Effect Of Ethanol, Hexane And Water Extracts Of Clinacanthus Nutans Leaves On Hone-1 Proliferation

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ABSTRACT

Background: Mortality from cancer has increased especially in the developing country. Global pattern shows the half of mortality occurred in Asia. One type of cancer occurring in Indonesia is nasopharyngeal cancer. Clinacanthus nutans is a species of herbal plants in the Acanthaceae family that grows in tropical Asia, especially Indonesia. This plant is considered as one of traditional medicinal plants, which is effective in treating several diseases, including cancer. Different polarity of solvent that used to extract this plant will have different dissolved compounds.

Objective: To determine the effect of ethanol, hexane, and water extract of C. nutans leaves on HONE-1 cell proliferation.

Method: This study conducted using experimental research in vitro laboratory. Three different extract, ethanol, hexane, and water extract of C. nutans leaves treated to HONE-1 cell. Cell proliferation was measured using MTT assay.

Result: The results showed significant differences (p < 0.05) of treatments group compared to negative control. Ethanol extract showed the highest inhibition of HONE-1 cell proliferation.

Conclusion: Ethanol, hexane, and water extract of C. nutans leaves could inhibit the HONE-1 cell proliferation.

Keywords: Clinacanthus nutans, ethanol, hexane, water, HONE-1 cell, proliferation.

INTRODUCTION

Nasopharyngeal cancer is one of cancer in head and neck that are most frequently occur.¹ The distribution of this cancer in Asia is quite high, namely 81%, while in Africa only reached 9%. Countries in southeast asia give a large contribution, up to 67%.² In Indonesia, this disease is number 4 tumor that occurred most frequently after cervical cancer, breast cancer and skin cancer. The incidence is expected at around 12,000 new cases per year, but many of the cases was not detected because of a shortage of consciousness and lack of hospital facilities in Indonesia.³ Men are at risk 3 times higher than women to suffer this disease. Incidence is the highest in persons with the age of 50 to 60 years old.⁴ Elderly people have higher risk to relapse and have low survival rate.⁵ Risk factor of nasopharyngeal cancer include smoking, salted fish consumption,⁶ nitrosamine in some of the Chinese traditional food,⁷ exposure of formaldehyde, wood dust and smoke.⁸ Nasopharynx cancer clinically characterized by low differentiation and high metastasis.⁹ Typically diagnosed at advanced stadium.¹⁰ Early identification of this disease is effecting the prognosis. At advanced stadium, the prognosis is very poor.¹¹ Management of nasopharyngeal cancer is currently carried out by surgery accompanied by radiation and chemotherapy. However, this therapy causes various side effects on normal tissue with various symptoms such as nausea, vomiting, anorexia, diarrhea, oral mucositis and numbness. Based on these side effects, as well as the relatively expensive costs, so far research on cancer therapies continues to be obtained from natural sources.¹² One of the natural source which has anti-cancer activity is Dandang gendis (Clinacanthus nutans).

Clinacanthus nutans is a plant species in the Acanthaceae family that grows in tropical Asia, especially Indonesia. The Acanthaceae family is considered as one of the sources of medicinal plants which provides effective traditional
medicines for several diseases. Until now this plant attracted many researchers because of the potential and pharmacological effects it has. All parts of the plant have been reported to have antiviral activity, anti-inflammatory properties, antibiotic films, antioxidants, and anti cancer.

In previous studies, ethanol extract of C. nutans leaves showed antitumor activity by increasing the regulation of the immune response. In addition, ethanol extract also showed anti-inflammatory, antioxidant and anti-dengue activities. Whereas hexane extract of C. nutans leaves showed anti-proliferation activity against A549 (lung cancer cell line), CNE1 (nasopharyngeal cancer cell line) and HepG2 (liver cancer cell line). The water extract of this plant also has been reported to have anti-angiogenic activity. The aim of this study is to determine the effect of ethanol, hexane and water extract of C. nutans leaves on HONE-1 (epithelial tumor cell lines) cell proliferation.

METHODS
This study is an in vitro experimental laboratory. Clinacanthus nutans leaves were extracted using ethanol, hexane and water, after which the three extracts were treated to HONE-1 cells.

Plant Material Preparation
Clinacanthus nutans leaves was washed thoroughly using water to remove dust particles attached. The leaves are dried for approximately 2 weeks. The dried leaves are then turned into a powder form with a blender. One hundred grams of C. nutans powder was extracted with 500 mL ethanol, hexane and water by maceration method using for 72 hours. After that, the extract residue is filtered and continued by concentrating with a rotary evaporator. The ethanol extract, hexane, and water obtained were then stored at -20°C until the extract was ready for use.

Cell Culture
HONE-1 cells were cultured in RPMI medium (Gibco), supplemented with 10% fetal bovine serum (Gibco), 250 IU/ml penicillin (Invitrogen) under standard culture conditions at 37°C in 5% CO₂ humidified incubator. The medium was changed regularly and the cells were sub-cultured every 3-4 days.

