Evaluation of North Sumatera Cardamom seed (Amomum compactum) Extract as Antibacterial and Anticancer

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Abstract. Cardamom seeds (Amomum compactum) or Kapulaga have been used as a flavoring spice in cooking. Besides, they are also useful in traditional medicine for oral health and gizzard, treating coughs and colds, maintaining kidney and urinary health, pain medication, preventing infections, as well as smoothing digestion. This study was aimed to evaluate the acetone extract of North Sumatera A. compactum as antibacterial and anticancer. The antibacterial activity test was carried out by the paper disc diffusion method. It was then followed by determination of minimum inhibitory concentration (MIC) and minimum kill concentration (MBC) against S. aureus ATCC 25923, S. mutans ATCV 35668 and E. faecalis ATCC 49619. The cytotoxic test was performed against MCF-7 cells. It has been found that acetone extract of A. compactum showed the best activity against S. aureus ATCC 25923 with a diameter of inhibition zone was of 8.3mm with MIC and MBC values of 625µg/mL. Acetone extract of A. compactum has anticancer breast activity with IC₅₀ value was found to be 44.7828 µg/mL.

Keywords: North Sumatera Cardamom Seed (Amomum compactum), acetone extract, antibacterial, and breast anticancer (MCF-7)

1. Introduction

Kapulaga is a native plant of Indonesia that grows wild in forests on the island of Java. The type of cardamom most commonly found in Indonesia is cardamom java (A. compactum). It is widely used for cooking spices, health drinks, traditional medicine and aromatherapy. So that now many have been cultivated in various regions in Indonesia. In traditional Chinese medicine, A. compactum seeds have been used to treat various diseases such as digestive disorders, stomach disorders, and antipyreticmenagogues. While in Malaysia A. compactum seeds are used as cough and fever medicines, in Indonesia cardamom is used as an aromatic ingredient, treating cough, bad breath, and itchy throat.

A. compactum is a type of spice that is quite popular so that many interesting researchers to explore its potential as a drug. Based on previous studies, A. compactum seed extracts have been tested for various biological effects. A. compactum seed extracts show biological effects, such as diethyl ether extract of cardamom seeds show antimicrobial activity, as well as methanol extracts from A.
compactum seeds and fruit, can inhibit the growth of Botrytis cinereal fungi, while methanol extracts show antibacterial activity. The same is true of ethyl acetate fraction which shows activity against S. aureus. In addition to the antimicrobial ethanol extract of A. compactum seeds showed anti-inflammatory and anti-asthma. There were no reports on the biological effects of acetone extract of A. compactum seeds. Therefore this article reports the acetone seed extract activity of A. compactum as an antibacterial and anticancer.

2. Methodology

Plants extract preparation
Samples were obtained from herbal medicine shop CV. Sempurna Sambu. A 100 g of dried A. compactum seed was mashed and macerated with 500 ml acetone solvent (v/v) for 3 x 24 hours at room temperature. Then filtered and evaporated at low pressure until a concentrated extract was obtained.

Antibacterial agents
The concentration of the extract for the antibacterial activity test was made in a concentration of 1% with a solvent of 10% DMSO (Sigma Aldrich) which is equivalent to 10,000 µg/ml. Antibiotic chloramphenicol at a concentration of 500 µg/ml was used for positive control.

Antibacterial strains and inoculums preparation
The test bacteria consisted of gram-negative bacteria, Streptococcus mutans ATCV (35668) (-), and two positive bacteria namely Enterococcus faecalis ATCC 49619 (+) and Staphylococcus aureus ATCC 25923 (+). Inoculums prepared with a turbidity of their suspensions are standardized with turbidity of 0.5 Mc. Farland.

Antibacterial activity
The M02-A11 paper disc diffusion method Clinical and Laboratory Standards Institute (CLSI) was used for preliminary test of antibacterial activity. A test solution of 15-20 µL was dropped on a paper disc (6 mm diameter disc, oxoid) placed on a Mueller Hinton Agar (MHA) plate which already contained an inoculum of a bacterial species. Then it was incubated aerobically at 37ºC for 24 hours.

Determination of the Minimum inhibitory concentration (MIC) value
MIC determination was conducted by the microdilution method (M07-A9). Briefly, into the microplate (96-well), 100 µL Muller Hinton Broth (MHB, oxoid) liquid media was inserted in first column as a negative control. The second column was filled with media and bacteria as positive control and the third column to 12th were filled with media, bacteria and samples with varying concentrations, the amount of solution in each well was 100 µL. Furthermore, Microplat was incubated at 37ºC for 24 hours.

