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FULL PAPER

Antioxidant activities and polyphenol compounds of kenaf leaves tea infusion after *in-vitro* gastrointestinal digestion and consumer perception survey

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Abstract

Kenaf leaves are one of the kenaf parts which has a high potential to be utilized as a tea leave infusion in Malaysia. Kenaf leaves are known to have high antioxidant properties. Kenaf leaves tea (KLT) can be prepared through a simple process, however, the consumer perception of the uses of KLT was not evaluated in the Malaysian market. In this study, the antioxidant properties were evaluated through *in-vitro* gastrointestinal digestion (GID). It was proven that KLT contains a high amount of total phenolic and flavonoid content, tested using DPPH, ABTS and FRAP assays. Although the antioxidant properties were digested throughout simulated gastro and intestine digestion, their content remained quite high after the digestion. Therefore, KLT should have a high potential to be introduced to the Malaysian market. The consumer perception survey obtained a total of 397 responses and it was clear that Malaysians frequently consume tea products at least once a week. Most consumers preferred a slightly sweet and light-coloured tea. While the majority of the respondent was not aware of the existence of KLT, their market acceptability was high as there were 47.4% of respondents chose to try KLT product once it is made available in the market. KLT made from the freeze-drying technique preserved the antioxidants properties, and KLT market potential was proven relatively high due to consumer perception of KLT was similar to commonly available tea products.

1. Introduction

Kenaf, also known as *Hibiscus cannabinus* L. is a valuable fibrous and herbaceous plant from the Malvaceae family and *Hibiscus* genus (Ayadi *et al.*, 2017). Kenaf is characterized as a multipurpose crop as it has a high number of industrial applications, for instance, fibre, biocomposite, bioenergy production, cooking oil, animal feedstock, oil and chemical absorbent, textile application, in the automotive industry and construction industry. Kenaf had once successfully drawn attention due to its potential utilization for the prevention of global warming and cleaning muddy water (Falasca *et al.*, 2014; Lim *et al.*, 2020).

Kenaf is useful in all its parts, including its fibres, seed oil (Goh *et al.*, 2021), and leaves. Kenaf seeds and kenaf leaves have been considered potential sources of industrial products, especially in the biopharmaceutical industry. However, their pharmacological effects and chemical compositions are still poorly studied. Literature has revealed that there is insufficient scientific evidence for polyphenolic compounds and the antioxidant activity of kenaf leaves. No doubt, tea leave infusions made from plant leaves and roots are highly consumed because of their potential health benefits (Metussin *et al.*, 2017). In some research, kenaf leaf is strongly suggested as a potential alternative antioxidant source in the food and biopharmaceuticals industry (Jin *et al.*, 2013; Wong *et al.*, 2014; Ryu *et al.*, 2017).

Kenaf leaves contain a comparable amount of antioxidant content and activity to other tea, such as green and black tea (Jin *et al.*, 2013). However, the evaluation of kenaf leaves as tea is not explored, especially in the Malaysian market. Therefore, this study aimed to determine the stability of antioxidant activity and polyphenolic compounds of freeze-dried kenaf leaves tea infusion after 2-stage gastrointestinal phases by evaluating the antioxidant properties (DPPH, FRAP, ABTS, TPC, and TFC analysis). Also, the evaluation of consumer awareness, perception and market potential of KLT will be conducted by an online self-administered questionnaire. This information will provide useful information for further product research and development.

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2. Materials and methods

2.1 Materials and chemicals

The 90 days after sowing (DAS) kenaf (*Hibiscus cannabinus* L.) leaves were obtained from Lembaga Kenaf and Tembakau Negara (LKTN). All the reagents and chemicals were purchased from a local supplier (Nextgene Sdn. Bhd) unless stated otherwise.

2.2 Preparation of kenaf leave and kenaf leaves tea

The kenaf leaves were frozen at −80°C for 24 hrs and dried using a freeze dryer at 0.0004 mbar for another 24 hrs. The dried leaves were grounded into fine particles and stored in sealed vacuum packing until needed.

