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ORIGINAL RESEARCH

Study of white frangipani flower and bitter grape stem ethanol extract combination on antibacterial and antioxidant activities

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Abstract. Frangipani flower (*Plumeria accuminata*) and bitter grape (*Tinospora crispa* L. Miers) stem extract alone shows antimicrobial and antioxidant activities. However, there is a limited report about the actions from the combination of both extracts. Combinations of ethanol extract of white frangipani flower (EFF) and ethanol extract of the bitter grape stem (EBS), each of 0, 1, 2, and 3% were tested for inhibition against *Staphylococcus aureus* growth and DPPH (1,1-diphenyl,2-picrylhydrazyl) free radicals. A yield of 3.17 and 2,62 g of EFF and EBS, respectively, were resulted from 100 g of dried powdered of the white frangipani flower and bitter grape stem using 200 mL of absolute ethanol. The antimicrobial test was assayed using a 10% stock solution of extract diluted in water. The combination of EFF and EBS showed a synergistic effect on antimicrobial activity, but it proved an antagonistic effect on antioxidant activity. The combination of EFF 3% and EBS 3% showed the highest inhibition index on the *S. aureus* growth , i.e. 2.02±0.06, which is higher than the inhibition index of *Amoxycillin* 2%, i.e. 1.85±0.09. The highest antioxidant activity showed by 1% EFF alone (57.5 ± 0.60%), while the lowest was from the combination of EFF 1% and EBS 3% (23.4±0.30%).

Keywords: *Plumeria*, bitter grape, *Tinospora*, *Staphylococcus aureus*

INTRODUCTION

Frangipani (*Plumeria* sp.) or commonly known as Kamboja in Indonesia (Fig. 1a) is a small lactiferous tree or shrub originated from tropical America [1], which is broadly grown in tropical countries, including in South-East Asia region. The shrub is used as traditional medicine for infections, digestive diseases, anti-inflammatory and antipyretic action, anti-tumor potential, and has antioxidant properties [2]. The well-known Frangipani types are *P. obtusa*, *P. acuminata*, *P. rubra*, *P. alba*, *P. lancifolea*, *P. drastic* and *P. phagidenica* [2, 3]. The frangipani flower shows an inhibition on microbial growth. The *P. alba* flower showed inhibition on *Escherichia coli*, *Staphylococcus saprophyticus*, *Proteus vulgaris*, *Serratia marcescens*, *Staphylococcus aureus* and *Streptococcus pyogenes* growth [4–6], as well as on the growth of tooth plaque bacteria (*Aggregatibacter actinomycetemcomitans*) [7]. In addition, ethanol extract of frangipani flower show antioxidant activity [8].

Another well-known medicinal plant in South-East Asia is bitter grape (*Tinospora crispa*) or brotowali in Indonesian (Fig. 1b.), is a small herb which grows in South East Asia such as Malaysia, Thailand, Indonesia, India, Philippines, Vietnam, including China. *T. crispa* also is known as *Menispermum crispum*, *M. rumphii* and *M. tuberculata* [9]. The bitter grape stem extract showed antimicrobial and antioxidant activity [10].

(a) (b)

Figure 1. White Frangipani (*Plumeria acuminata*) flower (a), Bitter grape (*Tinospora crispa*) (b).

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A combination of two plant extracts with pharmacological activity may result in a synergistic or antagonistic effect [11], e.g., antioxidant activity [12, 13] and antimicrobial activity [14]. There is little information about the synergistic or antagonistic effect by combining plant extract attributed with antioxidant and antimicrobial activities. In this study, an antagonistic and synergistic effect of the combination of ethanol extract of frangipani flower and bitter stem on antioxidant and antimicrobial activity, respectively, are reported. The idea in this study was to find an excellent synergistic effect by combining frangipani and bitter grape stem extract for cosmetics products possessing high antimicrobial and antioxidant activities.

METHODOLOGY

Materials

White frangipani flower and bitter grape stem were collected from Samarinda, Indonesia. Absolute ethanol was provided by Smart-Lab, 1,1 diphenyl-2-picrylhydrazyl (DPPH, Merck, Nutrient Agar (NA, Oxoid) and Nutrient broth (NB, Oxoid), and *Staphylococcus aureus* pure culture was obtained from Microbiology Laboratory of Department of Agricultural Products Technology, Faculty of Agriculture, Mulawarman University.

