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PRELIMINARY STUDY OF ANTIOXIDANT ACTIVITY OF DRAGONFRUIT, DATES AND KEFIR MIXTURES

STUDI AWAL AKTIVITAS ANTIOKSIDAN CAMPURAN BUAH NAGA, KURMA, DAN KEFIR

Research Report
Penelitian

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A B S T R A C T

Background: *Some previous studies showed that either dragon fruit, dates, or kefir has antioxidant activity. Nevertheless, there has not been any study that showed the activity of the combination of those three elements.* ***Purpose:*** *Therefore, the main purpose of this study is to examine the best combination of dragon fruit, dates, and kefir as an antioxidant activity.* ***Method:*** *The combination variation includes: dragon fruit-dates, dragon fruit-kefir, kefir-dates, and dragon fruit-dates-kefir. Then, the antioxidant activity was tested using the DPPH assay.* ***Result:*** *The variation of dragon fruit and dates, the highest value of antioxidant activity was obtained from the combination of 10: 2 of dragon fruits: dates; that was 76.33 ± 0.27 mg/dL. The combination of dragon fruit and kefir, the highest value was obtained from the variation of 10: 4 of dragon fruit: kefir. For this variation, the antioxidant activity was 80.9 ± 0.4 mg/dL. The combination of kefir and dates, the highest value was obtained from the variation of 10: 3 of kefir: dates. The combination of dragon fruit, dates, and kefir, the highest value of antioxidant activity was obtained from the combination of 4: 10: 1 of dragon fruit: kefir: dates; that was 80.94 ± 0.4 mg/dL.* ***Conclusion:*** *In summary, the highest antioxidant activity came from the combination variation of 4: 10: 1 of dragon fruit: kefir: dates.*

A B S T R A K

Latar belakang: Beberapa penelitian yang pernah dilakukan sebelumnya menunjukkan bahwa, baik buah naga, kurma atau kefir memiliki aktivitas sebagai antioksidan. Namun, belum pernah ada penelitian yang menyatakan tentang aktivitas antioksidan dari kombinasi ketiga bahan tersebut. **Tujuan:** Oleh karena itulah, penelitian ini dilakukan untuk mengevaluasi bioaktivitas antioksidan dari berbagai macam variasi kombinasi ketiga bahan tersebut. **Metode:** Variasi kombinasi tersebut meliputi: buah naga-kurma, buah naga-kefir, kefir-kurma, dan yang terakhir adalah buah naga-kurma-kefir. Aktivitas antioksidan dari kombinasi tersebut diuji menggunakan uji DPPH. **Hasil:** Empat macam kelompok variasi kombinasi diperoleh data, bahwa aktivitas antioksidan tertinggi adalah sebagai berikut, (1) kombinasi buah naga: kurma (10: 2) = 76,33 ± 0,27 mg/dL, (2) kombinasi buah naga: kefir (10: 4) = 80,9 ± 0,4 mg/dL, (3) kombinasi buah naga: kefir: kurma (4: 10: 1) = 80,94 ± 0,4 mg/dL. **Kesimpulan:** Kombinasi buah naga: kefir: kurma (4: 10: 1) memiliki aktivitas antioksidan tertinggi.

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INTRODUCTION

Indonesia is a tropical country that is gifted with a high number of natural-resources types. The biodiversity of plants growing in Indonesia is about more than 25,000 types. This natural wealth has been used to support the lives of the living beings, especially those with medicinal benefits. There are numerous potential medicinal plants that have not been published yet, especially from their biological activity aspect (Wulansari *and* Chairul, 2011).

Many studies reported that the biological activities of natural products focused on the variety of plant species. The majority of those biological activities include the ones that demonstrate anti-cancer, anti-inflammatory, hepatoprotective, and those having antioxidant activities. The latest biological activity known fragmentarily was reported in medicinal plants. Also, the biological activity, as antioxidant, is frequently reported in many plant species (Ekiert *and* Szopa, 2020).

Antioxidant activity plays an important role to help indicate the potential of the other biological activities. This condition is based on the special ability of antioxidant compound to overcome many metabolic disorders and many other patological conditions such as cardiovascular or respiratory disorder, inflammation, carcinogenesis, and aging (Vergara *et al*., 2021).

