ABSTRAK

EFEKTIVITAS FRAKSI, ISOLAT DAUN JATI CINA (*SENNA ALEXANDRINA* MILL.) DAN DAUN DELIMA (*PUNICA GRANATUM* L.) SEBAGAI ANTIOBESITAS DAN ANTISINDROMA METABOLIK SERTA MEKANISME KERJANYA

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Obesitas adalah salah satu masalah kesehatan didunia dan prevalensi obesitas terus meningkat pertahunnya. Terapi alternatif melalui penggunaan bahan alam menjadi sebuah pilihan yang seharusnya dipertimbangkan. Beberapa tumbuhan obat yaitu daun jati cina (*Senna alexandrina* Mill.) dan daun delima (*Punica granatum* L.) telah digunakan masyarakat untuk menangani bermacam-macam penyakit. Tujuan penelitian ini yaitu untuk menguji aktivitas daun jati cina dan daun delima sebagai antiobesitas dan antisindroma metabolik.

Pada penelitian ini, daun jati cina dan daun delima diekstraksi dengan metode refluks, selanjutnya dilakukan karakterisasi dan penapisan fitokimia ekstrak. Rendemen masing-masing ekstrak berturut-turut yaitu 18,93 % untuk daun jati cina dan 11,23 % untuk daun delima. Hasil penapisan fitokimia menunjukkan bahwa ekstrak daun jati cina dan daun delima memiliki kandungan kimia meliputi alkaloid, flavonoid, tanin, saponin, kuinon, dan steroid/triterpenoid.

Fraksinasi dilakukan dengan metode ekstraksi cair-cair, kemudian dilakukan penapisan fitokimia fraksi. Rendemen masing-masing fraksi daun jati cina adalah fraksi n-heksana (11,1 %), fraksi etil asetat (10,5 %), dan fraksi air (32,2 %), sedangkan rendemen fraksi daun delima adalah fraksi n-heksana (17,8 %), fraksi etil asetat (15,4 %), dan fraksi air (25,0%).

Hasil uji *in vitro* inhibisi ekstrak dan fraksi daun jati cina dan daun delima terhadap enzim lipase pankreas menunjukkan bahwa kekuatan inhibisi daun jati cina terhadap enzim lipase pankreas berdasarkan IC_{50} berturut-turut adalah ekstrak etanol (49,79 μg/ml) > fraksi etil asetat (52,51 μg/ml) > fraksi air (53,02 μg/ml) > fraksi n-heksana (57,49 μg/ml). Sedangkan kekuatan inhibisi daun delima terhadap enzim lipase pankreas berdasarkan IC_{50} adalah ekstrak etanol (33,74 μg/ml) > fraksi etil asetat (39,43 μg/ml) > fraksi air (42,88 μg/ml) > fraksi n-heksana (50,67 μg/ml). Hasil IC_{50} orlistat sebagai pembanding adalah 0,25 μg/ml.

Uji *in vitro* lainnya yang dilakukan yaitu uji enzim alfa-glukosidase, alfa-amilase, dan antioksidan. Inhibisi ekstrak dan fraksi daun jati cina dan daun delima terhadap enzim alfa-glukosidase menunjukkan bahwa kekuatan inhibisi daun jati cina

terhadap enzim alfa-glukosidase berdasarkan IC_{50} berturut-turut adalah fraksi air (46,93 μg/ml) > ekstrak etanol (49,03 μg/ml) > fraksi etil asetat (49,46 μg/ml) > fraksi n-heksana (78,13 μg/ml). Sedangkan kekuatan inhibisi daun delima terhadap enzim alfa-glukosidase berturut-turut adalah ekstrak etanol (45,31 μg/ml) > fraksi air (56, 88 μg/ml) > fraksi etil asetat (58,48 μg/ml) > fraksi n-heksana (60,00 μg/ml). IC_{50} akarbosa sebagai pembanding adalah 36,17 μg/ml. Hasil uji *in vitro* inhibisi ekstrak dan fraksi daun jati cina dan daun delima terhadap enzim alfa-amilase menunjukkan kekuatan inhibisi daun jati cina terhadap enzim alfa -amilase berdasarkan IC_{50} berturut-turut adalah fraksi air (40,68 μg/ml) > fraksi etil asetat (46,65 μg/ml) > fraksi n-heksana (49,98 μg/ml) > ekstrak etanol (60,11 μg/ml). Sedangkan, kekuatan inhibisi daun delima terhadap enzim alfa-amilase berdasarkan IC_{50} berturut-turut adalah ekstrak etanol (42,71 μg/ml) > fraksi air (55,18 μg/ml) > fraksi etil asetat (60,63 μg/ml) > fraksi n-heksana (62,63 μg/ml). IC_{50} akarbosa sebagai pembanding adalah 27,90 μg/ml.

