

## The infusion of goji berries and red dates ameliorates the overall qualities of kenaf leaves tea

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### Abstract

Kenaf (*Hibiscus cannabinus*) is an industrial crop in Malaysia, and especially used as a source for composite wood. Kenaf leaves as a by-product of the plantation can be consumed as food due to its high nutritional value. Kenaf leaves have high antioxidant properties, thus are suitable to be made into herbal tea. However, its flavour is considered sour, and becomes a challenge for product development. The main objective of the present work was to evaluate the physicochemical and functional (ascorbic acid content, calcium content, and anti-diabetic) properties of kenaf leave tea (KLT) with 0, 60, and 100% kenaf leaves used. KLT was prepared by steaming and drying the kenaf leaves, followed by sieving. Then, the powder was mixed with distilled water at 1% (w/v). Another portion of red dates and goji berries tea (RGT) was prepared by boiling the red dates:goji berries:water at 1:2.5:28.5 ratio. Two portions of tea were infused using 60% KLT and 40% RGT. Results showed that a 100% KLT (positive control) was always highest in terms of total phenolic content (TPC) and total flavonoid content (TFC). The antioxidant activities were positively correlated with DPPH and ABTS scavenging activities, and ferric reducing antioxidant power (FRAP). Contrary, the negative control (0% KLT) showed the highest  $\alpha$ -amylase inhibitory effect. The present work also evaluated the acceptance of consumers using the Hedonic sensory test among 50 panellists with balance male and female candidates. Since 100% KLT extract was in low pH values ( $2.17 \pm 0.26$ ), 60% KLT infused with goji berries and red dates gained the highest consumers' acceptance. Therefore, as a compromise between sensory and functional properties, a maximum of 60% KLT was a suitable formulation for the consumers.

### Keywords

kenaf,  
antioxidant,  
sensory,  
infusion tea

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### **Introduction**

Kenaf is an annual herbaceous species belonging to the family Malvaceae, and has a great potential to be used as fibre, energy, and feedstock. Kenaf has been domesticated as a fibre plant since 6,000 years ago, and it is a common wild plant in most of the African countries (Mariod *et al.*, 2010). Nowadays, kenaf has been widespread in the tropics and subtropics countries. In Malaysia, the National Economic Action Council has initiated a kenaf project in 1999 for the growth of kenaf as an industrial crop to minimise the issues of deforestation due to the increasing demand for paper and fibre-based products (Mohd *et al.*, 2014).

Kenaf is a versatile plant material where almost all of the components have their own usage. Kenaf fibre is strong enough to be made into fishnet (Ayadi *et al.*, 2017). Kenaf seed is used to produce cooking oil and as traditional medicine (Kamal, 2014). Kenaf leaves as one of the major components

of the plant are nutritionally healthy as compared to other leafy vegetables. Kenaf leaves are also used to treat stomach disorder, and possess erythrocyte protective activity against drug-induced oxidative stress when consumed as vegetables. Kenaf leaves are also applicable in treating dysentery and throat disorders. Kenaf leaves aqueous extracts have haematinic activity on haemolytic anaemic rats (Agbor *et al.*, 2005).

Owing to the aforementioned benefits, kenaf leaves have the potential to be made into herbal tea. Herbal tea is a generic term used among the Chinese when plant extract is used in beverage form, which serves as a traditional medicine (Shen *et al.*, 2020). Kenaf leaves have a natural sour taste, and are believed to have therapeutic and energising properties. It is also reported that kenaf leaves are good for digestive problem and immune system (Jung *et al.*, 2013). However, kenaf leaves tea may not appear as a mainstream beverage choice among consumers due to the underutilisation of this crop and

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the natural sour taste. Kenaf leaves tea should therefore be introduced to consumers in combination with other products that are well accepted. In this case, goji berries and red dates are chosen as common herbal tea products to be incorporated with kenaf leaves as a new combination. Therefore, the present work aimed to determine the consumers' preference, physicochemical properties, antioxidant activities, phenolic and flavonoid compounds, and hypoglycaemic effects ( $\alpha$ -amylase inhibitory effect) of the infused tea prepared from kenaf leaves, goji berries, and red dates.

