INTISARI

Kanker payudara merupakan jenis kanker mematikan pertama pada wanita. Penggalian obat baru dapat menggunakan *Ethnobotanical bioprospecting approach*. Tumbuhan Mekai (*Albertisia papuana* Becc.) telah lama digunakan masyarakat suku Dayak, Kalimantan Timur dalam pengobatan kanker.

Tujuan penelitian ini untuk mendapatkan senyawa toksik terhadap terhadap sel kanker payudara T47D dari *A. papuana* Becc., serta mekanisme penghambatannya, dan senyawa metabolit sekunder dalam fraksi paling toksik tersebut.

Obyek penelitian dibatasi pada organ akar, batang, daun tumbuhan *A. papuana* Becc., sel T47D, dan sel normal Vero. Ekstraksi sampel menggunakan pelarut kloroform dan etanol (metode maserasi) dan air (meode perebusan). Fraksinasi ekstrak dan fraksi paling toksik menggunakan *Vacuum Liquid Chromatography* (VLC), dilanjutkan dengan Kromatografi Lapis Tipis Preparatif (KLTP). Uji sitotoksisitas ekstrak/fraksi dengan metode *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra zolium bromide* (MTT) dan penentuan IC_{50} ekstrak/fraksi menggunakan SPSS (Probit/Logit). Identifikasi senyawa antikanker pada fraksi paling toksik menggunakan GC-MS. Uji mekanisme aksi senyawa bioaktif antikanker menggunakan metode imunositokimia untuk ekspresi Ki67, caspase-8, dan caspase-9. Analisis siklus sel menggunakan metode *flow cytometric*. Analisis apoptosis menggunakan metode *double staining.*

Ekstrak maserasi etanol akar (mAE) merupakan ekstrak paling toksik (nilai IC_{50} 20,2 µg/mL). Nilai IC_{50} ekstrak akar yang lain yaitu ekstrak maserasi kloroform akar (mAK) dan rebusan akar (rA) sebesar 32,8 dan 48,9 µg/mL. Nilai IC_{50} ekstrak batang yaitu ekstrak maserasi kloroform batang (mBK), maserasi etanol batang (mBE), dan rebusan batang (rB) sebesar 127,9; 234,0; dan $3{,}2x10^{6}$ µg/mL. Nilai IC_{50} ekstrak daun yaitu ekstrak maserasi kloroform daun (mDK) dan maserasi etanol daun (mDE) sebesar 203,5 dan 118,3 µg/mL. Pada perlakuan dengan ekstrak rebusan daun (rD) tidak terjadi kematian sel T47D.

Ekstrak mAE difraksinasi menggunakan teknik *Vacuum Liquid Chromatography* (VLC). Fraksinasi VLC-1 terhadap mAE menghasilkan sembilan fraksi (F1.1 – F1.9). Fraksi F1.1 tidak menunjukkan adanya kemampuan mematikan sel T47D. Nilai IC_{50} fraksi F1.2 – F1.9 berturut-turut 112,0; 140,7; 144,8; 67,7; 66,7; 4,7; 9,1; dan $3{,}6.10^{4}$ µg/mL. Sitotoksisitas F1.7 dan F1.8 tidak berbeda nyata secara statistik dan paling toksik dibandingkan fraksi yang lain sehingga kedua fraksi digabung dan mempunyai nilai IC_{50} sebesar 9,1 µg/mL. Fraksi tersebut merupakan fraksi etil asetat-etanol 3:2 (EaE3.2).

Fraksinasi VLC-2 terhadap EaE3.2 menghasilkan enam fraksi (F2.1 – F2.6) dengan nilai IC_{50} berturut-turut 25,1; 22,1; 61,1; 8,6; 26,2; dan 72,8 µg/mL. Fraksi F2.4 yang bersifat paling toksik (IC_{50} 8,6 µg/mL) merupakan fraksi heksana-kloroform-etil asetat-etanol 1:44:76:6 (HKEaE1.44.74.6).

Kromatografi Lapis Tipis Preparatif-1 (KLTP-1) terhadap fraksi HKEaE1.44.74.6 membentuk sepuluh pita (P1.1 – P1.10). Nilai IC_{50} pita P1- P8 dan P10 berturut-turut 64,9; 17,4; 7,2; 5,9; 21,4; 117,0; dan 117,1µg/mL. Pita P9 1 tidak menunjukkan adanya kemampuan mematikan sel T47D. Pita P3 (Fraksi H:K:Ea:E.1.44.76.6-3) dan pita 4 (Fraksi H:K:Ea:E.1.44.76.6-4) merupakan fraksi paling toksik dan keduanya tidak berbeda nyata dengan toksisitas doxorubicin (IC_{50} 8,5 µg/mL).

