Inhibitory effects on HepG2 cell proliferation and induction of cell cycle arrest by Chromolaena odorata leaf extract and fractions

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Abstract

Concern about the side effects of liver cancer treatment has driven studies on anticancer to find compounds from plants that can act as chemotherapy. The anticancer activity of Chromolaena odorata against colorectal cancer, lung cancer, leukemia, cervical cancer, breast cancer, and liver cancer has been proven. However, this plant’s mechanism that can inhibit liver cancer cell growth is still undetermined. This study aims to investigate the anticancer activity of C. odorata against HepG2 cells. Extraction of C. odorata leaves was done by maceration method using 80% ethanol and further fractionated. Total flavonoid and major compound of the crude extract were determined by aluminum chloride colorimetric assay and Liquid Chromatography-Mass Spectrometry method. The IC₅₀ and proliferation analysis was performed by MTT assay. Cell cycle was analyzed by using flowcytometry. Total flavonoid of 1.95% and compounds such as 5,7,8,3ʹ,4ʹ-Pentamethoxyflavonone, 1-Carboethoxy-β-carboline, 3-Methylcanthin-2, 6-dion, Canthin-6-one were found in C. odorata. The proliferation of HepG2 was significantly lower after 72 hours of incubation with ½ IC₅₀ of C. odorata fractions. HepG2 cells treated with C. odorata extract and fractions were accumulated in the G0-G1 phase. These results indicated that C. odorata leaves could inhibit the proliferation of HepG2 cells and induce cell cycle arrest.

Keywords

anticancer, C. odorata, HepG2 cells, liver cancer

Introduction

Liver cancer or hepatoma is a malignant and deadly cancer (Wahid et al. 2017). Generally, most liver cancer arises in humans against the background of chronic hepatitis or cirrhosis (Kimura et al. 2013). Most patients undergoing liver cancer therapy suffer from the disease and side effects of the treatment given, either because of radiotherapy or chemotherapy (Palumbo et al. 2013). Furthermore, this cancer is usually diagnosed at an advanced stage, making treatment difficult and even likely to fail (Befeler and di Bisceglie 2002). Therefore, the search for new compounds that can control the development of liver cancer becomes essential to reduce mortality from the disease (Zhang et al. 2013).

The main characteristic of cancer cells is the ability to trigger proliferation quickly, blocking the signals that stop their proliferation and induction of their progression at certain stages of the cell cycle pathway (Bailon-Moscoso...
et al. 2017). The ability to induce apoptosis, inhibit cancer cell proliferation, regulate its progression to cancer cells, and inhibit proliferation markers is vital in determining the success of anticancer therapy (Ren et al. 2017). Lately, anticancer research is more directed to compounds derived from plants for therapeutic intervention in various types of cancer (Mukherjee et al. 2001). This is possible because compounds derived from plants have a variety of chemical structures, which are thought to be better pharmacologically than synthetic compounds (Van Wyk and Wink 2018). Extracts, fractions, and pure compounds from natural ingredients believed and proven effective in treating liver cancer are often used, including in prevention efforts (Stagos et al. 2012).

Natural compounds play an important role in discovering new drugs for various therapeutic indications, including cancer therapy (Nobili et al. 2009). The failure of treatment with some anticancer has triggered sufferers or their families to look for alternative medicines from natural compounds (Bruno and Njar 2007). Compounds of plant origin are believed to have no side effects, inexpensive, and used for generations (Pal and Shukla 2003). Chromolaena odorata L (C. odorata) was one of the plants that have been previously studied as an anticancer against colorectal cancer (HT-29) (Adedapo et al. 2016), lung cancer (LLC), and leukemia (HL-60) (Hung et al. 2011), cervical cancer (HeLa) (Nath et al. 2015), breast cancer (Yusuf et al. 2020, 2021), and liver cancer (Prabhu 2012). Therefore, this study aims to determine the proliferation and cell cycle analysis of Chromolaena odorata L (C. odorata) extract and fractions against HepG2 cancer cells lines.

Materials and methods

Plant and chemicals material

Fresh leaves of Chromolaena odorata L was collected from Samahani in Aceh Besar Regency, Aceh Province, Indonesia. This plant was identified by the expert from the Biology Department, Faculty of Mathematics and Natural Sciences University of Syiah Kuala Banda Aceh with reference number of B/435/UN11.1.8.4/TA.00.01/2020 and the specimen was kept in herbarium.

