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Nutritional, Phytochemical, Antioxidant Activity and Sensory Attributes of Herbal Infusion from Sukun (*Artocarpus altilis*) Leaf

Siti Nur Shafiqah Azli[1,a)], Mohd Fadzelly Abu Bakar[1,2,b)], Shuaibu Babaji Sanusi[1,2,c)] and Fadzilah Awang-Kanak[1,d)]

[1]*Faculty of Applied Sciences & Technology (FAST), Universiti Tun Hussein Onn Malaysia (UTHM), Hub Pendidikan Tinggi Pagoh, KM1, Jalan Panchor,84600, Muar, Johor, Malaysia.*
[2]*Centre of Research- Sustainable Research of Natural Resources (CoR-SUNR), Universiti Tun Hussein Onn Malaysia (UTHM), 86400 Parit Raja, Batu Pahat, Johor, Malaysia*

a) sitinurshafiqah.azli@gmail.com
b) Corresponding author: fadzelly@uthm.edu.my
c) sanusishuaibu@gmail.com
d) akfadzilah@gmail.com

Abstract. "Sukun" or breadfruit, scientifically known as *Artocarpus altilis* contained several nutritive and pharmaceutical values. The objectives of this study were to determine the sensory attributes, phytochemicals, antioxidant activity, nutritional content, and physicochemical, of herbal tea prepared from *A. altilis* leaf at three different infusion temperatures (i.e. 45°C, 80 °C and 100°C). The samples were prepared by using hot water extraction method, and all extracts were later analysed for sensory attributes, phytochemical content, nutritional content, and also tested for antioxidant activity by using DPPH, FRAP, and ABTS assays. The sensory testing score where the highest score belonged to sample 357, infusion at 100°C with the score of 5.6±2.1, followed by sample 790, infusion at 80°C and 539, infusion at 45°C where both scored 4.9±1.9 and 4.2±2.1, respectively. Total phenolic (9.76±0.86) and total flavonoid (47.22±0.01) contents of *A. altilis* leaf tea was highest at 100°C of infusion temperature. As for antioxidant activity tests, the results demonstrated the same trend, that *A. altilis* leaf tea infused at 100°C of water displayed the highest antioxidant activity as for DPPH (8.84±0.04), FRAP (30.99±0.01), and ABTS (45.42±0.01) assays. Nutritional content for the highest infusion temperature (100°C) was 0.13% (carbohydrates), 0.1% (protein), 0.2% (fat), 0.04±0.01% (ash) and 99.53±0.23% (moisture content). Meanwhile, the concentration of sodium, calcium, potassium, and magnesium for that infusion was 0.34±0.03, 3.11±0.25, 1.18±0.05 and 0.81±0.06 mg/L, respectively. The physical properties were 0.66±0.01 for water activity, 0.2% total soluble solid and reading of color L*, a* and b* was 65.05±0.8, -0.87±0.08 and 20.66±0.1, respectively. As a conclusion, *A. altilis* dry leaf infused at 100°C displayed the highest antioxidant and phytochemicals properties and can be considered as functional food and nutraceutical.

Keywords: Antioxidant, herbal tea, sensory, phytochemical, physicochemical, nutritional compositions.

INTRODUCTION

People nowadays are concerned about the kind of food they eat; as such the demand for healthy food and beverages is very high. The occurrence of chronic diseases such as cardiovascular diseases, cancer, diabetes mellitus and ageing, were recognized to be associated with the oxidative stress, where the reactive oxygen species (ROS) and reactive nitrogen species (RNS) including free radicals were continuously produced in human cells and led to oxidative damage to cell components [1]. Tea and herbal infusions have been said to be the primary source of phenolic compounds in our diet, and the production of green tea was made to preserve the polyphenolic compounds [2-3]. Tea falls under beverages category and known as the most consumed beverage in the world, aside from water, in China and Japan, green tea has been consumed as primary beverage [3]. Tea leaf that prepared fresh through hot water infusion able to preclude up to 30% of flavanol such as catechins [3].

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Artocarpus altilis (Breadfruit), or known as "sukun" by Malaysian natives, is used in traditional medicine to treat various ailments. Not many know how useful this plant since it is an underutilized and neglected crop. The starchy nature of it fruit makes its rich source of carbohydrate, calcium, and phosphorus [4]. Furthermore, flavonoids isolated from *A. altilis* showed potential as antioxidants and skin-whitening agents [5]. A review on *Artocarpus* showed that this fruit exhibited diverse pharmacological properties [6]. Breadfruits leaf is believed to have potential health benefits to human.. The purpose of this study is to investigate the antioxidant activity, phytochemicals, nutritional content, physicochemical, and sensory attributes of herbal tea prepared from *A. altilis* leaf.