Antioxidants are divided into two groups: synthetic and natural, which most of them are phenolic compounds. Natural antioxidants are primarily phenolics that may be found in all parts of plants, such as fruits, vegetables, seeds, leaves, and roots (Lourenco *et al*., 2019). Those natural substances were soon replaced by synthetic chemicals which are easier to get and have greater antioxidant activity, but are still restricted to a few compounds. It became the main reason that natural products are still the best. Further, it should be highlighted that the major sources of natural antioxidants come from fruits, vegetables, whole grains, green and black tea, coffee, herbs and spices (Akbarirad *et al*., 2016).

Free radical continuously occurs in cells as a part of a normal cellular function. On the other side, the excessive free radical production from endogenous or exogenous sources might lead to disease. To prevent the activity of free radicals, the body needs compounds that are called antioxidants. Antioxidants block the free radical to induce tissue damage by preventing the radicals, scavenging them, or promoting radicals to decomposition (Young *and* Woodside, 2001). It can be concluded that the main performance of antioxidants is delaying, preventing, or even removing oxidative harms caused by oxidative stress (Banjarnahor *and* Artanti, 2014).

Dragon fruit (*Hylocereus spp*.) belong to the *Cactaceae* family in plant kingdom. The genus, *Hylocereus*, is nowadays included into Selenicereus group (Korotkova *et al*., 2017). Originally, dragon fruit were native to Mexico, Central America, and then spread worldwide. There are three common types of the plant that are cultivated worldwide including: *Hylocereus undatus*, *H*. *monachantus*, and *H*. *megalanthus* (Ortiz-Hernandez *and* Carrilo-Salazar, 2012).

Dragon fruit were first introduced in Indonesia around 2000. It came from Thailand and spread into many regions in Indonesia (Purba R., 2012). There are two peeled types of dragon fruit that are very common in Indonesia, white and red-flesh dragon fruit (Kristanto, 2009). Dragon fruit are usually consumed directly or being processed into juice (R. *et al*., 2010).

According to Park *et al.* (2008), polyphenols play the main role in contributing to their antioxidant activity. Polyphenols, specially the flavanoids, can be found mostly in the pulps, peels and seeds of the dragon fruit. On the other hand, Esquivel *et al.* (2007) found out that betalains that were containing both phenolic and non-phenolic structures became the main factor for the major antioxidant capacity of purple *Hylocereus* juices.

Dragon fruit are gaining much attention recently because their phenolic compounds that possess antioxidant activity. Betalains in pulps of purple *Hylocereus* species were responsible for the major antioxidant capacity (Esquivel *et al*., 2007). The antioxidant activity of dragon fruit using water extract with DPPH assay was 261.520 ± 0.980 µg/mL (Lourith *and* Kanlayavattanakul, 2013).

Phoenix dactylifera L., well known as dates, is the most important crop in Mauritania and all of North Africa. Nowadays, there are more than 5000 cultivars of date palm that are known in all over the world (Ibrahim, 2008). Date palm fruit are composed of a fleshy pericarp, a membranous endocarp, and seed (Lemine *et al*., 2014).

The date palm (*Phoenix dactylifera L*.) is grown in over 30 countries. Dates are usually consumed freshly or processed into juice, jam, and any other value-added products. The phytochemicals of dates are reported into several classes: phenolics, alkaloids, steroids, terpenes, and saponins. Those compounds have many bioactivities such as antimutagenic, antimicrobial, and antioxidant. Phenolic compounds are those who take responsibility to the antioxidant activity. Phenolic in dates can act as a hydrogen donor, a reducing agent, a metal chelator, and a singlet oxygen quencher. Studies on various date fruit cultivars demonstrated a linear relationship between antioxidant activity and phenolic content. Dates serve as a good source of natural antioxidants and could potentially be considered as functional food or ingredient (Al-Jasass *et al*., 2015).

Pyhtochemical screening reported that date fruit contained poliphenolic compounds, such as flavanoids, prosianidine, and antocianidine (Fahlevi, 2015). In Saudi Arabia, a report showed that antioxidant activity of ethanol extract of date fruit has IC_{50} for about 84.92 ppm and 600.3 mg/100 mg of d-cathecine (Al-Mamary *et al.*, 2014). Those two data were enough to state that date fruit have a strong antioxidant activity (Elisya *et al.*, 2017).

