ± 0.09	2.93 ± 0.21	0.99 ± 0.04
7	0.77 ± 0.10	0.41 ± 0.16	3.42 ± 0.14	1.04 ± 0.12
8	0.78 ± 0.15	0.44 ± 0.07	3.81 ± 0.16	1.04 ± 0.20
9	0.60 ± 0.25	0.45 ± 0.00	4.08 ± 0.25	1.73 ± 0.09
10	1.05 ± 0.29	0.54 ± 0.04	4.26 ± 0.11	2.15 ± 0.11

3.2.3 Total Soluble Solid (TSS). Based on Table 4, the result for TSS in coconut sap with and without chengal wood chips on week zero was 17.3 ± 0.05 and 13.2 ± 0.12 °Brix, respectively. The result for the sample with chengal wood chips at 25 °C and 4 °C decreased gradually until week ten. However, the result for the sample without chengal wood chips at 25 °C also decreased, but at 4 °C the TSS values fluctuated between 13.2 ± 0.12 and 15.1 ± 0.10 °Brix. The fluctuation of TSS might be due to the utilisation of sugar for energy, conversion into another substance, and effect from the presence of bacteria and yeast [20]. The previous study by Iwuoha and Eke [21] stated that TSS variation must depend on the different sources of palm sap, and the fermentation process occurs with the presence of microorganisms.

Table 4. Total soluble solids of coconut sap

Week	With Chengal wood chips		Without Chengal wood chips	
	25 °C (°Brix)	4 °C (°Brix)	25 °C (°Brix)	4 °C (°Brix)
0	17.3 ± 0.05	17.3 ± 0.05	13.2 ± 0.12	13.2 ± 0.12
1	9.4 ± 0.08	15.9 ± 0.08	12.3 ± 0.00	15.1 ± 0.10
2	9.3 ± 0.05	14.3 ± 0.05	11.9 ± 0.15	14.8 ± 0.00
3	9.2 ± 0.08	13.4 ± 0.05	11.6 ± 0.10	15.1 ± 0.06
4	9.0 ± 0.00	12.6 ± 0.00	11.6 ± 0.00	14.9 ± 0.06
5	8.9 ± 0.08	12.3 ± 0.08	11.5 ± 0.06	14.8 ± 0.06
6	8.8 ± 0.05	12.1 ± 0.05	11.6 ± 0.06	14.7 ± 0.00
7	8.8 ± 0.19	11.9 ± 0.08	11.4 ± 0.10	14.8 ± 0.06
8	8.5 ± 0.08	11.5 ± 0.25	11.3 ± 0.06	14.4 ± 0.06
9	8.3 ± 0.00	9.6 ± 0.08	10.8 ± 0.06	14.1 ± 0.00
10	8.3 ± 0.05	9.0 ± 0.05	10.4 ± 0.00	13.8 ± 0.06

3.2.4 Colour Analysis.

Based on Table 5, the results indicate that the lightness (L*) were ranged from 18.55 to 38.94, while redness (a*) were ranged from 0.84 to 7.05, and yellowness (b*) were ranged from 2.42 to 12.37. The main observation of this analysis is to determine the lightness of coconut sap using (L*) value instead of (a*) and (b*) value whereby they will help determine the redness and yellowness of the sample. The (L*) value of coconut sap sample with and without chengal wood chips was increasing from week zero to week one but continued to decrease gradually for both temperatures. Generally, fresh coconut sap was oyster white in colour and translucent. The decrease of (L*) value happens because of the reaction of the fermentation process, which lowered the intensity of the lightness of coconut sap. Furthermore, the decrease of (L*) value in coconut sap with chengal wood chips was due to enzymatic browning of coconut sap. The previous study reported that the colour of the palm sap might be affected by the pigment of Kiam wood that dissolves during the collection period. Moreover, enzymatic browning processes can also occur during the collection of sap [15].

