Evaluating antioxidant and anti-inflammatory activities of *Dracaena cambodiana* Pierre ex Gagne extracts on RAW 264.7 and SW 1353 cell lines

Huynh Cong Duy[1], Nguyen Trung Quynh Nhu[1], Mai Kieu Tien[1], Nguyen Phuc Thinh[1], Nguyen Thi Le Thuy[1], Le Thi Truc Linh[2], Tran Thi Van Anh[3], Pham Thi Kim Tram[1*]

[1]Division of Medical Biotechnology, Biotechnology Center of Ho Chi Minh City, Highway 1, Quarter 2, Trung My Tay Ward, District 12, Ho Chi Minh City, Vietnam
[2]Faculty of Biotechnology, Ho Chi Minh City Open University, 35-37 Ho Hao Hon Street, Co Giang Ward, District 1, Ho Chi Minh City, Vietnam
[3]Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, 41 Dinh Tien Hoang Street, Ben Nghe Ward, District 1, Ho Chi Minh City, Vietnam

Received 26 October 2023; revised 27 November 2023; accepted 18 January 2024

Abstract:

***Dracaenae cambodianae* Pierre ex Gagnep (Huyết Giác in Vietnamese) is widely utilised in traditional remedies to alleviate joint pain. Indeed, *D. cambodianae* contains chemical components with biological properties such as cytotoxicity, antimicrobial, and antioxidant activities. This research aims to determine the antioxidant and anti-inflammatory properties of *D. cambodianae* extract in RAW 264.7 and SW 1353 cell lines. The *D. cambodianae* was extracted with two solvents: dichloromethane (HGA) and methanol (HGB) and subsequently assessed for the total polyphenols and flavonoids content before evaluating its antioxidant and anti-inflammatory properties in RAW 264.7 and SW 1353 cell lines. The extract with HGB exhibited 1.73 times higher total polyphenol content than HGA. The antioxidant capacity (EC_{50}) of HGA was lower than that of HGB. HGA displayed significant cytotoxicity towards the cells, while HGB did not induce cell death. HGB effectively inhibited nitric oxide production, LPS-induced *iNOS*, and IL-1β-stimulated *MMP13* expression. These findings demonstrate that HBG derived from *D. cambodianae* Pierre ex Gagnep possesses antioxidant and anti-inflammatory properties, offering potential applications in the treatment of inflammation and osteoarthritis in the future.**

Keywords: **anti-inflammatory, antioxidant, *Dracaenae cambodianae,* osteoarthritis, RAW 264.7, SW 1353.**

Classification numbers: **3.3, 3.5, 3.6**

1. Introduction

Inflammation is defined as a biological response of the immune system to harmful stimuli such as irritants, pathogens, or damaged cells. Its progression involves the participation of immune cells, blood vessels, and molecular mediators. The primary purpose of the inflammatory response is to eliminate the original cause of cell injury, remove necrotic cells and damaged tissues, and initiate tissue repair.

Arthritis, particularly osteoarthritis, is characterised by the destruction of articular cartilage and local inflammation. It is one of the diseases that significantly impact our health and quality of life, especially in the elderly population. Statistics reveal that 61.1% of individuals over 60 years old are affected, whereas the range is 8-30% among those aged 40-59 [1]. However, there is currently no definitive treatment for these diseases, only several intervention methods to mitigate disease progression, including the application of traditional remedies. Given Vietnam's rich and diverse reservoir of medicinal herbs with potential therapeutic properties, we were determined to conduct research to identify their chemical composition and mechanism of action. Identifying the chemical components and biological activities of these herbs holds practical value for human health.

D. cambodiana Pierre ex Gagnep belongs to the genus Dracaena, which comprises more than 50 species, primarily distributed in tropical and subtropical Asia and Africa. In Vietnam, there are approximately 12 species [2]. According to Oriental medicine, *D. cambodiana* is documented to have effects on sprains, joint pain, and anticoagulation [2, 3]. Currently, several compounds found in *D. cambodiana* extract have exhibited antifungal activity against *Aspergillus niger* and cytotoxic activity against HepG2 at an IC_{50} dose of 2 µg/ml [4]. Of particular interest is the ethyl acetate fraction of *D. cambodiana* extract, which displays the highest antioxidant activity [5]. Three new flavonoid derivatives have demonstrated cytotoxicity against the K562 cell line and resistance to methicillin-resistant *Staphylococcus aureus* (MRSA) [6]. Moreover, the 96% ethanol extract of the *D. cambodiana* plant did not exhibit acute oral toxicity [7]. The identification of the chemical composition, antibacterial, antifungal, and cytotoxic activities of *D.*

*Corresponding author: Email: phamthikimtram@gmail.com

cambodiana extract underscores its potential for treating various diseases. In this study, we further investigate the *in vitro* antioxidative, anti-inflammatory activity, and the inhibition of joint degeneration of the *D. cambodiana* plant extract.

