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Article

Green Synthesis of Halogen Substituted Chalcone Against Cervical Cancer (HeLa) Cell Lines

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ABSTRACT

Cervical cancer is the second leading cause of death in women. Many cancer treatments currently provide toxic effects on normal cells. Therefore, alternative treatment using chalcone derivatives has potent anticancer properties that can help reduce cancer side effects. Chalcone derivatives with halogen and methoxy groups in ring B can potentially inhibit cancer cells. This research aimed to synthesize halogen-substituted chalcone by a green chemistry approach and determine activity against cervical cancer (HeLa) cell lines. The Claisen-Schmidt reaction was used to synthesize 2'-hydroxy-2-bromo-4,5-dimethoxychalcone using a grinding technique. The purity of the synthesized compound was determined using thin-layer chromatography and melting range. The compounds' structures were characterized using FTIR, MS ^{1}H, and ^{13}C-NMR. The result showed that the synthesized compound was yielded in 53% as a yellow solid. In vitro cytotoxicity of the synthesized chalcone was evaluated by the MTT assay method. The IC_{50} of the compound was 67,23 µg/mL as a moderate activity inhibiting cervical cancer (HeLa) cell lines. Based on the IC_{50} value, this compound can be a candidate for anticancer against Cervical cancer (HeLa) cell lines.

INTRODUCTION

Cancer is a disease when abnormal cells divide uncontrollably and destroy body tissues. The World Health Organization (WHO) reported that cancer had become the second most common cause of death worldwide after cardiovascular (Kristina et al., 2022). Cervical cancer was the world's fourth most common cancer in women, with 604.127 cases (Bray et al., 2018). In Indonesia, cervical cancer was the second-highest female cancer after breast cancer (Qorina et al., 2020). There are many types of cancer treatment, such as chemotherapy, surgery, radiotherapy, hormone therapy, and biological therapy (Fikroh et al., 2020). However, this treatment has adverse effects, such as killing normal cells around cancer cells, depression, and alopecia (Ranjit et al., 2013). Therefore, the development of new drugs with selective anticancer needs to develop. *Flavonoids* are phenolic compounds reported as anticancer agents

(Kopustinskiene et al., 2020). Flavonoid compounds are secondary metabolites with aromatic rings which have been reported to have activity as antioxidants, anticancer, antibacterial, anti-inflammatory, and cardioprotective agents and are exciting candidates for pharmaceutical and medical applications (Tungmunnithum et al., 2018). *Chalcone* is a flavonoid compound derivative that has the potential as a chemotherapeutic agent (Anwar et al., 2018).

Chalcones were synthesized by Claisen Schmidt condensation by reacting aldehyde and ketone derivatives to produce the aromatic compound α,β-Unsaturated ketone (Elkanzi et al., 2022). In the laboratory, chalcone synthesis using the conventional method, namely reflux, requires a long time and lots of solvents and catalysts, which makes this method hazardous to health and not environmentally friendly (Susanti & Setyowati, 2018). Therefore, modification of the synthesis of chalcone compounds needs to be carried out to support the principles of green chemistry, one of which is the removal of the solvent in the synthesis process. The chalcone synthesis method can be modified by using the grinding technique. The grinding method is a synthesis method that uses mortar without heating and uses excess solvent (Zangade & Mokle, 2011a). Prabawati et al. (2022) successfully synthesized a chalcone derivative of 4-dimethylamino-4-hydroxy chalcone using a grinding technique without solvents, a short reaction time of about 15 minutes, and a yield of 46.32%. Synthesis of chalcone derivative compounds using grinding techniques has been carried out to synthesize the compound of 2-acetyl-1-naphthol and benzaldehyde to produce chalcone. This synthesis is carried out without using organic solvents. The time is relatively fast, between 4-8 minutes, and the yield is around 84-95%, so it can be said to be eco-friendly compared to conventional methods (Zangade & Mokle, 2011a). Susanti & Setyowati, (2018) synthesized chalcone derivatives by grinding using a NaOH catalyst with a reaction time of 15 minutes and produced a yield of 64.79%.

One essential thing that acts as an anticancer agent in chalcone compounds is the presence of α,β-unsaturated ketone bonds (Bazzaro et al., 2011). In addition, the substituent on chalcone has important role in determining anticancer activity. Rao et al. (2004) reported that the methoxy group on the aromatic ring has antiproliferative activity in tumor cells without any toxic effects on normal cells. Oktavia et al. (2018) also supported the research statement that chalcone with a methoxy group has a more significant toxic effect than other groups in chalcone compounds. Chalcone derivatives with halogen groups, namely bromo, and chloro, have been reported to have anticancer activity in breast cancer cells (Ketabforoosh et al., 2014). Chalcone derivatives with halogen substituents such as bromo are beneficial to modulate the drug's electron and steric characteristics and affect the balance of the hydrophilic and hydrophobic molecules (Fikroh et al., 2020).