The kenaf leaves tea (KLT) was prepared using at 1% concentration (w/v) using boiling water for 5 mins. The residue was removed by filtration using filter paper.

2.3 In-vitro gastrointestinal digestion of kenaf leaves tea

A two-staged *in-vitro* simulated gastrointestinal digestion (GID) model was used according to the previously reported methods (Donlao and Ogawa, 2017). The two-stage GID model started with simulated gastric fluid (SGF) with 2% NaCl, 7% of HCL and 2% pepsin, pH adjusted to 1.5. 170 mL sample of KLT was mixed with 19 mL of SGF, incubated at 37°C with 100 rpm of stirring speed. Then, 0.5 mL of the gastric digested sample was mixed with 4.5 mL of 70% methanol, followed by centrifugation at 1800 × *g* for 10 mins. The sample was further adjusted to pH 6 using 3M NaOH solutions.

The small intestinal phase was initiated by adding 23 mL of simulated intestinal fluid (SIF), which consist of 6.8% KH_2PO_4, 8.7% NaCl, 5% bile salt and pH adjusted to 6.8. The sample was mixed with SIF and pH was further adjusted to 6.8 again using 0.5 M NaOH. The sample was then maintained at the intestinal condition for 150 mins with 100 rpm stirring. By the completion of incubation, 0.5 mL of sample were treated with 4.5 mL of 70% methanol, followed by centrifugation at 1800 × *g* for 10 min again and their remaining antioxidant content for both phases was tested.

2.4 Determination of polyphenolic content and antioxidant activity

The total phenolic content (TPC) was determined using Folin-Ciocalteu assay, while total flavonoid content (TFC) was determined using the aluminium trichloride colourimetric method (Sim and Nyam, 2019). Antioxidant activities were determined by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay and 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assay. Besides Ferric reducing antioxidant power (FRAP) was also conducted on KLT samples (Wootton-Beard *et al.*, 2011).

2.5 Consumer perception and market potential of kenaf leaves tea

A questionnaire was conducted to determine the consumer perception and market potential of KLT in Malaysia. The sampling subjects were youth and young adults in Malaysia. The questionnaire was developed to evaluate the consumers' perception and market potential of kenaf leaves tea based on several studies on different tea products (de Godoy *et al.*, 2013). There were sixteen questions divided into two sections such as demographic profiles, tea consumption behaviour, tea preferences and perceptions towards kenaf tea. All respondents were required to agree to participate in this study by clicking "Yes" in the informed consent section to indicate their voluntary participation, or else their responses will not be considered. All information was kept confidential and used solely for this research. The first section of the questionnaire was the socio-demographic profile. The socio-demographic profile included gender, age, race, nationality and marital status. The second section included tea consumption behaviour, tea preferences and perceptions towards kenaf leaves tea. All questions were assessed by a 7-point Likert scale and an 11-point hedonic scale.

2.6 Statistical analysis

All of the data obtained were expressed in mean±standard deviation (SD). One-Way ANOVA and followed by Tukey's HSD post hoc test was conducted using SPSS software.

3. Results and discussion

3.1 The polyphenols compounds stabilities and antioxidant activities of kenaf leaves tea after in vitro gastrointestinal digestion

Table 1 shows the descriptive statistics of polyphenol content and antioxidant activity of KLT infusion at the initial phase, after the gastric phase and after the small intestine phase. First, the bioactive compounds tested as total phenol or flavonoid compounds decreased after the gastric and intestine phases. The same trend was observed in DPPH and ABTS scavenging activities as well.

Among the parameters tested, TFC recorded was the highest (5545.83±275.38 mg QHE /100 g), while TPC was 3104.17±16.60 mg CAE/100 g. These results indicated that kenaf leaves bioactive compounds were preserved in the ground powder form of infusion tea and

Table 1. Descriptive statistics of polyphenol content and antioxidant activity of KLT infusion after *in-vitro* GID.

