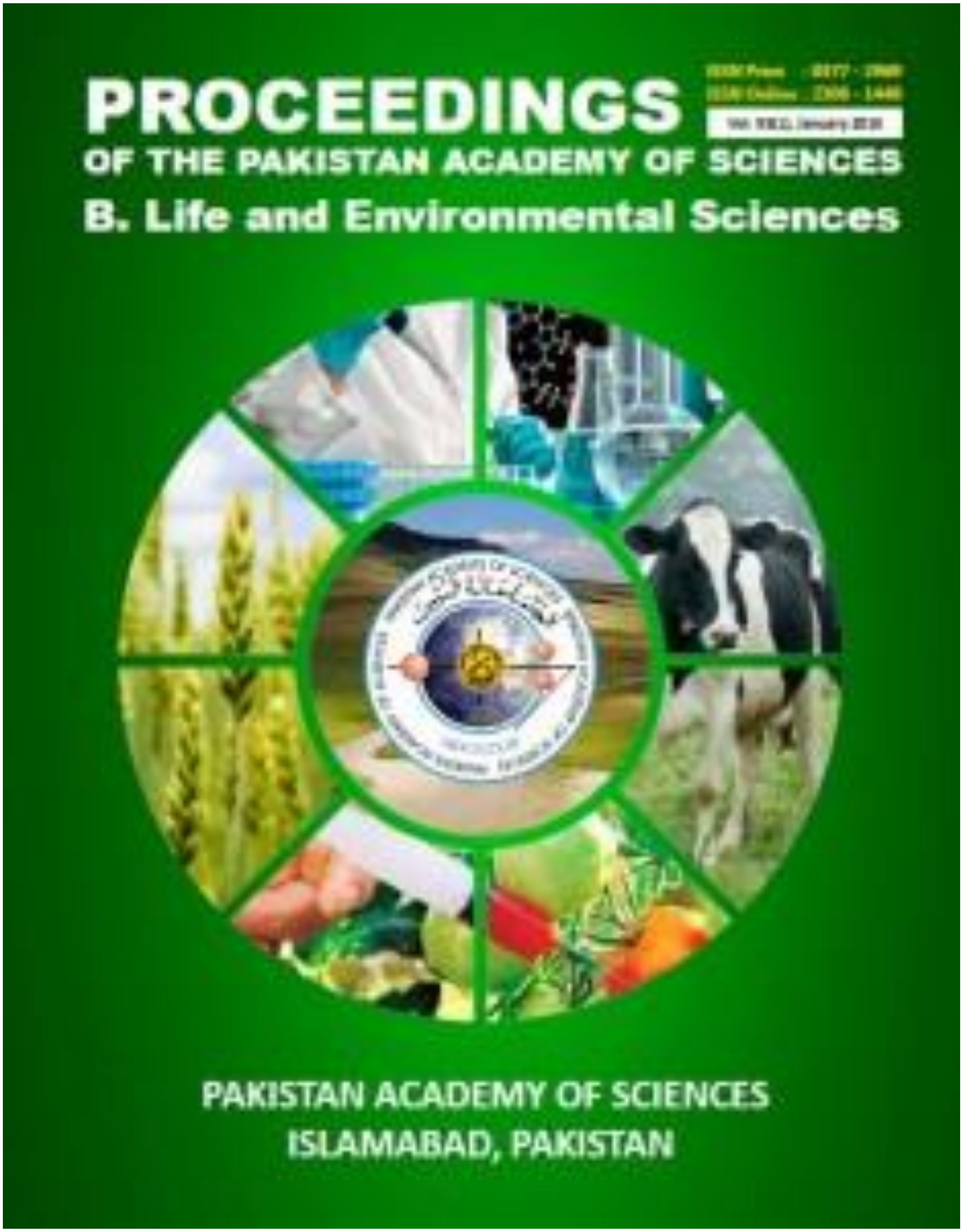
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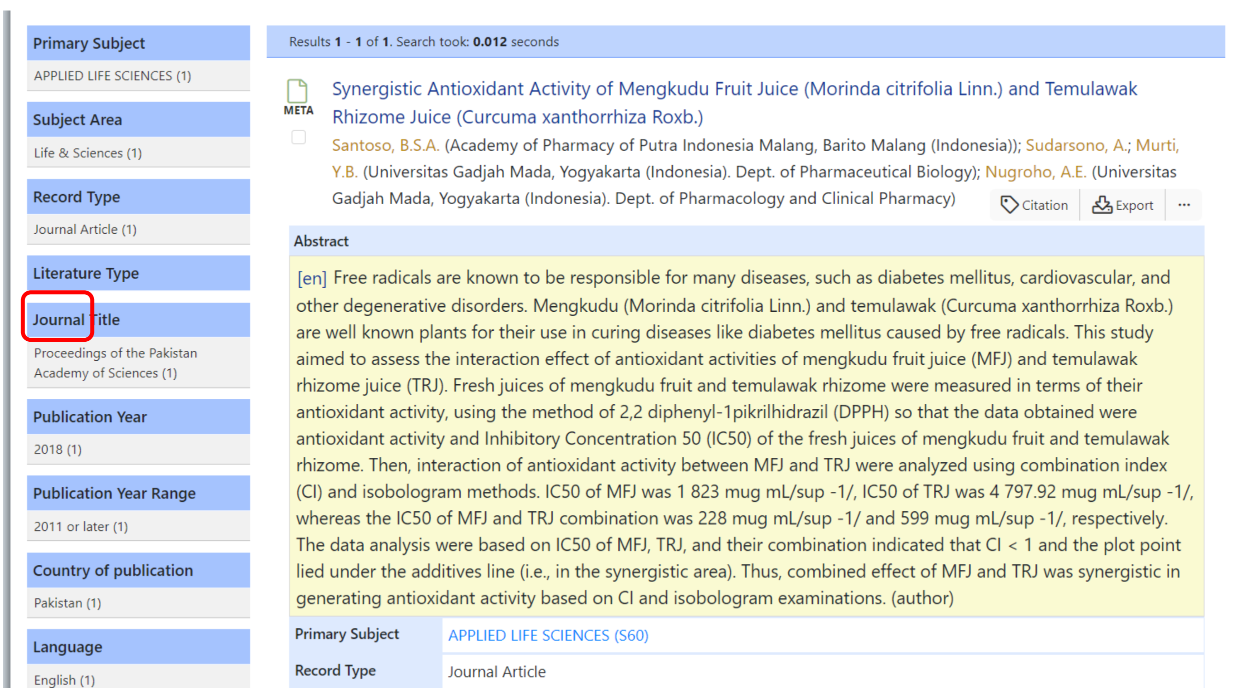

