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RESEARCH ARTICLE

Phytochemical Analysis and *In Vitro* Cytotoxicity of N-Hexane Extract of *Kaempferia pandurata* and its Nanoparticle to Breast Cancer MCF-7 Cells

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Abstract

Background: Finger root (Kaempferia pandurata) is a medicinal herb which has shown anticancer activity as potential targeted-therapy towards estrogen receptor positive breast cancer. Objective: This research was conducted to analyze the phytochemical contents of n-hexane extract of Kaempferia pandurata and its nanoparticle to the growth of estrogen positive breast cancer MCF-7 cells. Methods: Kaempferia pandurata rhizome was extracted in n-hexane, and its phytochemical contents was analyzed by simple phytochemical test and thin layer chromatography. Nanoparticle was then synthesized from n-hexane extract of Kaempferia pandurata. Finally, the n-hexane extract of Kaempferia pandurata and its nanoparticle were then tested using MTT Assay to MCF-7 cells in order to determine their inhibition rate and IC50. Results:. The extraction of Kaempferia pandurata rhizome in n-hexane extract was conducted successfully. Through simple phytochemical testing, n-hexane extract of Kaempferia pandurata contained flavonoids, triterpenoids, and tannins. Thin layer chromatography using non-polar eluent (n-hexane : ethyl acetate = 5 :1) showed eight spots with Rf values of 0,12; 0,18; 0,23; 0,29; 0,41; 0,55; 0,62; 0,80. MTT assay resulted in IC50 value of 94.387 ± 11.667 µg/mL and 31,298 ± 0,242 µg/mL for n-hexane extract of Kaempferia pandurata and its nanoparticle respectively. Discussion: The phytochemical contents of n-hexane extract of Kaempferia pandurata (flavonoid, triterpenoid, tannin) was shown to have anticancer activities on breast cancer cells n-hexane extract of Kaempferia pandurata and its nanoparticle has moderately active anticancer activities towards estrogen positive breast cancer MCF-7 cells. Nanoparticle enhances n-hexane extract of Kaempferia pandurata's entry to cancer cells. Conclusion: N-hexane extract of Kaempferia pandurata and its nanoparticle can be a potenial anticancer agent towards estrogen positive breast cancer.

Keywords: *Kaempferia pandurata, Nanoparticle, MCF-7, Estrogen Receptor, Phytochemical.*

Introduction

Breast cancer is the second largest cancer in the world, with 25% prevalence and 15% mortality [1,2]. Estimated half of breast cancer cases and mortality due to breast cancer is found in developing countries [2]. In 2013 in Indonesia, breast cancer is second in prevalence after cervical cancer, which is suffered by 0.5% or 61.110 Indonesian citizens [3].

Without the increase of efficacy in treatment, the number of breast cancer cases is predicted to increase by two-fold to 3.2 million cases per year in 2050 [4]. One of the contributing factor to high breast cancer mortality is the overexpression of estrogen receptor (ER) in 60-70% of breast cancer cases [5]. ER overexpresison, known as ER positive breast cancer, increases cell proliferation through ERα signalling, genetic mutation rates, and failure in DNA repair [6]. Efforts have been made in breast cancer therapy: chemotherapy, endocrine therapy, and biological therapy [6].

Hormonal receptor inhibition therapy with Tamoxifen is currently the drug of choice to treat ER positive breast cancer; [7] however,

repeated use of Tamoxifen emerged drug resistance problems and adverse side effects such as thromboembolic disease, endometrial hyperplasia, and vaginal bleeding [7]. These problems have encouraged researches to find safer and more effective alternative breast cancer therapy .Potential as alternative therapy is found in finger root (*Kaempferia pandurata),* Indonesian native plant which is commonly used to treat digestive problems [8].Organic compounds in finger root have shown cytotoxicity and anticancer effect, and through in vivo testing has inhibited ER positive breast cancer [8, 9].