3.3 Volatile Organic Compounds (VOCs)

3.3.1 Storage Temperature at 25 °C.

Based on the observation of volatile compounds (Tables 6 and 7), there were 10 compounds identified in coconut sap with chengal wood chips while 11 compounds in coconut sap without chengal wood chips. The classifications of volatile components were divided into organic compounds, aromatic hydrocarbon, fatty acids, alcohols, and alkane. The results were expressed in percentage (%) of the peak area of the detected compound. Other major VOCs (>30%) found in coconut sap with chengal wood chips were phenyl ethyl alcohol (52.58%), ethylbenzene (44.66%), and butylated hydroxytoluene (30.70%). For coconut sap without chengal wood chips, the volatile compounds found were ethylbenzene (100%), butylated hydroxytoluene (68.8%), p-xylene (58.82%), and phenyl ethyl alcohol (41.92%). Overall, the sample with chengal wood chips contains three volatile compounds, while without chengal contains four volatile compounds. Other minor VOCs fluctuated or were not detected.

3.3.2 Storage Temperature at 4 °C.

The observation of the volatile organic compounds (VOCs) based on Tables 8 and 9 were determined in the sample with and without chengal wood chips at 4 °C. Eight compounds were identified in the sample with chengal wood chips while nine compounds in the sample without chengal wood chips. The classifications of volatile components were the same as before, which were organic compound, aromatic hydrocarbon, fatty acids, alcohols, and alkane. The results were expressed in the percentage of peak area

on the detected compounds. The percentage of VOCs has been varied whether if the peak area of the compound has been at >60%. There are four volatile compounds in coconut sap with chengal wood chips including ethylbenzene (100%), butylated hydroxytoluene (91.57%), phenyl ethyl alcohol (72.07%) and pentacosane (68.17%) while coconut sap without chengal wood chips contains two major compounds known as ethylbenzene (100%) and butylated hydroxytoluene (77.66%).

3.4 Microbiological Analysis

3.4.1 Total Plate Count.

Based on Table 10 the coconut sap with chengal wood chips at week zero in MRS agar has recorded the value of total plate count of $1.55x10^3$ CFU/mL. In contrast, the coconut sap without chengal wood chips was recorded as $2.80x10^3$ CFU/mL, which showed acceptable plate count that is lower than the maximum limit (10^5 CFU/mL). The coconut sap with chengal wood chips on week zero in MEA agar has recorded the value of total plate count as too few to count (TFTC). Subsequently, the coconut sap without chengal wood chips was recorded at TFTC showed acceptable plate count that is lower than the maximum limit (10^5 CFU/mL).

Besides that, the coconut sap with chengal as preservatives in MRS agar, at conditions of 25 °C of total plate count was recorded in the range of $2.40x10^4$ CFU/mL to $2.37x10^5$ CFU/mL on week one until week six, which still indicated the acceptable plate count that is lower than the maximum limit (10^5 CFU/mL). Apart from that, on week seven, the total plate count measured resulted higher than the maximum limit with $2.46x10^6$ CFU/mL. From week eight to week ten, the coconut sap was recorded as too numerous to count (TNTC) as the colonies were filled on the agar surface. The coconut sap without chengal as preservatives in MRS agar, at 25 °C was recorded as TNTC from week one to week ten as the growth of colonies filled the agar surface.

Result for the coconut sap with chengal wood chips in MRS agar at 4 °C of total plate count recorded in the range of $1.19x10^4$ CFU/mL to $1.82x10^5$ CFU/mL in week one until week five still showed the acceptable plate count that is lower than the maximum limit (10^5 CFU/mL). Apart from that, on week six, the total plate count measured resulted higher than the maximum limit with $1.26x10^6$ CFU/mL. From week eight to week ten, the coconut sap was recorded as too numerous to count (TNTC) as the colonies were filled on the agar surface. The coconut sap without chengal wood chips in MRS agar, at 4 °C has recorded the total plate count ($1.17x10^6$ CFU/mL and $2.37x10^6$ CFU/mL) from week one to week two. From week three to week ten, the coconut sap was recorded as too numerous to count (TNTC) as the colonies were filled on the agar surface.

The coconut sap with chengal wood chips in MEA agar, at 25 °C of total plate count was recorded as TFTC from week one until week four, which still showed the acceptable plate count that is lower than the maximum limit (10^5 CFU/mL). From week five, the colonies start growing in the range of $1.93x10^4$ CFU/mL to $1.14x10^4$ CFU/mL until week seven. Apart from that, in week eight to week ten, the total plate count measured resulted in a decrease from $8.20x10^3$ to $3.50x10^3$ CFU/mL. The coconut sap without chengal wood chips in MEA agar, at the condition of 25 °C was recorded as TFTC from week one to week four which still showed the acceptable plate count that is lower than the maximum limit (10^5 CFU/mL). From week five, the colonies start growing in the range of $5.40x10^3$ CFU/mL to $1.15x10^5$ CFU/mL until week ten.

