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Short Communication

# THE COMBINATION OF ROSELLA (*HIBISCUS SABDARIFFA*, L) AND STEVIA (*STEVIA REBAUDIANA*) EXTRACTS INCREASE THE ANTIOXIDANT ACTIVITY AND STABILITY

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**ABSTRACT**

**Objective**: Antioxidant has been widely used for preventing many diseases. The objective of this study was to determine the effect of the combination of rosella (Hibiscus sabdariffa, L) and stevia (Stevia rebaudiana, L) leaf extract on antioxidant activity and stability.

**Methods**: Rosella and stevia were extracted by maceration using ethanol. The combinations of rosella and stevia extract prepared were 0:1; 3:1; 1:1; 1:3; and 1:0. The antioxidant activity was determined using the DPPH method. The antioxidant stability test was carried out by incubate the extract solution on the different temperature of 60,70, and 80 $^{o}$C and followed by DPPH assay.

**Results**: The results showed that antioxidant value expressed as the $IC_{50}$ of rosella and stevia extract of 0:1; 3:1; 1:1; 1:3; and 1:0 were 450.32±14.10; 418.8±18.48; 272.9±7.14; 246.39±4.96; and 148.29±3.81 ug/ml respectively. The antioxidant stability expressed as degradation constant (K) of rosella and stevia extract of 0:1; 3:1; 1:1; 1:3; and 1:0 were 1.804.10[24]; 2.825.10[14]; $2.704.10^{5}$; $2.708.10^{4}$; $2.089.10^{9}$; $2.062.10^{2}$/min.

**Conclusion**: The combination of rosella: stevia 1:3 had the most active antioxidant and the most stable composition. The combination increase the antioxidant activity and stability significantly (P<0.05).

**Keywords:** *Hibiscus sabdariffa*, *Stevia rebaudiana*, Antioxidant, DPPH method



Reactive oxygen species are highly reactive molecules in the body. Reactive oxygen species including superoxide anion, hydroxyl, and hydrogen peroxide could damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions [1]. Oxidative stress contributes to many pathological conditions and diseases, including cancer, neurological disorders, atherosclerosis, hypertension, ischemia/perfusion, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and asthma.

Rosella has been used widely as an antioxidant [2] antihypertension [3] and hepatoprotective [4,5]. This antioxidant activity due to the content of ascorbic acid, polyphenols, flavonoids, gossipetin, hi bis cetin, sabdaretin, glycosides flavonol, and some organic acids [6]. Anthocyanin, one of a major component of rosella, was known unstable and influenced by pH and temperature [7]. The un-stability of anthocyanin could affect the antioxidant activity of rosella extract.

Stevia (*Stevia rebaudiosida*) leaf contains eight terpene glycosides that stevioside, steviol biocides, rebaudioside (A, B, C, D, and E), dulcosida A, tannins, polyphenols, and flavonoids [8, 9]. The leaves of Stevia is reported as an antihypertensive, antihyperglycemic, antihyperlipidemic and antioxidant [10–13].

The use of herbal combinations (polyherbal) has been used in the practice of traditional medicines since thousands of years ago for increasing therapeutic effect. Some herbal has a synergistic effect with other herbal, and some have the complementary effect with others. A combination of plant extracts proven to increase the antioxidant properties than a single form [14, 15].

There is a very limited publication on a combination of herbal extract to increase the activity and stability. The objective of this research was to explore the increasing of antioxidant properties of the combination of rosella and stevia extract and the effect on the stability.

The rosella calyx was obtained from Kediri (East Java, Indonesia) and stevia leaves were obtained from Solo (Central Java, Indonesia). The plant was identified at the Laboratory of Biology, University of Ahmad Dahlan. Rosella calyx and stevia leaves were extracted using maceration method with ethanol and followed by evaporation to get the concentrated extract.