Finger root has also shown anti-inflammation, [10] antimicrobial, [11] and antioxidant [12] activities. Efforts to increase efficacy in breast cancer therapy was also done through development of drug cellular transport by nanoparticle system. Nanoparticle directly transports drug to the target cell, thus increasing drug cytotoxicity [13]. Problems of cancer cell resistance and adverse effects, balanced by the anticancer potential of finger root; encouraged the conduction of this study to find out the efficacy of finger root extract and its nanoparticle in inhibiting ER *in vitro* using ER positive model, MCF-7 cell line which expresses ER significantly [14]. In the clinical aspect, nanoparticle technology is developed to ease the binding of therapeutic compounds to target molecule, then to aid diagnosis and therapy in imaging and drug transport [13].

The use of nanoparticle in cancer therapy is aimed to increase cytotoxicity to cancer cells and decrease side effects to surrounding cells, tissues, or organs. Several types of nanoparticles could be designed to control the duration, amount, and site of release in the body [13, 15, 16].Organic nanoparticles, especially those constituting polysaccharides, are suited as adjuvant therapy and transport medium in breast cancer therapy [13, 15, 16]. Chitosan-alginate is one of the organic nanoparticle with low toxicity and easily degradable in the body; therefore it is commonly used as adjuvant therapy with anti ER agents and doxorubicin [13, 16]. Chitosan-alginate was also reported to have mucoadhesive characteristics, supporting entry from mucosal surface [13, 15, 16].

Method

This research was conducted in the Chemistry Department, Faculty of Medicine University of Indonesia on January-April 2019. This experimental study is aimed to know the phytochemical contents of *Kaempferia pandurata,* and the cytotoxicity of n-hexane extract of *Kaempferia pandurata* and its nanoparticle to ER positive breast cancer cell MCF-7. The four steps of this research were *Kaempferia pandurata* extraction in n-hexane solvent, phytochemical testing, thin layer chromatography, and cytotoxicity testing using MTT assay. Percentation of inhibition was analysed statistically by Shapiro-Wilk normality test, then by one-way ANOVA (parametric test) or Kruskal-Wallis (non-parametric) with its post-hoc tests. IC50 values were acquired from linear graph of percentation inhibition to the antilog of sample concentration.

Results

Phytochemical Analysis

N-Hexane extract of *Kaempferia pandurata* in this research has yield value of 13, 25%.Phytochemical analysis shown flavonoid, triterpenoid, and tannin as secondary metabolites in n-hexane extract of *Kaempferia pandurata.*

Table 1: Phytochemical Analysis Results of n-Hexane Extract of Kaempferia pandurata

No.	Metabolite	Observation Result	Interpretation
1	Flavonoid	Yellow-colored glow from extract when observed under 336 nm UV light	+
2	Saponin	Extract solution did not generate foam upon and after shaking	-
3	Triterpenoid	Brownish ring on the surface of the solution	+
4	Tanin	Extract changed color to black upon addition of reagent	+
5	Glikosida	Extract darkened to dark orange from orange	-

Thin Layer Chromatography

Thin layer chromatography using n-hexane: ethyl acetate (5:1) as eluent shows eight spots from eight compounds with Rf value ranging between 0.12 to 0.80 (Table 2).

Table 2: Rf Values of n-Hexane Extract of *Kaempferia pandurata*

No.	Rf Values	No	Rf Values
1	0.80	5	0.29
2	0.62	6	0.23
3	0.55	7	0.18
4	0.41	8	0.12

MTT Assay

Table 3: Inhibition Activity and IC50 Values of n-Hexane Extract of *Kaempferia pandurata*, its Nanoparticle, and Doxorubicin to MCF-7 Cells

