Quality Improvement in Dental and Medical Knowledge, Research, Skills and Ethics Facing Global Challenges – Widyarman et al. (Eds)

Cytotoxicity activity of *Allium sativum* extracts against HSC-3 cells

I.J. Pardenas
Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia

M.O. Roeslan
Department of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia

ABSTRACT: Background: One of the major issues with dental and oral health is oral cancer. One often used natural component, garlic (*Allium sativum*), has a wide range of pharmacological effects, including anticancer activity. *A. sativum*'s organosulfur compounds have the ability to hinder the development of cancer cells. Objectives: This research sought to determine the cytotoxicity activity of aqueous and ethanol extracts of *A. sativum* toward the HSC-3 cell line. Methods: *A. sativum* extract was applied to HSC-3 cells at doses of 50 g/mL, 100 g/mL, 200 g/mL, 400 g/mL, and 800 g/mL for 24 hours before cytotoxicity test using the MTT method and data analysis using one-way ANOVA and Post-Hoc test (Tukey HSD). Results: Cytotoxicity of aqueous and ethanol extracts of *A. sativum* varied. Aqueous extract of *A. sativum* was recognized to be more capable of reducing cell viability at a concentration of 400 µg/mL with a cell viability percentage of 40.229% ± 5.367% and 800 µg/mL with a percentage of 34.482% ± 3.448%. The ethanol extract of *A. sativum* with a concentration of 800 µg/mL had a cell viability percentage of 40.229% ± 3.448%. Conclusion: In comparison to the ethanolic extract of *A. sativum*, the aqueous extract significantly reduced the vitality of HSC-3 cells.

1 INTRODUCTION

Dental and oral health problems inflict a huge health concern in various countries. It causes pain, unpleasantness, disability, and death (World Health Organization 2020). Oral cancer is one of many dental and oral health problems. It is malignant cancer that attacks the lips, and oral cavity which includes the gingiva, tongue, cheek mucosa, roof of the mouth, and floor of the mouth (Lingen *et al.* 2008). Oral cancer is predicted to cause close to 180.000 fatalities in 2020, with Asia having the highest frequency (International Agency for Research on Cancer 2020).

Squamous cell carcinoma (SCC) is the term most frequently used to describe oral cancer since the majority of the oral cavity is lined by stratified squamous epithelium. The most typical SCC in the oral cavity is squamous carcinoma of the tongue. The highly aggressive characteristics of HSC-3 cell lines have made HSC-3 cells a suitable model for study or research on metastasis of SCC (Rivera 2015). Treatment of oral cancer is surgery, radiotherapy or a combination of chemo-radiotherapy (Shih *et al.* 2017). However, this treatment attacks normal cells and has severe side effects. Therefore, alternative treatments with minimal side effects are needed (Aslam *et al.* 2014).

Garlic (*Allium sativum*) is a natural ingredient that has an anticancer potential (Kooti *et al.* 2017). *A. sativum* methanol extract exhibited the capacity to decrease the viability of Jurkat Clone E6-1 cells and U-937 cells, according to earlier studies (Jasamai *et al.* 2016). Additionally, other research demonstrated that an aqueous extract of *A. sativum* causes

DOI: 10.1201/9781003402374-59

SCC-9 cells to undergo apoptosis (Vemuri *et al.* 2018). The cytotoxicity of aqueous and ethanolic extracts of *A. sativum* on HSC-3 cells, however, has not been investigated. The authors were therefore interested in comparing the differences between the cytotoxic potency of aqueous and ethanol extracts of *A. sativum* toward the HSC-3 cell line.

2 METHODS

2.1 *Plant material and extraction procedure*

A. Sativum was accumulated in Indonesia. The specimens were authenticated by the Indonesian Institute of Science. It was dried at a temperature of 30°C and then crushed to powder. Each extraction involved macerating 85 grams of powder for three days at room temperature with 425 mL of distilled water and ethanol. After that, filter paper (Whatman Int. Ltd., Maidstone, UK) was used to remove the solvent and powder residue. A rotary evaporator (Buchi, Switzerland) was then used to evaporate the solvent from the extracted *A. sativum*. A refrigerator set to 4°C was used to keep the extract.