Research on synthesizing chalcones with methoxy substituents on ring B has been widely reported. However, substituents with Bromo and Methoxy groups on ring B and their activity as anticancer agents of cervical cancer (HeLa) cells have not been reported yet. Therefore, this research focuses on synthesizing chalcone derivatives with halogen substituents and methoxy in the chalcone B ring using grinding method and the cytotoxicity test of halogen substituted chalcone against cervical cancer (HeLa) cells using the MTT assay method. Grinding method give high yield, eco-friendly, non-hazardous and give simple process (Zangade & Mokle, 2011)

METHODS

Synthesis of 6-bromoveratraldehyde

In a 150 mL three-necked flask with a magnetic stirrer, potassium bromate (1 g, 6 mmol), glacial acetic acid (30 mL), and veratraldehyde (2.5 g, 15 mmol) were combined. After rapidly stirring the mixture, 4 mL of 47% hydrobromic acid was added. A red-brown mixture appeared after each addition. At room temperature, a mixture was stirred for 60 minutes. After that, 50

mL of cool water was added and stirred for 10 minutes. Drop by drop, sodium thiosulfate anhydrous was added to a mixture until a gray precipitate formed, which was then filtered, washed with water, and crystallized from 50% ethanol. TLC monitoring, FTIR, and ^{1}H-NMR spectroscopy were used to measure the precipitated solid.

Green synthesis 2'-hydroxy-2-bromo-4,5-dimethoxychalcone

A mortar and pestle were used to grind 0.68 g of 2-hydroxy acetophenone (5 mmol), 1.22 g of 6-bromoveratraldehyde (5 mmol), and a solid of NaOH (20 mmol) for 5 minutes at room temperature. On completion of grinding as monitored by TLC and the solid mixture was diluted with cold water, acidified by 10% HCl until a yellow precipitate formed. To obtain pure compounds, the precipitate was crystallized from ethanol. The obtained product's melting point was determined and characterized using FTIR, MS, ^{1}H and ^{13}C-NMR.

In vitro anticancer activity assay

Cervical cancer cells (HeLa) were cultured in 96-well plates at a density of 10^6 cells per well and incubated for 24 hours in an incubator (37°C in 5% CO_2). In an incubated well plate, various concentrations of compound solution and doxorubicin were placed. After 24 hours, the culture medium was removed, washed with PBS, and treated with 100 L of MTT. For four hours, the plate was incubated. The purple formazan was dissolved in 0.1N HCl with 10% sodium dodecyl sulfate. The absorbance was measured at 595 nm with an ELISA reader after overnight incubation at room temperature. The viability percentage was computed as follows:

$$\frac{(\text{absorbance of sample} - \text{absorbance media controls})}{(\text{absorbance of cell controls} - \text{absorbance media controls})} \text{ x } 100$$

RESULTS AND DISCUSSION

Synthesis of 6-bromoveratraldehyde

The compound of 6-bromovertraldehyde has been synthesized from veratraldehyde, potassium bromates, and glacial acetate acid through a bromination reaction. The reaction was monitored with TLC until getting one dot in UV-Vis, which means the product has formed without another contaminant compound. the product was yielded in 82% as a white solid with melting point of 145°C. Spectra IR showed that a new peak at 655 cm^{-1} as C-Br vibration (stretch) and 1666 cm^{-1} as conjugated carbonyl vibration. It is indicated that bromine has been substituted for veratraldehyde.