METHODOLOGY

Preparation of Leaf Extracts

The leaves of *A. atilis* after collection were cleaned and then oven dried at 40°C for seven days. After that, the sample was coarsely grinded, sieved through 2 mm and then stored in an air-tight container till further used. Two commercial teas such as "BOH Green Tea" and "SABAH Black Tea" were purchased at local market were used for comparison with *A. altilis* herbal infusion. The hot water extraction method was used to prepare the herbal tea. 2.0 g of *A. altilis* leaves were infused in 200 ml of distilled water at the temperatures of 45°C, 80°C and 100°C for 3 minutes. Afterward, the sample infusion mixture was let cool for 5 minutes, filtered using filter paper and transferred into the volumetric flask.

Sensory Analysis

Acceptance test was conducted on three herbal teas infused at different temperature by 48 untrained panelists of students and staffs at Universiti Tun Hussein Onn Malaysia. Panellists used plain water between samples. The hedonic scale of 1 - 9 was used to evaluate the overall acceptability, flavour, aroma, aftertaste, mouthfeel, and color of the tea [7], where 1 = extremely dislike and 9 = extremely like. Scoresheet was given to each panelist. The analysis was done on each of the attributes by calculating the mean point from the result.

Determination of Phytochemical Contents

The phytochemical contents such as the total phenolic content and total flavonoids contents were determined using folin-ciocalteu [8] and aluminum-chloride methods [11] respectively.

Determination of Free radical Scavenging Activity

The free radical scavenging activities were determined using DPPH assay, FRAP assay, and ABTS assay according to the methods previously described previously [8-10].

Determination of the Nutritional Composition

The nutritional composition such as carbohydrate, protein, fat, moisture, ash and mineral content of the sample was determined by using AOAC method [12]. Atomic absorption spectroscopy (AAS) was used for identifying mineral content in the sample.

Determination of Physicochemical Properties

The physicochemical properties such as colour and water activity were determined by using the method from AOAC for water activity and colour [12]. On the other hand, TSS (total soluble solids) was determined by Refractometer (PAL-BX/RI, Atago, Japan) [13].

Statistical Analysis

The statistical analyses were performed by a one-way ANOVA using Microsoft Excel 2016. The results were expressed as means ± SD to show variations in the various experiments. Differences are considered significant when $p < 0.05$.

RESULTS & DISCUSSION

Sensory Analysis

The results of the sensory testing score where the highest score belonged to sample 357, infusion at 100°C with the score of 5.6±2.1, followed by sample 790, infusion at 80°C and 539, infusion at 45°C where both scored; 4.9±1.9 and 4.2±2.1, respectively (table 1).

There was an apparent score difference of samples attributes on color, aroma and overall acceptability ($p < 0.05$) as shown in table 1. For sensory attributes on aftertaste, mouthfeel, and flavor, there was only a slight difference in score between that three sample and all of them were below the score of five. Tea-infused at 100°C was more acceptable compared to 80°C and 45°C of infusions. Scores were low for flavor, mouthfeel and aftertaste attributes due to the astringency of the tea.

There was a colour difference between three samples, concluding that the higher the temperature of water infusion, the higher the intensity of tea color produced. Also, the aroma was more accepted by decreasing infusion temperature. *A. altilis* leaf infusion gave off green tea-like aroma, as that was the perceived aroma from panelists.

TABLE 1. Sensory score for *A. altilis* leaf infusions.

Attributes	Sample		
	357	790	539
Color	6.1±1.8	5.8±1.5	4.6±2.1
Aroma	5.9±1.7	5.5±1.8	4.4±2.1
Flavor	4.4±2.1	4.4±2.2	3.8±2.3
Mouthfeel	4.7±2.3	4.3±2.1	3.9±2.4
Aftertaste	4.8±2.3	4.4±2.2	4.0±2.2
Overall acceptability	5.6±2.1	4.9±1.9	4.2±2.1

Values are expressed as mean ± standard deviation.

Total Phenolic Content and Total Flavonoid Content.