Kromatografi Lapis Tipis Preparatif-2 (KLTP-2) fraksi HKEaE1.44.74.6-3 menghasilkan 4 pita (P2.3.1 – P2.3.4) sedangkan HKEaE1.44.76.6-4 menghasilkan 6 pita (P2.4.1 – P.2.4.6). Sitotoksisitas semua pita terhadap sel T47D lebih rendah dibandingkan pita sebelumnya sehingga HKEaE1.44.74.6-3 dan HKEaE1.44.74.6-4 disebut sebagai fraksi paling toksik.

Nilai IC_{50} ekstrak/fraksi paling toksik terhadap sel vero berturut-turut adalah 229,6 µg/mL (mAE), 35,0 µg/mL (EaE3.2), 17,4 µg/mL (HKEaE1.44.74.6), 78,3 µg/mL (HKEaE1.44.74.6-3), dan 548,9 µg/mL (HKEaE1.44.74.6-4).

Uji imunositokimia menunjukkan bahwa fraksi HKEaE1.44.76.6-3, HKEaE 1.44.76.6-4 dan doxorubicin menurunkan ekspresi Ki67 berturut-turut 21, 37, dan 15%, meningkatkan ekspresi caspase-8 sebesar 17, 6, dan 11%, dan caspase-9 sebesar 35, 26, dan 15% dibandingkan kontrol sel T47D. Pola siklus sel T47D akibat pemberian fraksi HKEaE1.44.76.6-4 serupa dengan pemberian doxorubicin yaitu menurunkan fase G0-G1, S, dan M sedangkan fase G2-M meningkat. Pengaruh fraksi HKEaE1.44.76.6-3 menurunkan fase G0-G1 dan G2-M, menaikkan fase S, dan fase M tetap. Pengamatan menggunakan metode *double staining* menunjukkan adanya kematian sel oleh fraksi HKEaE1.44.76.6-3 (33%) dominan pada *early apoptotic*, fraksi HKEaE1.44.76.6-4 (69%) dominan pada nekrosis, dan doxorubicin (55%) dominan pada *late apoptotic*.

Fraksi paling toksik HKEaE1.44.76.6-3 dan HKEaE1.44.76.6-4 merupakan senyawa campuran. Lima senyawa metabolit sekunder yang sama yang memiliki potensi antikanker pada kedua fraksi yaitu *1,2-benzenedicarboxylic acid, diisooctyl ester*, *squalene, β/γ-tocopherol*, *stigmasterol*, dan *γ-sitosterol*. Senyawa lain ada fraksi HKEaE1.44.76.6-3 yaitu alkaloid *3,4-dihydro-6,7-dimethoxy isoquinoline 2-oxide* sedangkan pada fraksi HKEaE1.44.76.6-4 yaitu alkaloid *2-methyl-3-phenyl-1H-indole; 1H-indole, 5-methyl-2-phenyl; 1H-1,2,3-triazole-4-carbonyl chloride; 1H-4(p-methoxy phenyl)-1,2,3-triazole;* dan *quinoline-7-ethyl*.

Kata kunci: *A. papuana* Becc., metabolit sekunder, sel T47D, *Bioassay-guided isolation*, apoptosis, proliferasi

ABSTRACT

Breast cancer is the first leading cause of cancer death in women today. The search of new drugs usually used Ethnobotanical bioprospecting approach. Mekai (*Albertisia papuana* Becc.) has been used by Dayak poeple, East Kalimantan to treat cancer.

The purpose of this research was to determine the most toxic fraction of *A. papuana* Becc. and its inhibitory mechanism on the T47D breast cancer cells lines, and its secondary metabolites.

A. papuana Becc. plant, T47D cancer cell lines, and Vero cell lines were used in this studies. The extraction of sample (root, stem, and leaf) using maceration method (chloroform and ethanol as solvent) and boiling method using water. Fractination by vacuum liquid chromatography (VLC) followed by preparative thin layer chromatography (PTLC) for detection of the potential fraction. The cytotoxic assay used 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method while the calculation of IC_{50} value used of SPSS program (Probit/Logit). The identification of anticancer bioactive compounds used Gas Chromatography-Mass Spectrophotometry (GC-MS) method. The anticancer mechanism of bioactive compounds determined by identification of Ki67, caspse-8, and caspase-9 expression (immunocytochemistry method), cell cycle analysis (flow cytometric method), and apoptotic analysis (double staining method).