The coconut sap with chengal wood chips in MEA agar, at 4 °C total plate count was recorded as TFTC from week one

Table 5. Colour analysis of coconut sap with and without chengal wood chips at different storage conditions

Week	With Chengal wood chips						Without Chengal wood chips					
	25 °C			4 °C			25 °C			4 °C		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
0	22.75 ± 1.19	7.05 ± 1.20	9.07 ± 0.10	22.75 ± 1.19	7.05 ± 1.20	9.07 ± 0.10	25.98 ± 1.23	4.33 ± 2.67	6.62 ± 0.73	25.98 ± 1.23	4.33 ± 2.67	6.62 ± 0.73
1	44.13 ± 0.40	1.86 ± 0.07	9.55 ± 0.12	38.85 ± 0.79	3.34 ± 0.45	10.98 ± 2.83	38.72 ± 1.44	1.58 ± 0.13	5.36 ± 0.15	28.32 ± 5.29	1.66 ± 0.06	4.14 ± 0.15
2	41.33 ± 1.40	1.69 ± 0.15	8.31 ± 0.42	36.44 ± 3.55	3.97 ± 0.36	12.37 ± 1.36	36.81 ± 2.97	3.38 ± 0.28	7.77 ± 0.90	26.31 ± 2.52	2.26 ± 0.47	6.93 ± 1.35
3	44.12 ± 0.77	1.79 ± 0.11	8.91 ± 0.42	38.94 ± 3.00	3.83 ± 0.20	12.01 ± 0.57	33.91 ± 2.54	2.66 ± 0.41	8.53 ± 1.25	26.11 ± 0.41	2.10 ± 0.19	6.74 ± 0.95
4	35.50 ± 0.35	1.25 ± 0.05	6.50 ± 0.13	30.53 ± 0.47	2.84 ± 0.20	6.63 ± 0.96	32.79 ± 3.13	2.31 ± 0.47	7.44 ± 1.41	24.50 ± 0.39	2.33 ± 0.04	4.76 ± 0.18
5	33.29 ± 3.10	1.32 ± 0.24	6.20 ± 0.41	27.92 ± 1.77	2.46 ± 0.20	7.42 ± 0.36	33.94 ± 0.37	1.56 ± 0.05	7.10 ± 0.11	24.59 ± 0.72	2.35 ± 0.27	6.96 ± 0.35
6	34.01 ± 1.78	1.86 ± 0.17	4.53 ± 0.82	24.30 ± 0.26	2.23 ± 0.03	6.82 ± 0.04	28.83 ± 1.77	1.93 ± 0.14	5.37 ± 0.40	20.86 ± 0.80	1.09 ± 0.03	2.84 ± 0.01
7	30.64 ± 0.76	0.94 ± 0.10	3.22 ± 0.30	23.75 ± 0.50	1.18 ± 0.09	3.38 ± 0.13	26.19 ± 0.49	1.59 ± 0.34	3.41 ± 0.63	20.00 ± 1.03	1.10 ± 0.02	2.42 ± 0.09
8	29.07 ± 0.30	0.99 ± 0.08	4.14 ± 0.01	25.44 ± 1.25	1.81 ± 0.19	4.87 ± 0.41	25.03 ± 1.84	0.84 ± 0.12	2.68 ± 0.26	21.93 ± 0.10	1.86 ± 0.12	5.96 ± 0.08
9	26.72 ± 2.32	1.17 ± 0.28	3.84 ± 0.68	23.10 ± 0.55	1.91 ± 0.31	3.89 ± 0.64	23.31 ± 0.80	0.91 ± 0.04	2.68 ± 0.18	18.55 ± 0.32	1.01 ± 0.11	2.59 ± 0.19
10	25.84 ± 0.80	0.88 ± 0.11	3.69 ± 0.12	21.95 ± 1.96	1.34 ± 0.13	3.13 ± 0.24	22.18 ± 2.69	0.91 ± 0.08	2.76 ± 0.11	19.40 ± 0.50	0.93 ± 0.04	2.80 ± 0.04