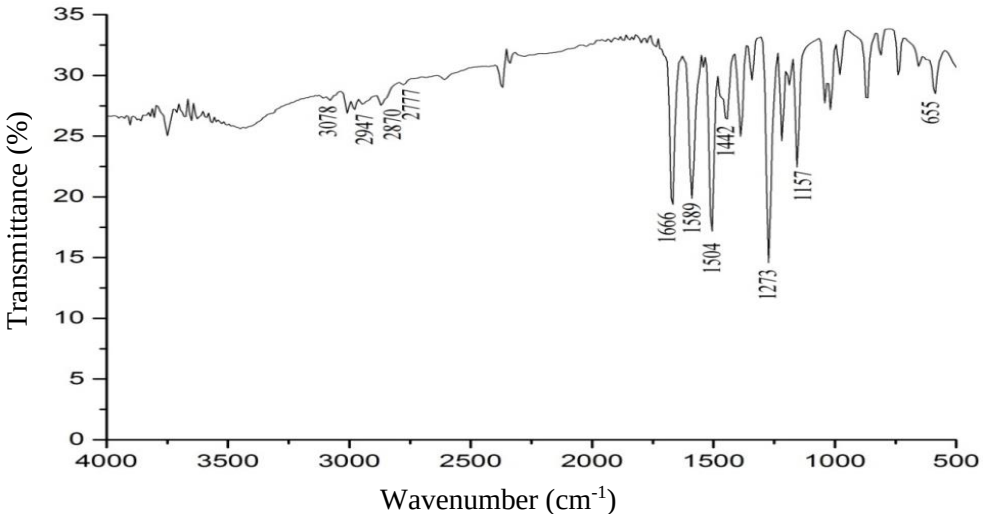

Figure 1. The spectra FTIR of 6-bromoveratraldehyde

The scheme synthesized is presented in figure 2.

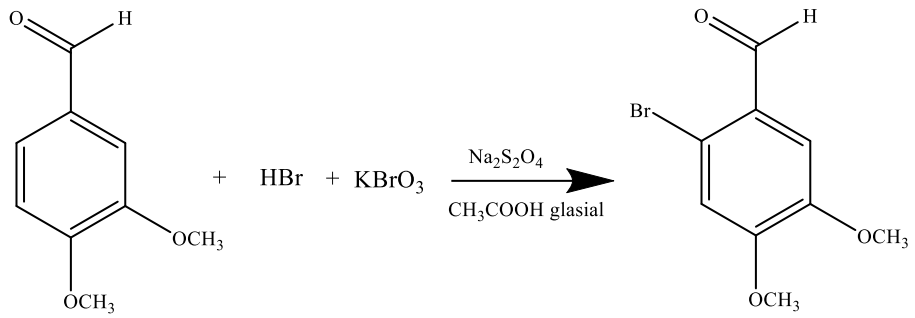

Figure 2. Synthesis 6-bromoveratraldehyde

According to 1H-NMR, the product has 5 protons depending on the target product. Aromatic compounds appeared as singlet peaks at 7.05 and 7.41 ppm, while aldehyde appeared as a singlet peak at 10.18 ppm. Methoxy Protons were detected as a singlet peak at 3.92 and 3.96 ppm. The 1H-NMR spectra are shown in Figure 3.