Kefir is a soured, frothy and mild alcoholic dairy drink produced from acid and alcohol fermentation. Consequently, kefir tastes refreshing, slightly acidic, yeasty and consistently creamy. The traditional kefir is produced by the addition of small kefir grains to fresh milk. Kefir differs from any other fermented dairy products because kefir is the product of fermentation by a mixed group of microflora confined to a matrix of discrete "kefir grains" (Liu *et al.*, 2005). The function of those groups of microorganism of the kefir grains may include the production of lactic acid, bactericides, or natural antibiotics. Because of those abilities, kefir grains are able to inhibit the growth of undesirable and pathogenic microorganisms in kefir milk (Angulo *et al.*, 1993).

In several countries, kefir has been widely considered as a beverage and a medication to treat various illnesses. Previous studies have shown that fermented soy products performed better antioxidative activity. Furthermore, soymilk bioprocessing by active kefir cultures has been shown to cause phenolic antioxidant mobilization (Kesenkas *et al.*, 2011) .

Those three food materials (fruits and fermented milk) could be consumed either fresh or in the form of juice. Nevertheless, those three materials were never made into a combination. Therefore, there is no research examining the antioxidant activity of those three natural product combinations. Beside that, the combination of those natural products was never made into a healthy fresh beverages or juices although the benefit is already mentioned, with each antioxidant activity of those materials, we hope that the total antioxidant activity of combination could synergize one another, so that the antioxidant activity would rise higher (Crespo *et al.*, 2019). Those three materials were combined into many patterns with different ratio. Antioxidant activity of each pattern has been measured, then analyzed to determine which pattern was better than the others.

MATERIAL AND METHOD

The materials were collected from different cities in Indonesia, because the main process was conducted in Banjarmasin. The dragon fruit were collected in Banjarmasin, South Kalimantan in December 2018. The dragon fruit were at their optimal harvest time in October-April at that time. Then, they were washed with tap water, wiped to dry, and peeled into small pieces. Dates were collected from Surabaya, East Java because there was a special area that collected original dates from Saudi Arabia. The dates were then treated the same way like the dragon fruit. Besides, kefir grains were also obtained from Surabaya, East Java.

• Preparation of fruit peels

Small pieces of dragon fruit peel were blended with distilled water until smooth and turning into juice (10 g of fruits in 100 mL of distilled water). The same preparation was done to the dates. The combination was soluted with distilled water because the combination is expected to become a drinkable healthy beverage.

• Production of kefir

The kefir grains were obtained from East Java, then activated using pasteurized cow milk. The kefir grains in milk is set into 3% concentration, so for 2 litres of milk, 60 grains of kefir were needed and incubated for 48 hours. Then, This kefir was then used for the combination.

• Preparation of combination

After the samples were ready in smooth form, the next step was the preparation for the combination. The various combinations are shown on Table 1 . The various combinations (A-T) could be stored in a refrigerator at 16°C for 3-4 days. Samples were placed in centrifuge tubes which are 20 mL of M

• Antioxidant activity assay

Antioxidant activity was assesed by using DPPH radical scavenging activity. Samples (the various combinations) and standart were prepared in MeOH (Merck, Germany). Before the measurement of antioxidant activity of samples, the determination of standart curve of DPPH was done by calculating the wavelength of standart solutions (10, 20, 30, 40, 50, 60, and 70 ppm). The wavelength obtained was used to calculate the antioxidant activity of samples (combinations).

First, 20 mL of DPPH in MeOH was mixed into each sample (A-T) with 2: 1 ratio of comparison. The samples were stirred for 20 minutes at 200 rpm in a waterbath shaker, followed by centrifugation for 10 minutes. Supernatans of samples were collected then incubated at 37°C for 30 minutes, then poured into the cuvette. Further, the absorbance at the λ_{maks}= 517 nm was calculated by using BK-D560 Double Beam UV/Vis Spectrofotometer. The absorbance data that were obtained were used to determine the IC_{50} value of samples (A-T) by using linear regression with the equation (1).

$$\%\text{inhibition} = \frac{(\text{The absorbance of control} - \text{The absorbance of sample})}{\text{Absorbance of control}} \times 100\% \quad ...(1)$$

The IC_{50} value showed the ability to scavenge free radicals (DPP). The IC_{50} value was calculated from the absorbance of blank, standart, and samples. All measurement of samples (A-T) were done in three replications.

• Statistic analysis

The data from three replications were analyzed to find out the normality and mean by using *Kolmogorov-Sminorv* Test. The mean of each sample was then statistically analyzed by using analysis of variance (ANOVA) F-tabel with the α=0.05 in SPSS software program.

RESULT

The various combinations are shown on Table 1. The antioxidant activity from sample A-T was shown as IC_{50} value (mg/dl) on Table 2.