Determination of the Minimum Bactericidal Concentration (MBC)
The mixture in each well on the microtiter plate MIC test results were inoculated into the MHA plate by dripping 10 L of the mixture from each well into the surface of the MHA, then incubated at 37ºC for 24 hours.

Cytotoxic testing of MCF-7 cells
The cytotoxic activity test was carried out by the method as described by Anon with a slight modification. In summary, the testing workflow starts from incubating cell culture to be used in 96 well plates in incubation (at 37°C and 5% CO2 gas until the percentage of cell growth reaches 70%). Then the cells were treated with samples and then incubated (for 48 hours at 37°C and 5% CO2 gas), and presto blue working reagents was added to the cells. Absorbance measurement using Multimode Reader.
3. Results and Discussion

**Antibacterial activity**

*A. compactum* seed acetone extract was tested for its potential against cavities-causing bacteria (caries teeth) namely *S. mutans* ATCV 35668 and *E. faecalis* ATCC 49619 and bacteria that cause skin diseases namely *S. aureus* ATCC 25923. Paper disc diffusion test results showed that the colony seed extract of *A. compactum* has activity against *S. mutans* ATCV (35668) and *S. aureus* ATCC 25923 with almost the same inhibitory zone diameter of 8.2 and 8.3 mm. The results of this study were consistent with previous studies, the results are consistent which shows the presence of antibacterial activity, although there are differences in the diameter of the inhibitory zone with the results of the study Ağaoğlu, *et al.*5 Antimicrobial activity on several types of microbes including *S. aureus* with 30 mm inhibition zone, *E. faecalis* with 15 mm inhibition zone. The difference in these results was because the extractor used was different, so there are differences in the secondary metabolites that were extracted. Furthermore, quantitative testing through MIC and MBC measurement. MIC and MBC measurement results are summarized in Table 1.

**Table 1.** MIC and MBC values of *A. compactum* seeds acetone extract

| Bacteria   | MIC µg/mL | MBC µg/mL | MIC µg/mL | MBC µg/mL | MIC µg/mL | MBC µg/mL |
|------------|-----------|-----------|-----------|-----------|-----------|-----------|
| Chloramphenicol | 0.97       | 125       | 0.48      | 7.8       | 7.8       | 250       |
| *A. compactum* acetone extract | 625       | 2500      | 312.5     | 312.5     | ND        | ND        |
| ND : not detested |           |           |           |           |           |           |

acetone seed extract acetone showed the best antibacterial activity against *S. aureus* ATCC 25923 with moderate category.15

**Anticancer Activity**

Based on searches through various Web, Scopus, Pubmed and on-line media used for publication. Research related to the anticancer of *A. Compactum* was still very limited. Anticancer studies that have been reported that *A. compactum* can reduce the side effects of postoperative chemotherapy for carcinoma of the large intestine and improve the immune function of patients so that it can increase the effectiveness of chemotherapy.16 The results of absorbance measurements of *A. compactum* acetone seed extract against MCF-7 breast cancer cells are summarized in Table 2.

**Table 2.** Absorbance Test Results of *A. compactum* acetone seed extract against MCF7 cells

| Wavelength /nm | Media | Media -Sel | Cisplatin | DMSO 1.00% | Concentration of sample (µg/mL) |
|----------------|-------|------------|-----------|-------------|---------------------------------|
| 570            | 0.4785 | 0.7512     | 0.6094    | 0.7554      | 0.5208                          |
|                | 0.4639 | 0.7616     | 0.6218    | 0.7495      | 0.5008                          |
|                | 0.6186 | 0.2137     | 0.4388    | 0.2241      | 0.6718                          |
|                | 0.5989 | 0.2290     | 0.4455    | 0.3234      | 0.6469                          |
| Corrected Absorbance | 0.1371 | 0.6746     | 0.3076    | 0.6685      | 0.0139                          |
|                | 0.6696 | 0.3133     | 0.6541    | 0.0090      | 0.4586                          |
Based on the data in Table 2, Figure 1 shows the linear regression chart with $y = 0.1014x - 7.5034$