Time	DPPH (%)	ABTS (%)	FRAP (mg TEAC/100 g)	TPC (mg CAE/100 g)	TFC (mg QHE/100 g)
Initial phase	24.31±1.14[a]	67.84±1.15[a]	2780.28±4.81[a]	3104.17±16.60[a]	5545.83±275.38[a]
Gastric phase	13.5±1.34[b]	21.95±0.97[b]	756.39±33.39[b]	602.50±73.78[b]	1520.83±62.92[b]
Small Intestine phase	3.93±0.88[c]	3.71±1.29[c]	488.06±38.63[c]	367.50±119.46[c]	687.50±66.14[c]

Values are presented as mean±SD based on triplicate readings (n = 6). Values with different superscripts within the same column are significantly different ($p<0.05$) measured by Tukey's HSD test.

presented antioxidants properties. The trend of TFC being digested was in agreement with previously reported studies (Ryu *et al.*, 2017; Omenna and Ojo, 2018). On the other hand, the TPC results obtained were also comparable with the previously reported study on freeze-dried KLT infusion (Kho *et al.*, 2019; Sim and Nyam, 2019). The loss of polyphenol after *in-vitro* digestion was expected due to the extreme digestion in both gastric and intestinal conditions (Chew *et al.*, 2015). These results also indicated that phenolic compounds in KLT are highly sensitive to the extreme pH conditions in the stomach and small intestine. Some authors suggested that these secondary metabolites could be degraded into respective by-products after the digestion process occurs in the gastrointestinal tract (Ryan and Prescott, 2010; Wong *et al.*, 2014). The DPPH scavenging activity of KLT infusion was found to be unstable after *in-vitro* GID conditions ($p<0.05$) and left only 16% of the scavenging power compared to initial phase KLT.

Similarly, the antioxidative activities evaluated using DPPH, FRAP and ABTS assay were all showing aligned trends with the decrease in total phenol and/or total flavonoid content. A similar trend of decreasing DPPH scavenging power was observed in kenaf extract (Wong *et al.*, 2014) and green tea (Donlao and Ogawa, 2018). Also, DPPH scavenging activities were recorded the lowest as compared to ABTS and FRAP assay. This result was in agreement with other studies comparing the value obtained from a variety of fruits, vegetables and beverages that the DPPH assay always exhibits lower activities as compared to the ABTS assay (Floegel *et al.*, 2011; Shannon *et al.*, 2017). Fresh KLT had a FRAP assay value which was in agreement with finding from frozen kenaf leaves again (Sim and Nyam, 2019). The antioxidative activities reduction was significantly reduced after stimulated digestion. However, the reduction was tally with the digestion of phenolic and flavonoid compounds.

3.2 Correlation among the polyphenols compounds stabilities and antioxidant activities of kenaf leaves tea after in vitro gastrointestinal digestion

Table 2 shows the Pearson correlation among the test and it was clear that all the antioxidants and phenolic content stabilities were highly correlated and significant. The reduced antioxidant compounds were related to their scavenging activities while the content of TPC and TFC remained at concentrations of 367.50±119.46 mg CAE/100 g and 687.50±66.14 mg QHE/100 g, respectively. The digestive enzymes are effective to degrade the phenolic and flavonoid compounds and similar observations were observed in other plant tea, for instance, Pereskia bleo (Johari and Khong, 2019) and green tea leaves (Donlao and Ogawa, 2017).

Table 2. Pearson Correlation Coefficient among five antioxidant analyses

Traits	DPPH	ABTS	FRAP	TPC
ABTS	0.972*	-	-	-
FRAP	0.920*	0.984*	-	-
TPC	0.909*	0.978*	0.998*	-
TFC	0.936	0.990	0.996*	0.993*

*Significant level at $p<0.05$

3.3 Respondents' socio-demographic profiles

A total of 397 respondents were recruited for this study and the detail is shown in Table 3. There demographic variables obtained by the questionnaire included age, gender, nationality, race and marital status. Based on the data collected, the majority (46.6%) of them were aged between 18-23 years old. There were 61.2% of them were female and 38.8% were male. Among all respondents, there were 77.3% of them who were single while 20.4% were married. The race of respondents was Malay (25.9%), Chinese (65.6%), Indian (5.8%), and others (2.8%).