2. Materials and methods

Extract and cell lines: The extracts of *D. cambodiana* Pierre ex Gagnep were provided by the Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City. The redwood part of the tree was collectively extracted using HGA and HGB. The crude extract of each sample was weighed and then dissolved in 100% dimethyl sulfoxide (DMSO) (final concentration was 10 mg/ml) and stored at -20°C for further experiments. RAW 264.7 mouse macrophage cell line (ATCC TIB-71) and human SW 1353 chondrocytes cell line (ATCC HTB-94).

Equipment: Biological safety cabinet class II (ESCO), CO_2 incubator (Panasonic Healthcare), inverted microscope (Carl Zeiss), 4-decimal balance (Mettler Toledo), microplate reader VersaMax (Molecular Devices), cooling micro-centrifuge (Eppendorf), Realtime Lightcycler 480 (Roche), PCR cabinet (Biosan), vortexer (Cleaver Scientific).

Chemicals and reagents: Folin-Ciocalteu (Merck), gallic acid (Sigma), $AlCl_3$ (Merck), quercetin (Sigma), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Merck), 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) (Biobasic), Dulbecco's Modified Eagle Medium (DMEM) high glucose (GE), heat-inactivated fetal bovine serum (FBS) (Sigma), Lipopolysaccharide (LPS) (Sigma), DMSO (Merck), Trizol (Thermo), RevertAid First Strand cDNA Synthesis Kit (Thermo), Maxima SYBR Green qPCR Master Mix (Thermo), and other chemicals.

Quantification of total polyphenols in the extract: The total polyphenolic content of the extract was analysed using the Folin-Ciocalteu method as follows: 10 µl of the extract was mixed with 50 µl of Folin-Ciocalteu reagent. Then, 40 µl Na_2CO_3 (10% w/v) was added, and the reaction mixture was incubated in the dark at room temperature for 2 hours on a shaker. The absorbance of the sample was measured at 760 nm using a spectrophotometer. The total polyphenolic content was determined based on the gallic acid standard curve.

Quantification of total flavonoid in the extract: Total flavonoid content was determined using the $AlCl_3$ colorimetric method as follows: 50 µl of the extract was mixed in a reaction mixture consisting of 25 µl $AlCl_3$ 2%, 25 µl H_2O, 25 µl HCl 1M, and 25 µl CH_3COONH_4 1M. The reaction mixture was incubated in the dark at room temperature on a shaker for 15 min. Absorbance of the sample was measured at 425 nm using a spectrophotometer. The total flavonoid content was determined based on the quercetin standard curve.

Antioxidative activity evaluation by DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity assay: 100 µl of 0.3 mM DPPH radical solution was added to 100 µl of the extract. The reaction mixture was incubated for 30 min. The absorbance was then measured at 517 nm using a spectrophotometer. The radical scavenging activity of the extract was calculated using the following equation:

$$\%\,SC = \left(1 - \frac{As - Ab}{Ac}\right) x100\%$$

where A_c is the absorbance at 517 nm of DPPH, A_b is the absorbance at 517 nm of the negative control sample, and A_s is the absorbance at 517 nm that contains the test sample.

Evaluation of cytotoxicity effect of the extract on RAW 264.7 cells by MTT assay: RAW 264.7 cells were inoculated at a density of 2×10^4 cells/well in DMEM high glucose medium supplemented with 10% FBS, incubated for 24 hours at 37°C, 5% CO_2 on a 96-well plate. Then, cells were treated with extracts at concentrations of 10, 20, and 50 µg/ml (the extract was diluted in DMEM high glucose medium supplemented with 10% FBS). The treated cell plate was incubated for 24 hours at 37°C and 5% CO_2. Then, 10 µl of MTT solution (5 mg/ml) was added to all wells, followed by incubation for 3 hours. After treatment with MTT, the old medium was removed, and 100 µl of DMSO was added to each well. The plate was incubated in the dark at room temperature for 10 min to dissolve the formazan crystals. Finally, the percentage of surviving cells was determined by comparing the absorbance at 540 nm of the test well (with extract treatment) with the absorbance value of the negative control (not treated with extract).

Evaluation of the ability to inhibit nitric oxide secretion induced by Lipopolysaccharide (LPS) on RAW 264.7 model: RAW 264.7 cells were prepared similarly to the MTT assay. Then, cells were treated with extracts at concentrations of 10 and 20 µg/ml. Next, LPS (diluted in PBS at pH 7.4) was added to all the wells at a final concentration of 1 µg/ml after 1 hour of treatment with the extract. A negative control (cells not treated with extract and LPS), a positive control (treated with dexamethasone and LPS), and a control treated with LPS only (no extract treatment) were also included. After 24 hours, cultures were collected to measure nitric oxide (NO) secretion using the Griess reagent. Briefly, 100 µl of cell culture fluid was mixed with the corresponding volume of the Griess reagent. The reaction mixture was incubated for 10 min at room temperature, and the absorbance was then measured at 540 nm. The experiment was repeated three times. The procedure was adapted from the study of G. Yang, et al. (2012) [8].