Concentration of test samples (µg/mL)	Percentation of Inhibition (Mean ± S.D %)		
	n-Hexane Extract of *Kaempferia pandurata*	Nanoparticle of n-Hexane Extract of *Kaempferia pandurata*	Doxorubicin
0.781	-	-	48.671 ± 1.517
1.562	-	-	62.870 ± 2.237
3.125	-5.053 ± 1.053	-72.427 ± 7.830	69.754 ± 1,.671
6.25	0.818 ± 5.816	-31.475 ± 3.384	77.373 ± 1.139
12.5	17.540 ± 0.527	3.716 ± 2.878	84.637 ± 1.823
25	32.664 ± 2.774	24.303 ± 3.635	87.800 ± 0.232
50	40.060 ± 1.012	57.636 ± 2.460	-
100	48.766 ± 1.758	132.667 ± 2.572	-
IC50 Values	94.387 ± 11.667	31.298 ± 0.242	0.616 ± 0.083

Generally, inhibition percentage of n-hexane extract of *Kaempferia pandurata*, its nanoparticle, and doxorubicin is increasing in a dose-dependent manner. Negative values of inhibition percentage indicate test sample stimulates growth of MCF-7, while positive values of inhibition percentage indicate test sample inhibits growth of MCF-7. 6.25, 12.5, 25, 50, 100 µg/mL n-Hexane extract of *Kaempferia pandurata* inhibited MCF-7 growth; while 3.125 µg/mL n-Hexane extract of *Kaempferia pandurata* stimulated MCF-7 growth. 12.5, 25, 50, 100 µg/mL nanoparticle inhibited MCF-7 growth; while 3.125 and 6.25 µg/mL n-Hexane extract of *Kaempferia pandurata* stimulated MCF-7 growth.

Statistical analysis has shown a significant difference in mean inhibition percentage between concentration groups of n-hexane extract of *Kaempferia pandurata*, its nanoparticle, and doxorubicin. Descriptively, lower IC50 values indicate better anticancer activity of the samples. The smallest IC50 value out of three samples is doxorubicin (0.616 ± 0.083 µg/mL), followed by nanoparticle of *Kaempferia pandurata* (31. 297 ± 0.242 µg/mL) and n-hexane extract of *Kaempferia pandurata* (94.387 ±11.667 µg/mL).

One-way ANOVA resulted in statistically significant difference of IC50 values between the three test samples.

Discussion

Phytochemical Contents of n-Hexane Extract of *Kaempferia pandurata*

Phytochemical analysis of n-hexane extract of *Kaempferia pandurata* in this study showed phytochemical contents of flavonoid, triterpene, and tannin. Compared to other studies [17, 18] there are a difference in phytochemical contents of *Kaempferia pandurata* rhizome and extract; [17] also a difference in the phytochemical contents between extracts with different solvents (alkaloid was found in rhizome and ethanolic extract of *Kaempferia pandurata*) [18]. This is in accordance to the selective solubility pinciple of solvents (*like-dissolves-like):* polar solvents solve polar compounds and non-polar solvents solve non-polar compounds [19].

The solvent used in this research is n-hexane, a non-polar solvent which solves non-polar organic compounds in *Kaempferia pandurata* rhizome [19]. Glycon flavonoid has hydroxyl groups which bind with sugar, thus having a

polar characteristic. Aglycon flavonoid (without sugar) such as flavonone, isoflavone, flavanone, and flavonol, has less hydroxyl groups and non-polar characteristics. Pinostrobin and Panduratin A are flavonoids which have non-polar characteristics [20]. Kirana ET. Al. reported that Panduratin A showed cytotoxicity towards MCF-7 with IC50 value of 3, 75 µg/mL [21]. Pinostrobin has antiaromatase effect; therefore, it was able to inhibit the growth of estrogen-induced breast cancer cells such as MCF-7 [7, 22].

Triterpene dissolved in n-hexane and was found in n-hexane extract of finger root, though its low polarity caused majority of it to be poorly absorbable by the digestive tract [23, 24]. Triterpene has shown potent cytotoxicity activity to ER positive breast cancer cells (MCF-7) and ER negative breast cancer cells (MDA-MB-231). Triterpene inhibit the growth of cancer cells by pro-apoptotic protein binding with proapoptotic enzymes, and shortening replication phase of cell [22].