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Synergistic Antioxidant Activity of Mengkudu Fruit Juice (Morinda citrifolia Linn.) and Temulawak Rhizome Juice (Curcuma xanthorrhiza Roxb.)

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Citation Export ···

Abstract

[en] Free radicals are known to be responsible for many diseases, such as diabetes mellitus, cardiovascular, and other degenerative disorders. Mengkudu (Morinda citrifolia Linn.) and temulawak (Curcuma xanthorrhiza Roxb.) are well known plants for their use in curing diseases like diabetes mellitus caused by free radicals. This study aimed to assess the interaction effect of antioxidant activities of mengkudu fruit juice (MFJ) and temulawak rhizome juice (TRJ). Fresh juices of mengkudu fruit and temulawak rhizome were measured in terms of their antioxidant activity, using the method of 2,2 diphenyl-1pikrilhidrazil (DPPH) so that the data obtained were antioxidant activity and Inhibitory Concentration 50 (IC50) of the fresh juices of mengkudu fruit and temulawak rhizome. Then, interaction of antioxidant activity between MFJ and TRJ were analyzed using combination index (CI) and isobologram methods. IC50 of MFJ was 1 823 mug mL/sup -1/, IC50 of TRJ was 4 797.92 mug mL/sup -1/, whereas the IC50 of MFJ and TRJ combination was 228 mug mL/sup -1/ and 599 mug mL/sup -1/, respectively. The data analysis were based on IC50 of MFJ, TRJ, and their combination indicated that CI < 1 and the plot point lied under the additives line (i.e., in the synergistic area). Thus, combined effect of MFJ and TRJ was synergistic in generating antioxidant activity based on CI and isobologram examinations. (author)

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Research Article

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Keywords: Antioxidant activity, DPPH, IC_{50}, mengkudu, synergistic, temulawak

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Keywords: Antioxidant activity, DPPH, IC_{50}, mengkudu, synergistic, temulawak

1. INTRODUCTION

Antioxidants are molecules that can neutralize free radicals that are toxic to body by protecting the cellular components in tissues [1] and slowing the tissue damages caused by oxidative stress [2]. Based on the source there are two kinds of antioxidants: endogenous and exogenous. Endogenous antioxidant is an antioxidant that comes from inside of the body. The examples of antioxidant include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) [3]. While exogenous antioxidant is an antioxidant that comes from outside of the body, for examples, metabolites in plants like flavonoid and phenolic compounds [4]. Mengkudu (*Morinda citrifolia* Linn.) and temulawak (*Curcuma xanthorrhiza* Roxb.) are plants have been proven to have antioxidant activity [5, 6]. In Indonesia, those plants are widely used as traditional herbal medicine (Jamu), sold in the street by hawkers carrying refreshing drinks, usually bitter in taste but they are usually sweetened with honey or palm sugar. Based on the above explanation then researcher want to assess the interaction of antioxidant activity of combination of juices from mengkudu fruit juice and temulawak rhizome juice.