**Figure 1. Inhibition curves of A. acetone seed extract acetone against MCF-7 cells**

Based on this equation IC$_{50}$ values 567.09 μg/mL were obtained. Anticancer activity criteria according to the National Cancer Institute (NCI), if an extract has an IC$_{50}$ value of less than 30 μg/mL, the extract has a strong anticancer activity, moderate if it has a value of 30 <IC$_{50}$ <100 μg/mL and is inactive if it has an IC$_{50}$ value > 100 μg/mL. Based on NCI criteria, seed acetone extract was not active against MCF-7 cells. Although less active against MCF-7 cells, based on observations using a microscope (in Fig.1) showed the potential as an anticancer, which is seen from the number of dead cells and at a concentration of 500 μg/mL. The morphology of the dead cells appears dark, non-radiant and the cell membranes look broken or somewhat faint while the living cells appear to shine brilliantly and the membrane boundaries with the media were visible (Fig.2)

**Figure 2. MCF7 Cell Morphology Documentation Test Results for A. compactum Seed Extract**
The failure of *A. Compactum* acetone seed extract to inhibit the growth of MCF-7 cancer cells might be due to the strong antagonistic effect of the compound that exists together in the extract. The biological activity of *A. compactum* seeds was determined by their secondary metabolite content. The phytochemical test results of *A. compactum* seeds contain essential oils, flavonoids, saponins, steroids, and triterpenoids. The components of *A. Subulatum* seed essential oil were 1,8-cineol (60.8%), β-pinene (8.3%), and α-pinene (6.4%), while the fruit peels were 1,8-cineol (39.0%), β-pinene (17.7%), and α-pinene (4.8%). Thus active against antibacterial was a compound group of flavonoids, terpenoids and steroids. Flavonoids can cause bacterial cell wall permeability damage whereas terpenoids inhibit bacterial growth by interfering with membrane function. Steroids cause brittle cells and lysis. While the most important role in anticancer activity was compounds that can inhibit many oxidation reactions such as flavonoids and triterpenoids whose structure contains a hydroxyl group that can donate hydrogen atoms to free radicals.

4. Conclusions
Acetone extract of *A. compactum* showed antibacterial activity against *S aureus* and *S. mutans* bacteria with MIC of 312.5 and 625 µg/mL and MBC values of 312.5 and 2500 µg/mL as well as potential anticancer with IC₅₀ 567.09 µg/mL.

Acknowledgments
Thank you to the Directorate of Research and Community Directorate General of Strengthening Research and Development of the Ministry of Research, Technology and Higher Education for the Decentralization of “Penelitian Desentralisasi Unggul Perguruan Tinggi (PDUPT), 2019 scheme with contract number 0190/SP2H/LT/DRPM/2019.

References
[1] De Guzman, CC and Siemonsma, JS. (eds.) *Spices*. Plant Resources of Southeast Asia 13; 1999; Leiden: Backhuijs
[2] Setyawan, AD., Wiryanto, Suranto, Bermawie, N and Sudarmono. Comparisons of isozyme diversity in local Java cardamom (*Amomum compactum*) and true cardamom (*Elettaria cardamomum*). *Nusantarabioscience*; 2014; 6(1) 94-101.
[3] De Padua, L.S., Bunyapraphatsara and Lemmens, RHMJ. *Plant resources of South-East Asia no 12(1).* 1999; Backhuys Publishers, Leiden.
[4] Suratman, E. dan Djauhariai, (1997), *Flasma Nutfah Kapulaga dalam Buletin Penelitian Tanaman Rempah dan Obat; 1997; 3(1), Balai Penelitian Tanaman Rempah dan Obat, Semarang*
[5] Agaoglu, S.I., Dostbil, N., Alemdar, S., 2005, Antimicrobial Effect of Seed Extract of Cardamom (*Elettaria cardamomum* Maton), *YYÜ VetFakDerg* 2005, 16 (2): 99-101
[6] I. Prasasty, Suranto & R. Setyaningsih. Aktivitas Anticendawan Biji dan Buah Kapulaga Lokal (*Amomum cardamomum* Willd.) terhadap Botrytis cinerea Pers. Als Buah Anggur (*Vitis* sp.), BioSMART; 2003; 5(1), 61–64 ISSN: 1411–321 X.
[7] Islam, S., Rahman, A., Sheikh, M.I., Rahman, M., Jamal, A.H.M dan Alam, F., *Invitro Antibacterial Activity of Methanol Seed Extract of Elettaria cardamomum* (L.) Maton. *Agriculturae ConpectusScientificus*; 2010, 75(3): 113–117.
[8] Sukandar, D., Hermanto, S., Amelia, R.E dan Zaenudin, M., Aktivitas Antibakteri Ekstrak Biji Kapulaga(*Amomum cardamomum* Sol.Ex Maton, JKTII; 2015;17(2) : 119-129, ISSN 0853-2788.
[9] Lee Lee, J. A., Lee, M. Y., Shin, I. S., Seo, C. S., Ha, H., and Shin, H. K., *Anti-inflammatory Effects of Amomum cardamomum RAW 264.7 cells via induction of heme oxygenase-1, Archives of Pharmacal Research; 2012; 35(4) : 739–746, Doi 10.1007/s12272-012-0419-x.
[10] Lee, J. A., Lee, M. Y., Seo, C. S., Jung, D. Y., Lee, N. H., Kim, J. H., and Sin, H. K., Anti-Asthmatic Effects of an *Amomum compactum* Extract on an Ovalbumin (OVA)-Induced Murine Asthma Model, *Biosci. Biotechnol., and Biochemistry;* 2010; 74(9): 1814–1818, Doi:10.1271/bbb.100177.