## Materials and methods

### Materials and chemicals

Kenaf (*Hibiscus cannabinus* L.) leaves were obtained from the Lembaga Kenaf and Tembakau Negara (LKTN). Dried goji berries and red dates were purchased from a local Chinese traditional medicine retail shop (Eu Yan Sang Company). All chemicals used were purchased from a local supplier (Merck, Malaysia) based on the purity and grade required.

### Dry kenaf leaves preparation

Fresh kenaf leaves were washed and steamed for 35 s at 100°C. Then, the leaves were baked in a convection oven for 1 h at 120°C. Finally, the dried leaves were ground and sieved through 154  $\mu$ m mesh. The ground leaves were vacuum-packed and stored at -20°C until further use.

### Infusion tea preparation

Kenaf leaves tea (KLT) was prepared using dried kenaf leaves powder and boiling water at a ratio of 1:100. The kenaf leaves powder was allowed to sit in boiling water for 5 min with constant stirring before filtration using filter paper. Red dates and goji berries tea (RGT) was prepared at a ratio of red dates:goji berries:water of 1:2.5:28.5. Ingredients were rinsed with lukewarm (60°C) water, drained, boiled, and simmered for 30 min at 100°C. Then, it was filtered to obtain a clear solution. Lastly, the infused tea was prepared by combining both liquid portions.

### Colour, pH, and refractive index measurements

The colour of tea samples was measured using a Hunter Lab colorimeter with the Easy Match QC software version 4.60. The pH of tea samples was measured using a calibrated pH meter (Jenway, United States). The refractive index of tea samples was measured using a refractometer (Kimble,

United States).

### Calcium content

The calcium content was determined using atomic absorption spectrometry (AAS). Briefly, 5 mL of tea sample was mixed with  $\text{LaCl}_3$  (5% w/v) solution, and absorbance was read at 493 nm. The calcium content was obtained by comparing with calcium carbonate ( $\text{CaCO}_3$ ) standard calibration curve ranging from 0 to 200 mg/L (Sowmya *et al.*, 2015).

### Ascorbic acid content

The ascorbic acid content was determined using titration against the iodine solution. Briefly, 25 mL of tea sample (50 mg/mL) was added with 0.1 mL of 1% starch solution as an endpoint indicator. Ascorbic acid content was calculated based on the titrate volume of standard ascorbic acid of 1 mg/mL (Keenan *et al.*, 2012).

### Antioxidant properties

Antioxidants properties including 2-2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities, 2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging (Jin *et al.*, 2013), ferric reduction antioxidant power (FRAP) (Wootton-Beard *et al.*, 2011), total phenolic content (TPC) (Lim *et al.*, 2007), and total flavonoid content (TFC) (Ogbunugafor *et al.*, 2011) of tea samples were determined following previously described procedures.

### $\alpha$ -amylase inhibitory assay

A 50  $\mu$ L phosphate buffer (100 mM, pH = 6.8), 10  $\mu$ L  $\alpha$ -amylase (2 U/mL), and 20  $\mu$ L tea sample were mixed in 96-well plates, and pre-incubated at 37°C for 20 min. Then, 20  $\mu$ L of 1% starch solution was added into the mixture, and incubated for another 30 min at 37°C. After that, 100  $\mu$ L of DSA (dinitrosalicylic acid) reagent was added into the mixture, and the 96-well plate was placed into a water bath for 15 min at 85°C. The absorbance was read at 510 nm. All the results were expressed as percentage inhibition using Eq. 1:

$$\text{Inhibition percentage} = (1 - A_s/A_c) \times 100 \quad (\text{Eq. 1})$$

where,  $A_s$  and  $A_c$  = absorbance value of the sample and control, respectively (Telagari and Hullatti, 2015).

### Sensory evaluation

Each panellist was given three samples (60,

80, and 100% KLT) randomly labelled as three-digit code generated from a random number table. Each panellist was required to evaluate the samples, and rate the degree of liking in terms of taste, colour, aroma, and overall acceptance. A nine-point Hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely) was used.

#### Experimental design and statistical analysis

The experiment was started by comparing a formula consisting of 60% KLT and 40% of RGT, with both positive (100% KLT) and negative control (0% KLT) in terms of their physicochemical properties. Then, the sensory evaluation involving 50 panellists was tested using increased KLT concentration (60, 80, and 100% KLT) to evaluate the likeliness of high percentage KLT consumption by consumers. All values were expressed as mean  $\pm$  standard deviation (SD). All the values were obtained with a total of four replicates ( $n = 4$ ).