Preparation of extract and fractions of C. odorata leaves

Five kg of dry leaf powder C. odorata was macerated by soaking it in 80% ethanol with a ratio of the leaf powder to ethanol 1:3. This mixture was stirred regularly, and after 24 hours, it was filtered. This process was repeated three times (3 × 24 hours), and all the filtrate obtained was collected and evaporated using a rotating vacuum evaporator. The remaining ethanol in the crude ethanolic extract of C. odorata leaves (CECO) was evaporated in the oven at a temperature of 50 °C until a constant weight was obtained (yielded 110.15 gr of CECO).

About 50 gr of CECO was then dissolved in ethanol and then put into a separating funnel and partitioned through regular shaking using a non-polar solvent n-hexane. Then the n-hexane layer was separated, and this process was repeated until the n-hexane layer was colorless. Furthermore, the residue was processed similarly using semipolar solvent ethyl acetate and ethanol as a polar solvent. The n-hexane (HECO), ethyl acetate (ETACO), and ethanol (ECO) fractions obtained were evaporated and weighed, which resulted in 10.50 gr, 30.90 gr, and 25.10 gr, respectively.

Determination of total flavonoids in crude ethanolic extract of C. odorata leaves

The total flavonoid in CECO was determined by the aluminum chloride colorimetric assay method which was adapted from Chatatikun et al. 2013 (Chatatikun and Chiabchalard 2013), with slight modification in ethanol used (96% of ethanol was used). A total of 50 μL of extract (1 mg/mL) or standard solution was added to 10 μL of 10% of the aluminum chloride solution followed by 150 μL of 96% ethanol. Then 10 μL of 1M sodium acetate was added to the mixture in a 96 well plate. 96% ethanol was used as a reagent blank. All reagents were mixed and incubated for 40 min at room temperature and protected from light. The absorbance was measured at 415 nm with a microplate Elisa reader. All tests were performed in triplicate.

Liquid Chromatography-Mass Spectrometry (LC-MS) of the crude ethanolic extract of C. odorata leaves

LC-MS was used to identify the major compounds in the crude ethanolic extract of C. odorata leaves. Chromatographic analysis of extract and fractions was carried out by reverse phase elution (LC:18 column 250 × 4.6 mm, 5 μm) on Agilent 6500 Series Accurate-Mass Quadrupole Time-of-Flight (Q-TOF; Agilent Santa Clara, CA, USA) which was detected by using LC/MS system with Agilent 1200 Series Diode Array Detector. The mobile phase consisted of (A) formic acid / WA (0.1%, v/v); (B) acetonitrile + 0.1% formic acid; gradient (in solvent B), flow rate: 0.2 ml/min; injection volume 3 L; ESI parameters: both negative and positive ion mode; mass range 50–1200 m/z; spray voltage 4 kV; gas temperature 325 °C; gas flow 10 L/min; Nebulizer 40 psi and the mass was analyzed by using Agilent technologies Mass-Hunter software.

The IC_{50} of C. odorata extract and fractions

The IC_{50} of C. odorata extract and fractions was determined by using the MTT colorimetry method. HepG2 and Vero cells with a density of 10,000 cells/well were put into a 96 well microplate, then incubated for 24 hours in a 5% CO_{2} incubator at 37 °C. After 24 hours, cells were treated with extract and fractions of Chromolaena odorata at
concentrations of 7.8, 15.63, 31.25, 125, 250, and 500 μg/mL, respectively. The partitions treatment was incubated for 24 hours in a 5% CO₂ incubator at 37 °C. Then, 100 μL culture media with 10 μL MTT 5 mg/mL was added to each well and then incubated for 4 hours at a 5% CO₂ incubator at 37 °C. Intact cells will react with MTT to form purple formazan crystals. Crystals were dissolved with the addition of a reagent stopper (SDS 10% in 0.01N HCL), and left aside in a dark place overnight. The optical density was then read by using an ELISA reader on a wavelength of 595 nm and converted into a percentage of living cells by using the formula below:

\[
\% \text{Viable cells} = \frac{(\text{Control of normal cells} - \text{Control of media}) - (\text{Treatment cells} - \text{Control of media})}{(\text{Control of normal cells} - \text{Control of media})}
\]

The IC₅₀ of HepG2 was determined using non-linear regression analysis and defined as a concentration of each extract and fractions that cause a 50% decrease of viable cells. Meanwhile, viability of Vero cells was calculated using linear regression analysis. We also calculated the selectivity index using the following formula:

\[
\text{Selectivity index (SI)} = \frac{\text{IC}_{50} \text{ of Vero cells}}{\text{IC}_{50} \text{ of Cancel cells}}
\]