Total phenolic content (TPC) of teas decreases in the following order: green tea > black tea > *A. altilis* leaf tea with temperature. The obtained results were in agreement with previously published data, where they found that the higher the temperature of the water, the higher the TPC and TFC extracted, which reached to their maximum values at 100^0C [18]. The differences in TPC among teas could simply be attributed to the plant source *as C. sinensis* may have a larger quantity of polyphenols than the plants and herbs that are used to make the herbal and rooibos teas [19].

The antioxidant properties of plants and polyphenol content depend on many factors, such as nutrient in the soil and climate conditions in which plant was cultivated, harvest seasons, methods of processing and storage [20]. The trend of total flavonoid content (TFC) of teas was decreasing in the following order: green tea > black tea > A. altilis leaf tea, which is directly proportional with decreasing of temperature. Quercetin value in *A. altilis* leaf tea is probably the lowest that only small amount of TFC found in the tea compared to both green and black tea. This was perhaps due to the maturity of *A. altilis* leaf. As the leaf aged, flavonoids may lose due to metabolic conversion to other secondary phenolic compounds or degradation via enzyme action, which lowers the level of flavonoid compounds [21]. The summary of total phenolic and total flavonoid contents are summarized in table 2.

Free radical Scavenging Activity

The effect of different infusion temperature used for *A altilis* leaf infusion, green tea and black tea on free radical scavenging activity in the DPPH, FRAP and ABTS assay was presented in Table 2. At 100°C, the highest percentage inhibition of DPPH for *A altilis leaf* infusion, green tea and black tea (8.84±0.04, 92.3±0.02 and 91.3±0.02%), followed by 80°C (6.06±0.18, 91.7±0.02 and 80.2±0.11%) and 45°C (6.07±0.28, 90.8±0.73 and 68.9±0.10%). For FRAP assay, the highest ferric ion reduction also at 100°C of infusion, followed by 80°C and 45°C. The same trend of result also showed in ABTS free radical scavenging assay.

However the highest reading was belonged to black tea sample, followed by green tea and *A.altilis* leaf infusion. For TPC and TFC, both showed the same trend where at 100°C of infusion had the highest TPC and

TFC, followed by 80°C and 45°C of infusion. The results showed that the DPPH radical scavenging capacity of the tested samples is not as a result of a single phytochemical compound, but instead widely distributed amongst the phenolic constituents [14] and that the polyphenols identified in this paper represent a part of the total polyphenols found in tea. Also, the reason why the percentage inhibition for *A. altilis* was lowest among all samples tested was due to the less brewing temperature since. The result is in line with a study conducted for *A. borneensis,* which found that brewing leaf for 3 minutes yielded low antioxidant compared to brewing it for 1 hour [15].

TABLE 2. Summary of result for TPC, TFC, DPPH, FRAP and ABTS assay.

Sample	Temp (°C)	Antioxidant assays				
		DPPH (%)	FRAP (mM/g)	ABTS (µ/ml)	TPC (µg /ml)	TFC (µg /ml)
*AAT	45	6.07±0.28	20.59±0.78	42.48±0.18	5.28±0.37	23.05±0.01
	80	6.06±0.18	20.94±0.01	42.77±0.00	6.67±0.33	29.07±0.01
	100	8.84±0.04	30.99±0.01	45.42±0.01	9.76±0.86	47.22±0.01
**GT	45	90.8±0.73	201.89±0.01	42.80±0.00	27.86±0.42	100.98±0.01
	80	91.7±0.02	205.43±0.01	46.53±0.01	36.52±0.86	108.67±0.01
	100	92.3±0.02	209.13±0.03	47.35±0.00	53.37±0.57	125.82±0.01
***BT	45	68.9±0.10	182.08±0.01	47.40±0.00	15.96±1.21	58.08±0.01
	80	80.2±0.11	195.41±0.01	47.59±0.00	20.03±0.82	97.65±0.01
	100	91.3±0.02	203.06±0.02	48.12±0.00	27.76±1.70	100.90±0.01

*AAT = *A. alitis* tea, **GT = Green tea, ***BT = Black tea.

Values are expressed as mean ± standard deviation of three replicate measurements

For *A. altilis* leaf tea, the lowest reducing ability was seen at 45°C, followed by 80°C and 100°C. This is in line with the previous study on *C. asiatica* where it was found that high FRAP value was obtained when brewed at 100°C [16]. Reaction of phenolics with ABTS radical is rapid when compared with DPPH depending on the reducing potential. DPPH radical reacts only with lipophilic antioxidants, whereas the ABTS radical reacts with both hydrophilic and lipophilic antioxidants, thus covering a more significant share of antioxidants in a sample and resulting in a higher correlation between these two assays [17].