## Results and discussion

#### Colour, pH, and Brix values

From Table 1, we can observe that the addition of red dates and goji berries was effective in influencing the colour of the flavoured KLT. The colour of positive control (100% KLT) showed significant highest values of lightness ( $L^*$ ), and lowest  $a^*$  and  $b^*$  values among the samples. The colour attributes of 100% KLT were in light, green to yellow tone as compared to the sample with the addition of red dates and goji berries tea (RGT). The addition of RGT reduced the lightness, and increased the redness ( $a^*$ ) and yellowness ( $b^*$ ) of the product, thus producing a darker appearance. The difference in lightness was significantly different ( $p < 0.05$ ).

The acidity of 100% KLT was significantly ( $p < 0.05$ ) lower (2.17). This is in agreement with other studies which indicated that kenaf leaves are naturally perceived as sour taste, similar to roselle (*H. sabdariffa* L.) (Fasoyiro et al., 2005). The

increased pH values upon the addition of RGT (60 and 0% KLT) proved that flavouring with RGT could neutralise the acidity of kenaf leaves extract.

The Brix $^\circ$  values of KLT increased when a higher percentage of RGT was used. This indicated that kenaf leaves were low in sugar. The total soluble content (mainly soluble sugar) was contributed by the RGT addition.

#### Antioxidant activities of KLT

Table 2 shows the DPPH and ABTS scavenging activities (%), as well as total phenolic content, total flavonoid content, and ferric reduction. 100% KLT showed the highest antioxidant properties in all tests. It was believed that kenaf leaves extract prepared by oven drying improved the antioxidant properties. This result is in accordance with a previous study (Brozkova et al., 2016) which stated that oven drying of kenaf leaves can serve as a post-harvest storage method while improving the antioxidant activities (Mediani et al., 2014; Leng et al., 2017). A more recent study suggested that fresh kenaf leaves had the lowest value in the antioxidants activities test when compared to dried kenaf leaves (Sim and Nyam, 2019). The oven-drying process to produce KLT could have increased the antioxidative properties. Other research analysed the tamarind leaves (Leng et al., 2017), *Cosmos caudatus* leaves (Mediani et al., 2014), olive leaves (Boudhrioua et al., 2009), and Lamiaceae herbs (Hossain et al., 2010) and also showed that drying plant leaves had positive effects in enhancing the antioxidants properties (especially in TPC content). 100% KLT formation was expected to have the greatest antioxidant properties since kenaf leaves had been proven to contain higher antioxidants properties as compared to other parts of the kenaf plant (Ryu et al., 2006; 2017).

#### Pearson correlation test among antioxidants properties

Phenolic and flavonoid compounds are

Table 1. Colour ( $L^*$ ,  $a^*$ , and  $b^*$ ), pH, and Brix values of different kenaf leaves tea (KLT) formulations.

Sample	$L^*$	$a^*$	$b^*$	pH	Brix $^\circ$
100% KLT	41.58 $\pm$ 0.07 <sup>a</sup>	-1.863 $\pm$ 0.050 <sup>c</sup>	10.74 $\pm$ 0.06 <sup>c</sup>	2.17 $\pm$ 0.26 <sup>c</sup>	nil
60% KLT	39.33 $\pm$ 0.08 <sup>b</sup>	-0.183 $\pm$ 0.013 <sup>b</sup>	12.38 $\pm$ 0.06 <sup>b</sup>	3.38 $\pm$ 0.11 <sup>b</sup>	4.5 $\pm$ 0.57 <sup>b</sup>
0% KLT	29.41 $\pm$ 0.06 <sup>c</sup>	9.967 $\pm$ 0.065 <sup>a</sup>	36.56 $\pm$ 0.02 <sup>a</sup>	4.46 $\pm$ 0.13 <sup>a</sup>	9.5 $\pm$ 0.57 <sup>a</sup>

Values are the mean of two determination from two replicate experiments  $\pm$  standard deviation ( $n = 4$ ). Means followed by different lowercase superscripts in the same column are significantly different at  $p > 0.05$  by ANOVA and Tukey's test.  $L^*$  = lightness,  $a^*$  = red-green tone, and  $b^*$  = yellow-blue tone.

Table 2. Antioxidant activities of different kenaf leaves tea (KLT) formulations.