Experimental design and data analysis

A factorial experiment (4x4) arranged in a Completely Randomized Design was conducted in this research. Each treatment was replicated two times. The first factor was the concentration of ethanol extract of frangipani flower (EFF), and the second factor was ethanol extract of the bitter grape stem (EBS). Each combination consisted of four levels of each factor, i.e., 0, 1, 2, and 3% v/v of the solid ethanol extract stock solution (10 % w/v, one gram of ethanol extract in 10 mL of water). Antimicrobial tests on *S. aureus* and antioxidant tests against DPPH were the parameters observed. Both assays were prepared using a 10% stock solution extract in water. The data were analyzed descriptively instead of using ANOVA because they failed to fulfill the requirement for the ANOVA. The ANOVA in rank (non-parametric statistics) for the factors was also not appropriate because of the limited number of replications.

Preparation of ethanol extract of white frangipani flower (EFF) and bitter grape stem (EBS)

EFF and EBS were prepared using the method suggested by Farhan [15] with a slight modification. The samples were sorted and washed, then dried in an oven at 50^{o}C for 13 h. The dried samples were powdered using a blender. The powdered samples (each sample of 100 g) were macerated in 200 mL absolute ethanol for 24 h at room temperature. The maceration process was repeated, and both ethanol extracts were collected. The ethanol extracts were then vaporized using a Vacuum rotary evaporator at 78^{o}C, 227 mbar until the sample becomes solid. The solid extract ethanol of both samples then stored at -20^{o}C until being used for analysis.

Antimicrobial activity assay

The antimicrobial activity was assayed using the disk diffusion (Kirby-Bauer) method, as suggested by Furtado [16]. A log phase growth of the bacteria was prepared by inoculating a colony of the pure culture of *S. aureus* in 10 mL Nutrient Broth and incubated with shaking at 150 rpm at room temperature for 18 h. The *S. aureus* suspension at the log phase then diluted six times in 0.90% NaCl solution to reach the turbidity standard of 0.5 by McFarland or equal to 1-2x10^{8} cfu.mL^{-1}. A 100 µL of the diluted *S. aureus* suspension was pipetted and spread over 2% of nutrient agar in Petri dish using a sterile cotton bud and let it at room temperature for 3-5 min. The paper disks were then placed on the media following submerged the paper disks in the combination of EFF and EBS, as showed in treatments. After 24 h, the formed clear/inhibition zones were determined. Amoxycillin 2% and ethanol 95% were used as positive and negative control, respectively. The following equation developed an inhibition index on microbial growth.

$$\text{Inhibition index} = \frac{(\text{Clear zone diameter} - \text{Paper disk diameter})}{\text{Paper disk diameter}}$$

Antioxidant activity assay

Antioxidant activity assay was measured against DPPH (1,1-diphenyl,2-picrylhydrazyl) [15] with slight modification. Combination of EFF and EBS of 0.5 mL was added to 0.5 mL DPPH (0.15 mM in ethanol) in 1.5 mL Eppendorf cap. The mixtures were vortexed and then incubated in a dark room for 30 minutes, followed by measuring the absorbance at 517 nm. The ascorbic acid was used as positive control and ethanol was used as blank. The free radical inhibition was calculated by the following equation,

$$\text{Free radical inhibition (\%)} = \frac{(\text{Control absorbance} - \text{Sample absorbance})}{(\text{Control absorbance})} \times 100$$

Note: Control/blank absorbance was the absorbance of DPPH + ethanol; Sample

absorbance was the absorbance of DPPH + sample

RESULTS AND DISCUSSION

A 1,471 g fresh white frangipani flower gave about 198 g dry sample, while a dry bitter grape stem of about 196 g was provided from 1,277 g fresh stem. Using the absolute ethanol, the yield of the solid extract of white frangipani flower and bitter grape stem from fine dried samples were 3.12 and 2.73%, respectively (Table 1).

Table 1. Characteristic of extract ethanol of Frangipani Flower and Bitter Grape Stem

Sample	Form	Yield (%)*	Color
Frangipani Flower	Thick	3.12±0.08	Yellow brownish
Bitter Grape Stem	Thick	2.73±0.15	Dark green

Note: *) Yield (mean ± SD) were calculated from two replications.

The yields were lower than the previous reports. The yields of about 8.7 and 2.9% were achieved from extracting of white frangipani flower using ethanol 70% and n-hexane [17, 18]. While bitter grape stem extraction using ethanol 96%, n-hexane, ethyl acetate, and water yielded 12.02, 2.28, 3.83, and 5.68%, respectively [19]. The solvent used in the maceration process was one of the factors affecting the yield of the extract [4, 20].

