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Utilization of Antioxidant *Fagraea fragrans* fruit as Phytocosmetics

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Abstract

The objective is to test the antioxidant activity of methanol (MeOH) extracts, ethyl acetate (EtOAc) fractions, and an isolated compound of *F. fragrans* (*tembesu*) fruitby using DPPH method respectively. Antioxidant is one of the main components of phytocosmetic beside anti-inflammatory, anticancer, and antimicrobial activities while phytocosmetics of *F. fragrans* fruit is the cosmetics that mainly use extracts or components derived from this fruit only, without preservatives, such as products that do not contain water, oils, dry ointments, or hydro-alcoholic solutions. As a result, The IC_{50} values of the MeOH extracts, EtOAc fractions, and the isolated compound were 186.5 ± 0.52µg/mL, 357.2 ± 0.59 µg/mL, and 5.658 µg/mL respectively. The MeOH extracts exhibited moderate antioxidant activity while the isolated compound exhibited strong antioxidant activity. The total flavonoid content of the MeOH extracts and EtOAc fraction was measured by a colorimetric assay, using reagents of 1 M sodium acetic and 10% aluminum chloride. The EtOAc fraction had a total flavonoid content of 4.505 mgQE/g-extract and total phenolic content of 13.732 mgGAE/g-extract while the MeOH extracts had a total flavonoid content of 9.088 mg QE/g-extract and total phenolic content of 23.34 mgGAE/g-extract respectively. Therefore, the utilization of MeOH extracts or the isolated compound of these fruit may be beneficial for developing skincare phytocosmetics and personal care products.

Keywords: Phytocosmetics, antioxidant, Fagraea fragrans, tembesu fruit

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Tujuan adalah untuk menguji aktifitas antioksidan ekstrak metanol (MeOH), fraksi etil asetat (EtOAc), dan senyawa hasil isolasi dari buah tembesu (*F. fragrans)* dengan mengunakan metoda DPPH. Antioksidan adalah suatu komponen utama fitokosmetika disamping aktifitas anti-inflamasi, antikanker, dan antimikrobial, sedangkan fitokosmetika dari buah tembesu adalah kosmetika yang hanya ekstrak atau komponen yang berasal dari buah tembesu semata, tanpa pengawet, air, minyak, bahan salep, atau larutan hidrokhlorida. Hasilnya, nilai IC_{50} ekstrak MeOH, fraksi EtOAc, dan senyawa isolasi masing-masingnya adalah 186.5 ± 0.52µg/mL, 357.2 ± 0.59µg/mL, and 5.658 µg/mL. Ekstrak MeOH memiliki aktifitas antioksidan yang moderat sedangkan senyawa isolasi menunjukan aktitas antioksidan yang kuat. Total flavonoid kontent dari ekstrak MeOH, fraksi EtOAc yang diukur secara kolorimetri using reagensia 1 M natrium asetat dan 10% aluminum klorida. Fraksi EtOAc memiliki kandungan flavonoid total = 4.505 mg QE/g-ekstrak dan kandungan fenolik total = 13.732 mg GAE/g-ekstrak sedangkan ekstrak MeOH memiliki kandungan flavonoid total = 9.088 mg QE/g-ekstrak dan kandungan fenolik total = 23.34 mgGAE/g-ekstrak. Oleh karena itu, pemanfaatan ekstrak MeOH atau senyawa hasil isolasi yang berasal dari buah tembesu dapat memberikan keuntungan untuk mengembangkannya menjadi fitokosmetika perawatan kulit dan wajah dan sebagai produk perawatan yang bersifat personal.

Kata Kunci: fitokosmetika, Antioxidan, Fagraea fragrans, buah tembesu

INTRODUCTION

Such as edelweiss and sakura flowers, the goal of this work is to evaluate the antioxidant activity of *Fagraea fragrans* Roxb. fruit in order to be used in herbal level application for skin care. This plant species, locally named *Tembesu*, grows vigorously along east coast of the Sumatran Island as well as its fruit. This fruit of a size of corn with red color that can be harvested from March to April or September to October. There are no yet phytochemical and pharmaceutical reports dealing with the application this fruit for phytocosmetics. So that the fruitis a great resource for searching natural active ingredients without injuring the plant body, when this raw material is used for phytocosmetics and herbal cosmetics having various properties such as antioxidant, anti-aging, and anti-wrinkle properties.