The proliferation analysis of HepG2 cells after C. odorata extract and fractions treatment

The proliferation test used CECO, HECO, ETACO, and ECO with a concentration equal to the IC₅₀ value. After 24, 48, and 72 hours, 10 μL of MTT reagent (5 mg/mL) was added to each well and again incubated at 37 °C for four hours. Then the purple crystals of formazan formed were dissolved by adding 100 μL of 10% SDS and incubation was continued at room temperature overnight. The absorbance value was read at a wavelength of 595 nm with an ELISA reader. The result was presented as the percentage of living cells.

Cell cycle analysis of HepG2 cells after C. odorata extract and fractions treatment

HepG2 cell lines (1 × 10⁶ cells/well) were seeded into a 6-well plate and incubated for 24 hours to synchronize them in the G2/M phase. After that, the cells were treated with IC₅₀ of CECO, HECO, ETACO, and ECO and incubated for 24 h. Both floating and adherent cells were collected in a conical tube with trypsin 0.025%. The cells were washed three times with cold PBS and centrifuged at 2500 rpm for 5 min. The supernatant was separated, while the sediment was collected and fixed in cold 70% ethanol in PBS at 4 °C for 1 h. The cells were washed 3 times with cold PBS and resuspended, then centrifuged at 3000 rpm for 3 min, and PI kit (containing PI 40 μg/mL and RNAse 100 μg/mL) was added to the sediment and resuspended and incubated at 37 °C for 30 min. The samples were analyzed using FACS Calibur, Becton Dickinson, CA, USA and the percentage of cells in each phase of the cell cycle was evaluated using the ModFit LT 3.0 (Nurrochmad et al. 2011).

Statistical analysis

The statistical evaluations were performed using the Graph Pad Prism v.8.02 software (San Diego, CA, USA). The results are presented as the mean ± standard deviation (SD). Two-way analysis of variance (ANOVA) determined the differences among multiple groups with p < 0.05 was considered statistically significant.

Results and discussions

Flavonoids in crude ethanolic extract of C. odorata leaves

Maceration of dry powder of C.odorata leaves was carried out using 80% ethanol, followed by liquid-liquid fractionation of the crude extract to obtain a flavonoid-rich fraction with various solvent polarities. The total flavonoid content in CECO was 1.95%.

LC-MS analysis of crude ethanolic extract of C. odorata leaves

The results LCMS assay on the crude ethanolic extract of C. odorata was presented in Table 1.

Our findings showed that the CECO contained alkaloids and flavonoids. The flavonoid in CECO is 5,7,8,3',4'-Pentamethoxyflavonone, and the alkaloids 1-Carboethoxy-β-carboline, 3-Methylcanthin-2,6-dione, Canthin-6-one. This flavonoid (5,7,8,3',4'-Pentamethoxyflavonone) contains an aromatic ring and belongs to the group of methoxylated flavonones. Several studies have shown that polymethoxylated flavonones have anticancer activity through increased antiproliferative activity in

| No | Component name              | Observed m/z | Neutral mass (Da) | Observed RT (min) | Adduct | Formula       |
|----|------------------------------|--------------|-------------------|-------------------|--------|---------------|
| 1  | 1-Carboethoxy-β-carboline   | 241.0969     | 240.0899          | 4.31              | +H     | C14H12N2O2    |
| 2  | 3-Methylcanthin-2,6-dione   | 251.0814     | 250.0742          | 6.88              | +H     | C13H10N2O2    |
| 3  | 5,7,8,3',4'-Pentamethoxyflavonone | 375.1439 | 374.1366          | 4.07              | +H     | C20H22O7      |
| 4  | Canthin-6-one               | 221.0708     | 220.0637          | 6.76              | +H     | C14H8N2O      |
colorectal cancer cells (Pereira et al. 2019). On the other hand, the potency of the alkaloids 1-Carboethoxy-β-carboline as anticancer was also reported by Xu et al. 2016, where the compound selectively suppresses the proliferation of hepatocellular carcinoma cells (SMMC-7721 and Hep G2) (Xu et al. 2016).