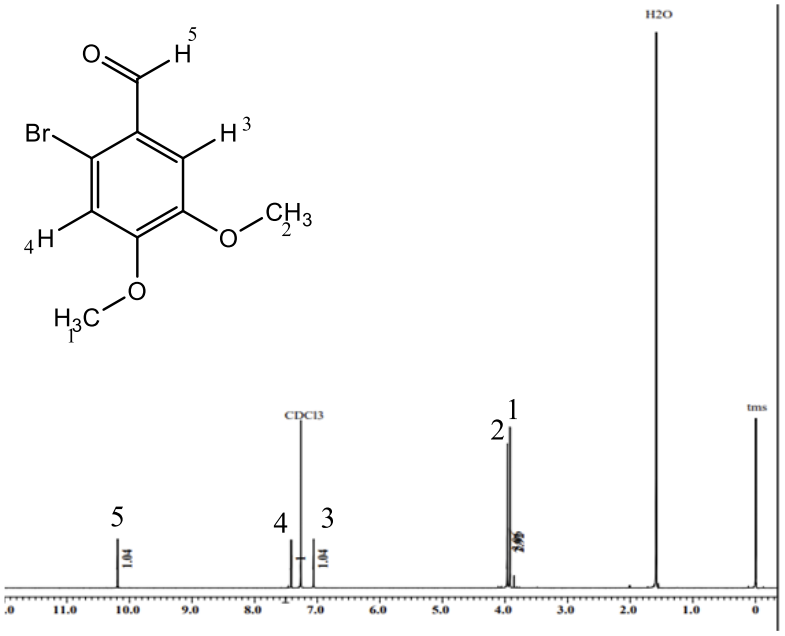

Figure 3. The spectra [1]H-NMR of 6-bromoveratraldehyde

Green synthesis 2'-hydroxy-2-bromo-4,5-dimethoxychalcone

Claisen Schmidt condensation has been used to produce the compound 2'-hydroxy-2-bromo-4,5-dimethoxychalcone by adapting the process from Shivshankar et al., (2016). The Chalcone derivative was synthesized through grinding methods between 6-bromoveratraldehyde and 2-hydroxy acetophenone, and the chalcone derivative's synthetic scheme is presented in Figure 4. The grinding method used in chalcone synthesis is a green chemistry alternative that includes reducing minimum hazards and making simple reaction procedures to design new chalcone synthesis. The grinding method is used at room temperature in a solvent-free environment using only mortar and pestle and giving a short synthesis reaction (Zangade & Mokle, 2011a). The grinding method was efficient (Kumar et al., 2008) compared to the conventional method, which requires a longer reaction time of about 24 hours (Fikroh et al., 2020).

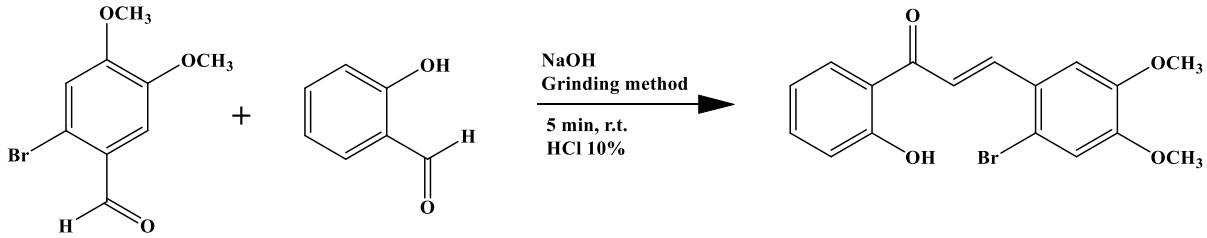

Figure 4. Synthesis of Chalcone by grinding method

The result showed that the chalcone derivative compound was yielded in 53% as a yellow solid with melting point of 169-170 °C. Spectral data of IR, MS, and NMR confirmed the structure of the chalcone. The IR spectra showed a band at 3441 cm^{-1} due to OH stretch and characteristic of conjugated carbonyl (C=O stretch) band near 1635 cm^{-1} (Anwar et al., 2018). The compound also showed a disappearing peak at 2777 cm^{-1} (aldehyde). The new peak shows nearly 972 cm^{-1} due to C-H trans-bending and nearly 663 cm^{-1} due to the C-Br stretch. The identification compound with MS shows that the mass spectrum was equivalent to theoretical molecule massa at m/z 362 and 364 for M^+. The ^{1}H-NMR spectra showed that peaks at 7.45 and 8.41 with J coupling 15.55 Hz due to formation of trans alkene from α, β-unsaturated carbonyl. from ^{13}C-NMR spectra clearly showed the presence of α, β-unsaturated carbonyl (Cα and Cβ) resonating at 118.79 and 142.96 ppm. The result showed that grinding method was simple and highly efficient for synthesis of chalcone under solvent free conditions in high yields. The spectra ^{13}C-NMR was presented in Figure 5.