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2. MATERIALS AND METHODS

2.1 Herbal and Chemical Materials

Mengkudu (*Morinda citrifolia* Linn.) and temulawak (*Curcuma xanthorrhiza* Roxb.) plants were obtained from Balai Materia Medika Batu, Malang, East Java, Indonesia. DPPH was purchased from Sigma-Aldrich, USA. Ethanol was purchased from E. Merck, USA.

2.2 Methods

Mengkudu (*M. citrifolia*) fruit and Temulawak (*C. Xanthorrhiza*) rhizome were made into juices beforehand using a juicer. The antioxidant activity of MFJ (mengkudu fruit juice), TRJ (temulawak rhizome juice) and the combination of MFJ and TRJ were determined by using DPPH method. This was the simplest method, wherein the prospective compound was mixed with DPPH solution and absorbance was recorded after a defined period. Each of MFJ and TRJ was dissolved in ethanol and prepared into various concentrations ranging from 500 µg mL^{-1} to 5000 µg mL^{-1}. A volume of 1 mL from each of MFJ and TRJ concentration were taken and then put into test tubes. The amount of 1 mL of DPPH solution were added into each tubes (15 mg DPPH dissolved in ethanol ad to 100 mL) and 3 mL of ethanol, then vortex and left for 30 min in dark room at room temperature [7, 8], next the maximum wavelength of DPPH in the range of λ 500 nm to 520 nm was determined DPPH solution added with ethanol beforehand was used as control [9]. The IC_{50} values were calculated using regression equation. The IC_{50} values of MFJ and TRJ were used as the basis for analyzing the mixture combination of MFJ and TRJ [10]. MFJ was made into several concentrations: 1/16 IC_{50}, ⅛ IC_{50}, ¼ IC_{50}, ½ IC_{50}, 1 IC_{50} and 2 IC_{50} whereas TRJ also made into several concentrations: 1/16 IC_{50}, ⅛ IC_{50}, ¼ IC_{50}, ½ IC_{50}, 1 IC_{50} and 2 IC_{50}. The combination of concentrations of MFJ and TRJ were arranged using the checkerboard [11].

2.3 Data Analysis

Data were obtained as the absorbance values from MFJ and TRJ, as well as control; then the percentage of antioxidant activity was calculated using formula (1) [12]:

$$\%\ \text{Antioxidant activity} = \frac{\text{control absorbance - sample absorbance}}{\text{control absorbance}} \times 100\ \% \quad (1)$$

After obtaining the percentage of antioxidant activity from each concentration, the calculation was further followed by linear regression calculation (*x, y*) to obtain IC_{50} values, where x is the concentration (µg mL^{-1}) and y is the percentage of antioxidant activity (%). The IC_{50} values of MFJ and TRJ samples were calculated by the formula $y = Bx + A$. The IC_{50} value was obtained from x value after replacing the y value by 50.

The data analysis was made based on CI. The interaction of antioxidant activity of MFJ and TRJ was analyzed based on the obtained IC_{50} values. After MFJ and TRJ were made into several concentrations and combinations, the combination of juice concentration that gave IC_{50} value was found, which was then followed by the calculation of CI values using formula (2), formula (3) and formula (4) by Chou-Talalay [13]:

$$\text{CI} = \frac{(D)1}{(Dm)1} + \frac{(D)2}{(Dm)2} = 1,$$

which shows additive effect (2)

$$\text{CI} = \frac{(D)1}{(Dm)1} + \frac{(D)2}{(Dm)2} > 1,$$

which shows antagonistic effect (3)

$$\text{CI} = \frac{(D)1}{(Dm)1} + \frac{(D)2}{(Dm)2} < 1,$$

which shows synergistic effect (4)

Annotations:

(D)1 and (D)2 were concentrations of MFJ and TRJ which were combined to produce some specific effects (50 % inhibition).

(Dm)1 and (Dm)2 were concentrations of MFJ and TRJ that were given alone and had the same effect (50 % inhibition).

