Anticancer potential of *Syzygium aromaticum L.* in MCF-7 human breast cancer cell lines

Parvinnesh S. Kumar[1], Raden M. Febriyanti[2,3], Ferry F. Sofyan[2], Dimas E. Luftimas[3,4], Rizky Abdulah[1,3]

[1]Departments of Pharmacology and Clinical Pharmacy, [2]Biological Pharmacy, Faculty of Pharmacy, [4]Medical Nutrition, [3]Laboratory of Cell Culture and Cytogenetic, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

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ABSTRACT

Background: The common treatment for cancer is unfavorable because it causes many detrimental side effects, and lately, there has been a growing resistance toward anticancer drugs, which worsens the future of cancer treatment. Therefore, the focus has now shifted toward natural products, such as spices and plants, among many others, to save the future of cancer treatment. Cloves (*Syzygium aromaticum L.*) are spices with the highest antioxidant content among natural products. Besides acting as an antioxidant, cloves also possess many other functions, such as anti-inflammatory, antibacterial, and antiseptic, which makes them an ideal natural source to be developed as an anticancer agent. **Objective:** This study aims to evaluate the cytotoxic activity of cloves toward MCF-7 human breast cancer cell lines. **Materials and Methods:** Different concentrations of water extract, ethanol extract, and essential oil of cloves were investigated for their anticancer potential *in vitro* through a brine shrimp lethality test (BSLT) and an MTT assay. **Results:** In both BSLT and MTT assays, the essential oil showed the highest cytotoxic effect, followed by ethanol and water extract. The LD_{50} concentration of essential oil in the 24 hours BSLT was 37 µg/mL. Furthermore, the IC_{50} values in the 24 hours and 48 hours MTT assays of the essential oil were 36.43 µg/mL and 17.6 µg/mL, respectively. **Conclusion:** Cloves are natural products with excellent cytotoxicity toward MCF-7 cells; thus, they are promising sources for the development of anticancer agents.

Key words: Antioxidant, brine shrimp lethality test, clove, cytotoxicity, MCF-7

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INTRODUCTION

The war against the highly malicious public enemy called "cancer" has been going on for ages, and humans are often at the losing end. Cancer is often likened to age because every year the numbers just keep escalating and do not seem to go down. It is well-known as one of the leading causes of death worldwide, accounting for 7.6 million deaths (around 21,000 cancer deaths a day) in 2008.[1] The probability of death caused by cancer is 1 in 8 deaths worldwide, and a huge wave of new cases amounting to 12.7 million was diagnosed in 2008.[2] Cancer is the general classification for more than 200 different diseases of the human body caused by normal cells going wild, resulting in abnormal cell growth that spreads in or invades the body and eventually causes death when not controlled.[3]

The current treatment for cancer usually involves a series of combined therapies to combat and curb the disease. The treatment options available for cancer patients are surgery, radiation, chemotherapy, hormone therapy, biological therapy, and targeted therapy. This cocktail of treatment methods and drugs is highly toxic and has multiple side effects. Not only is it troublesome but its unconventional approach, with its fair share of side effects, always acts as a barrier and thus limits the treatment for cancer patients.[4]

Many of us are still unaware that diet is one of the main contributing factors to cancer. The foods that we consume contain a lot of chemicals, preservatives, and coloring, which makes us more susceptible to cancer. Spices, such as ginger, garlic, cloves, and turmeric, among many others, have dominated our kitchens for ages. However, until this very moment, most of us still do not fathom the benefit

Address for correspondence:
Dr. Rizky Abdulah, Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung Sumedang KM. 21, Jatinangor - 45363, Indonesia
E-mail: abdulahrizky@gmail.com

and medicinal values of these spices; in "our" opinion, their usage is merely meant to add aroma and taste to food.[5-7]