Tannin is constituted of non-polar aromatic phenol and polar hydroxyl froup [23]. Therefore, tannin is extracted using mixture of polar and less polar solvents, which are water and acetone [23]. Tannin gives anticancer effects by increasing apoptosis of breast and prostate cancer cells [24]. ER positive breast cancer cells is targeted by tannin in a more specific manner than ER negative breast cancer, by inducing apoptosis of cancer cells and promoting adipocyte survival [24].

Thin Layer Chromatography Profile of n-Hexane Extract of *Kaempferia pandurata*

The Ministry of Health of Indonesia in *Farmakope Herbal Indonesia* has constituted a standard to perform thin layer chromatography on ethanolic extract of *Kaempferia pandurata* using n-hexane: ethyl acetate = 4: 1, and had found seven compound components (RF values 0.10-0.87, Rf 0.64 is pinostrobin) [25, 26]. TLC on this research used n-hexane extract of *kaempferia pandurata* using n-hexane: ethyl acetate = 5: 1 and found eight major compounds with Rf values of 0.12, 0.18, 0.23, 0.29, 0.41, 0.55, 0.62, 0.80.

N-Hexane and ethyl acetate acted as a non-polar eluent; n-hexane with a lower polarity. Compound components with higher RF value have non-polar characteristics, for it dissolved in non-polar eluent and moves further on the alumunium plate. Pinostrobin, panduratin A, and pinocembrin chalcone are non-polar flavonoids; triterpene is also a non-polar compound, while Tannin is a polar compound [19]. However, in order to further identify the types of compound, mass spectrophotometry, FTIR, and NMR tests should be conducted.

Anticancer Activity of n-Hexane Extract of *Kaempferia pandurata* and Its Nanoparticle to MCF-7 Cells

Percentage of inhibition values showed growth inhibition effect of n-hexane extract of *Kaempferia pandurata* and its nanoparticle to MCF-7 in higher concentrations, and growth stimulation in lower concentrations. N-hexane extract of *Kaempferia pandurata* and its nanoparticle are moderately active anticancer to MCF-7 (20-100 µg/mL) [27].Inhibition of n-hexane extract of *Kaempferia pandurata* and its nanoparticle to MCF-7 is not as good as doxorubicin as potent anticancer agent; however, nanoparticle synthesis increased the efficacy of finger root extract to ER positive breast cancer MCF-7.

Bioavailability of organic compounds in n-hexane extract of finger root is relatively low in the human body, for it is dominantly non-polar. N-Hexane extract of finger root also works on estrogen signalling pathways and ER as a nuclear receptor [15, 22]. In order to have an inhibition effect on MCF-7 growth, n-hexane extract of finger root must pass through the cell membrane and cytoplasm.

Based on molecular docking and dynamic simulation this Pinostrobin and pinocembrin from *Kaempferia pandurata* can interact with ER and VEGF, having a potential for specific ER-treatment for breast cancer [28]. Nanoparticle plays a role in extract transport to MCF-7 cells through modification of size and chemical structure of the nanoparticle surface in respect to cell membrane selectivity [27]. Chitosan-alginate nanoparticle synthesis created a micelle structure on n-hexane extract of finger root; the inner part of the nanoparticle is non-polar and binds to finger root molecules and the surface of nanoparticle is polar.

The polar surface of nanoparticle binds with the phosphate head of phospholipid bilayer in cell membrane and nuclear membrane, and then releases its finger root contents, which diffuses through the hydrophilic part of phospholipid bilayer and enter the cytosol. Chitosan-alginate nanoparticles also have mucoadhesive properties in non-parenteral route of entry, such as through the mucosal surface of digestive tract, nasal, vagina, and lungs. A Mucoadhesive property of chitosan-alginate is caused by the difference of electrical charge between nanoparticle surface (positive charge) and cell membrane (negative charge).

Different electrical charge creates a driving force, so that nanoparticle is drawn near to the cell membrane and progressively releases its active compound.