**The IC\textsubscript{50} of *C. odorata* extract and fractions**

We found that the CECO was more potent as an anticancer against HepG2 cells with an IC\textsubscript{50} value of 23.44 g/mL than the HECO, ETACO, and ECO (IC\textsubscript{50}= 84.52 μg/mL; 167.49 μg/mL; and 88.51 μg/mL, respectively). The IC\textsubscript{50} for Vero cells after treatments with CECO, HECO, ETACO and ECO were 954.99 μg/mL, 3265.87 μg/mL, 2951.20 μg/mL, and 4365.15 μg/mL, respectively. These results were then used to calculate the selectivity index of each extract and fractions as follows; CECO (40.74), HECO (38.64), ETACO (17.62) and ECO (49.31). A potential drug can be further analysed if the SI value ≥10 (Peña-Morán et al. 2016). Another criteria of prospective anticancer drug was proposed by Weerapreeyakul et al. by using a lower SI value (≥3) (Weerapreeyakul et al. 2012). The ideal drug should not affect the normal cell while killing the cancer cells (Lopez-Lazaro 2015).

**The proliferation of HepG2 cells after *C. odorata* extract and fractions treatment**

The better way to treat complex diseases such as cancer is to aim for several targets at once. The search for potential natural compounds that can control the growth and progression of cancer cells with low toxicity in normal healthy cells has become an important requirement in cancer treatment (Newman and Cragg 2016). All plant extract or fractions from plant parts have been used as medicine, including isolates. Various alkaloids and flavonoids from medicinal plants have shown antiproliferative and anticancer effects on numerous types of cancer, both in vitro and in vivo (Mondal et al. 2019).

The antiproliferative activity of *C. odorata* leaves extract and fractions against HepG2 cells was presented in Fig. 1. Viable cells of HepG2 were significantly lower after 72 hours incubation with ½ IC\textsubscript{50} of ECO, ETACO and HECO (56.7±1.84%, 58.4±1.78%, and 55.2±3.19%, respectively) compared to HepG2 cells after 24 hours incubation with the same concentration (96.5±4.5%, 98.3±2.6% and 95.8±2.46, respectively), p-value < 0.0001 in all comparisons. The most significant decrease in viable cells was observed after 72 hours incubation with ½ IC\textsubscript{50} of ECO (40.6%), while the smallest decrease was observed after 72 hours incubation with ½ IC\textsubscript{50} CECO (21.7%). Significantly lower viable cells after 24 hours incubation with a concentration of ½ IC\textsubscript{50} were observed after treatment with CECO (76.9±4.05%) than the other fractions with p-value < 0.0001. However, no significant differences were observed among ½ IC\textsubscript{50} ECO, HECO, and ETACO when compared to each other after 24, 48, or 72 hours.

Significant increases of viable cells were observed after 48 hours treatment with IC\textsubscript{50} of all *C. odorata* extract and fractions compared to 24 hours incubation, with ETACO as the highest (51.7±2.35% to 64.30±2.88%), p-value < 0.05 in all comparisons. Then, after 72 hours of treatment with IC\textsubscript{50} of ECO, ETACO, HECO, and CECO, all viable cells were significantly decreased than 48 hours, with ETACO having the sharpest decline (64.3±2.26% to 28.6±2.26%, p-value < 0.0001).

Treatment with 2×IC\textsubscript{50} of all fractions (HECO, ECO and ETACO) resulted in a significant rise in viable cells after 48 hours incubation, than 24 hours incubation, p-value < 0.0001. The highest increase was after treatment with 2×IC\textsubscript{50} of ECO, where 28.2±3.5% HepG2 viable cells

![Figure 1. The proliferation analysis of HepG2 cells after 24, 48 and 72 hours incubation with ½ IC\textsubscript{50} and 2×IC\textsubscript{50} of extract and fractions of *Chromolaena odorata* leaves.](image-url)
(24 hours) increased to 47.3±6.5% (48 hours). But then, HepG2 intact cells gradually declined below 30% after 72 hours treatment with 2×IC_{50} of all C. odorata extract and fractions compared to 48 hours, p-value < 0.0001, although no significant difference observed if we compared it to 24 hours incubation.

**Cell cycle profile of HepG2 after administration of crude ethanol extract and leaf fraction of *Chromolaena odorata* for 24 hours**

The cell cycle is a cell reproduction process that mediates the growth and development of living things, both normal cells and cancer cells (Yang et al. 2014). It is known that cell cycle arrest and stimulation of apoptosis are essential keys for cancer therapy, primarily to determine the mechanism of cell death and cancer cell cycle progression after administration of the tested drug (Pistritto et al. 2016; Otto and Sicinski 2017).

