ABSTRACT

Tamarillo fruit is unique because it had anthocyanin (polar) and carotenoid (nonpolar) together in the fruit. They were natural antioxidant. Antioxidant needed in type 2 diabetes to defeat oxidative stress. Tamarillo extraction could be performed by the method of maceration, but had disadvantage at long extraction time. Sonication method was recognized to be able to repair the weaknesses of maceration method, but it really be determined by on material matrix. Application of sonication method on extraction tamarillo using ethanol solvent to get anthocyanin-rich extract and acetone solvent to obtain carotenoid-rich extract that have potential as lowering blood sugar in rats *Sprague Dawley* (SD) type 2 diabetes induced STZ-NA had never been reported.

The aims of the research were as follow: (1) to analyze chemical composition of the tamarillo fruit (nutriton compounds, anthocyanin, carotenoid and antioxidant activity); 2) proved the method sonication on the extraction tamarillo of ethanol and acetone solvents could increase antioxidant potential caused by the more cell damage than maceration; 3) to determine ability to inhibit the α-glucosidase enzyme *in vitro assay* of extract tamarillo obtained from sonication method in the form of ethanol extract (EE), acetone extract (EA) and thier mixtured (CEEA); 4) determine the ability of lowering blood sugar of EE, AE and MEAE in rats SD type 2 diabetes induced STZ-NA and 5) determining the mechanism of decreasing blood sugar of anthocyanin EE and carotenoid EA of tamarillo in rats SD type 2 diabetes induced STZ-NA.

The research was conducted in three steps: the firs step 1 begun to analysis of chemical composition of the tamarillo fruit (nutrition compounds, levels of anthocyanin, carotenoid, and antioxidant activity), followed by the extraction tamarillo of ethanol and acetone solvents using the extraction method of maceration and sonication. Parameters analysis were yield, antioxidant activity (DPPH), the microstructures of surface cells of materials and residuce (on EE and EA), levels of anthocyanin, phenolic, types of anthocyanin (on EE), levels of carotenoids, a type of carotenoid (on EA). Data analysis used independent t test (maceration with sonication). The second step was in *in vitro* assay of extracts tamarillo obtained from the sonication method in the form of EE, EA and CEEA with acarbose as a control. Parameters analysis were percentage of α-glucosidase enzyme inhibition, IC_{50} and inhibition mode. Data analysis used ANOVA test and advanced test DMRT. The third step was *in vivo* bio-assay was used rats SD that divided into six groups of each 5 rats. They were three control groups: the first healty rats as KON; two diabetic rats, untreatment as KON and metformin drug as KOP; three diabetic groups: EE treatment as DEE, EA treatment as DEA and their mixture as DCEEA. Parameters analysis were feed intake, weight, feed efficiency, fasting blood sugar, fasting insulin, HOMA-IR, HOMA-β, GLUT4, SOD, MDA and histopathology of the pancreas. Data analysis used ANOVA and advance test DMRT.

The results of the research showed tamarillo fruit had low fat and potential as antioxidant that indicated levels of anthocyanin and carotenoid: 83.90 ± 1.40 mg/100 g; 1.61 ± 0.01 mg/100 g respectively, and also antioxidant activity: 195.09 ± 14.01 mg AAE/100 g (DPPH). Sonication extraction method could be applied to