2.2 *Cell culture*

HSC-3 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) under standard culture conditions at 37°C in a 5% CO_2 humidified incubator, supplemented with 10% fetal bovine serum (Gibco, USA) as food for cells and antibiotics, namely 1% amphotericin B and 1% penicillin-streptomycin (Invitrogen, USA) (Memmert, Germany). Until the cells are 80% confluent, the media was routinely changed, and the cells were sub-cultured every 3–4 days. An enclosed biosafety cabinet served as the site of the entire cell culture procedure.

2.3 *Cytotoxicity assay*

The cytotoxicity assay, which gauges how long HSC-3 cells can survive after being exposed to the extract, was used to determine the cytotoxicity impact. *A. sativum* extracts at concentrations of 50 g/mL, 100 g/mL, 200 g/mL, 400 g/mL, and 800 g/mL were applied to HSC-3 cells that had been put in 96-well plates and cultured for 24 hours at 37°C and 5% CO_2. After incubation, the cytotoxicity test was carried out using the MTT method. Each well plate was filled with MTT solution (Sigma Aldrich, USA), and cells were then incubated for 4 hours at 37°C and 5% CO_2. The formazan crystals will be dissolved by adding acidified isopropanol to the mixture after 4 hours of incubation. At a wavelength of 570 nm, the absorbance value was determined using a microplate reader (Tecan, Salzburg, Austria).

2.4 *Statistical analysis*

The Shapiro-Wilk method was employed to conduct the normality test. One-way ANOVA and Tukey's post-hoc were used to assess differences between experimental groups. A p-value ($p < 0.05$) was considered statistically significant.

3 RESULTS

From the outcome of a qualitative phytochemical assay, it is known that the aqueous extract of *A. sativum* contains triterpenoids, flavonoids, tannins, and alkaloids. Meanwhile, the ethanol extract of *A. sativum* contains triterpenoids, flavonoids, steroids, tannins, and alkaloids (Table 1).

Table 1. Qualitative phytochemical test of aqueous and ethanol extracts of *A. sativum*.

Extract	Compound	Results
Aqueous extract of *A. sativum*	Triterpenoid	+
	Flavonoid	+
	Steroid	–
	Tannin	+
	Alkaloid	+
	Quinone	–
Ethanol extract of *A. sativum*	Triterpenoid	+
	Flavonoid	+
	Steroid	+
	Tannin	+
	Alkaloid	+
	Quinone	–

This study used an aqueous extract of *A. sativum* and an ethanol extract of *A. sativum* with concentrations of 50 µg/mL, 100 µg/mL, 200 µg/mL, 400 µg/mL, and 800 µg/mL. The negative control was HSC-3 cells without treatment, while the positive control was HSC-3 cells and doxorubicin µM. All variables will be tested for cytotoxicity against HSC-3 cells by putting them into the well plate that contains HSC-3 cells. The results of the cytotoxicity test were depicted in Figure 1.

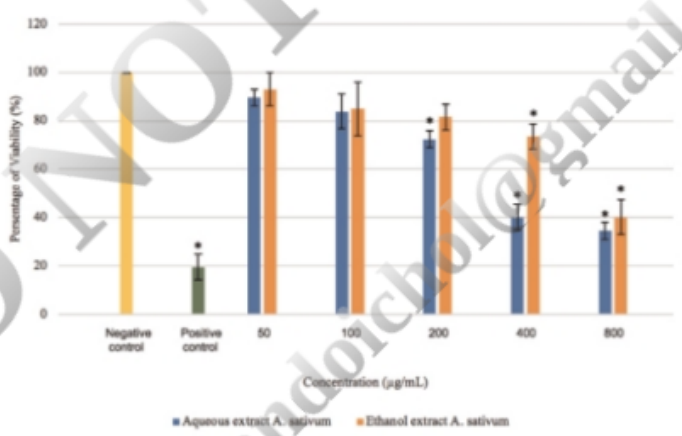

Figure 1. Viability percentage of HSC-3 cell line. The data are expressed as mean ± SD (n = 3). *Shows a significant difference compared to the negative control ($p < 0.05$)

The outcome of the normality test revealed that all data were normally distributed ($p > 0.05$). The one-way ANOVA test revealed significant differences between experimental groups with $p < 0.001$. Tukey's post-hoc test was then carried out, $p < 0.05$ denotes a significant difference.