The antioxidant survey on natural active ingredients for phytocosmetics has to be done on this genuine plant of Sumatran island, such as this *F. fragrans* fruit. Phytocosmetics are a part of cosmetology that consists of the use of plants in cosmetics. In addition, numerous works have shown that fruit crude extracts are considerably active skincare agents, as they provide anti-inflammatory, antioxidant, antimicrobial, and anti-oncogenic activities against different types of cancer [1, 2]. Phytocosmetics that use the dried-fruit extract only or extract combining with excipients to prevent wrinkle and aging have to be developed in order to protect skin from microorganism and UV radiation exposure because they can result in many types of damage, such as oxidative damage. Under normal conditions, the pro-oxidant and antioxidant systems are in their best state of balance. However, the balanced state can be disrupted when there is an overwhelming production of free radicals owing to repeated UV radiation exposure effects [1, 2, 3].

As for the present work, a local seasonal fruit such as this *F. fragrans Roxb* fruit, *Loganiaceae* family, was tested for anti-oxidant activity including identification of their metabolite secondary [5,13]. This plant mainly found in East Cost of Sumatera Island, Indonesia[4]. The red fruits usually blossom during the end of the year from September to November, see Fig.1a. and sometimes they appeared on April to March. The fruits were extensively studied for their phytochemical content and biological activities previously [5,6]. The fruit contains ursolic acid [its isomer oleanolic acid] which makes up 3.1% of in composition [6]. Ursolic and oleanolic acids are lead compounds in this inedible *F. fragrans* fruit. These triterpenic acids have wide range spectrum in biological activities, so they become useful also in cosmetic industries [7]. Beside those triterpenic acids, *F. fragrans Roxb* fruit also contains alkaloid [8], and phenolic compounds, flavonoids, tannin, and coniferol as bioactive compounds for phytocosmetics [5]. As a conclusion, it becomes our interest to disclose the potentiality of the *F. Fragraea* fruit methanolic extracts as a potential and halal skincare agent through its antioxidant profile. The stability methanol extracts to sunlight radiation has also been tested. Sortly in this study the *F. Fragraea* fruit methanolic extract and its ethyl acetate fraction had shown significantly antioxidant activity and they also relatively have stabiblity UV-A and UV-B absorbtion pattern after 5 hours' sun light exposure [9].

In addition to its UV stability, the antioxidant property of methanol extracts of the above fruit also plays an important role in the preservation of phytocosmetic formulation because they can neutralize free radicals [10]. So there is now a trend toward the use of natural substances present in plant to be phytocosmetics such as MeOH extracts of *F. frangrans* fruit rich in antioxidant to pamper our skin [11, 12]. Oxidative stress and inflammation are significant contributors to aging and age-related conditions, including skin aging. Antioxidants, substances that can prevent or slow cell damage caused by free radicals, have been identified as potential agents toalleviate these effects. Despite this, the use of natural ingredient such as *F. fragrans* fruit in the form of phytocosmetics, and herbal cosmeceuticals largely not yet exploited or used [15].

MATERIALS AND METHODS

Materials

F. fragrans fruit (fresh), methanol (technical), ethyl acetate (technical), *n*- hexane (technical), Sulphuric acid p.a., TLC Plate G 60 F_{254}, Silica gel - 60-120 mesh, for column chromatography, DPPH, DMSO, acetone, and methanol p.a.

Instruments

Glasswares, measuring pipette, micro pipette aluminum foil, percolator, rotary evaporator, cuvettes, Orion AquaMate 8000 UV-Vis Spectrophotometer, and LC-MS/MS of Mass Spectrometer with Specification of Xevo G2-S QT of (waters, USA), Two Generation Quadrupole time-of-flight mass spectrometer.

Methods

Fruit collection

F. fragrans fruit (15.6 kg) was collected from low land rain forest of Ogan Ilir (OI), South of Sumatra on March 2022 (**Figure** 1(a)), and dried for one week

under sunlight and three weeks at room temperature. The dried fruit was then milled to be powder (4.86 kg) [13].