the extraction of tamarillo (ethanol and acetone solvents) and could increase antioxidant potential than maceration. Tamarillo extracts obtained from the sonication method had the anthocyanin level and antioxidant activity: 386.48 ± 19.82 mg/100 g and 68.28% (DPPH) of EE respectively. Levels of carotenoids and antioxidant activity: 50.80 ± 3.02 mg/100 g and 55.48% (DPPH) of EA respectively. Types of anthocyanis included of: *Dp-3-rutinoside, Dp-3-glucoside-5-rhamnoside; Pg-3-rutinoside, Cy-3-rutinoside, Cy-3-O-glucosyl-rutinoside,* and *Dp-3-(6-z-ρ-coumaroylglucoside)-5-(6-malonylglucoside*). Types of carotenoids included of: *β-carotene, lutein, zeaxanthin, neoxanthin,* and *β-cryptoxanthin*. The largest type of anthocyanin and carotenoid respectively: *Pg-3-rutinoside* and *β-cryptoxanthin*. Damage microstructures of cell surface of residues more severe than maceration. In *in vitro* EE (concentration 40 µg/mL), EA (concentration 50 µg/mL), and the CEEA (20 µg EE/mL and 25 µg EA/mL) could inhibit the α-glucosidase enzyme. The highest percentage of inhibition of α-glucosidase enzym on EE of 48.08 ± 1.87% with IC_{50} 43.51 µ g/mL, followed CEEA and EA of: 42.14 ± 1.47% and 1.72 ± 30.59% respectively. The percentage inhibition of EE is still lower than acarbose. Mode of inhibition of EE, EA, and CEEA were mixed inhibitor that different to acarbose (competitive inhibitors). EE diet (19.3 mg anthocyanin/kg BW), EA diet (2.5 mg carotenoid/kg BW), and CEEA diet (9.65 mg anthocyanin/kg BW and 1.25 mg carotenoid/kg BW) for 28 days colud be lower blood sugar in SD rats induced STZ-NA. The best results as lowering blood sugar and insulin resistance were on the DEE with the percentage decrease in blood sugar and HOMA-IR of: 50.20% and 2.28 ± 0.45 respectively. Best results increase the insulin and pancreatic SOD were on the DCEEA of: 10.84 ± 0.88 µ U/ml; 0.81 ± 0.17 ng/mL respectively. The mechanism of decreasing blood sugar of the anthocyanin EE and carotenoid EA of tamarillo in SD rats induced STZ-NA through several ways including: 1) α-glucosidase enzyme inhibition; 2) increased uptake of glucose g; 3) increased levels of insulin; and 4) a decrease in oxidative stress (antioxidant function).

Keywords: anthocyanin, carotenoids, extract, tamarillo, type 2 diabetes

INTISARI

Tamarillo merupakan buah yang unik karena memiliki antosianin yang bersifat polar dan karotenoid yang bersifat non polar secara bersama pada bagian buah. Kedua senyawa tersebut merupakan antioksidan alami. Antioksidan diperlukan pada diabetes tipe 2 untuk menekan stres oksidatif. Ekstraksi tamarillo dapat dilakukan dengan metode maserasi, namun memiliki kekurangan seperti waktu ekstraksi yang lama. Metode ekstraksi sonikasi diketahui dapat memperbaiki kekurangan dari metode maserasi, namun sangat tergantung pada matrik bahan. Aplikasi metode sonikasi pada ekstraksi tamarillo untuk mendapatkan ekstrak etanol kaya antosianin dan ekstrak aseton kaya karotenoid yang memiliki potensi sebagai penurun gula darah pada diabetes tipe 2 menggunakan tikus *Sprague Dawley* (SD) induksi *steptozotocin-nicotinamida* (STZ-NA) belum pernah dilaporkan.

Tujuan penelitian meliputi: 1) menentukan komposisi kimia buah tamarillo (proksimat, serat pangan dan komponen antioksidan: vitamin C, antosianin, karotenoid); 2) membuktikan metode sonikasi pada ekstraksi tamarillo pelarut etanol dan aseton dapat meningkatkan potensi antioksidan yang disebabkan oleh kerusakan sel yang lebih parah daripada maserasi; 3) mengevaluasi secara *in vitro* kemampuan ekstrak tamarillo yang diperoleh dari metode sonikasi dalam bentuk ekstrak etanol (EE), ekstrak aseton (EA) dan campurannya (CEEA) sebagai penghambat enzim α-glukosidase 4) mengevaluasi secara *in vivo*, kemampuan EE, EA dan CEEA tamarillo sebagai penurun gula darah pada tikus SD diabetes induksi STZ-NA; dan 5). menentukan mekanisme penurunan gula darah dari antosianin EE dan karotenoid EA pada tikus SD diabetes induksi STZ-NA.

Penelitian dilakukan dalam 3 tahap yaitu: tahap 1 diawali dengan analisis komposisi kimia buah tamarillo (proksimat, serat pangan dan vitamin C, antosianin, karotenoid serta aktivitas antioksidan), dilanjutkan dengan ekstraksi tamarillo pelarut etanol dan aseton menggunakan metode ekstraksi maserasi dan sonikasi. Parameter uji meliputi: rendemen, aktivitas antioksidan (DPPH), mikrostruktur permukaan sel bahan dan residu (pada EE dan EA), kadar antosianin, kadar fenolik, jenis antosianin (pada EE), kadar karotenoid, jenis karotenoid (pada EA). Analisis data dengan uji t independen. Tahap 2 menguji secara in *vitro* ekstrak tamarillo yang diperoleh dari metode sonikasi dalam bentuk EE, EA dan CEEA dengan kontrol acarbose. Parameter uji meliputi: persentase penghambatan enzim α-glukosidase, IC_{50} dan mode penghambatannya. Analisis data dengan uji ANOVA dan uji lanjut DMRT. Tahap 3, menguji secara *in vivo* menggunakan tikus SD dengan 6 kelompok masing-masing 5 tikus meliputi; satu kontrol sehat (KOS); dua kontrol diabetes dengan tanpa perlakuan sebagai kontrol negatif (KON) dan obat metformin sebagai kontrol positif (KOP) dan tiga kelompok diabetes dengan perlakuan diet EE (DEE), diet EA (DEA) dan diet CEEA (DCEEA). Tikus diabetes dengan cara induksi STZ-NA. Intervensi dilakukan selama 28 hari. Parameter uji meliputi: konsumsi pakan, berat badan, efisiensi pakan, gula darah puasa, insulin puasa, HOMA-IR, HOMA-β, GLUT4, MDA, SOD dan histopathologi pankreas. Analisis data dengan ANOVA dan uji lanjut DMRT.