4 DISCUSSION

Based on the phytochemical test, it is known that *A. sativum* contains non-organosulfur content which is flavonoids. Flavonoids have anti-cancer properties via inhibiting protein

tyrosine kinase, an enzyme involved in controlling cell proliferation and transforming cells into malignant forms (Steiner 1997). In addition, *A. sativum* contains organosulfur compounds that play a major role in inhibiting cancer cell growth, γ-glutamyl-S-alk(en)yl-L-cysteine, and alliin. These two organosulfur compounds can produce other compounds such as allicin, S-allyl cysteine (SAC), S-allyl mercaptocysteine (SAMC), diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), vinyl dithiin and ajoene (Pandrangi 2015).

The solvents used in this study were distilled water and ethanol. Distilled water and ethanol are considered solvents because they are polar solvents, capable of extracting compounds such as allicin which have an anticancer role (Bajac *et al.* 2018). The difference between these two solvents is in the polarity value. Water has a polarity value of 10.2. Meanwhile, ethanol has a lower polarity value, which is 4.3 (Haerudin *et al.* 2017). The difference between water and ethanol as a solvent based on the polarity value lies in the content of *A. sativum* compounds that can be extracted.

Based on the research that has been done, it can be seen that the greater the concentration of aqueous and ethanol extracts of *A. sativum*, the lower the viability of the HSC-3 cell line. This indicates that the greater the concentration of aqueous and ethanol extracts of *A. sativum*, the greater the cytotoxicity effect it has on HSC-3 cells.

Research on the impact of methanol extract of *A. sativum* on cell survival and apoptosis in U-937 cells and Jurkat Clone E6-1 cells provided support for this investigation (Jasamai *et al.* 2016). This study has shown that the cell viability decreased with increasing concentrations of the methanol extract of *A. sativum*. According to the study's findings, the maximum concentration that can decrease cell viability is an *A. sativum* methanol extract at a concentration of 800 g/mL. On the other hand, methanol extract of *A. sativum* at a concentration of 50 µg/mL was the smallest concentration that is capable of reducing cell viability. When compared with research conducted with an aqueous extract of *A. sativum*, the smallest concentration that was able to reduce the viability of HSC-3 cells was 200 µg/mL. Meanwhile, in the ethanol extract of *A. sativum*, the smallest concentration that was able to reduce cell viability was 400 µg/mL.

This difference can occur due to differences in incubation time. In the previous study, the incubation time was 48 hours, whereas, in the current study, the incubation time was 24 hours. Research on the cytotoxicity effect of *A. sativum* extract on HT-29 cell viability that had previously been carried out showed that the number of cell viability at 24 hours of incubation was less than that of 48 hours of incubation (Ghazanfari *et al.* 2011). Therefore, in this study, the incubation time used was 24 hours. The solvents used also have different polarity values. In previous studies, methanol with a polarity value of 5.1 was used as a solvent, while in the current study, distilled water and ethanol were used as solvents. Methanol is not used as a solvent because methanol can be toxic in the liver when converted to formate in the presence of an alcohol dehydrogenase enzyme (Joshi & Adhikari 2019). In addition, there are differences in the cancer cells used, so further research is needed to compare the *A. sativum* extracts of the three solvents on the same cancer cells.