Sample	DPPH (%)	ABTS (%)	FRAP (TEAC) (mg/g)	TPC (CAE) (mg/g)	TFC (QHE) (mg/g)
100% KLT	41.58 ± 0.07 <sup>a</sup>	57.34 ± 1.181 <sup>a</sup>	1671.54 ± 11.85 <sup>a</sup>	1606.77 ± 16.44 <sup>a</sup>	5.371 ± 0.130 <sup>a</sup>
60% KLT	39.33 ± 0.08 <sup>b</sup>	40.05 ± 0.708 <sup>b</sup>	254.20 ± 5.96 <sup>b</sup>	1241.65 ± 12.30 <sup>b</sup>	0.872 ± 0.094 <sup>b</sup>
0% KLT	29.41 ± 0.06 <sup>c</sup>	25.75 ± 1.039 <sup>c</sup>	95.71 ± 10.10 <sup>c</sup>	828.36 ± 12.62 <sup>c</sup>	0.400 ± 0.061 <sup>c</sup>

Values are the mean of two determination from two replicate experiments ± standard deviation ( $n = 4$ ). Means followed by different lowercase superscripts in the same column are significantly different at  $p > 0.05$  by ANOVA and Tukey's test. TEAC = Trolox equivalent, CAE = chlorogenic equivalent, and QHE = quercetin hydrate equivalent.

Table 3. Pearson correlation coefficient ( $r$ ) among total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacities of different kenaf leaves tea (KLT) formulations.

Trait	DPPH	ABTS	FRAP	TPC
ABTS	0.982*			
FRAP	0.966*	0.928*		
TPC	0.957	0.863*	0.893*	
TFC	0.910*	0.993*	0.976*	0.820*

\*significant at  $p < 0.05$ .

believed to act as scavengers of free radicals produced during oxidation. The radical-scavenging power is positively correlated to the total phenolic and flavonoid contents. Therefore, Pearson's correlation analysis was performed (Table 3) which showed that all the antioxidant analyses were positively correlated at significant level ( $p < 0.05$ ); total phenolic and flavonoid contents were responsible for the DPPH and ABTS radical scavenging and ferric reduction power, respectively.

#### Functional properties of KLT

The ascorbic acid content from the three

samples were not significantly different ( $p > 0.05$ ), and ranged from 1.08 to 1.22 mg/100 mL (Table 4). Although kenaf plant is considered as one of the plants containing high ascorbic acid (Ayadi *et al.*, 2017), more data are still scarce. Nevertheless, the ascorbic data in red dates (Visnjevec *et al.*, 2019) and goji berries (Niro *et al.*, 2017) are widely available. It is known that a dried fruit (especially red dates and goji berries) have lower ascorbic acid content, and further boiling of the red dates and goji berries tea could cause the loss of ascorbic acid. Ascorbic acid in the food matrix could be lost due to heat and heating duration (Herbig and Renard, 2017). The oven-drying method applied to kenaf leaves was believed to be the main reason for its low ascorbic acid content. Interestingly, the low ascorbic acid had indirectly proven that high antioxidative power presented by 100% KLT was due to the high phenolic content. Although ascorbic acid generally acts as an antioxidant (Ibuki *et al.*, 2020), it did not contribute highly to our samples.

Calcium content was the highest in the positive control ( $p < 0.05$ ). Calcium content of kenaf leaves has been reported to be higher than the other parts of kenaf plants (Kim *et al.*, 2018). This indicated that the oven drying of kenaf leaves did not cause calcium content loss. Similarly, pumpkin leaves (Namadi and Sarah, 2017) and bitter leaves

Table 4. Ascorbic acid and calcium contents, and  $\alpha$ -amylase inhibitory activity of different kenaf leaves tea (KLT) formulations.

Sample	Ascorbic acid (mg/100 mL)	Calcium (mg/100 mL)	$\alpha$ -amylase inhibitory (%)
100% KLT	1.22 ± 0.05 <sup>a</sup>	1.026 ± 0.0123 <sup>a</sup>	10.58 ± 1.25 <sup>b</sup>
60% KLT	1.08 ± 0.15 <sup>a</sup>	0.354 ± 0.007 <sup>b</sup>	19.17 ± 1.62 <sup>a</sup>
0% KLT	1.09 ± 0.09 <sup>a</sup>	0.076 ± 0.002 <sup>c</sup>	21.18 ± 1.33 <sup>a</sup>

Values are the mean of two determination from two replicate experiments ± standard deviation ( $n = 4$ ). Means followed by different lowercase superscripts in the same column are significantly different at  $p > 0.05$  by ANOVA and Tukey's test.