Antimicrobial activity

Ethanol extract of the white frangipani flower (EFF) and bitter grape stem (EBS) showed an antimicrobial effect. The EFF alone showed better antimicrobial activity compared to the EBS. The EEF and EBS of 1-3% alone showed an inhibition zone against *S. aureus* of 12.76-16.51 and 9.63-13.63 mm, respectively, which showed an inhibition index of 1.13-1.75 and 0.61-1.27, respectively (Table 2). The column and row with average title at Table 2. (the shadow areas) figure the effect of EFF or EBS alone, respectively, at each level of treatment on the antimicrobial activity.

The ethanol extract of white frangipani at a concentration of 5% showed an inhibition zone of 8 mm in diameter against *E. coli*, while a 22 mm of inhibition zone was detected when ciprofloxacin of 5 μg/mL was applied as a positive control [17].

The hexane extract of the red frangipani flower showed antimicrobial effect against *E. coli* and *S. aureus* [4]. Ethanol dan chloroform extract of bitter grape stem showed an antimicrobial effect against *E. coli, S. pneumonia* dan *C. albicans* [9], while the water extract was effective as an inhibitor against *E. coli* and *S. aureus* [21]. Mohammed et al. [9] showed that ethanol extract of the bitter grape stem was the best antimicrobial compared to three other extracts of the bitter grape stem, i.e., water, methanol, and chloroform against *E. coli*, *S. pneumonia* and *C. albicans*.

The combination of EEF and EBS showed a low synergistic effect on antimicrobial activity against *S. aureus*. In the combination of both ethanol extract, the EFF gave a better synergistic effect (Table 2). The data derived from antimicrobial activity do not fit the requirement for statistical analysis. However, the descriptive analysis using two-dimensional graphic clearly shows the trend of the synergistic effect of the combination of EFF and EBS on the antibacterial activity (Figure 2).

Table 2. Effect of combination of ethanol extract of Frangipani Flower (EFF) and Bitter Grape Stem (EBS) on antimicrobial activity.

EFF (%)	EBS (%)				Average
	0	1	2	3	
0	0.00±0.00	1.13±0.03	1.24±0.04	1.75±0.15	0.66±0.48
1	0.60±0.03	1.25±0.03	1.41±0.04	1.85±0.03	1.27±0.11
2	0.75±0.00	1.28±0.04	1.48+0.03	1.93+0.04	1.42±0.13
3	1.27±0.00	1.42±0.00	1.55±0.02	2.02±0.06	1.89±0.12
Average	1.03±0.69	1.28±0.48	1.36±0.45	1.57±0.30	

Note: Data are expressed as (mean ± SD) of inhibition index. Each sample was replicated two times for antibacterial experiment and two times for the antioxidant. Absolute ethanol and 0.2% Amoxicillin were used as a negative and positive control, and they showed an inhibition index of 1.06±0.18 and 1.85±0.09, respectively. For the assay, the paper disks were submerged in absolute ethanol, Amoxycillin 2%, EFF, EBS or combination of EFF and EBS for 5 minutes then let it dried at room temperature in a sterile condition. The data do not fit to the requirement of statistical analysis then a descriptive analysis is performed (please see Figure 2.).

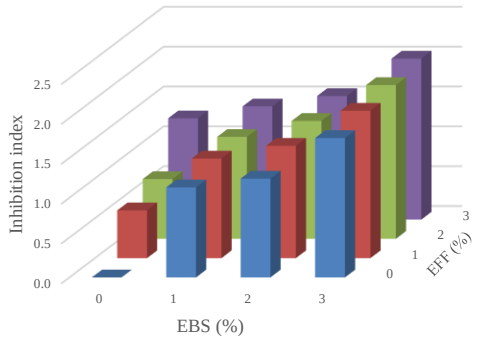

Figure 2. The trend of synergistic effect on antimicrobial activity of ethanol extract combination of Frangipani Flower (EFF) and Bitter Grape Stem (EBS). *Please refer to note of Table 2 for the experiment condition.*

Synergistic on the therapeutic effect of many herbal occur through pharmacokinetic and/or pharmacodynamic interaction. However, the mechanism remains open to be elucidated [11]. The clear zone around the paper disk resulted from the inhibition effect of the combination of EEF and EBS were presented in Figure 3.