Analysis of data based on isobologram. MFJ and TRJ concentrations with particular activity (IC50) were plotted at coordinates (MFJ, 0) and (0, TRJ). The two points were then connected by a line called the hypotenuse additive line. The combination of both juices that give the same effect were written in one point coordinate (MFJ, TRJ). The combination is synergistic if (MFJ, TRJ) is under the additive line, the combination is additive if (MFJ, TRJ) is right in the additive line and the combination is antagonistic if (MFJ, TRJ) is above the additive line [13].

3. RESULTS AND DISCUSSION

M. citrifolia and *C. xanthorrhiza* were identified by Balai Materia Medika Batu, Malang, Indonesia. The advantage of taking the material plants from Balai Materia Medika Batu was that quality and authenticity could certainly be guaranteed because it is one of government institutions that seek to foster the farmers to cultivate some plants that are widely used as herbal raw materials [14]. The maximum wavelength of DPPH was obtained at λ 515 nm in accordance with previous research by Bondet et al. [15] with the absorbance of control was 0.869 for the measurement of MFJ IC_{50}¬¬ and the absorbance of control was 0.862 for the measurement of TRJ IC_{50}. Results of absorbance and antioxidant activity of MFJ and TRJ can be seen entirely in Table 1 and Table 2 below:

The IC_{50} values were obtained from Table 1 and Table 2, after calculating the regression equation of concentration data and percentage of antioxidant activity. The concentrations of MFJ and TRJ that produce the antioxidant activity for 50 % (IC_{50}) respectively were 1 823 µg mL^{-1} and 4 797.92 µg mL^{-1}. Antioxidant activity of combination concentrations of MFJ and TRJ were arranged using the checkerboard. Concentrations of MFJ were 1/16 IC_{50} (114 µg mL^{-1}), ⅛ IC_{50} (228 µg mL^{-1}), ¼ IC_{50} (456 µg mL^{-1}), ½ IC_{50} (912 µg mL^{-1}), 1 IC_{50} (1 823 µg mL^{-1}) and 2 IC_{50} (3 646 µg mL^{-1}) whereas concentrations of TRJ were 1/16 IC_{50} (299 µg mL^{-1}), ⅛ IC_{50} (599 µg mL^{-1}), ¼ IC_{50} (1 199 µg mL^{-1}), ½ IC_{50} (2 399 µg mL^{-1}), 1 IC_{50} (4 797 µg mL^{-1}) and 2 IC_{50} (9 594 µg mL^{-1}). Analysis of the effect of MFJ-TRJ combination juice was done in two ways, i.e., calculation based on Combination Index (CI) and isobologram [11].

Figure 1 shows that the result of the combination that produces the IC_{50} value is a combination of ⅛ IC_{50} (228 µg mL^{-1}) concentration of MFJ with ⅛ IC_{50} (599 µg mL^{-1}) concentration of TRJ. The CI values of the data were calculated using Chou-Talalay formula and yielded Cl < 1. Based on the result of calculations, based on Combination Index obtained CI value < 1, it can be stated that combined effect of MFJ-TRJ juices was synergistic. CI is used to determine the effect of two combinations of juices, whether synergistic, additive or antagonistic [16]. When compared with each single juice without a combination, it can be said that the combination of

Table 1. Results of absorbance and antioxidant activity of MFJ.

Concentration (µg mL^{-1})	**Absorbance**			**Antioxidant activity (%)**		
	1	**2**	**3**	**1**	**2**	**3**
Control		0.869				
504.2	0.705	0.704	0.702	18.9	18.9	19.2
1 008.4	0.581	0.585	0.586	33.1	32.7	32.6
1 210.1	0.561	0.563	0.567	35.4	35.2	34.7
2 016.8	0.384	0.386	0.384	55.8	55.6	55.8
3 025.2	0.201	0.202	0.204	76.9	76.7	76.5

Table 2. Results of absorbance and antioxidant activity of TRJ.

Concentration (µg mL^{-1})	**Absorbance**			**Antioxidant activity (%)**		
	1	**2**	**3**	**1**	**2**	**3**
Control		0.862				
1 005.2	0.611	0.609	0.589	29.1	29.3	31.7
2 010.4	0.565	0.567	0.547	34.5	34.2	36.5
3 015.6	0.513	0.515	0.514	40.5	40.3	40.4
4 020.8	0.468	0.462	0.467	45.7	46.4	45.8
5 026.0	0.426	0.419	0.417	50.6	51.4	51.6