Typically, the role of spices in our kitchen is limited to adding color to food or enhancing the taste and aroma of our cooking with the help of the essential oils and oleoresins present in them.[8,9] Some of these spices are loaded with flavonoids that can act as barricades against carcinogenesis. The extremely vital perks of some of these spices, on top of their chemopreventive activity, are their anti-inflammatory, antibacterial, and antioxidant activities; thus, they serve combined functions for those who consume them.[10-14] Flavor-enhancing spices are a special group because of their ability to defend against cancer, heart diseases, and other chronic illnesses.[15] In one study, spices were also observed to inhibit lipid peroxidation, which simply adds to their benefits. As the benefits of their consumption accumulate, spices are emerging as clear candidates for an alternative approach toward cancer treatment.[11]

The use of spices as mediators is gaining popularity, vastly due to their safe toxicity profiles and their potential as chemosensitizers. *Syzygium aromaticum* (clove) ranks highest in terms of antioxidant properties, followed by cinnamon, pepper, ginger, and garlic.[11,16-18] Cloves are well-known in Ayurveda, a form of Indian traditional medicine, due to their potency as a chemopreventive agent and ability to treat several ailments.[19]

MATERIALS AND METHODS

Plant material

Fresh cloves were purchased from the local market of Subang, West Java Province, Indonesia. The botanical identification was made in the Laboratory of Plant Taxonomy, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia.

Preparation of clove extracts

The cloves were milled to fine powder by using a blender. Then, 50 mg of clove powder was soaked in 200 mL of distilled water to prepare the aqueous extract; the same method was used to prepare the ethanol extract but 200 mL of 96% ethanol was used instead of distilled water. Both extracts were allowed to stand for 24 hours before being filtered with Whatman no. 1 filter paper. The filtrate was then allowed to evaporate with the aid of a rotary vacuum evaporator before being transferred to a water bath for further drying.[20] The clove essential oil was prepared through the steam distillation process.[21]

Brine shrimp lethality test

Brine shrimp lethality test (BSLT) is a simple pharmacologic guide for toxicity screening of compounds by using *Artemia salina* Leach nauplii as a convenient monitor. BSLT was done following the method described by Meyer *et al*.[22] Briefly, the hatching eggs were prepared in artificial seawater (3.8% NaCl solution) for 48 hours to obtain mature shrimp called nauplii. The test samples (extract) were prepared by dissolving them in dimethyl sulfoxide (DMSO) (not more than 50 μL in 5 mL solution) added with artificial seawater to attain variable concentrations of each extract. A vial containing 50 μL DMSO diluted to 5 mL was used as control. The nauplii of *A. salina* were applied to each experimental and control vials. After 24 hours of exposure, the dead nauplii were counted as percent of mortality. From these data, the percent of lethality of the brine shrimp nauplii and the concentration variations were plotted on a graph, and LC_{50} was calculated.

Cell culture and treatment

MCF-7 human breast cancer cell lines (ATCC, Manassas, VA, USA) were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Sigma, MO, USA) supplemented with 10% fetal bovine serum and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin). For cell treatments, various concentrations of extracts were added to the cell culture medium for 24 hours.

Drug sensitivity assay

Cell proliferation analysis was performed in the presence of multiple concentrations of various extracts through a colorimetric MTT assay. Cells (2 × 104 in 50 μL/well) were placed in 96-well plates. After the initial cell seeding, different concentrations of each extract were added and incubated for 24 hours. Ten microliters of water-soluble tetrazolium (WST)-8 assay cell-counting solution (Dojindo Lab., Tokyo, Japan) was added to each well and incubated at 37°C for 3 hours. After the addition of 100 μL/well of 1 N HCl, the cell proliferation rate was determined by measuring the absorbance at a wavelength of 450 nm, with a reference wavelength of 650 nm. The absorbance was read with a microtiter plate reader (Becton-Dickinson, NJ, USA). The results were derived from triplicate experiments.