Hasil penelitian menunjukkan buah tamarillo memiliki komposisi kimia zat gizi rendah lemak dan potensial sebagai antioksidan karena adanya antosianin dan karotenoid. Kadar antosianin dan kadar karotenoid masing-masing sebesar: 83,90±1,40 mg/100 g dan 1,61±0,01 mg/100 g, serta aktivitas antioksidannya sebesar 195,09±14,01 mg AAE/100 g (DPPH)). Metode ekstraksi sonikasi dapat diterapkan pada ekstraksi tamarillo (pelarut etanol dan aseton) dan dapat meningkatkan potensi antioksidan dibandingkan maserasi. Ekstrak tamarillo yang diperoleh dari metode sonikasi memiliki kadar antosianin dan aktivitas antioksidan EE masing-masing sebesar: 386,48±19,82 mg/100 g dan 68,28% (DPPH). Kadar karotenoid dan aktivitas antioksidan EA masing-masing sebesar: 50,80±3,02 mg/100 g dan 55,48% (DPPH). Jenis antosianin meliputi: *Dp-3-rutinoside, Dp-3-glucoside-5-rhamnoside; Pg-3-rutinoside, Cy-3-rutinoside, Cy-3-O-glucosyl-rutinoside* dan *Dp-3-(6-z-ρ-coumaroylglucoside)-5-(6-malonylglucoside*). Jenis karotenoid meliputi: *β-caroten, neoxantin, zeaxantin, lutein dan β-cryptoxantin.* Jenis antosianin dan karotenoid terbesar masing-masing: *Pg-3-rutinoside* dan *β-cryptoxantin.* Kerusakan mikrostruktur pemukaan sel residu sonikasi lebih parah daripada maserasi. Secara *in vitro* EE (40 µg/mL), EA (50 µg/mL) dan CEEA (20 µg EE/mL dan 25 µg EA/mL) dapat menghambat enzim α-glukosidase. Persentase penghambatan enzim α-glukosidase tertinggi pada EE sebesar 48,08±1,87% dengan IC_{50} 43,51 µg/mL, diikuti CEEA dan EA masing-masing sebesar: 42,14 ±1,47% dan 30,59 ±1,72%. Persentase penghambatan EE masih lebih rendah dari acarbose. Mode penghambatan EE, EA dan CEEA adalah mixed inhibitor berbeda dengan acarbose (kompetitif inhibitor). Diet EE (19,3 mg antosianin/kg BB), EA (2,5 mg karotenoid/kg BB) dan CEEA (9,65 mg antosianin/kg BB dan 1,25 mg karotenoid/kg BB) selama 28 hari dapat menurunkan gula darah pada tikus SD diabetes induksi STZ-NA. Hasil terbaik sebagai penurun gula darah dan resistensi insulin pada DEE dengan persentase penurunan gula darah dan HOMA-IR masing-masing sebesar: 50,20 % dan 2,28±0,45. Hasil terbaik meningkatkan insulin dan SOD pankreas pada DCEEA dengan kadar insulin dan SOD masing-masing sebesar: 10,84±0,88 µU/ml; 0,81 ± 0,17 ng/mL. Mekanisme penurunan gula darah dari antosianin EE dan karotenoid EA tamarillo pada tikus SD diabetes induksi STZ-NA melalui beberapa cara meliputi: 1) penghambatan enzim α-glukosidase 2) peningkatan *uptake* glukosa; 3) peningkatan kadar insulin; dan 4) penurunan stres oksidatif (fungsi antioksidan).

Kata kunci: antosianin, diabetes tipe 2, ekstrak, karotenoid, tamarillo