Table 3. Descriptive socio-demographic profiles of respondents (n = 397)

Variables		No. of subjects	Percentage (%)
Age	< 18	72	18.1
	18-23	185	46.6
	24-29	86	21.7
	30-35	46	11.6
	>35	8	2.0
Gender	Male	155	38.8
	Female	243	61.2
Race	Malay	103	25.9
	Chinese	260	65.5
	Indian	23	5.8
	Others	11	2.8
Marital Status	Single	307	77.3
	Married	81	20.4
	Other	9	2.3

3.4 Consumer perception

Table 4 shows the frequency distribution of tea consumption of respondents. There were 132 out of 397 the participants who consumed tea once a week which occupied the most percentage of 33.2%. There were also a large number of the respondent (27.7%) who enjoyed tea two-three times a week and 25.4% consumed tea once a month or less. The remaining participants who consumed tea once a day, more than two times a day were 10.8% and 2.8% respectively. The increased consumption of tea beverages among Malaysians was aligned with the findings obtained by Norimah *et al.* (2008). Besides, it was reported that the daily caffeine source was contributed by tea consumption, which recorded 72.7 %, while coffee was the second biggest portion (Muhammad *et al.*, 2019).

Table 4. Frequency distribution of tea consumption frequency (n = 397)

	Variables	Frequency	Percentage
Tea consumption frequency	Once a month or less	101	25.4
	Once a week	132	33.2
	Two-three times a week	110	27.7
	Once a day	43	10.8
	More than two times a	11	2.8

*Significant level at $p<0.05$

On the other hand, based on the descriptive statistics of respondents for flavours and colour intensity of tea measured by a 7-point Likert scale were tabulated in Table 5. It was apparent that the majority of the respondents (33.2%) preferred their tea to be neither too bitter nor too sweet, followed by 32.0% of the respondents who preferred their tea to be just slightly sweet. Only a minority (5.5%) of respondents had chosen for scale 7 (extremely sweet) and proven the trends with increasing consumer health awareness towards sweetened beverages (Saleh *et al.*, 2018). The average scale for sweetness and bitterness was 4.89±1.01 indicating that consumer preference towards a tea flavour was between neutral and slightly sweet flavours. While the preference of colour, it was found that the mean was 3.63±1.11, indicating the consumers preferred a slightly light to neutral tea colour.

3.5 Consumer perception and awareness towards kenaf tea

Furthermore, consumers' perceptions and awareness of kenaf tea were also recorded throughout data collection. The results were tabulated as shown in Table 6. It was expected that the majority of the respondents (80.9%) were not aware of the market availability of kenaf tea. Similarly, up to 90.2% of the respondent did not know about the general health benefits of drinking kenaf leaves tea, therefore as high as 85.6% were not aware that kenaf leaves contain high antioxidant activities. This finding was not surprising due to the fact that Malaysia is not practising the culture of consuming kenaf leaves. Conversely, kenaf is used for industrial crops and is less reputable to the public as compared to other functional crops like palm oil and rubber.

From another point of view, there were 47.4% of the respondents chose to try the KLT product once it is made available in the market, 14.4% of them mentioned that they were not likely to try kenaf tea despite its various health benefits. In some cases, targeted consumers did not purchase the product due to unwillingness to pay even though they were interested in the products (Joshi and Rahman, 2015). In this study, it was observed that many of the respondents (42.8%) have a half-chance of paying more for a healthier tea option, while a great number of them (33.8%) were willing to pay more for a healthier tea beverage.