RESULTS

Brine shrimp lethality test

BSLT was the first step taken to evaluate the hidden potential of cloves as an antiproliferative agent. This test allowed us to determine whether cloves have the ability to inhibit cell growth and undergo further procedures en route to becoming a probable candidate for anticancer medicine. The data presented in Figure 1 show that the lowest potential lies in the water extract of cloves, which has the lowest percentage of lethality. Only the water extract had 0% lethality, which was observed at concentrations

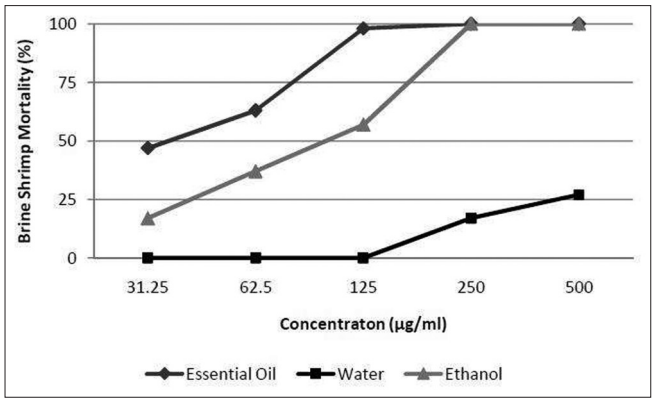

Figure 1: Comparative BSLT analysis of water extract, ethanol extract, and essential oil of cloves

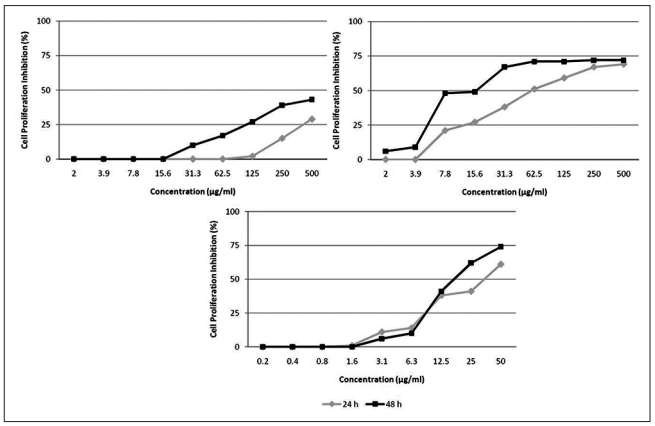

Figure 2: MTT assay on MCF-7 cells with the use of water extract, ethanol extract, and essential oil of cloves

of 31.25 µg/mL, 62.5 µg/mL, and 125 µg/mL. At a concentration of 250 µg/mL, the lethality percentage was 17%; at 500 µg/mL concentration, the lethality was 27%. The ethanol extract of cloves showed a much better result, with a 100% death rate observed at concentrations of 500 µg/mL and 250 µg/mL. The percentages of death at the other concentrations were 17% at 31.25 µg/mL, 37% at 62.5 µg/mL, and 57% at 125 µg/mL. The highest reading of potential bioactivity was found in the clove essential oil. Similar to the ethanol extract, 100% death was observed at concentrations of 500 µg/mL and 250 µg/mL. However, the essential oil had a higher lethality percentage at three other concentrations: 47% at 31.25 µg/mL, 63% at 62.5 µg/mL, and 98% at 125 µg/mL; in comparison, the ethanol extract has much lower lethality at these concentrations.

In 24 hours of experimentation, the clove essential oil reached LD_{50} concentration at 37 µg/mL, whereas the etanol extract reached LD_{50} at 103 µg/mL. The water extract, however, failed to reached LD_{50} concentration even at the highest concentration tested.

MTT assay

Because cloves showed some promising results during BSLT, further testing was done on the clove extract and the essential oil by using an MTT assay to evaluate the cytotoxic potential of cloves on MCF-7 cells. As indicated in Figure 2, the water extract of cloves had no cytotoxic activity on the MCF-7 cancer cells. The ethanol extract showed a promising result, with cell inhibition observed after 24 hours and 48 hours of incubation. To compare, 48 hours incubation resulted in a lower IC_{50} of 16.71 µg/mL, whereas 24 hours has achieved an IC_{50} of 61.29 µg/mL. The essential oil of cloves, however, showed the highest potential as an anticancer source with its lower IC_{50} concentrations in both the 24 hours and the 48 hours MTT assay (36.43 µg/mL and 17.6 µg/mL, respectively).