Proliferation Assay
Confluent cells in 96 wellplates were treated with ethanol, hexane and water extracts at concentration of 100 μg/mL. After 24 hours, each well was given 50 μL MTT (mitochondrial-dependent reduction of 3-(4,5-dimethyl-2-thiazolyl) -2,5-diphenyl-2H-tetrazoliumbromide) and incubated for 4 hours. Each well was added with 100 μL acidified isopropanol and placed in an incubator for 1 hour. The absorbance was measured at 570 nm using a microplate reader (Tecan, Salzburg, Austria). The cell viability (%) relative to the control wells containing cell culture medium without test samples as a vehicle was calculated using [A]_test / [A]_control x 100. Where [A]_test is the absorbance of the test sample and [A]_control is the absorbance of the control.

Statistical Analysis
Shapiro-Wilk test was used for normality test. The differences between experimental groups were analyzed using a one-way ANOVA with Post-Hoc test. A p-value (p < 0.05) was considered statistically significant.

RESULTS
The positive control group showed a decrease in proliferation by 91.8% ± 2.68%, ethanol extract showed a decrease in proliferation by 89% ± 4.85%, hexane extract showed a decrease in proliferation by 61.8% ± 27.2% and water extract showed a proliferation decreased by 56.7% ± 26.8% (Figure 1). The results of the normality test (Shapiro-Wilk) obtained a value p > 0.05, which means normal data distribution, with these results it can be followed by a one-way ANOVA test.

One-way ANOVA test results showed a significant difference between groups (p < 0.05). Then continued with the Dunnet post-hoc test, the results showed a significant difference between each group (positive control, ethanol, hexane and water) with negative control group (p <0.05).

DISCUSSION
Clinacanthus nutans is a traditional medicinal plant that is often used by people in Asia. The ability of C. nutans leaves extract as a natural remedy is often used because of its efficacy and...
benefits mainly due to the content of bioactive compounds in it. One of them is purpurin-18 phtyl ester isolated from chloroform extract of C. nutans leaves which has the potential to be a therapeutic agent for gingivitis and ulceration.14

In previous studies, C. nutans was shown to have antitumor activity against Hela cells, MCF-7, A549, K-562, IMR-32, HSC-4, and HONE-1.25

The results of this study indicate that ethanol extracts have the highest inhibitory activity against HONE-1 cell proliferation compared to two other solvents, hexane and water. Ethanol extract was not significantly different from positive control, meaning ethanol extract’s ability to inhibit HONE-1 cell proliferation is equivalent to doxorubicin 3 μM. Meanwhile, there is no significant difference between hexane and water extract. Hexane extract could inhibit HONE-1 cell proliferation up to 61%, while water extract up to 56%. It also showed that the ability of both extracts is good enough, because it could inhibit proliferation up to 50%. Since water is easily obtained to extract C. nutans leaves, water extracts can be easily obtained in remote areas. People in rural areas can extract C. nutans leaves with water for the treatment of nasopharyngeal cancer, but still must be under the supervision of an authorized doctor.

The high ability of ethanol extract to inhibit proliferation is thought due to the high ethanol polarity, so that it is able to dissolve active compounds. Ethanol solvents are effectively able to dissolved flavonoid compounds, phenols, alkaloids, terpenoids, saponins, tannins, anthocyanins.24 The possibility is these compounds have high anticancer activity. Based on previous research, flavonoids are the main active compounds in C. nutans.17

Hexane is the solvent with the lowest polarity index among the three solvents used in this study. Hexane is able to dissolve non-polar compounds such as lignin, waxes, lipids, aglycon, sterols and terpenoids.25 Terpenoids are probably compounds that have anticancer activity in hexane extracts. Terpenoids is the largest class of natural products. There are about 25,000 chemical structures of terpenoid that so far have been used in various fields, including medicine. Parts of terpenoids which are known to have anticancer activity are vincristine, irinotecan, etoposide, paclitaxel, limonene, cantharidin, artemisinin, andrographolide, alisol, lycopene, celestrol and many more.26

Water has the highest polarity index, which is 9. Water is able to dissolve alkaloids and glycoside compounds.24 proteins, sugars, tannins.27 In previous studies, tannins showed anticancer activity against HeLa cells, MCF-7 (breast cancer cell lines), HCT-116 (intestinal tumor cell strain), CCD-18Co (non-tumor intestinal cell strain), M220 (pancreatic cancer cell strain) and many more.28 This study is not in line with research conducted by Ng et al., which states that water extracts of C. nutans leaves are unable to inhibit A549 cell proliferation (lung cancer cell lines), CNE1 cells (nasopharyngeal cell lines) and HepG2 cells (liver cancer cell lines).16 However, compared to the research of Ng et al., there is a fundamental differences with current study, namely in terms of the extraction method. Although both use maceration techniques, Ng’s study used warm water, while this study uses water at room temperature. This can cause differences in soluble compounds.

CONCLUSION
Ethanol, hexane and water extract of C. nutans leaves can inhibit HONE-1 cell proliferation. Ethanol extract showed the highest activity, even showed activity that equivalent to positive control (doxorubicin 3 μM). However, further research is still needed to find out what compounds are responsible for the anticancer activity in each extract.

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CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest.

REFERENCE