L* representing the lightness of color, a* is red while, and b* is yellow

Table 6. Volatile organic compounds in coconut sap with chengal wood chips at 25 °C

With Chengal wood chips 25 °C												
		Area (%) / Week										
Volatile Organic Compounds	RT	0	1	2	3	4	5	6	7	8	9	10
Ethylbenzene	4.17 ± 0.01	nd	44.66	38.91	1.19	17.66	14.64	3.33	18.69	13.87	10.97	14.6
p-Xylene	4.34 ± 0.00	nd	25.03	22.47	nd	9.73	8.08	1.87	10.49	6.7	5.84	7.56
Phenylethyl Alcohol	12.72 ± 0.15	nd	52.58	24.36	1.38	16.07	17.78	6.42	30.9	31.44	20.66	25.89
Ethyl hydrogen succinate	16.21 ± 0.06	nd	nd	nd	nd	nd	nd	nd	nd	22.86	16.02	3.13
Butylated Hydroxytoluene	29.59 ± 0.01	12.88	30.7	24.5	nd	15.87	13.17	3.02	18.46	15.5	9.8	11.97
Dodecanoic acid	32.06 ± 0.03	nd	nd	18.6	1.25	39.44	34.55	5.45	29.88	25.61	15.63	17.49
Tetradecanoic acid	40.13 ± 0.01	nd	nd	8.47	nd	12.17	9.69	2.22	13.85	9.33	11.47	10.68
n-Hexadecanoic acid	45.99 ± 0.07	nd	nd	nd	nd	10.29	9.48	nd	14.62	8.92	2.13	nd
Oleic Acid	46.04 ± 0.01	nd	nd	nd	nd	nd	nd	1.11	7.89	nd	nd	6.38
(E)-9-Octadecenoic acid	52.19 ± 0.00	nd	nd	nd	nd	nd	nd	nd	nd	6.51	nd	nd

nd = not detected

Table 7. Volatile organic compounds in coconut sap without chengal wood chips at 25 °C

Without Chengal wood chips 25 °C												
	Retention Time	Area (%) / Week										
Volatile Organic Compounds	(RT)	0	1	2	3	4	5	6	7	8	9	10
Ethylbenzene	4.17 ± 0.00	nd	100	93.73	38.38	nd	2.93	20.18	42.61	13.61	1.75	9.87
p-Xylene	4.34 ± 0.01	nd	58.82	51.96	20.73	3.36	1.55	11.25	19.25	7.37	nd	4.61
Dodecane	10.21 ± 0.06	nd	nd	nd	4.16	nd	nd	1.61	3.34	nd	nd	2.88
Phenylethyl Alcohol	12.68 ± 0.06	nd	11.39	40.18	23.2	41.66	1.9	4.76	12.12	2.31	nd	41.92
Nonane	19.23 ± 0.82	nd	nd	11.54	nd	nd	nd	nd	3.31	nd	nd	nd
Butylated Hydroxytoluene	29.59 ± 0.01	10.26	68.8	57.4	31.88	5.27	2.73	18.1	47.55	12.1	1.37	7.6

n-Decanoic acid	31.98 ± 0.00	nd	nd	nd	nd	nd	nd	2.66	nd	nd	nd	nd
Dodecanoic acid	32.86 ± 0.02	nd	nd	nd	2.98	nd	nd	2.83	nd	3.07	nd	nd
n-Hexadecanoic acid	45.88 ± 0.01	nd	nd	nd	nd	nd	nd	5.4	nd	4.68	nd	1.27
Nonanoic acid	46.19 ± 0.52	nd	nd	nd	2.39	nd	nd	nd	19.62	2.53	nd	1.7
(E)-9-Octadecenoic acid	51.99 ± 0.00	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.58

nd = not detected

Table 8. Volatile organic compounds in coconut sap with chengal wood chips at 4 °C.