DISCUSSION

Being one of the major causes of death around the world, cancer is fast becoming a ghastly disease. To make things worse, recent observations show an increase in cases of resistance toward anticancer drugs. This has given rise to very serious issues surrounding the efficacy and future of cancer treatment.[23] Subsequently, this has led to research on natural products, which have undeniably emerged as the next best treatment option available to overcome the resistance faced by anticancer drugs. One of the many reasons why natural products can be the key is that, long before the emergence of any modern medicine, many natural products, such as plants, herbs, fruits, and spices, have been used in traditional medicine to cure multiple ailments.[5]

In recent years, considerable research has been done with very positive results indicating that several fruits and vegetables have the ability to inhibit cell proliferation and reduce the spread of cancer. Spices are considered as the golden compounds in these researches because they seem to have the major dietary phytochemicals that have the ability to suppress inflammatory processes, hyperproliferation, and the initiation of carcinogenesis. All these activities have made spices the most prominent natural products that could help combat the extensive growth of cancers worldwide.[24]

In this study, the primary measure taken to identify the capabilities of cloves as an anticancer agent was BSLT. From Figure 1, it can be concluded that the water extract of cloves has the lowest capability among all three extracts. Only the water extract showed 0% lethality, which indicates that it has insufficient ability to inhibit cell proliferation. The ethanol extract was observed to have intermediate capability, whereas the essential oil of cloves showed the highest potential as an anticancer agent. Although a 100%

death rate was observed at concentrations of 500 μg/mL and 250 μg/mL for both the ethanol extract and the essential oil, the ability to cause shrimp death was much higher for the essential oil. Thus, it can be concluded that the essential oil of cloves has an advantage over the water and ethanol extracts, based on the preliminary results obtained through BSLT.

The second test done to further strengthen the results and prove the viability of clove extracts as a potential anticancer agent, particularly for breast cancer, was an MTT assay on MCF-7 cells. Figure 2 presents the data obtained for all three extracts after 24 hours and 48 hours of incubation. The results show that the water extract had no activity on MCF-7 cells; this is supported by BSLT results in Figure 2, which indicate that only the water extract had a 100% survival rate among all three extracts. The extract with the lowest IC_{50} has the higher potential to be developed as a possible anticancer agent. The essential oil showed an IC_{50} of 36.43 μg/mL at 24 hours and of 17.6 μg/mL at 48 hours of incubation. When both extracts were incubated at a higher concentration or for a longer period of 48 hours, the IC_{50} values of the ethanol extract and essential oil became much lower compared with those at 24 hours of incubation. This suggests that the ethanol extract and essential oil of cloves inhibit the MCF-7 human breast cancer cell lines in a time- and dose-dependent manner. These finding are supported by Prashar *et al.* (2006), who reported that clove oil exhibits cytotoxicity towards human fibroblast and endothelial cells.[25]

The results of other researches on cloves further support the ability of this spice as an excellent cytotoxic agent. Cloves are being hailed as the future of cancer treatment because of their capability to induce apoptosis and work in various cancer cells.[5] Cloves are also a source of betulinic acid and other triterpenes, which can act as chemopreventive agents against breast cancer.[26] Various researches have concluded that cloves are ideal for cancer treatment because they enhance apoptosis and inhibit cell proliferation [19,24,27]–the two key properties that are ideally required for cancer treatment.

CONCLUSION

In conclusion, cloves have a highly promising future of being developed as an anticancer agent. This research has unlocked the hidden potential held within the tiny spices known as cloves. With such promising results as presented above, the possibility of a natural product being the key to the future of cancer treatment, in particular for breast cancer, becomes real.

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