[11] Clinical Laboratory Standards Institute. Reference method for Antimicrobial Disk Susceptibility Tests; Approved Standard M02-A11; 2012, Eleventh Edition. Clinical and Laboratory Standards Institute, Wayne.

[12] Clinical and Laboratory Standards Institute, Reference method for microdilution antibacterial susceptibility testing; approved standard CLSI document M07-A9; 2012; Clinical and Laboratory Standards Institute, Wayne.

[13] Igbinoso OO, Igbinoso EO, Aiyegoro AA, Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas*(Linn). *African Journal of Pharmacy and Pharmacology;* 2009; Vol. 3(2). pp. 058-062.

[14] Anon, 2018. Antimicrobial, antioxidant, cytotoxic and apoptotic activities of Saturejakhuzeastanica; 2018; *Gazi Medical Journal,* 29(3). Available at: http://dx.doi.org/10.12996/gmj.2018.75

[15] Droyem J.P., Nkute A.H.L., Kuete, V., Tala, M. F., Wabo, H.K., Guru, S.K., Rajput, V. S., Sharma, A., Tane, P., Khan, I. A., Saksena, A.K, Laatsch, H., Tan, N. H., Cytotoxicity and Anti Microbial Activity of The Methanol Extract and Compound from Polygonumlimbatum; 2012; Plantamedica, 78, 787-792

[16] Deng S, Hu B, An H-M. Traditional Chinese Medicinal Syndromes and Treatment in Colorectal Cancer. *Journal of Cancer Therapy [Internet].* Scientific Research Publishing, Inc.; 2012;03(06):888–897. Available from: http://dx.doi.org/10.4236/jct.2012.326114

[17] Suffness, M. &Pezzuto, J.M. Assays related to cancer drug discovery. *Methods in plant biochemistry;* 1990; assays for bioactivity. 6: 71-133.

[18] Susanti, D., Sirat, H. M., Ahmad, F., Ali, R. M., Aimi, N., &Kitajima, M. Antioxidant and cytotoxic flavonoids from the flowers of *Melastoma malabathricum* L. *Food Chemistry;* 2007; 103(3), 710–716. doi:10.1016/j.foodchem.2006.09.011

[19] Bamu’min, N., Djamil, R danKrtiningsih., (2013), *Skrining Fitokimia dan Formulasi Sediaan Tablet Hisap Ekstrak Kering Kapulaga Jawa (Amomumcardamomum Willd.) dengan PVP sebagai Pengikat*, 1-10.

[20] Satyal, P., Dosoki, A. S., Kincer, B. L and Setzer, W. N., (2012), Chemical Compositions and Biological Activities of *Amomumsubulatum* Essential Oils from Nepal, *Natural Product Communications,* 7(9), 1233-1236.

[21] Cushnie, T.P.Tim. Lamb, Andrew J. Antimicrobial Activity of Flavonoids. *International Journal of Antimicrobial Agents;* 2005;26: 343-35

[22] Saleem M, Nazir M, Ali MS, Hussain H, Lee YS, Riaz N, et al. ChemInform Abstract: Antimicrobial Natural Products: An Update on Future Antibiotic Drug Candidates. *ChemInform [Internet].* Wiley; 2010 May 25;41(21)

[23] Ahmed, Bahar. 2007. *Chemistry Of Natural Products.* New Delhi: Department of Pharmaceutical Chemistry Faculty of Science Jamia Hamdard.

[24] Kandaswami, C and Middleton, E. 1997. Flavonoids as antioxidant, In F. Shahidi (Ed). *Natural Antioxidant Chemistry, Health Effects and Applications.* Champaign Illions : AOCS Press.