The ethanolic root extract (mAE) was the most toxic extract (IC_{50} value of 20.2 µg/mL). The IC_{50} value of other root extracts were 32.8 µg/mL for chloroform root extract (mAK) and 48.9 µg/mL for boiled root extract (rA). The IC_{50} value of stem extracts were 127.9, 234.0, and $3.2x10^6$ µg/mL for chloroform stem extract (mBK), ethanolic stem extract (mBE), and boiled stem extract (rB) respectively. The IC_{50} value of chloroform leaf extract (mDK) and ethanolic leaf extract (mDE) were 203.5 and 118.3 µg/mL. The boiled leaf extract (rD) did not cause cell death.

The mAE extract fractionated using Vacuum Liquid Chromatography (VLC) technique. VLC-1 of mAE produced nine fractions (F1.1 – F1.9). F1.1 did not show the ability to kill T47D cells. The IC_{50} value of F1.2 – F1.9 fractions were 112.0, 140.7, 144.8, 67.7, 66.7, 4.7, 9.1, and $3.6.10^4$ µg/mL respectively. The cytotoxicity of F1.7 and F1.8 did not differ significantly so both of them were merged and called the most fraction ethyl acetate-ethanol 3:2 (EaE3.2) with the IC_{50} value of 9.1 µg/mL.

The VLC-2 of EaE3.2 produced six fractions (F2.1 – F2.2) with the IC_{50} value of 25.1, 22.1, 61.1, 8.6, 26.2, and 72.8 µg/mL respectively. Fraction F2.4 which the most toxic ftaction (IC_{50} value of 8.6 µg/mL) were hexane-chloroform-ethyl acetate-ethanol 1:44:76:6 (HKEaE1.44.74.6).

The PTLC-1 of H:K:Ea:E.1:44:76:6 produced ten fractions. The IC_{50} value of P1.1 – P1.8 and P1.10 were 64.9, 17.4, 7.2, 5.9, 21.4, 117.0, and 117.1µg/mL respectively. The most toxic fraction were band number 3 (H:K:Ea:E.1:44:76:6-3; IC_{50} value of 7.2 µg/mL) and band number 4 (H:K:Ea:E. 1:44:76:6-4; IC_{50} value of 5.9 µg/mL). The strength of both fractions did not significantly different from doxorubicin (IC_{50} value of 8.5 µg/mL).

The PTLC-2 of fraction HKEaE1.44.74.6-3 produced four bands (P2.3.1 – P2.3.4) while HKEaE1.44.74.6-4 produced six bands (P2.4.1 – P2.4.6). The cytotoxicity of all bands on T47D cell lines were less than

The IC_{50} value of the most extract/fraction on T47D cell lines were 229.6 µg/mL (mAE), 35.0 µg/mL (EaE3.2), 17.4 µg/mL (HKEaE1.44.74.6), 78.3 µg/mL (HKEaE1.44.74.6-3), dan 548.9 µg/mL (HKEaE1.44.74.6-4).

The immunocytochemistry assay showed that fraction HKEaE1.44.76.6-3, HKEaE1.44.76.6-4, and doxorubicin decreased the expression of Ki67 respectively 21%, 37%, and 15%, increased the expression of caspase-8 17%, 6%, dan 11%, dan increased the expression of caspase-9 35%, 26%, dan 15% compared to cell control T47D. Fraction HKEaE1.44.76.6-4 decreased the G0-G1 phase, S phase, M phase, and increased the G2-M phase of T47D cell cycle, similar to the effect of doxorubicin. Fraction HKEaE1.44.76.6-3 decreased the G0-G1 and G2-M phase, increased the M phase, and no effect on the M phase. The double staining method showed that the cell death by fraction HKEaE1.44.76.6-3, fraction HKEaE1.44.76.6-4, and doxorubicin were 33%, 69%, and 55% respectively. The influence of HKEaE1.44.76.6-3 fraction was dominant on early apoptotic, HKEaE 1.44.76.6-3 on necrosis, doxorubicin on late apoptotic.

The most toxic fractions, HKEaE1.44.76.6-3 and HKEaE1.44.76.6-4 were not a single compound. There were five secondary compounds which have anticancer activity that were present in both fractions, namely 1,2-benzenedicarboxylic acid, diisooctyl ester, squalene, β/γ.-tocopherol, stigmasterol, and γ-sitosterol. The other compounds in fraction HKEaE1.44.76.6-3 was 3,4-dihydro-6,7-dimethoxyisoquinoline 2-oxide alkaloid while in fraction HKEaE1.44.76.6-4 were 2-methyl-3-phenyl-1H-indole; 1H-indole, 5-methyl-2-phenyl; 1H-1,2,3-triazole-4-carbonyl chloride; 1H-4(p-methoxy phenyl)-1,2,3-triazole; dan quinoline-7-ethyl alkaloid.

Keyword: *A. papuana* Becc., secondary metabolite, T47D cell lines, Bioassay-guided isolation, apoptosis, proliferation