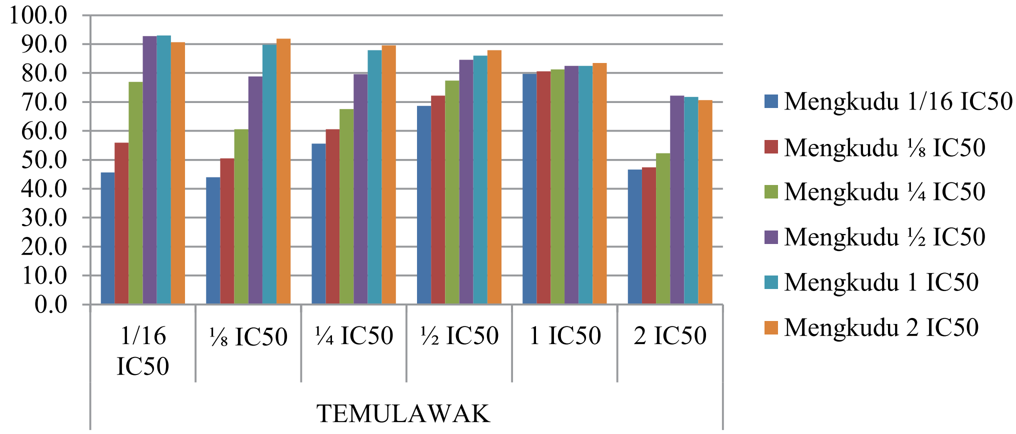

Fig. 1. Mengkudu and Temulawak combinations in various concentrations.

MFJ-TRJ juices were better.

The ordinate point was in the synergistic area (Fig. 2). The results of combination of ⅛ IC50 (228 µg mL^{-1}) concentration MFJ and ⅛ IC50 (599 µg mL^{-1}) concentration TRJ may be seen at ordinate point (¤) in isobologram. Isobologram is a commonly used method for evaluating drug combinations. The isobologram analysis begins with the effective concentration of each drug applied as a single agent then plotted on the X and Y axes. These two points are then connected to form a linear line called the additive line. Furthermore, the combination concentrations of the two drugs that produced the same effect were plotted into the graph. It says the effect is synergistic when the plot point is below the additive line, it is said the additive effect when the combination drug plot is right on the additive line, and it is said to antagonistic effect when the combination plot is above the additive line [17, 18]. This is similar to that of a former researcher who states that the isobologram for a particular effect (e.g., 50 % of the maximum) in which a single dose of A drug is 20 and a single B drug is 100, after combined A + B produces an effect under the line drawn between a dose of 20 and a dose of 100 as a synergistic area [19]. This isobologram analysis has been used in various fields of health especially related to drug interactions including used in interaction studies among antioxidant agents [20, 21]. The analysis using this isobologram was performed to compare the dose of drug as a single agent and the dose of drug combination to determine the type of drug interaction [17].

The obtained IC50 of MFJ was 1 823 µg mL^{-1} and IC50 of TRJ was 4 797.92 µg mL^{-1}. This value can be considered to have weak antioxidant activity [22] but when both of them were combined, the concentration of each of them only needs 228 µg mL^{-1} of MFJ and 599 µg mL^{-1} of TRJ. A small combination of concentration of MFJ and TRJ has been produced IC_{50}. This proved that combination of both of them is better than single concentration. The synergistic interaction was expected to reduce the dosage in order to minimize the occurrence of toxicity and resistance from medicinal plant.

4. CONCLUSIONS

This study revealed that combination of mengkudu

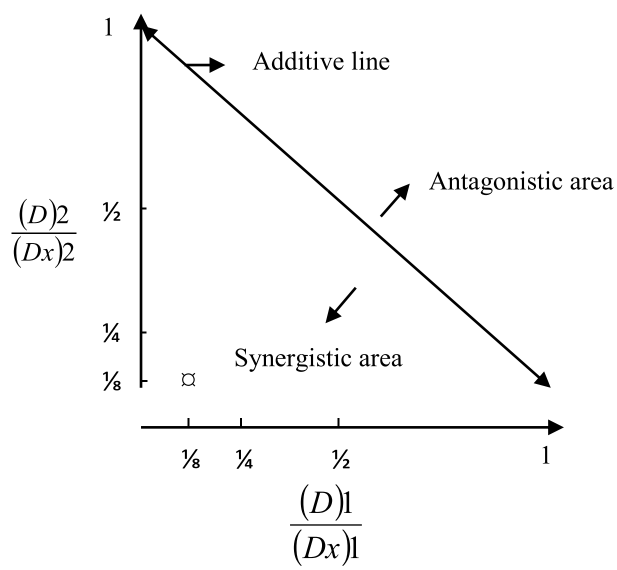

Fig. 2. Isobologram of Mengkudu and Temulawak combinations.

fruit juice (MFJ) and temulawak rhizome juice (TRJ) has synergistic antioxidant effect.

5. ACKNOWLEDGEMENTS

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