Based on the results of the cytotoxicity test, which can be measured by looking at the percentage of cell viability, it showed that the positive control (19,539% ± 5,266%) had a significant difference from the negative control. In another experimental group, the aqueous extract of *A. sativum* at concentrations 400 µg/mL (40.229% ± 5.367%) and 800 µg/mL (34.482% ± 3.448%) and the ethanol extract of *A. sativum* at concentration 800 µg/mL (40.229% ± 3.448%) showed that there is no significant difference with the positive control (Figure 1). This indicated that the aqueous extract of *A. sativum* at concentrations 400 µg/mL and 800 µg/mL and the ethanol extract of *A. sativum* 800 µg/mL had a cytotoxic effect similar to the positive control.

The aqueous extract of *A. sativum* 400 µg/mL (40.229% ± 5.367%) had a significant difference with the ethanol extract of *A. sativum* 400 µg/mL (73.562% ± 5.267%). This indicated that at the same concentration of 400 µg/mL, there was a difference in cytotoxic

effectiveness against the HSC-3 cell line, the aqueous extract of *A. sativum* was more potent against HSC-3 cells than the ethanol extract of *A. sativum*. The aqueous extract of *A. sativum* 800 µg/mL (34.482% ± 3.448%) had no significant difference with the ethanol extract of *A. sativum* 800 µg/mL (40.229% ± 3.448%). However, there are still differences in the percentage of cell viability, an aqueous extract of *A. sativum* 800 µg/mL was able to lessen cell viability by 6% more than an ethanol extract of A. sativum 800 µg/mL.

The difference in effectiveness between aqueous and ethanol extracts of *A. sativum* is supported by research on the determination of organosulfur compounds of *A. sativum* and the optimization of the solvent mixture in the extraction of bioactive compounds of *A. sativum*. Based on these two studies, it is said that distilled water solvent is more capable of extracting allicin (Bajac *et al.* 2018; Cavalcanti *et al.* 2021). This explains why the aqueous extract of *A. sativum* 400 µg/mL is better than the ethanol extract of *A. sativum* 400 µg/mL. However, based on the phytochemical test, the ethanol extract of *A. sativum* has steroids, while the aqueous extract does not have steroids. Based on this fact, the ethanol extract of *A. sativum* with steroid content could be more able to reduce cell viability, but the phytochemical tests carried out were qualitative so it was not known how much each compound contained quantitatively and also the main protective effect of *A. sativum* comes from organosulfur compounds (Asemani *et al.* 2019). Although the ethanol extract of *A. sativum* contains steroids, the aqueous extract of *A. sativum* contains more organosulfur compounds than the ethanol extract of *A. sativum*. This research demonstrated that there were discrepancies in the cytotoxic effectiveness of aqueous and ethanol extracts of *A. sativum* against HSC-3 cells. When compared to the ethanol extract of *A. sativum*, the aqueous extract was better able to lower cell viability.

5 CONCLUSION

Aqueous extract of *A. sativum* at concentrations of 400 µg/mL and 800 µg/mL and ethanol extract 800 µg/mL were cytotoxic to HSC-3 cells. The amount of aqueous and ethanol extract of *A. sativum* that was applied to HSC-3 cells directly correlated with their cytotoxicity. Both the 400 and 800 mg/mL aqueous extracts of *A. sativum* and the 800 mg/mL ethanol extract did not substantially vary from the positive control. Additionally, it was discovered that the aqueous extract of *A. sativum* was more cytotoxic to HSC-3 cells than the ethanol extract.

ACKNOWLEDGMENT

The authors would like to acknowledge Dr. Indra Kusuma, M. Biomed from the Faculty of Medicine, YARSI University, Jakarta, Indonesia for providing the HSC-3 cell line. The authors would also like to convey their gratitude to the Head of the BioCORE laboratory, Faculty of Dentistry, Trisakti University, Jakarta, Indonesia for providing facilities used in the completion of this research.

REFERENCES

Asemani, Y., Zamani, N., Bayat, M., & Amirghofran, Z. 2019. Allium vegetables for possible future of cancer treatment. *Phytotherapy Research* 33(12): 3019–3039.

Aslam, M.S., Naveed, S., Ahmed, A., Abbas, Z., Gull, I., & Athar, M.A. 2014. Side effects of chemotherapy in cancer patients and evaluation of patients opinion about starvation based differential chemotherapy. *Journal of Cancer Therapy* 2014.