Strong adhesive properties of chitosan-alginate also play a role in creating a protective barrier from variative environment pH [29].

Conclusion

Kaempferia pandurata extraction in n-hexane was successfully conducted. Phytochemical contents of n-hexane extract of *Kaempferia pandurata* found in this research are flavonoid, triterpene, and tannin. TLC found eight major compound components with RF values of 0.12; 0.18; 0.23; 0.29; 0.41; 0.55; 0.62; 0.80.

N-Hexane extract of *Kaempferia pandurata* and its nanoparticle inhibited the growth of ER positive breast cancer cell line MCF-7. Synthesis of nanoparticle from n-hexane extract of *Kaempferia pandurata* increased its inhibition effect to MCF-7.

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References

1. Globocan (2012) Breast Cancer: Estimated Incidence, Mortality and Prevalence Worldwide in 2012 [Internet]. France: IARC; 2019 [cited 2019 Jan 17]. Available from: http://globocan.iarc.fr/old/FactSheets/cancers/breast-new.asp
2. Alvarez RH, Cortes J, Falzon M, Gandy M, Gianni L, Harbeck N, Piccart M (2016) Handbook of HER2- Targeted Agents in Breast Cancer, 2nd ed. Switzerland: Springer International Publishing.
3. Tao Z, Shi A, Lu C, Song T, Zhang Z, Zhao J (2015) Breast Cancer: Epidemiology and Etiology. Cell Biochem Biophys., 72(2):333-8. doi: 10.1007/s12013-014-0459-6.
4. Mitri Z, Constantine T, Regan RO (2012) The HER2 Receptor in Breast Cancer: Pathophysiology, Clinical Use, and New Advances in Therapy. Chemotherapy Research and Practice, 743193:1-7.
5. Manavathi B, Dey O, Gajulapalli VNR, Bhatia RS, Bugide S, Kumar R (2013) Derailed Estrogen Signaling and Breast Cancer: An Authentic Couple. Endocr. Rev., 34(1): 1-32. doi: 10.1210/er.2011-1057.
6. Osborne CK, Schiff R, Fuqua SA, Shou J (2001) Estrogen receptor: current understanding of its activation and modulation. Clin Cancer Res., 7(12):4338s-4342s; discussion 4411s-4412s.
7. Lumachi F, Brunello A, Maruzzo M, Basso U, Basso SMM (2013) Treatment of Estrogen Receptor-Positive Breast Cancer. Current Medicinal Chemistry, 20:596-604.
8. Engel RH, Kaklamani VG (2007) HER2-positive breast cancer: current and future treatment strategies. Drugs, 67(9): 1329-1341.
9. Poerwono H, Sasaki S, Hattori Y, Higashiyama K (2010) Efficient microwave-assisted prenylation of pinostrobin and biological evaluation of its derivatives as antitumor agents. Bioorganic & Medicinal Chemistry Letters, 20(7):2086-2089.
10. Fahey JW, Stephenson KK (2002) Pinostrobin from honey and Thai ginger (Boesenbergia pandurata): a potent flavonoid inducer of mammalian phase 2 chemoprotective and antioxidant enzymes. J. Agric. Food Chem., 4: 50(25):7472-6.
11. Panthong A, Kanjanapothi D, Tuntiwachwuttikul P, Pancharoen O,

Reutrakul V (1994) Anti-inflammatory activity of flavonoids. Phytomedicine, 1(2):141-4. doi: https://doi.org/10.1016/S0944-7113(11)80032-2.