With Chengal wood chips 4 °C												
	Retention Time	Area (%) / Week										
Volatile Organic Compounds	(RT)	0	1	2	3	4	5	6	7	8	9	10
Ethylbenzene	4.17 ± 0.01	nd	nd	68.67	2.97	87.71	81.85	100	78.44	100	100	60.74
p-Xylene	4.33 ± 0.01	nd	nd	30.19	1.67	46.52	50.84	54.68	41.43	52.04	54.89	32.12
Phenylethyl Alcohol	12.65 ± 0.01	nd	nd	nd	Nd	nd	nd	nd	72.07	26.45	nd	49.95
Butylated Hydroxytoluene	29.59 ± 0.01	12.88	nd	21.4	1.33	61.42	69.35	63.37	70.63	91.57	63.64	56.37
Dodecanoic acid	32.03 ± 0.01	nd	nd	nd	Nd	nd	nd	nd	55.75	52.63	nd	34.65
Tetradecanoic acid	40.12 ± 0.00	nd	nd	nd	Nd	nd	nd	nd	nd	nd	nd	7.4
n-Hexadecanoic acid	45.92 ± 0.01	nd	nd	nd	Nd	32.19	nd	nd	25.18	nd	nd	9.11
Pentacosane	47.31 ± 0.00	nd	nd	nd	68.17	nd	nd	nd	nd	nd	nd	nd

nd = not detected

Table 9. Volatile organic compounds in coconut sap without chengal wood chips at 4 °C

Without Chengal wood chips 4 °C												
		Area (%) / Week										
Volatile Organic Compounds	Retention Time (RT)	0	1	2	3	4	5	6	7	8	9	10
Ethylbenzene	4.16 ± 0.01	nd	nd	100	nd	26.52	100	4.45	4.68	100	100	nd

p-Xylene	4.33 ± 0.01	nd	13.85	53.64	34.44	17.66	52.3	2.15	2.3	44.75	47.62	nd
Nonane	10.42 ± 0.35	nd	9.91	16.27	18.04	nd	11.79	nd	nd	2.73	4.3	nd
Phenylethyl Alcohol	12.69 ± 0.04	nd	16.77	nd	nd	4.79	nd	nd	nd	9.47	nd	nd
Dodecane	19.72 ± 0.23	nd	5.89	11.02	6.18	nd	5.89	nd	nd	nd	nd	nd
Butylated Hydroxytoluene	29.59 ± 0.02	10.26	100	73.44	42.85	19.83	69.19	3.35	3.23	54.71	77.66	15.19
Hexadecane	30.77 ± 0.09	nd	15.42	5.62	nd	nd	nd	nd	nd	nd	nd	nd
Dodecanoic acid	32.67 ± 0.43	nd	2.6	6.15	1.32	4.1	nd	nd	nd	nd	nd	nd
n-Hexadecanoic acid	45.93 ± 0.00	nd	nd	nd	nd	5.13	nd	nd	nd	nd	nd	nd

nd = not detected

Table 10. Microbiological counts of coconut sap with and without chengal wood chips in two different conditions from week zero to week ten

			CFU/mL										
Sample	**Media Growth**	**Temp**	**W0**	**W1**	**W2**	**W3**	**W4**	**W5**	**W6**	**W7**	**W8**	**W9**	**W10**
With Chengal	**MRS Agar**	**25 °C**	1.55x10^3	2.40x10^4	2.25x10^4	1.55x10^4	1.16x10^5	2.05x10^5	2.37x10^5	2.46x10^6	TNTC	TNTC	TNTC
Without Chengal			2.80x10^3	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
With Chengal		**4 °C**		1.19x10^4	8.50x10^3	5.60x10^3	4.70x10^3	1.82x10^5	1.26x10^6	1.78x10^6	TNTC	TNTC	TNTC
Without Chengal				1.71x10^6	2.37x10^6	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
With Chengal	**MEA Agar**	**25 °C**	TFTC	TFTC	TFTC	TFTC	TFTC	1.93x10^4	1.45x10^4	1.14x10^4	8.20x10^3	5.40x10^3	3.50x10^3
Without Chengal			TFTC	TFTC	TFTC	TFTC	TFTC	5.40x10^3	2.40x10^4	8.50x10^4	1.62x10^5	1.33x10^5	1.15x10^5
With Chengal		**4 °C**		TFTC	TFTC	TFTC	TFTC	2.38x10^4	1.40x10^4	1.60x10^3	TFTC	TFTC	TFTC
Without Chengal				TFTC	TFTC	TFTC	TFTC	1.15x10^4	2.35x10^4	8.30x10^4	3.30x10^4	TNTC	TNTC

