in vitro Antidiabetic, Antioxidant and Cytotoxic Activities of *Syzygium cumini* Fractions from Leaves Ethanol Extract

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Abstract

Ethanol extract of *Syzygium cumini* (jamblang) leaves reveal its significant α-glucosidase inhibitory activity which perform the potential activity of this plant extract as antidiabetic agent. However, other bioactivities of this plant extract have been reported. The aim of current study was to evaluate the comparison of *in vitro* antidiabetic, antioxidant and cytotoxic activity of *S. cumini* fractions. The *S. cumini* leaves ethanol extract was fractionated by using column chromatography with n-hexane, ethyl acetate and methanol as eluents. Seven fractions were obtained from column chromatography (F1-7). The results showed that the best antidiabetic activity was found in F5 (93% α-glucosidase inhibitory activity), the best antioxidant activity was found in F4 (83% inhibition of DPPH free radical) and the best cytotoxic activity was found in F2 (69% growth inhibition of T47D breast cancer cell line). Therefore besides *in vitro* antidiabetic activity, fractions of *S. cumini* leaves ethanol extract also showed antioxidant and cytotoxic activities. Since the results showed that the most active fractions for antidiabetic, antioxidant and cytotoxicity were differed, it could be possible to isolate the different potential active compounds for each activity.

Keywords : *Syzygium cumini, a-glucosidase inhibitor, DPPH, cytotoxicity*

INTRODUCTION

Syzygium cumini (L.) Skeels (Myrtaceae) is a traditional medicinal plant that has been used to treat a number of illnesses. This medicinal plant is native to the subtropical Himalayas, India, Sri Lanka, Malesia and Australia, where it is also widely cultivated. At present it is grown throughout the tropics and subtropics. This plant have synonyms as *Eugenia jambolana* Lam., *Myrtus cumini* Linn., *Syzygium jambolana* DC., *Syzygium jambolanum* (Lam.) DC., *Eugenia djouant* Perr., *Calyptranthes jambolana* Willd., *Eugenia cumini* (Linn.) Druce. and *Eugenia caryophyllifolia* Lam. Whereas the common names are jambolan, black plum, jamun, java plum, Indian blackberry, Portuguese plum, Malabar plum, purple plum, Jamaica and damson plum (Ayyanar and Subash-Babu, 2012).

S. cumini extracts prepared from its different parts, especially seed, and pulp-fruit has been

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studied especially for antidiabetic and metabolic-related activity. However, phytochemical profile and biological properties of its leaf is a lack of studies. Our previous studies on 70% ethanol extract of *S. cumini* leaves found significant *in vitro* (α-glucosidase inhibitory activity) and *in vivo* (animal model) antidiabetic activity which present the potential of this plant extract for diabetes treatment. Antioxidant activity of *S. cumini* is reported by Mohamed, *et al*., (2013) and Ruan, *et al*., (2008). Fruit extract of *S. cumini* shows cytotoxic activity on MCF-7 breast cancer cells (Tripathy and Pradhan, 2015). However, the comparison of antidiabetic, antioxidant and cytotoxic activity from *S. cumini* leaves fraction has never been reported yet. The aim of current study was to evaluate the comparison of *in vitro* antidiabetic, antioxidant and cytotoxic activity of *S. cumini* leaves fractions obtained from column chromatography of the leaves ethanol extract using n-hexane, ethyl acetate and methanol as eluents.

MATERIALS AND METHODS

Material

S. cumini leaves EtOH extract (6 gr) was fractionated in silica gel vacuum column chromatography using n-hexane, ethyl acetate and methanol as eluents (Hexane 100%; Hexane: Ethyl Acetate 50%; Ethyl Acetate 100%; Ethyl Acetate: Methanol 25%; Ethyl Acetate: Methanol 50%; Ethyl Acetate: Methanol 75%; Methanol 100%). The dryed fractions obtained were subjected to antidiabetic, antioxidant and cytotoxicity assays.

Antidiabetic assay

Measurement of α-glucosidase inhibition activity was performed according to the method of Kim, *et al*., (2004) with slightly modification. Solution of p-nitrophenyl-α-D-glucopyranoside (Sigma) 5 mM (25 µL) and 0.1 M phosphate buffer pH 7 (50 µL) was added to a microplate containing 5 µL sample solution in DMSO (Merck, New Jersey, USA) to give final concentration 5 µg/mL. The solution was preincubated for 5 min at 37°C, the reaction was initiated by addition of 25 µL solution of 0.063 units α-glucosidase (TCI, Tokyo, Japan), and incubated for the next 15 minutes. The reaction was stopped by addition of 95 µL of 0.2 M Na_2CO_3 (Merck). The enzyme activity was measured by reading absorbance of p-nitrophenol formed at λ 400 nm (Varioskan Flash, Thermo Fisher, Massachusetts, USA).