12. Melliou E, Chinou J (2004) Chemical analysis and antimicrobial activity of Greek propolis. Planta Med., 70: 515-9.
13. Yehelyev MV, Gao X, Xing Y, Al-Hajj A, Nie S, O'Regan RM (2006) Emerging use of nanoparticles in diagnosis and treatment of breast cancer. Lancet Oncol., 7(8):657-67.
14. Hou J, Yu X, Shen Y, Shi Y, Su C, Zhao L (2017) Triphenyl Phosphine-Functionalized Chitosan Nanoparticles Enhanced Antitumor Efficiency Through Targeted Delivery of Doxorubicin to Mitochondria. Nanoscale Research Letters, 12: 158.
15. Kumar N, Patel AN, Kumari N, Kumar A (2014) A review on chitosan nanoparticles for cancer treatment. International Journal of Nanomaterials and Biostructures, 4(4):63-65.
16. Pratiwi E (2009) Aktivitas Antioksidan Ekstrak dan Fraksi Aktif Temu Kunci (*Boesenbergia Pandurata Roxb.*) [skripsi]. Bogor: Departemen Kimia Institut Pertanian Bogor.
17. Handayani S, Mursiti S, Wijayati N (2018) Uji Aktivitas Antibakteri Senyawa Flavonoid dari Rimpang Temu Kunci (*Kaempferia pandurata* Roxb.) terhadap *Streptococcus mutans*. Indo. J. Chem. Sci., 7(2):146-152.
18. Montes I, Lai C, Sanabria D (2003) Like Dissolves Like: A Guided Inquiry Experiment for Organic Chemistry. Journal of Chemical Education, 80(4):447.
19. Panche AN, Diwan AD, Chandra SR (2016) Flavonoids: an overview. J. Nutr. Sci., 5: e47.
20. Kirana C, Jones, GP, Record IR, McIntosh GH (2007) Anticancer properties of panduratin A isolated from Boesenbergia pandurata (Zingiberaceae). Journal of Natural Medicines, 61: 131-7.
21. Bishayee A, Ahmed S, Brankov N, Perloff M (2011) Triterpenoids as potential agents for the chemoprevention and therapy of breast cancer. Front Biosci., 1(16): 980-996.
22. Wang TY, Li Q, Bi KS (2018) Bioactive flavonoids in medicinal plants: Structure, activity, and biological fate. Asian Journal of Pharmaceutical Sciences, 12(1):12-23.
23. Serrano J, Pimia RP, Dauer A, Aura AM, Calixto SF (2009) Tannins: current knowledge of food sources, intake, bioavailability, and biological effects. Mol. Nutr. Food Res, 53 (2): S310-29.
24. Putra AY, Chahyadi A, Yaman E (2014) Production of Panduratin A, Cardamomin, and Sitosterol Using Cell Cultures of Finger root (Boesenbergia pandurata (Roxb.) Schlecter)). Biosciences Biotechnology Research Asia, 11(1):43-52.
25. Depkes RI (2008) Farmakope Herbal Indonesia, 1st ed. Jakarta: Departemen Kesehatan RI.
26. Atjanasuppat K, Wongkham W, Meepowpan P, Kittakoop P, Sobhon P, Bartlett A, Whitfield PJ (2009) In vitro screening for anthelmintic and antitumour activity of ethnomedicinal plants from Thailand. J. Ethnopharmacol., 123:475-482.
27. Nurrachma MY, Lintangsari G, Tika LN, Luthfiananda B, Adiningsih W, Hermawan A. Ethanolic Extract of Fingerroot *(Boesenbergia pandurata)* Inhibits *Aldehyde Dehydrogenase* in 4T1 Breast Cancer Cells. CCRC Farmasi UGM. 2017; 10(2): 45-54.
28. Fadilah F, Edina BC, Rahmawati RA, Wiyono L, Erlina L, Paramita RI, Tedjo A (2018) Molecular Dynamic of Pinostrobin and Pinocembrin from Kaempferia pandurata Roxb. towards Estrogen Receptor Positive (ESR) and Estrogen Receptor Negative (VEGFR) of Breast Cancer. Asian Journal of Pharmaceutics, 12 (4):1473.
29. Contini C, Schneemilch M, Gaisford S, Quirke N (2018) Nanoparticle-membrane interactions. Journal of Experimental Nanoscience, 13(1):62-81.