Fig. 2 shows the distribution of cell cycles of HepG2 treated with extract and fractions of *C. odorata* leaves at IC_{50} concentrations for 24 hours. HepG2 cells treated with CECO, HECO, ETACO, and ECO mainly were accumulated in the G0-G1 phase with results as follows; CECO (49.9±0.44%), HECO (47.9±1.02%), ETACO (46.1±0.78%), and ECO (47.05±0.15%). These results were significantly lower than control (62.3±0.24%, p-value < 0.05 in all comparisons). On S phase, all HepG2 cells treated with CECO, HECO, ETACO, and ECO (8±0.5, 7.75±0.57, 7.44±0.73, and 7.91±0.46, respectively) were significantly lower than control (35.14±1.4) with p-value < 0.05 in all comparisons. However, the cells treated with ethanol partitions of *Chromolaena odorata* leaves on the G2/M phase were significantly higher than control cells (1.97±0.02) with p < 0.05 in all comparisons.

The accumulation of HepG2 cells in the G0-G1 phase of the cell cycle shows the cells experience inhibition in preparing the DNA material to be synthesized, thereby disrupting the proliferation of HepG2. Termination of the cell cycle in the G0-G1 phase provides an opportunity for cells to repair damaged DNA and provides an opportunity for damaged cells to be recognized and then proceed to the process of apoptosis (Doan et al. 2019). Errors in the checkpoint will allow cells to reproduce even if there is DNA damage or incomplete replication or chromosomes that are not completely separated to result in genetic damage. Genetic damage is an important thing related to the emergence of cancer; therefore, the cell cycle regulation process can play a role in cancer prevention (Meeran and Katiyar 2008). The accumulation of cells in the G0-G1 phase means the progression of HepG2 proliferation was prevented. Growth factors usually regulate cell proliferation progression through the restriction point in late G1. However, cells cannot pass the restriction point without these growth factors and frequently get into the resting state /G0 (Zheng et al. 2019).

The SubG1 phase is a phase where the DNA content is in a hypodiploid state, and the G2/M phase, as complete replication, has formed the 4n set of chromosomes. In this phase, the percentage of the cell population increases after being given the tested drug. It is estimated that an increase in the SubG1 phase and G2/M phase will occur to prevent uncontrolled division ((Choi et al. 2009; Haneef et al. 2012). The increased inhibition of the S phase means that the extracts or fractions prevent DNA synthesis of HepG2.

The increase in the percentage of cell accumulation in the

![Figure 2](image-url). The effect of crude ethanolic extract (CECO), n-hexane (HECO), ethyl acetate (ETACO), and ethanol fractions of *Chromolaena odorata* L leaves on the HepG2 cell cycle distribution.
G2/M phase compared to the control indicates DNA repair (Haneef et al. 2012).

The cell cycle is the process of chromosomal duplication followed by cell division (Cooper and Hausman 2007). The cell cycle consists of four phases, namely the G1 gap phase, the S phase (DNA synthesis phase), the G2 gap phase, and the M phase (nuclear and cell division phase) (Tan et al. 2017). In addition, there is another phase, namely the G0 phase. In this phase, the cell is at rest and does not divide. Cells in the G0 phase can rest for a long time or even permanently (Alberts et al. 2002).

Cyclin-dependent kinases (CDKs) regulate and tightly control the cell cycle process. The cell cycle regulation includes induction by mitogenic signals and inhibition by activation of cell cycle checkpoints in response to DNA damage (Otto and Sicinski 2017). In an adult animal, most cells are stable cells and reside in the G0 (gap) cell cycle phase. When division is necessary, cells capable of doing so enter the G1 phase of the cell cycle. In most cells, the DNA in the nucleus is replicated during only a limited portion of the cell cycle called the S (synthesis) phase. After the S phase, the cells enter a second gap phase called the G2 phase. Finally, the M (mitosis) phase, the contents of the nucleus condense to form visible chromosomes, which through an elaborately orchestrated series of movements, are pulled apart into two equal sets. The cell itself then splits into two daughter cells (Yang et al. 2014).