In addition, the majority of the respondents agreed that kenaf leaves tea is good for health (45.1% slightly agree, 14.1% agree, 5.5% strongly agree) while there were nearly half of the respondents agreed that kenaf leaves tea could be a better tea option than other commercial tea products (31.7% of slightly agree, 12.8% agreeing, 4.8% strongly agree).

In the last section of the questionnaire, the likelihood of respondents purchasing kenaf tea products was obtained by an 11-point scale (Lewis and Erdinç, 2017). Most of the respondents voted that they are likely to purchase and try kenaf leaves tea (16.4% slightly likely, 23.7% moderately likely, 22.4% very much likely, 14.1% extremely likely, 8.3% greatest imaginable likely). There were 9.6% of them were uncertain on if they would buy and try kenaf leaves tea. Fortunately, only a minority of respondents were not likely to purchase and try kenaf leaves tea. This result indicated that most of the study population who participated in this study were adventurous to try new tea beverages in the market.

4. Conclusion

This study proved that KLT prepared using the freeze-drying technique was at high bioavailability access by *in-vitro* GID conditions. The consumer preference survey revealed that the KLT had the potential to be commercialized as a tea product in Malaysia. The majority of the consumer reflected that slightly sweet and light-coloured tea was preferred.

Table 5. Descriptive statistics of preference and behavior (n = 397)

Variables	7-point Likert scale	Frequency	Percentage (%)	Mean±SD
Flavor intensity	1 – Extremely bitter	0	0.0	4.89±1.01
	2 – Moderately bitter	1	0.3	
	3 – Slightly bitter	23	5.8	
	4 – Neutral	132	33.2	
	5 – Slightly sweet	127	32.0	
	6 – Moderately sweet	92	23.2	
	7 – Extremely sweet	22	5.5	
Colour Intensity	1 – Extremely bitter	3	0.8	3.63±1.11
	2 – Moderately bitter	61	15.4	
	3 – Slightly bitter	129	32.5	
	4 – Neutral	104	26.2	
	5 – Slightly sweet	87	21.9	
	6 – Moderately sweet	13	3.3	
	7 – Extremely sweet	0	0.0	
Do you agree that Kenaf leaves tea is good for health?	1 – Extremely bitter	3	0.8	4.76±1.08
	2 – Moderately bitter	9	2.3	
	3 – Slightly bitter	27	6.8	
	4 – Neutral	101	25.4	
	5 – Slightly sweet	179	45.1	
	6 – Moderately sweet	56	14.1	
	7 – Extremely sweet	22	5.5	
Do you agree that Kenaf leaves tea could be a better tea than other commercial tea products?	1 – Extremely bitter	5	1.3	4.43±1.24
	2 – Moderately bitter	21	5.3	
	3 – Slightly bitter	56	14.1	
	4 – Neutral	119	30.0	
	5 – Slightly sweet	126	31.7	
	6 – Moderately sweet	51	12.8	
	7 – Extremely sweet	19	4.8	

Table 6. Frequency distribution of consumer perception and awareness towards kenaf tea (n = 397)

Variables		Frequency	Percentage (%)
Have you heard of kenaf leaf tea?	Yes	76	19.1
	No	321	80.9
Do you know the general health benefits of drinking kenaf leaf tea?	Yes	39	9.8
	No	358	90.2
Do you know that Kenaf leaf tea has high antioxidant activity and polyphenolic compounds?	Yes	57	14.4
	No	340	85.6
Would you like to try this product if it's available in the market and has a lot of health benefits?	Yes	188	47.4
	No	57	14.4
	Maybe	152	38.3
Would you pay more to buy for a healthier option for tea beverages?	Yes	134	33.8
	No	93	23.4
	Maybe	170	42.8

Although using kenaf leaves as tea is not a culture in Malaysia, the survey also showed that the market potential is high because the majority (< 50%) of the respondents showed a high acceptance rate of KLT after being educated about its health benefits and are willing to try once it is made available.

Conflict of interests

The authors declare no conflict of interest.

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