Antioxidant assay

The α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging activity was conducted according to Yen and Chen (1995). Sample was dissolved in methanol (Merck), 5 µL sample was pipette into the microplate (Nunc, Roskilde, Denmark). Then, 40 µL methanol solution of 1 mM DPPH (Sigma, Missouri, USA) was added to the sample solution, and followed with 155 µL methanol was added to give final concentration of the sample was 5 µg/mL. Absorbance was measured at 515 nm (Varioskan Flash, Thermo Fisher).

Cell culture and *in vitro* cytotoxic activity examination

Breast cancer cell line T47D was kindly provided by Prof. Dr. Edy Meiyanto (Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta) and maintained in Research Center for Chemistry, Indonesian Institute of Sciences (Lembaga Ilmu Pendidikan Indonesia/LIPI). The cell lines were cultivated at 37°C with 5% CO_2 in RPMI-1640 (GIBCO, Massachusetts, USA) medium supplemented with 10% v/v Fetal Bovine Serum (Sigma), 1% antibiotic-antimycotic (GIBCO). Cell suspensions ($5x10^4$ cells/mL) were seeding to each well and the cells were incubated for at 37°C under 5% CO_2. After 24 hours, samples were added and microscopic observation was conducted after 24 hour incubation, AlamarBlue reagent (Thermo Fisher) was added to the sample for quantitative fluorometric measurement of cytotoxicity activity (Varioskan Flash, Thermofisher).

RESULTS

Seven fractions (F1-7) were obtained from column chromatography using various solvents (Table 1). F1 was not analyzed for bioactivities since the yield obtained only 2.9 mg (0.06%), which suggest that 70% ethanol extract of *S. cumini* might only has small amount of non-polar compounds. The semi-polar to polar fractions (F5-7) was considered as the major content that gives yield 66.33% whereas the more semi-polar fractions (F2-4) were only 36.61%. Table 1 showed the comparison of antidiabetic, antioxidant and cytotoxic activities of F2-7. The results showed that F2-F4 exhibited a good cytotoxic activity, F5-F7 exhibited a good antidiabetic activity and F4-F7 exhibited a good antioxidant activity. The best antidiabetic activity was found in F5 (93% α-glucosidase inhibitory activity), the best antioxidant activity was found in F4 (83% inhibition of DPPH free radical) and the best cytotoxic activity was found in F2 (69% growth inhibition of T47D breast cancer cell line) which also shown in Figure 1. T47D cells on F2-F4 treatment showed morphological alteration compared to untreated cells. Most of dead cells was observed on F2-F4 treatment as black dot that not attached to the well that showed its good cytotoxic activity toward cancer cells. But then, treatment of F5-F7 did not alter the morphology of T47D cells compared to untreated cells.

DISCUSSION

Although the highest antidiabetic activity as α-glucosidase inhibitor were found on F5, activities of F6 and F7 were also significantly high (88% inhibitory activity to α-glucosidase). Therefore further attempt to isolate the active antidiabetic compounds from these 3 fractions currently still in progress which will support our previous finding that the 70% ethanol leaves extract has in vitro and in vivo antidiabetic activities. This finding was in accordance with studies reported by Sanches, *et al*., (2016).

Besides its antidiabetic activity, fraction F5-7 also showed excellent antioxidant as DPPH free radical scavenged. This suggest F5-7 containing compounds that have both activities such as flavonoid and phenolic compounds. Whereas F4 containing compound that having the best antioxidant activity and average cytotoxic activity. One of mechanism of action of anticancer drug is

Table 1. Antidiabetic, antioxidant and cytotoxic activities of *S.cumini* fractions.