Preparation of F. fragrans methanol extract and ethyl acetate fraction

Dried powder of *F. fragrans* fruit (1.2 kg) was extracted with MeOH (3 x 4L) at room temperature for 3 x 30 hours. The total MeOH (12 L) were dried to be residue (67.8 g) under reduced pressure by using rotary evaporator and it is called as MeOH extracts. EtOAc fraction was made by dissolving the above residue (60.1 g) with MeOH (250 mL) in separating funnel and water 300 mL was added and then extracted with *n*-hexane (3 x 500 mL), and EtOAc (3 x 500 mL) respectively. The total EtOAc (1.5 L) were dried to be residue (18.5 g) under reduced pressure by using rotary evaporator (**Figure** 1(b)). Keep the residue in desiccator provided with silica as adsorbent for 2 weeks and then its Antioxidant activity test was performed by DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging method, and compare to reference standard ascorbic acid [3,14].

Isolation

Isolation has been done shortly from concentrated acetone coming from EtOAc fraction-residue by chromatography methode with 10% ethyl acetate in n-hexane.

DPPH Assay [9]

DPPH Solution Preparation

DPPH solution (0.05 M) was prepared by dissolving (1.95 mg) DPPH in a 100 mL dark volumetric flask, adding methanol to the mark and then homogenized.

Determination of Maximum Wavelength (λ_{max})

DPPH solution (3.8 mL) was added with 0.2 mL of methanol and then the absorbance was measured at a wavelength of 400-600 nm with a UV-Vis spectrophotometer and methanol was used as a blank.

Determination of antioxidant activity of MeOH extract of F. fragans

The antioxidant activity of MeOH Extract was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay [9,14] which has maximum absorbance at 515 nm (A = 0.874). This DPPH assay was carried out on MeOH extract without precipitating compound. The stock solution of MeOH extract and MeOH fraction without precipitating compound was prepared at 1000 µg/mL in methanol, and then gradually diluted into several concentrations (500, 250, 125, 62.5, 31.25 and 15.625 µg/mL). The assay consisted of the reaction of 3.8 mL DPPH solution and 0.2 mL sample solution at each dilution. After 30 minutes of reaction, the absorbance of each solution was measured at 515 nm using UV-Vis spectrophotometer (Thermo Scientific Orion AquaMate 8000 UV-Vis). The percentage of DPPH inhibition was calculate using the following equation:

$$\%\ Inhibition = \left(\frac{A_0 - A_1}{A_0}\right) x\ 100\% \quad (1)$$

where, A_0 is control absorbance and A_1 is absorbance after 30 minutes' incubation.

Through linear regression, the inhibition concentration (IC_{50}), the sample concentration necessary for the inhibition of 50 % of DPPH free radicals, was determined. The same method was also carried out for Ascorbic acid at various concentration (1.0, 2.5, 5.0, 7.5 and 10.0 µg/mL) as standard. The same prosedure was also used to determinate antioxidant activity for both EtOAc fraction and the isolated compound (Rf = 0.35) respectively. where the concentration of the isolated compound (Rf = 0.35) in this experiment was prepared at 1000 µg/mL in methanol, and then gradually diluted into several concentrations (500; 250; 62,5; 31,25; 15,625; 7,825 µg/mL).

(a) (b)

Figure 1. *F. fragrans* fruit (a), and Phytocosmetics of the fruit with rice as excipients (b)

Determination of total flavonoid content

The total flavonoid content was determined by spectrophotometry based on reaction. EtOAc fraction sample (3 mL) was added to a measure flash containing methanol (2mL) and CH_3COONa 1 M (0.5 mL), it was mixed and left for 5 min. Into this mixture, AlCl3 10% (0.2 mL) added and then homogenized for 5 min. Quercetin standard curves were made with a concentration series 5.0, 6.0, 7.0, 8.0, 9.0, and 10 µg/mL and treated the same as the samples described above. Absorbance fractions and standards were measured at λmax 420-430 nm (abs.=0.662) [9,14]. The blanks are defined as all reagents used without quercetin or sample i.e. methanol, water, and CH_3COONa 1 M, and the measurements were replicated triplicate. This method is also used for MeOH extracts [3,9].

Data analysis

The findings pertaining to the IC_{50} value of DPPH radical including the computation of the total flavonoid content (TFC) and total phenolic content (TPC) for both of the EtOAc fraction and MeOH extracts have been calculated according to the standard prosedure of DPPH method [3,9,14].