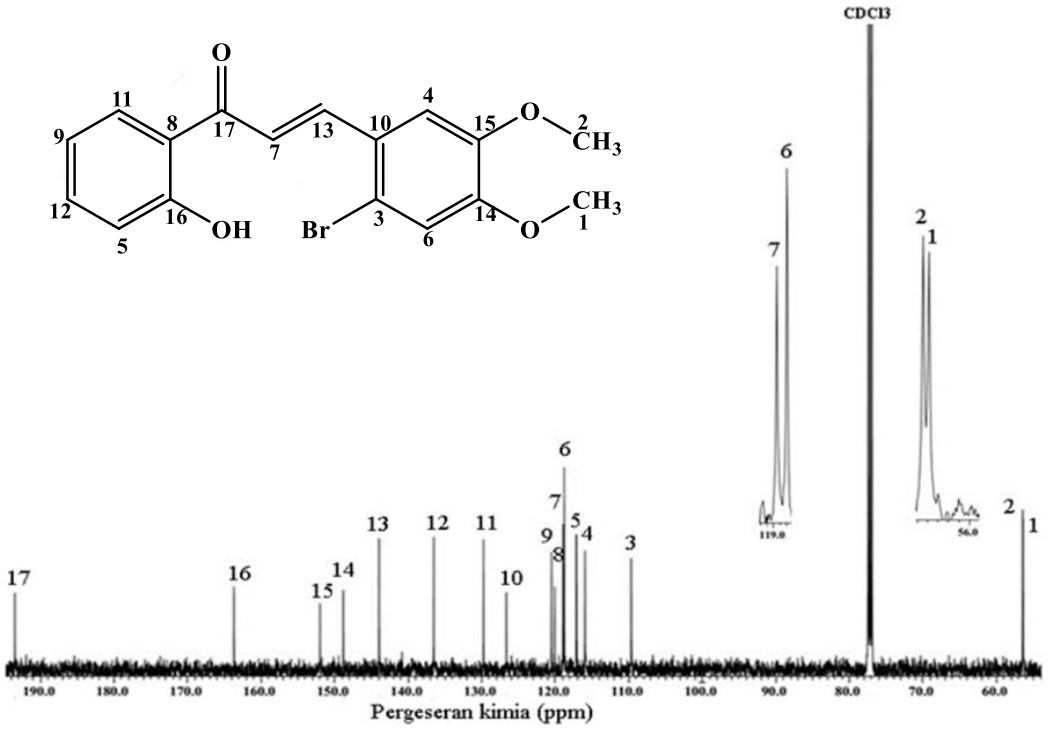

Figure 5. The ^{13}C-NMR spectra of 2'-hydroxy-2-bromo-4,5-dimethoxychalcone

In vitro anticancer activity assay

2'-hydroxy-2-bromo-4,5-dimethoxychalcone was tested as an anticancer agent against cervical cancer (HeLa) cells using an MTT assay. Chalcone has an active compound to inhibit cell growth of cancer cells if the IC_{50} value is less than 20 μg/mL, IC_{50} between 20-100 μg/mL has moderate activity, and IC_{50} more than 100 had not actively inhibited the growth of cancer cells (Tanamatayarat et al., 2003). Based on this research shows that chalcone derivative has moderate activity with IC_{50} 67.23 μg/mL to inhibit cervical cancer (HeLa) Cells line. The results are similar to the research conducted by Suryani et al. (2020), which states that chalcone

with methoxy substituents in ring b and hydroxy in ring A has activity in inhibiting cervical cancer (HeLa) with an IC_{50} value of around 74.24 μg/mL.

Chalcone structure consisting of two aromatic rings with a substituted hydroxyl group on ring A and a methoxy group on ring B acts as an active site in inhibiting cancer cell growth (Venkateswararao et al., 2012). According to research, Mao et al. (2016) demonstrate that the ring B bromo group substituents and the α,β-unsaturated carbonyl of the chalcone actively inhibit cervical cancer cells. Ik-kB, an IKK of the protein-mediated NF-kB activation pathway, attacks the α,β-unsaturated carbonyl of chalcone. Due to the proliferation of cancer cells in humans, the bond between the ik-kB protein and unsaturated carbonyl from chalcone can stop the NF-kB pathway (Yadav et al., 2011). It is assumed that the chalcone's active ingredient, which can be found using an in vitro MTT assay test, can block the NF-kB pathway (Suryani et al., 2020).

The compound's activity is selective because its activity against normal cells (vero) has an IC_{50} of 1170 μg/mL with a high selectivity index of 17.3. The selectivity index is an essential factor in developing new chemotherapeutic agents because it provides a calculation to evaluate the toxicity of the studied compounds to normal cells and predict their therapeutic potential (Ramalho et al., 2013). A high SI value results from the significant difference between cytotoxicity against cancer and normal cells, which means that cancer cells will be killed at a higher rate than normal ones (Krzywik et al., 2020). Masriani et al. (2014) showed that compounds with a selectivity index > 2 are active as anticancer drugs, and selectivity < 2 is considered to have a toxic effect on normal cells. Our research shows that selectivity is more than two, so it can be said to have low cytotoxicity to normal cells. The compound could be considered an anticancer agent due to its IC_{50} and high selectivity index value.

CONCLUSION

The grinding technique successfully synthesized halogen-substituted chalcone through the Claisen-Schmidt condensation reaction. A yellow solid with a yield of 33% and a melting point of 144°C, respectively, is the end product. The compound 2'-hydroxy-2-bromo-4,5-dimethoxychalcone has moderate activity toward cervical cancer (HeLa) cells with an IC_{50} of 67.23 μg/ml and for normal cells (Vero) has IC_{50} of 1170 g/ml. The selectivity index (S1) is approximately 17.3. The compound can be recommended as an anticancer candidate against cervical cancer (HeLa) cell lines based on its IC_{50} and selectivity index (SI) value. This study was limited to only using bromo as halogen substituent. Further research is suggested to other halogen substituent.

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