Note: TFTC: Too few to count, TNTC: Too numerous to count

until week four which still showed the acceptable plate count that is lower than the maximum limit (10^5 CFU/mL). From week five and week six, the colonies started growing in the range of $2.38x10^4$ to $1.40x10^4$ CFU/mL while in week seven, the total plate count decreased again until week ten resulted in TFTC. The coconut sap without chengal wood chips in MEA agar, at the conditions of 4 °C was recorded as TFTC from week one until week four which still showed the acceptable plate count that is lower than the maximum limit (10^5 CFU/mL). From week five the colonies started growing in the range of $1.15x10^4$ CFU/mL to 2.35^4 CFU/mL until week eight, and in week nine and week ten resulted in TNTC for the colonies.

3.4.2 Determination of Inhibition Zone.

The result of the antibacterial and antifungal activities of ethanol and methanol extracts are presented in Table 11. The negative control (sterile distilled water (SDW)) did not exert any inhibition on the strains tested. In contrast, the positive control (trimethoprim) of *S. aureus* and *E. coli* has shown great zone of inhibition. The positive control (fluconazole) of *C. albicans* and cycloheximide for *A. brasiliensis* has shown substantial zone of inhibition.

The ethanol and methanol extracts of chengal wood chips have successfully inhibited the growth of tested microorganisms at a concentration of 100 mg/mL. This result is in a good agreement with the previous study where it was reported that methanol extract of chengal wood chips for antimicrobial activity at 100 mg/mL using Kirby-Bauer disc diffusion method was enough to inhibit the gram-positive and gram-negative organisms including *E. coli*, *Klebsiella pneumonia, Proteus vulgaris*, *Pseudomonas sp.*, *Salmonella typhimurium*, *Bacillus subtilis*, and *S. aureus* [22]. In addition, the previous study by Kawamura et al. [14] reported that methanol extract of chengal wood showed antifungal activities against *C. albicans* and *A. brasiliensis*.

Table 11. Average zone of inhibition (mm) of chengal wood ethanolic and methanolic extracts against test microorganisms using the paper disc diffusion method

Micro-organisms	**Control**		**Extracts (100 mg/mL)**	
	Positive	Negative	Ethanol	Methanol
E. coli	23±0.57	-	16±0.47*	16±0.50*
S. aureus	19±0.48	-	19±0.49	20±0.43
C. albicans	15±0.45	-	13±0.50*	12±0.46*
A. brasiliensis	13±0.50	-	11±0.50*	12±0.47

Note: (-) indicates no zone of inhibition.*For all the results; the significance levels were showed as $P<0.001$.

4. Conclusion

In conclusion, the coconut sap with the presence of chengal wood chips as natural preservative showed positive results toward its physicochemical and antimicrobial properties compared to coconut sap without the presence of chengal wood chips. The pH analysis toward coconut sap with and without chengal wood chips shows a decreasing value. In contrast, the total percentage of acidity in coconut sap with and without chengal wood chips shows increasing values due to the presence of the fermentation process in coconut sap. Colour analysis based on lightness (L*) value of coconut sap, which also shows decreasing value for both coconut sap with and without chengal wood chips. In general, the physicochemical properties for coconut sap with and without chengal wood chips show a continuous degradation as the total sugar drop due to the conversion of lactic acid from glucose in coconut sap. However, with the presence of natural preservative (chengal wood chips) in coconut sap, it has helped to slow down the conversion of lactic acid and fermentation process. Next, in microbiological analysis, the methanol extract has shown bigger zone inhibition, which proved that methanol is the best solvent to extract the chengal wood compared to ethanol extract. The highest diameter of the inhibition zone indicated that the extracted plant have the highest antimicrobial activity. The microbial test of coconut sap with and without chengal wood chips resulted in different recorded of total plate count at 25 °C and

4 °C. The total viable count from coconut sap with chengal wood chips shows a slow fermentation rate that occurs in the coconut sap due to the presence of chengal wood chips that contain antimicrobial compounds. Thus, this study proved that the addition of chengal wood chips as natural preservative during the harvesting of coconut sap improved the coconut sap properties and its shelf life.

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