(Garba and Oviosa, 2019) were revealed to retain their calcium contents even after drying including air, sun, oven, and microwave drying.

Kenaf has been reviewed to have several functional properties including the ability to inhibit  $\alpha$ -amylase enzyme, which is often associated with anti-diabetic property (Cheng *et al.*, 2016). A study demonstrated that kenaf leaves exhibited anti-diabetic effects in rats (Kumar *et al.*, 2011). In the present work, however, the inhibitory effect of  $\alpha$ -amylase enzyme was lowest in 100% KLT sample. No doubt, goji berries were tested to possess anti-diabetic properties due to the presence of *Lycium barbarum* polysaccharides (Cai *et al.*, 2015; Chen *et al.*, 2019). From this, we can deduce that KLT was lower in relevant polysaccharides which could contribute to inhibit  $\alpha$ -amylase enzyme. This was also explained by significantly lower Brix° value in 100% KLT. However, we did not reject that the high content of phenolic and/or flavonoid compounds in KLT could also contribute to the inhibitory effects (Oyedemi *et al.*, 2017). In addition, KLT flavoured by goji berries and red dates can synergistically exhibit an inhibitory effect of  $\alpha$ -amylase enzyme because there was no significant difference between 60 and 0% KLT samples.

### Sensory evaluation

The 60, 80, and 100% KLT were used in sensory analysis involving 50 panellists since higher percentage of kenaf leaves had more antioxidant properties. Negative control (0% KLT) was removed from the experimental design. Figure 1 shows the likeliness score of three samples evaluated by the

Hedonic test. It is apparent that 60% KLT was most accepted by the panellists (overall acceptance scores between 7 to 8, thus indicating “like moderately” to “like very much”). Moreover, the scores of all three samples decreased with increasing percentage of KLT; 100% KLT scored significantly lower ( $p < 0.05$ ) in terms of all attributes (colour, aroma, taste, and overall acceptance) as compared to the formula flavoured with RGT. These data suggested that 100% KLT was less accepted by the panellists because of the low pH values (sour flavour).

Both kenaf and roselle are from the Malvaceae family. A study showed that water extraction of roselle had a pH value of 2.4 (Jung *et al.*, 2013). This is in agreement with the present work which showed that 100% KLT had a pH value of 2.17. Additionally, a sensory analysis conducted using roselle revealed a similar trend as in the present work where a lower percentage of roselle yielded higher acceptance by the panellists (Fasoyiro *et al.*, 2005).

### Conclusion

In conclusion, the antioxidative properties of kenaf leaves was preserved even after oven-drying. Results were in agreement with previous findings regarding the antioxidative properties and high calcium content. Although the ascorbic acid content and  $\alpha$ -amylase inhibitory effect were lower in 100% KLT, the high phenolic content should be appreciated. Besides, flavouring the 60% KLT using RGT at 40% was highly recommended, as evaluated from the sensory evaluation which revealed high acceptance in terms of colour, aroma, and taste.

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Sensory attributes (colour, aroma, taste, overall acceptance) of 100% KLT, 80% KLT and 60% KLT.

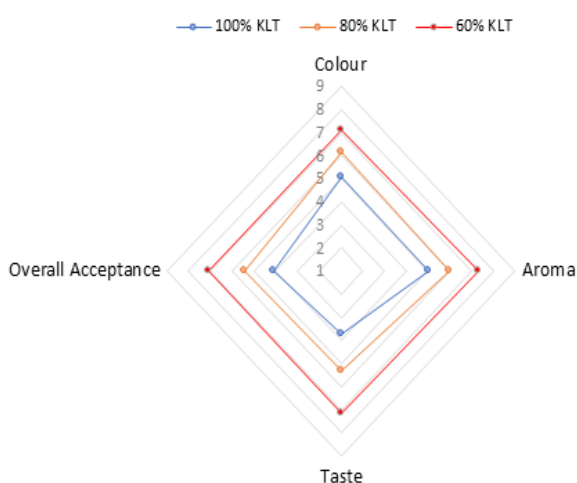


Figure 1. Sensory attributes (colour, aroma, taste, overall acceptance) of 100% KLT, 80% KLT and 60% KLT.

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