The activation of a cyclin-dependent kinase (CDK) associated with its subunit regulatory protein (cyclin) and via phosphorylation by CDK-activated kinase (CAK) affects each phase of the cell cycle (Jhaveri et al. 2021). Several cyclins such as cyclins A, B, C, D, E will be expressed periodically at a specific cell cycle phase. Cyclin D has a central role because its expression is regulated by growth factors and the cyclin D-cyclin complex. This CDK4 will phosphorylate the retinoblastoma protein (pRB). Phosphorylation of pRB results in the release of E2F transcriptional actor which will mediate the transcription of several genes encoding proteins that determine the continuity of the cell cycle (Singh et al. 2021). This indicates that cyclin D is a starter of the cell cycle. The cessation of the cell cycle can occur due to the presence of CDK inhibitors (CDKI), including the INK4 family (inhibitors of CDK4) (Ivanchuk and Rutka 2006). INK4 protein, especially p16INK4, will compete with cyclin D for binding to CDK4/6, thereby preventing pRB phosphorylation. The Rb-cyclin D-CDK4-p16 pathway is the main pathway that controls cell growth (Goel et al. 2018). In addition, the transcription factor p53 also plays a role in cell cycle arrest. Generally, the increase in p53 expression is triggered by various cellular stresses that will induce the expression of p21, which is a strong inactivator of the cyclin-CDK complex (Chen 2016). The p21 protein is a tumor suppressor protein that regulates cell cycle progression. Cell cycle progression will be achieved by blocking CDK binding to cyclins (Abbas and Dutta 2009). This protein is known to be able to block the binding between CDK2 and cyclin E. Besides, it is also able to bind to CDK2, CDK3, CDK4, and CDK6. The CDK and p21 bonds are strengthened by cyclins corresponding to these CDKs (Abukheir and Park 2008). The preference of p21 to bind to CDK associated with the G1/S phase transition makes it an important role as a checkpoint for cell cycle progression in preventing replication and degradation of damaged DNA (Satyanarayana et al. 2008).

Flavonoids in extract and fractions of *C. odorata* or *E. odoratum* leaves have a methoxyflavones group, playing an important role in various pharmacological activities, including antiproliferative and anticancer. In Artemisia species, Eupatin (5,7-dihydroxy-3′,4′,6-trimethoxyflavone) has antioxidant, anti-inflammatory, anti-allergic, and neuroprotective activities (Du et al. 2017). Eupatorin (3′,5-dihydroxy-4′,6,7-trimethoxyflavone) is a potent antiproliferative in prostate cancer (Salmela et al. 2012) antiproliferative and cytostatic effects on MDA-MB-468 human breast cancer cells due to CYP1-mediated metabolism (Androutsopoulos et al. 2008). The outcome of this study proves that the extract and fractions of *C. odorata* leaves might be responsible for the anticancer and antiproliferative effect of HepG2, and CECO is the most specifically active because it contains the methoxyflavones, as mentioned above. Testing the influence of extract and fractions of *C. odorata* leaves against the proliferation of HepG2 could highlight the responsible compounds for this effect. While the non-polar fraction, rich in methoxylated flavones, had a moderate cytotoxic effect after 24 hours of exposure on most of the cell lines tested. So further analytical studies are needed to identify the key responsible compounds and the biochemical pathways for their action.

**Conclusions**

These results indicated that the compounds contained in the extract and leaf fraction of *C. odorata* could inhibit the proliferation of HepG2 cells and induce cell cycle arrest in the G0/G1 phase. Both polar and non-polar compounds exert these effects, but further analytical studies are needed to identify the responsible compounds and the biochemical pathways for their action. In other words, these results suggested that CECO, HECO, ETACO, and ECO fractions could inhibit the proliferation of HepG2 cells by arresting in the G0/G1 and G2/M phase.

Although the biochemical mechanism involved in the above activity is not known with certainty whether, from pure flavonoids or alkaloids, it is important to note that one of the flavonoid compounds contained in *C. odorata* leaves (5,7,8,3′,4′-pentamethoxyflavonone) is a new type of flavonone that was first discovered in this plant. More detailed research on this flavonone compound on HepG2 cells is warranted and still ongoing both *in vitro* and *in vivo* in our research group.
Conflict of interest

The authors declared that there was no conflict of interest regarding the publication of this paper.

Author contributions

Conceptualization, HY; Methodology, HY; MF, CM; Software, MF, RDM; Validation, HY, MF, CM; Formal Analysis, MF, RDM; Investigation, HY, MF; Resources, HY; Data Curation, HY, MF, RDM; Writing – Original Draft Preparation, HY, MF, CM; Writing – Review & Editing, HY, MF, CM; Visualization, MF; Supervision, HY.

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