Free radical scavenging activities of different extracts were measured by 1, 1-diphenyl-2-picryl hydrazyl (DPPH). In brief, the concentrations series of an extract of 1.0 ml were taken and put into a test tube. Each test tube was added with 1.0 ml of 0.15 mM DPPH in ethanol. The mixture is homogenized and allowed to stand in a dark place during the operating time. The absorption of the solution is measured at a wavelength of 517 nm. The compositions of rosella and stevia extract used were: 1:0, 1:1, 3:1, 1:3, and 0:1.

$$\text{DPPH scavenging activity} = \frac{(A0 - A1) \times 100}{A0}$$

A0 was absorbance of control, and A1 was absorbance of the sample.

The antioxidant stability assay was done using the concentration of each extract and combinations which have the greatest value of % inhibition. The 25 ml of each concentration of extracts was put in a test tube (protected from light). The extract was incubated with varying temperature (60, 70, and 80 °C). Sampling was done for 5, 15, 30, 45, and 60 min respectively and followed by measuring antioxidant activity by DPPH method.

The antioxidant properties of rosella were highly related by the content of anthocyanin as the main active substance. Anthocyanins are unstable, easily damaged by heat, pH and light. The studies reported the increasing of color stability of anthocyanin in a combination of several herbals [7, 16]. The antioxidant capacity could reduce by the reducing anthocyanin content. This study aims were to provide a solution on the instability of *H. sabdariffa* anthocyanins through a combination with other samples. The antioxidant activity of rosella, stevia, and the combination were shown on table 1.

**Table 1: The antioxidant activity of Extract of rosella, stevia and the combination**

| Composition | $IC_{50}$ |
|---|---|
| Rosella | 450.321±14.101 |
| Stevia | 418.795±18.481 |
| Rosella: Stevia (3:1) | 272.899±7.139 |
| Rosella: Stevia (1:1) | 246.388±4.964 |
| Rosella: Stevia (1:3) | 148.286±3.807 |

The data presented as mean±SD from 3 experiments

The antioxidant activities of extract combinations were higher than the single form, and the composition of rosella: stevia (1:3) give the highest antioxidant activity. The previous report was also found the increasing antioxidant activity with a combination of polyherbal [17]. The combination of rosella, tiger nut, and Moringa leaves extract to increase the inhibition of free radicals [14].

Anthocyanin one of major compounds of rosella is a member of flavonoids group. The stability of anthocyanin may affected by temperature and pH [18]. The heating treatment in the manufacturing process can cause anthocyanin (flavonoids) to tend a colorless, including alkaline carbinol and chalcon. The structural changes of anthocyanin caused by heating can occur in two stages. The first, glycosidic bond hydrolysis occurs in anthocyanin resulting glycone and aglycone product. Second, the aglycone ring change to open form i. e chalchon and carbinol group. This degradation can occur further if there is an oxidant. The combination with another herbal extract could also change the pH value and will make the anthocyanin more stable and had higher antioxidant activity. The antioxidant stability of rosella and stevia combination was performed by a constant of degradation value as shown on table 2. The result showed that the combination increases the stability as shown by increasing the $t_{1/2}$ value.

**Table 2: The value of $t_{1/2}$ of extract solution of rosella, stevia, and the combination**

| Sample | $t_{½}$ (h)±SD |
|---|---|
| Rosella (R) | $9,5 \times 10^{-}$[12]±$2,8 \times 10^{-}$[13] |
| Stevia (S) | $0,6±1,8 \times 10^{-2}$ |
| R: S (3:1) | $1,5 \times 10^{-}$[11]±$4,4 \times 10^{-}$[13] |
| R: S (1:1) | $0,5±9,9 \times 10^{-3}$ |
| R: S (1:3) | 4,6±0,1 |

The data presented as mean±SD from 3 experiments

The overall result showed that combination of rosella with stevia extract could increase the antioxidant activity. The stability also was found to increase. The increasing of antioxidant and stability give benefit for the lifetime of the product.

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**CONFLICT OF INTERESTS**

The authors declare there is no conflict of interests

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