RESULTS AND DISCUSSION

Results of the extraction stage

In this context, we report the antioxidant activity, flavonoid and phenolic contents of MeOH extracts and EtOAc fraction derived from *F. fragrans* fruits. The MeOH extract yielded an IC_{50} DPPH value of 186.5± 0.52 µg/mL with an R-squared (R^2) value of 0.998 (see **Figure** 2 and **Table** 1), and the EtOAc fraction yielded an IC_{50} DPPH value of 357.2 ± 0.59 µg/mL with an R-squared (R^2) value of 0.995 (**Table** 1), while the isolated compound yielded an IC_{50} DPPH value of 5.658 µg/mL ± 0.57 µg/mL with an R-squared (R^2) value of 0.915 (see **Figure** 3 and Table 1), Additionally, the IC_{50} value of ascorbic acid as standard graph for the estimation of IC50value was found to be 24.1 µg/mL with an R-squared (R^2) value of 0.999.

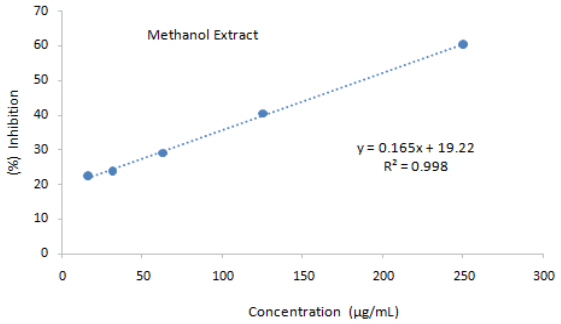


Figure 2. Graph of IC_{50} value of DPPH radical by MeOH extract of *F. fragrans*fruit (186.5 µg/mL), control absorbance = 0.567.

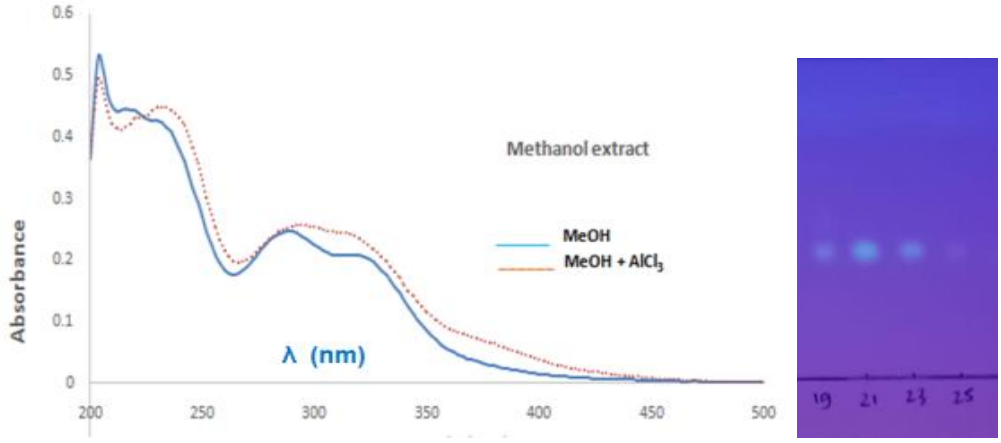
The dried MeOH extracts of *F. fragrans* fruits are rich in ursolic and its isomeric oleanolic acids [5,6,20]. However, these triterpenic acids are very difficult to dissolve in MeOH, which affects the inhibitory capability of the MeOH extracts in the DPPH test. Despite this limitation, the MeOH extract exhibited higher antioxidant activity (IC_{50} DPPH = 186.5 ± 0.52 µg/mL) than the EtOAc fraction (IC_{50} DPPH = 357.2± 0.59 µg/mL), although both values are lower than that of ascorbic acid (IC_{50} DPPH = 24.1 µg/mL). The MeOH extract is classified as a moderate antioxidant activity because its IC_{50} value is lower than 200 µg/mL, while the EtOAc fraction is categorized as a weak antioxidant activity because its IC_{50} value is higher than 200 µg/mL [16]. Moreover, the total flavonoid and phenolic contents of the MeOH extracts and EtOAc fraction were measured using a colorimetric assay with reagents of 1 M sodium acetate and 10% aluminum chloride. The EtOAc fraction had a total flavonoid content of 4.505 mgQE/g-extract and a total phenolic content of 13.732 mgGAE/g-extract, while the MeOH extracts had a total flavonoid content of 9.088 mgQE/g-extract and a total phenolic content of 23.342 mgGAE/g-extract, respectively (see **Table** 1).The absorbance of standard quercetin λ_{max} = 440 nm for EtOAc fraction, and MeOH extracts, and its Standard Graph of quercetin for the estimate of total flavonoid content have R-squared (R^2) value of 0.999. While Theabsorbance of standard gallic acid is λmaks = 740 nm for EtOAc fraction, and MeOH extract and its Standard Graph of gallic acid for the estimate of total phenolic content have R-squared (R^2) value of 0.997.