Berdasarkan uji antioksidan ekstrak etanol dan fraksi daun jati cina dan daun delima dengan metode DPPH menunjukkan bahwa aktivitas antioksidan tertinggi diberikan oleh fraksi air daun jati cina dengan IC_{50} 36,36 μg/ml, sedangkan ekstrak etanol daun delima menunjukkan aktivitas antioksidan lebih kuat dari pada fraksinya (IC_{50} DPPH 23,39 μg/ml). IC_{50} asam askorbat pembanding adalah 5,26 μg/ml.

Uji *in vivo* menggunakan *zebrafish* obese menunjukkan bahwa ekstrak, fraksi daun jati cina dan fraksi daun delima memiliki potensi yang baik dalam memperbaiki BMI, menurunkan perlemakan liver, dan menurunkan ekspresi sitokin pro-inflamasi bila dibandingkan dengan kelompok kombinasi ekstrak. Pada penelitian ini dosis ekstrak dan fraksi adalah 100 μg/ml dan 50 μg/ml.

Berdasarkan hasil uji *in vitro* dan uji *in vivo*, fraksi etil asetat daun jati cina dipilih untuk dilanjutkan ke tahap subfraksinasi. Proses subfraksinasi dilakukan dengan metode kromatografi cair vakum. Subfraksi diuji secara *in vitro* dengan menggunakan enzim lipase pankreas. Subfraksi terpilih dilanjutkan dengan proses pemurnian. Selanjutnya dilakukan uji kemurnian isolat, kemudian dikarakterisasi dan identifikasi dengan metode Resonansi Magnet Inti (RMI). Isolat diuji secara *in vitro* dengan menggunakan enzim lipase pankreas.

Hasil penelitian ini menunjukkan bahwa, ekstrak etanol daun jati cina, ekstrak etanol daun delima, fraksi etil asetat daun jati cina, dan fraksi air daun delima memiliki efektivitas yang menjanjikan sebagai antiobesitas dan antisindroma metabolik secara *in vitro* dan *in vivo*. Hasil penelitian juga menunjukkan bahwa isolat daun jati cina yang didapat merupakan senyawa butein, sub-golongan kalkon dan memiliki aktivitas yang kuat dalam uji *in vitro* terhadap enzim lipase pankreas bila dibandingkan dengan ekstrak dan fraksinya. Oleh karena itu, dapat disimpulkan bahwa daun jati cina dan daun delima memiliki efektivitas yang baik sebagai antiobesitas dan antisindroma metabolik.

Kata kunci: Delima, ekstrak, fraksi, jati cina, obesitas, sindroma metabolik

ABSTRACT

EFFECTIVENESS OF FRACTIONS, ISOLATE OF SENNA LEAVES (SENNA ALEXANDRINA MILL.) AND POMEGRANATE LEAVES (PUNICA GRANATUM L.) AS ANTIOBESITY AND ANTIMETABOLIC SYNDROME WITH THE MECHANISM OF ACTIONS

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Obesity is one of the health problems in the world and the prevalence of obesity always increased in every years. Alternative treatment by natural product use became a choice should be considered. Several medicinal plants such as senna (Senna alexandrina Mill.) and pomegranate (Punica granatum L.) have been used by community to treat various diseases. The objective of this research is to assay activities of senna leaves and pomegranate leaves as antiobesity and antimetabolic syndrome.

In this research, senna leaves and pomegranate leaves were extracted by reflux method, then characterization and phytochemical screening were performed. The yields of each extract were 18.93% for senna leaves and 11.23% for pomegranate leaves, respectively. The results of phytochemical screening showed that senna leaves and pomegranate leaves extracts contained alkaloid, flavonoid, tannin saponin, quinone, dan steroid/triterpenoid.

Fractionation was carried out using liquid-liquid extraction method, then phytochemical screening was conducted. The yields of senna fractions were n-hexane fraction (11.1%), ethyl-acetate fraction (10.5%), and water fraction (32.2%), meanwhile pomegranate fractions were n-hexane fraction (17.8%), ethyl-acetate fraction (15.4%), water fraction (25.0%).