Nutritional Content

Meanwhile, for nutritional composition, the A. altilis infusion at 100°C contained carbohydrates, proteins, fats, ash and moisture content with 0.13, 0.1, 0.2, 0.04±0.01 and 99.53±0.23% values respectively (table 3).

TABLE 3. Nutritional composition of *A. altilis* leaf infusion.

Nutritional content	Values
Carbohydrates (%)	0.13
Proteins (%)	0.1
Fats (%)	0.2
Ash (%)	0.04±0.01
Moisture content (%)	99.53±0.23

Values are expressed as mean ± standard deviation of three replicate measurements.

Fats content obtained in the sample indicated that there are phytosterols and steroid in the leaf of *A. altilis*, which is supported by the previous study that reported their presences in methanol and ethyl acetate extract of *A. altilis* leaf [22]. The moisture content in *A. altilis* tea infusion was 99.53±0.23. The tea was made up of almost 100% of water since it was an infusion. Even though herbal tea is healthy, it should not be consumed every day in other to prevent any toxicity of minerals. Mineral analysis in *A. altilis* leaf infusion at 100°C mg/L recorded calcium (Ca) as the highest mineral at 3.11±0.25 mg/L, and the infusion of tea at this temperature shows that the lowest sodium (Na) with the concentration of 0.34±0.03 mg/L (Table 4).

Thus, average dietary intake of mineral content must be considered when drinking *A. altilis* tea and not exceeding recommended daily intake. The RDA for calcium and magnesium are 1000 and 310 mg/day, meanwhile the AI for sodium and potassium are 1500 and 4700 mg/day. Hence, *A. altilis* tea could not be a good source to provide minerals for consumers since none of them could provide enough minerals to

individuals. To reach RDA/AI of sodium, calcium, potassium, and magnesium, one must consume 4411, 321, 3983 and 382 mg/L of *A. altilis* infusion/day.

TABLE 4. Minerals content of *A. altilis* leaf infusion.

Minerals	Mineral content (mg/L)	RDA/AI (mg/d)
Sodium (Na)	0.34±0.03	1500[a]
Calcium (Ca)	3.11±0.25	1000[b]
Potassium (K)	1.18±0.05	4700[a]
Magnesium (Mg)	0.81±0.06	310[b]

Values are expressed as mean ± standard deviation of three replicate measurements.

Physicochemical Properties

The physical properties of *A. altilis* leaf infusion at 100°C where the a_w of the sample was 0.66±0.01. Water activity TSS in the sample was 0.2 and the L*, a* and b* result were 65.05±0.80, -0.87±0.08 and 20.66±0.10, respectively (table 5). The water activity value for dry *A. altilis* leaf was 0.66±0.01 which is in the safe margin [23]. According to a previous study, the longer the infusion time, the higher the TSS. In this study, tea was infused for about three minutes, giving the TSS value 0.2%. Also, higher values of TSS act as an indicator for a better quality of material and low TSS value is probably due to respiratory losses and partly to a polyphenolic oxidation product combined with protein during manufacturing [24].

TABLE 5. Physical properties of *A. altilis* leaf infusion.

Physical properties	Values
Water activity	0.66±0.01
TSS (%)	0.2
Color:	
L*	65.05±0.80
a*	-0.87±0.08
b*	20.66±0.10

Values are expressed as mean ± standard deviation of three replicate measurements.

The color of *A. altilis* leaf tea diverges to green color since the value of a* was negative and in low intensity which is only 20.66. The tea color will be more diverge to red color if the value of a* is positive. Also, the positive value of b* indicated yellowish color at the value of 20.66 instead of blue colour. The light color A. altilis leaf tea is probably ascribed to very small polyphenolic molecules since a previous study showed that dark color of black tea infusions was from the oxidation and condensation of catechins during fermentation that formed larger polyphenolic molecules such as the aflavins and the arubigins [25].

CONCLUSION

In conclusion, *A. altilis* leaf infusion at 100°C displayed highest antioxidant activity for all assays; DPPH, FRAP, and ABTS. and phytochemical content, as compared to lower infusion temperature of 80°C and 45°C. Sensory testing scores also follow the same trend. Meanwhile, the nutritional content was not affected by the infusion temperature of leaf. However, *A. altilis* tea could not be a good source to provide minerals for consumers since at all temperatures of infusion, it could not provide sufficient minerals needed for individual.

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