Tabel 1. Various combinations of Dragon fruit (DF), Kefir (K), and Dates (D)

Sample Code	Combination (in mL)		
	Dragon fruit (DF)	Kefir (K)	Dates (D)
Combination of DF and D			
A	10	-	1
B	10	-	2
C	10	-	3
D	10	-	4
E	10	-	5
Combination of DF and K			
F	10	1	-
G	10	2	-
H	10	3	-
I	10	4	-
J	10	5	-
Combination of K and D			
K	-	10	1
L	-	10	2
M	-	10	3
N	-	10	4
O	-	10	5
Combination of DF: K: D			
P	1	10	1
Q	2	10	1
R	3	10	1
S	4	10	1
T	5	10	1

Tabel 2. Various combinations of Dragon fruit (DF), Kefir (K), and Dates (D)

No	Samples	IC_{50} value (mg/dl)
1.	A	$69,94 \pm 1,06^{b}$
2.	B	$76,33 \pm 0,27^{d}$
3.	C	$74,2 \pm 0,26^{c}$
4.	D	$71,19 \pm 0,155^{b}$
5.	E	$67,55 \pm 0,53^{a}$
6.	F	$79,08 \pm 0,31^{a}$
7.	G	$79,61 \pm 0,15^{a}$
8.	H	$79,81 \pm 0,3^{ab}$
9.	I	$80,9 \pm 0,4^{bc}$
10.	J	$80,6 \pm 0,3^{c}$
11.	K	$64,1 \pm 1,33^{b}$
12.	L	$76,06 \pm 0,53^{d}$
13.	M	$77,39 \pm 1,33^{d}$
14.	N	$71,27 \pm 0,53^{c}$
15.	O	$32,1 \pm 2,66^{a}$
16.	P	$79,08 \pm 0,31^{a}$
17.	Q	$79,61 \pm 0,15^{a}$
18.	R	$79,87 \pm 0,3^{ab}$
19.	S	$80,94 \pm 0,4^{bc}$
20.	T	$80,67 \pm 0,3^{c}$

*The data above show means with three replications ± standart error. Means with different letters in the same columns are significantly different with p = 0.05.

DISCUSSION

The first group of combinations (A-E) consisted of the combinations between dragon fruit and dates fruit peel with various concentrations. The dates concentration was made in a lower ratio because dates only function as sweetener. The Table 2, we might discover that the highest IC_{50} value was found in sample B. It showed that sample B indicated the highest radical scavenging activity than the other samples. Furthermore, the results of *One-Way* ANOVA showed that there was a significant difference or effect from dates concentration. Samples C, D, and E showed the decreasing pattern of IC_{50} value as dates concentration increased.

The second group consisted of samples F-J. That group was comprised of the combinations between dragon-fruit peel and kefir. The kefir concentration was made in a lower ratio than dragon-fruit peel concentration because kefir was always used as complementary food. The Table 2, we might find out that the highest IC_{50} value was sample I. The higher the concentration of kefir, the higher the IC_{50} value.

The various combinations of kefir and dates (samples K-O), we might conclude that the highest IC_{50} value was sample M (kefir: dates = 10: 3). The higher the concentration of dates, the lower the IC_{50} value. The *One-Way* ANOVA analysis concluded that various dates concentration gave the significant effect to IC_{50} value.

From the last group (samples P-T), the highest IC_{50} value was sample S. It showed that the higher the concentration of dragon-fruit peel, the higher the IC_{50} value. The result of *One-Way* ANOVA indicated that the variation of dragon fruit: kefir: dates from samples P, Q, and R were not significantly different to any other but different with samples S and T.

The IC_{50} value represents the minimal concentration of a drug that is required for 50% inhibition in vitro. Traditionally, this value is expressed as a molar concentration. The purpose of making various combinations was to examine the different effects from different concentrations of the combinations. All combinations, we may discover that the greatest effect of concentration to the IC_{50} value was from the dragon-fruit peel (samples I and S), followed by kefir, then dates.

CONCLUSION

From this study, it could be concluded that the mixture having the highest antioxidant activity was from the sample O with the formula (kefir: dates = 10: 5). The antioxidant of the sample O is about 32.1 mg/dl.

From the results of the study, future research that correlates to the main kinds of dragon fruit is highly suggested to be conducted as there are several types of dragon fruit. The different kinds of dragon fruit may indicate different antioxidant activities.

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