It was clearly shown that methanol extract with IC_{50} = 186.5 µg/mL that containing an isolated compound IC_{50} = 5.66 µg/mL is preferred then ethyl acetate fraction for phytocosmetics application . In accordance with data analysis in **Table** 1 that the methanol extract with TFC value = 9.0881 ± 0.000816497 mgQE/g extract and TPC value = 23.34164 ± 0.001633 mgGAE/g extract is more rational to be developed and used as ingredient of phytocosmetics. as it was supported by its anti-bacterial, anti-inflammatory, and anti-cancer activities [5,6,20].

Table 1. Flavonoid and phenolic total contents, and IC_{50} DPPH of *F. fragrans* fruit

No	Extracts	Flavonoid content (mg QE/g-extract)	Phenolic content (mg GAE/g-extract)	IC_{50} DPPH (µg/mL)
1	MeOH	9.088[(a)]	23.342[(b)]	186.5 ± 0.52
2	EtOAc	4.505[(a)]	13.732[(b)]	357.2 ± 0.59
3	Isolated compound (*Rf = 0.35*)	NA	NA	5.658

Note: (a) = Quercetin, (b) = Galic acid, values are expressed as means ± SD of triplicate measurements

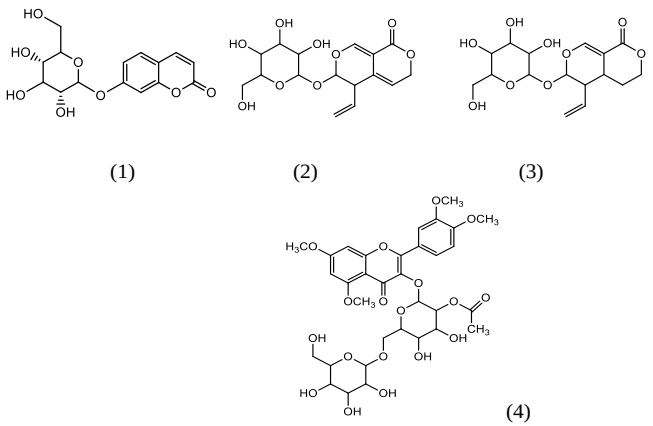


(a) (b)

Figure.4. (a) UV spectral of the MeOH extract and (b). TLC of isolated compound, Rf= 0.35 in 20% ethyl acetate in *n*-hexane

In addition to the data presented in **Table** 1, the IC_{50} DPPH value, total flavonoid, and phenolic contents, this work is also provided with UV of MeOH extracts (**Figure** 4(a)), and the TLC chromatogram of isolated compound under 254 nm UV light (**Figure** 4(b)), while LC-MS/MS of EtOAc fraction (**Table** 2) as compound indentity of F fragrans fruit extracts to predict constituent playing in role to the antioxidant property of this fruit. The EtOAc fraction showed a positive test with the $AlCl_3$ reagent and the UV spectral of the MeOH extract is given in **Figures** 4(a) respectively. The MeOH extract exhibited UV absorbance with two peaks observed 240-250 nm (benzoyl band) and 280-340 nm (cinnamoyl band). These spectral patterns are relatively closed to the UV spectral of flavonoid UV. So that the MeOH extracts is also expected to have photoprotective property to the skin from UV B and UV A wavelengths of sun ray. Therefore, the flavonoid or phenolic compounds present in *F. fragrans* fruits appear to contribute to their antioxidant activity, as indicated by the IC_{50} DPPH value. Thus, the MeOH extract, EtOAc fraction, and also isolated compound from *F. fragrans* fruits may serve as raw materials for phytocosmetic and herbal cosmetics for skincare, such as anti-aging products that combat the effects of reactive oxygen species and sun exposure.