Fraction	Eluent	Dry Weight (mg)	Antidiabetic** (α-glucosidase inhibitory activity) (%)	Antioxidant** (DPPH free radical scavenging activity) (%)	Cytotoxicity*** (against breast cancer cell line T47D) (%)
F1	Hexane 100%	2.9	Not determined	Not determined	Not determined
F2	Hexane: Ethyl Acetate 50%	978	36	9	68
F3	Ethyl Acetate 100%	627	41	31	56
F4	Ethyl Acetate: Methanol 25%	245	44	83	50
F5	Ethyl Acetate: Methanol 50%	2000	97	81	8
F6	Ethyl Acetate: Methanol 75%	2000	88	82	7
F7	Methanol 100%	1200	88	79	7

Notes: Sample concentration **=5μg/mL; ***=100μg/mL

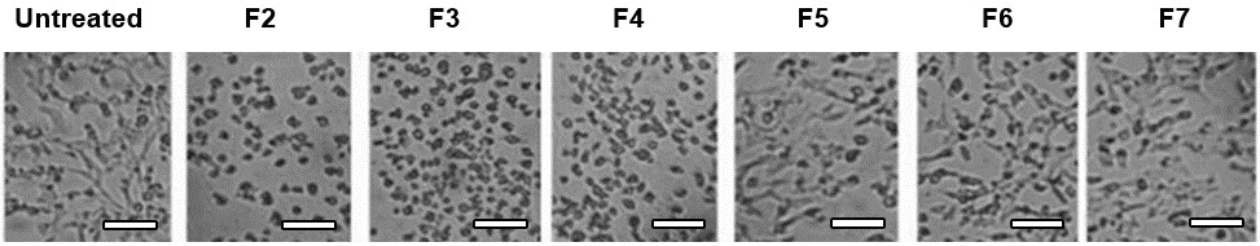

Figure 1. Morphological alteration of S. cumini fractions on T47D cells. Cells were treated with and without 100 µg/mL of *S.cumini* fractions for 24 h and followed with alamarBlue cytotoxic assay. Scale bar=100 µm.

related with mechanism of action of antioxidant (Lewandowski and Gwozdzinski, 2017). Then, it is reasonable that F4 exhibit antioxidant and also cytotoxic activity, but not all of fractions of S. cumini that show antioxidant activity also perform cytotoxic activity. Antioxidant activity as DPPH free radical scavenging activity of *S. cumini* leaves also reported by Ruan, *et al*., (2008) in methanol extract and its fractions (water, ethyl acetate (EaF), chloroform (CfF), and n-hexane (HxF) fractions); by Mohamed, *et al*., (2013) in methanol extract, dichlormethane extract and essential oils; by Ecker, *et al*., (2015) in aqueous extract.

As shown in fluorometric measurement and microscopic observation, the good cytotoxic activity against T47D breast cancer cell line were only shown in fraction F2-4 (more than 50%), whereas fraction F5-7 only showed very low activity (less than 10%). Anticancer activity of *S. cumini* was also reported by Aqil, *et al*., (2016); Barh and Viswanathan, (2008); Goyal, *et al.*, (2010); and Parmar, *et al*., (2010). Interestingly, this study revealed that most of *S. cumini* active fraction for antidiabetic and antioxidant had differ polarity compared with its active fraction for anticancer. *S. cumini* leaves contains non polar compounds, such as β-sitosterol, essential oil (pinocarveol, myrtenol), triterpene (betulinic acid), semi polar compounds such as flavonoid (quercetin, myricetin) and polar compounds such as glycoside derivatives of flavonoid (Subramanian, *et al*., 2013). Essential oil, β-sitosterol, betulinic acid and semi-polar flavonoid from other plant show cytotoxic activity on various cancer cells (Baskar, *et al*., 2010; Raja, *et al*., 2017; Urban, *et al*., 2012; Zorzetto, *et al*., 2015). Flavonoid quercetin and its glycoside had been studied for its antioxidant and antidiabetic activities (Eid, *et al*., 2010; Panat, *et al*., 2015; Zheng, *et al*., 2017). This study come to a suggestion that the potential anticancer compounds of *S. cumini* leaves might be differed from its potential antidiabetic and antioxidant compounds. Non polar and semi-polar flavonoid of *S. cumini* may have a role on its cytotoxic activity. On the other hand, the more polar compounds of *S. cumini* may have a role on its antidiabetic and antioxidant activity. Nevertheless, the advance study need to be done to explore the active compounds that have a role on each activity.

CONCLUSION

The results showed that besides the antidiabetic activity, the fractions from *S. cumini* leaves EtOH extract also showed antioxidant and cytotoxic activities. Since the most active fractions for antidiabetic, antioxidant and cytotoxicity were differed, it could be possible to isolate the different potential active compounds for each activity.

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