The results of in vitro assay of senna and pomegranate leaves extract and its fractions inhibition against pancreatic lipase enzyme showed that inhibition strength of senna leaves based on the IC_{50} were ethanol extract (49.79 μg/ml) > ethyl-acetate fraction (52.51 μg/ml) > water fraction (53.02 μg/ml) > n-hexane fraction (57.49 μg/ml), respectively. While inhibition strength of pomegranate leaves against pancreatic lipase enzyme based on the IC_{50} were ethanol extract (33.74 μg/ml) > ethyl-acetate fraction (39.43 μg/ml) > water fraction (42.88 μg/ml) > n-hexane fraction (50.67 μg/ml). the result of IC_{50} of orlistat as standard was 0.25 μg/ml.

Others in vitro assays were performed such as alpha-glucosidase, alpha-amylase, and antioxidant assays. The results of in vitro assay of senna and pomegranate leaves extract and its fractions inhibition against alpha-glucosidase enzyme denoted that inhibition strength of senna leaves based on the IC_{50} were water fraction (46.93 µg/ml) > ethanol extract (49.03 µg/ml) > ethyl-acetate fraction (49.46 µg/ml) > n-hexane fraction (78.13 µg/ml), respectively. Meanwhile inhibition strength of pomegranate leaves against alpha-glucosidase enzyme based on the IC_{50} such as ethanol extract (45.31 µg/ml) > water fraction (56.88 µg/ml) > ethyl-acetate fraction (58.48 µg/ml) > n-hexane fraction (60.00 µg/ml). IC_{50} of acarbose as standard was 36.17 µg/ml.

The results of in vitro assay of extract and fractions of senna and pomegranate leaves against alpha-amylase enzyme presented that inhibition strength of senna leaves based on the IC_{50} such as water fraction (40.68 µg/ml) > ethyl-acetate fraction (46.65 µg/ml) > n-hexane fraction (49.98 µg/ml) > ethanol extract (60.11 µg/ml), respectively. While, inhibition strength of pomegranate leaves against alpha-amylase enzyme based on the IC_{50} such as ethanol extract (42.71 µg/ml) > water fraction (55.18 µg/ml) > ethyl-acetate fraction (60.63 µg/ml) > n-hexane fraction (62.63 µg/ml). IC_{50} of acarbose as the standard is 27.90 µg/ml.

Based on the antioxidant activity of ethanol extract and fractions of senna and pomegranate leaves by DPPH method exposed that higher antioxidant activity was given by water fraction of senna leaves with IC_{50} was 36.36 µg/ml, while pomegranate leaves extract showed stronger antioxidant activity (IC_{50} DPPH 23.39 µg/ml) compared to its fractions. IC_{50} of ascorbic acid as the standard was 5.26 µg/ml.

In vivo assay using obese zebrafish expressed that extract, senna fractions, and pomegranate fractions had good activities to improve BMI, decrease fat accumulation in liver, and decrease pro-inflammatory cytokines expression compared to extracts combination. In this research, the doses of extracts and fractions were 100 µg/ml dan 50 µg/ml.

Based on results of in vitro and in vivo assays, the ethyl-acetate fraction of senna leaves was selected to continue through subfractionation. Subfractionation process was performed by vacuum liquid chromatography. Subfraction was tested by in vitro assay using pancreatic lipase enzyme. The selected subfraction of senna was followed by purification process. Then purity test of isolate was carried out, and characterization and identification of isolate was done by Nuclear Magnetic Resonance (NMR). The isolate was tested by in vitro assay using pancreatic lipase enzyme. The results of this research showed that ethanol extract of senna leaves, ethanol extract of pomegranate leaves, ethyl-acetate fraction of senna leaves, and water fraction of pomegranate leaves had promising effectiveness as antiobesity and antimetabolic syndrome by in vitro and in vivo. This research also presented that isolate of senna leaves which obtained was butein, sub-group of chalcones and had strength activity on pancreatic lipase enzyme by in vitro compared to extract and its fractions. Therefore, it can be concluded senna leaves and pomegranate leaves has good activity as antiobesity and antimetabolic syndrome.

***Keywords**: Extract, fraction, metabolic syndrome, obesity, pomegranate, senna*