To support the antioxidant activity such as flavonoid, and phenolic contents that could be found in this fruit. LCMS/MS analysis of EtOAc fraction which can be simpler than the LCMS/MS of MeOH extract is reported in this manuscript as additional data. EtOAc fraction is revealing 16 chromatogram peaks; there are 7 peaks having high intensity. Four compounds representing those peaks are reported in **Figure** 5. The four compounds were identified as coumarin compounds, namely skimmin ($C_{15}H_{16}O_8$), see compound 1 with m/z = 324 $[M]^+$ and rt. = 3.46 minutes; secoiridoid [18] namely gentiopicroside ($C_{16}H_{20}O_9$), see compound 2 with m/z = 356 $[M]^+$ and RT = 4.18 minutes; secoiridoid [18]. namely sweroside ($C_{16}H_{22}O_9$), see compound 3 with m/z = 358 $[M]^+$ and rt. = 4.47 minutes; and flavonoid glycoside, namely 2-((2-(3,4-dimethoxyphenyl)-5,7-dimethoxy-4-oxo-4H-chromen-3-yl)oxy)-4dihydroxy-6-(((3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2yl)oxy)methyl)tetrahydro-2H-pyran-3-yl acetate ($C_{33}H_{40}O_{18}$), see compound 4 with m/z = 722.3 $[M-2H]^+$, m/z = 723.2 $[M-H]^+$, m/z = 724.3 $[M]^+$, and m/z =725.3 $[M+H]^+$, and RT = 7.36 with molecular formula of $C_{33}H_{40}O_{18}$, see **Table** 3. This Table provides the precise calculated ion, selected ion, and precursor ion data of the four compounds, which closely match the experimental data. These LCMS/MS-identified compounds are likely to contribute to the UV spectral and antioxidant activity of the EtOAc fraction and MeOH extracts of *F. fragrans* fruits [18].



(1) (2) (3) (4)

Figure 5. Chemical structure identified by LCMS/MS from EtOAc fraction.

Table 2. Four compound classes identified by LCMS/MS from EtOAc fraction.

RT (min)	Compound class (structure number)	Formula	ion	Experimental	
				Selected ion	Precursor ion
3.46	Coumarin (1)	$C_{15}H_{16}O_8$	324	$[M+H]^+$	325(100%), 326(20%), 228(23%), 197(50%), 163(60%)
4.18	Gentiopicroside (2)	$C_{16}H_{20}O_9$	356	$[M+H]^+$	357(7%), 215(8%), 177(100%), 359(26%),
4.47	Sweroside (3)	$C_{16}H_{22}O_9$	358	$[M+H]^+$	197(100%), 127(29%), 717(21%) $[2M+H]^+$
7.36	Flavonoid glycoside (4)	$C_{33}H_{40}O_{18}$	722	$[M-2H]^+$	722(100%), 723(45%) $[M-H]^+$, 724(23%) [M], 725(6%) $[M+H]^+$

The antioxidant properties of MeOH extracts and EtOAc fraction of *F. fragrans* fruits have gained as well as some compounds found in EtOAc fraction. They are coumarin glucoside, iridoid glucoside, and flavonoid glucoside. Such as ascorbic acid which is rich with hydroxyl group (-OH) which is adjacent to double bond (-C=C-) conjugating with carbonyl (C=O), those four compounds reported in **Table** 5 could also play in role to contribute for antioxidant property by capturing free radicals [2,18]. As a result, various cosmetics and phytocosmetics products in modern urban society including intensive phytochemistry research can be made on *F. fragrans* fruit. Factors such as ultraviolet rays, fine dust, and blue light are known to induce the formation of active oxygen species, which contribute to the ageing process might be overcome by daily using *F. fragrans* fruit phytocosmetics [15,17,19].

CONCLUSION

Based on the results of this antioxidant study, the isolated compound (Rf = 0.35) give IC_{50} value 5.66 ± 0.57 µg/mL as strong antioxidant activity. The IC_{50} value of 186.5 ± 0.52 µg/mL suggests that the methanol extract of *F. fragrans* fruits has moderate antioxidant properties, while the total flavonoid (9.088 mgQE/g-extract) and phenolic (23.342 mgGAE/g-extract) content values indicate its potential as an antioxidant. Overall, these findings recomended that *F. fragrans* fruit methanol extract may be useful for its antioxidant properties in various applications.

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