The increasing of EFF concentration affected higher inhibition capacity on *S. aureus* than the increase of EBS concentration. The combination of EFF 3% and EBS 3% showed the highest activity on the inhibition growth of *S. aureus* of 18.13±0.25 mm, while the positive control (Amoxycillin 2%) showed inhibition of 17.13±0.32 mm. This report gives new sight of the synergetic effect between two plant extracts on antimicrobial activity. The previous reports only showed a synergistic effect of different plant extracts with different antibiotics [14, 22]

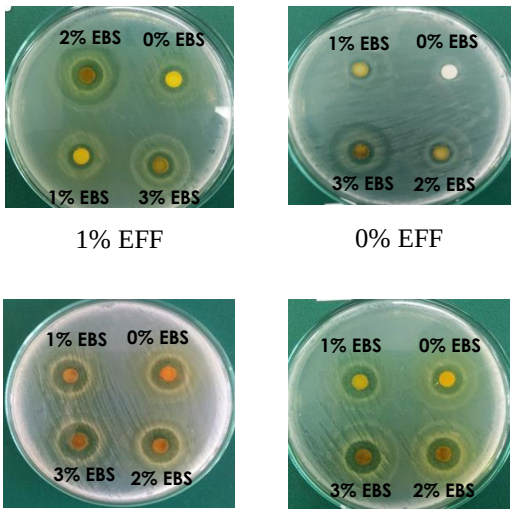

Figure 3. Effect of ethanol extract of frangipani flower (EFF) and ethanol extract of the bitter grape stem (EBS) combination on antimicrobial activity against S. aureus. The assay was applied on 2% Nutrient Agar. The paper disks were submerged for 5 min and left to dry at room temperature in a sterile condition.

Antioxidant activity

The EFF showed a better effect on antioxidant activity than EBS. The increase of EBS concentration until 3% in combination with EFF did not reach the IC_{50} of the antioxidant activity-based DPPH test (Table 3). The column and row with average title at Table 3. (the shadow areas) figure the effect of EBS or EFF alone, respectively, at each level of treatment on the antioxidant activity.

Table 3. Effect of combination of ethanol extract of Frangipani Flower (EFF) and Bitter Grape Stem (EBS) on antioxidant activity (%).

EFF (%)	EBS (%)				Average
	0	1	2	3	
0	1.05±0.00	26.00±0.16	23.69±0.15	26.43±0.15	19.29±11.31
1	57.90±0.60	30.96±0.01	25.58±0.15	23.58±0.30	34.51±14.37
2	47.66±0.41	43.37±0.30	30.85+0.45	28.63+0.30	37.63±8.63
3	44.63±0.30	40.84±0.30	47.06±0.75	26.00±0.45	39.63±8.75
Average	37.81±23.29	35.29±7.58	31.80±9.84	26.16±1.93	

Note: Data are expressed as (mean ± SD). Because of the data do not fit to the requirement of statistical analysis, a descriptive analysis is performed (please see Fig. 4.).

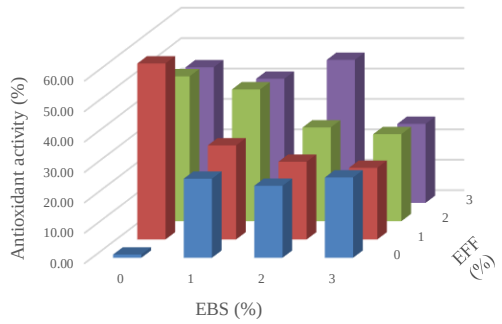

Figure 4. The trend of antagonistic effect on antioxidant activity of combination of ethanol extract of Frangipani Flower (EFF) and Bitter Grape Stem (EBS). *Please refer to note of Table 2 for the experiment condition*.

The EBS is responsible on the antagonistic effect of antioxidant activity of the combination between EFF and EBS. The increase of EBS concentration in the combination of EFF and EBS will decrease the antioxidant activity (Figure 4).

In advance, studies to find out the simultaneous synergistic effect on antimicrobial and antioxidant activity from the different extract solvents for both plants become an interesting theme and wide open to be solved.

CONCLUSION

The combination of ethanol extract from frangipani flowers and bitter grape stems shows a synergistic effect on antibacterial activity, but shows antagonistic effects on antioxidant activity. This evidence becomes a new sight of information to find the appropriate macerate agents resulted extracts that their combination shows a simultaneous synergetic effect on antimicrobial and antioxidant activities.

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