Bajac, J., Nikolovski, B., Kocić-Tanackov, S., Tomšik, A., Mandić, A., Gvozdanović-Varga, J., Vlajić, S., Vujanović, M., & Radojković, M. 2018. Extraction of different garlic varieties (Allium sativum L.)– Determination of organosulfur compounds and microbiological activity. *International Congress Food Technology. Quality and Safety*.

Cavalcanti, V.P., Aazza, S., Bertolucci, S.K.V., Rocha, J.P.M., Coelho, A.D., Oliveira, A.J.M., Mendes, L. C., Pereira, M.M.A., Morais, L.C., & Forim, M.R. 2021. Solvent mixture optimization in the extraction of bioactive compounds and antioxidant activities from garlic (Allium sativum L.). *Molecules* 26(19): 6026.

Ghazanfari, T., Yaraee, R., Rahmati, B., Hakimzadeh, H., Shams, J., & Jalali-Nadoushan, M.-R. 2011. In vitro cytotoxic effect of garlic extract on malignant and nonmalignant cell lines. *Immunopharmacology and Immunotoxicology* 33(4): 603–608.

Haerudin, A., Lestari, T.P., & Atika, V. 2017. Pengaruh jenis pelarut terhadap hasil ekstraksi rumput laut Gracilaria sp. sebagai zat warna alam pada kain batik katun dan sutera. *Dinamika Kerajinan Dan Batik* 34 (2): 83–92.

International Agency for Research on Cancer. 2020. *Global Cancer observatory: Cancer Today*. https://gco.iarc.fr/today/data/factsheets/cancers/1-Lip-oral-cavity-fact-sheet.pdf

Jasamai, M., Hui, C.S., Azmi, N., & Kumolosasi, E. 2016. Effect of Allium sativum (garlic) methanol extract on viability and apoptosis of human leukemic cell lines. *Tropical Journal of Pharmaceutical Research* 15(7): 1479–1485.

Joshi, D.R., & Adhikari, N. 2019. An overview on common organic solvents and their toxicity. *J. Pharm. Res. Int* 28(3): 1–18.

Kooti, W., Servatyari, K., Behzadifar, M., Asadi-Samani, M., Sadeghi, F., Nouri, B., & Zare Marzouni, H. 2017. Effective medicinal plant in cancer treatment, part 2: Review study. *Journal of Evidence-Based Complementary & Alternative Medicine* 22(4): 982–995.

Lingen, M.W., Kalmar, J.R., Karrison, T., & Speight, P.M. 2008. Critical evaluation of diagnostic aids for the detection of oral cancer. *Oral Oncology* 44(1): 10–22.

Pandrangi, A. 2015. Cancer chemoprevention by garlic-a review. *Hereditary Genet* 4(2): 1–7.

Rivera, C. 2015. Essentials of oral cancer. *International Journal of Clinical and Experimental Pathology* 8(9): 11884.

Shih, Y.-L., Hung, F.-M., Lee, C.-H., Yeh, M.-Y., Lee, M.-H., Lu, H.-F., Chen, Y.-L., Liu, J.-Y., & Chung, J.-G. 2017. Fisetin induces apoptosis of HSC3 human oral cancer cells through endoplasmic reticulum stress and dysfunction of mitochondria-mediated signaling pathways. *In Vivo* 31(6): 1103–1114.

Steiner, M. 1997. The role of flavonoids and garlic in cancer prevention. *Food Factors for Cancer Prevention*: 222–225.

Vemuri, S.K., Banala, R.R., Subbaiah, G.P. V, AV, G.R., & Thekkumalai, M. 2018. Apoptotic efficiency of aqueous extracts of turmeric, garlic and their active compounds in combination with Tamoxifen in lung and oral cancers: A comparative study. *Beni-Suef University Journal of Basic and Applied Sciences* 7(2): 184–197.

World Health Organization. 2020. *"Oral Health."* https://www.who.int/news-room/factt-sheets/detail/oral-health