Evaluation of the expression of pro-inflammatory genes iNOS induced by LPS on RAW 264.7 model: Cells were grown on 6-well plates at a density of 8×10^5 cells/well. After overnight proliferation, cells were treated with the extract at different concentrations and incubated at 37°C, 5% CO_2. 1 hour after treatment with the extract, LPS was added to the wells at a final concentration of 1 µg/ml to induce inflammation. A negative control (cells not treated with extract and LPS), a positive control (treated with dexamethasone and LPS), and a control treated with LPS only (no extract treatment) were also included. Total RNA from cells was isolated after 24 hours of treatment to evaluate the expression of inflammatory genes. Total RNA from cells was obtained using the Trizol kit according to the manufacturer's instructions. NanoDrop was used to quantify RNA concentration and purity by spectrophotometry. RNA after extraction was synthesised into cDNA using the RevertAid First Strand cDNA synthesis kit according to the instructions. The expression of *iNOS* genes was evaluated by real-time PCR reaction with SYBR Green fluorescence, and the internal control was the GAPDH gene. The specific primer sequences and thermal cycles of the real-time PCR reaction were referenced from S. Bose, et al. (2013) [9].

Evaluation of cytotoxicity effect of the extract on SW 1353 cells by MTT assay: Experiments were arranged similarly to the toxicity test of the extract on RAW 264.7 cells. SW 1353 cells were seeded at a density of 5×10^3 cells/well in DMEM high glucose medium supplemented with 10% FBS, incubated for 24 hours at 37°C, and 5% CO_2 on a 96-well plate. Then, cells were treated with extracts at concentrations of 50, 20, and 10 µg/ml (extracts were diluted in DMEM medium supplemented with 10% FBS). The toxicity of the extract on SW 1353 cells was tested using the same MTT assay as previously described.

*Evaluation of MMP13 gene expression in SW 1353 cells stimulated by IL-1*β: SW 1353 cells were cultured on a 6-well plate at a density of 2×10^5 cells/well in DMEM high glucose medium supplemented with 10% FBS, incubated for 24 hours at 37°C and 5% CO_2. Then, cells were treated with HGB extracts. After 1 hour of treatment, IL-1β was added to each well at a final concentration of 10 ng/ml, excluding the negative control wells (treated with medium only). A negative control (cells not treated with extract and IL-1β), a positive control (treated with dexamethasone and IL-1β), and a control treated with IL-1β only (no extract treatment) were also included. Cells were then incubated for 24 hours. Total RNA extraction was performed as previously described. A real-time PCR reaction was performed to check the expression of the *MMP13* gene with GAPDH as the internal control. The specific primer sequences and thermal cycling of real-time PCR with SYBR were referenced from J.E. Huh, et al. (2014) [10].

Statistical analysis of target gene expression: The quantification cycler value (the Qq) generated by real-time PCR constituted the initial quantitative data for the relative expression study [11]:

$$R = 2^{-\Delta\Delta Cq},\ \Delta\Delta C_q = (C_{q.target} - C_{q.GAPDH})_{treat} - (C_{q.target} - C_{q.GAPDH})_{untreat}$$

where R is the relative expression of target gene.

3. Results and discussion

Quantification of total polyphenols and flavonoids: The analysis of total polyphenolic content in the extract using HGB revealed a concentration of 234.47±5.55 GAE/g DW, which was 1.73 times higher than that obtained using HGA (135.92±0.32 GAE/g DW). However, the quantification of total flavonoids in the two extracts indicated that HGA (31.11±0.86 mg QE/g DW) had a higher flavonoid content than HGB (9.28±0.36 mg QE/g DW). This preliminary assessment of the chemical composition suggests that the extract from *D. cambodiana* Pierre ex Gagnep contains numerous compounds with potential applications as a medicinal herb.

Antioxidative activity evaluation: The *in vitro* antioxidative effect of HGA and HGB extracts was assessed by their ability to neutralise DPPH free radicals, using the EC_{50} value as a measure. The EC_{50} value (EC_{50} - half maximal effective concentration) represents the highest concentration of the extract that inhibits 50% of DPPH free radicals. The extract of *D. cambodiana* Pierre ex Gagnep in HGB displayed an EC_{50} value of 159.1 µg/ml, which was lower than that of HGA (242.2 µg/ml). However, the EC_{50} values of both solvents were lower when compared to vitamin C (EC_{50}=8.196 µg/ml). According to Y. Luo, et al. (2010) [5], the EC_{50} values of *D. cambodiana* extractions using petroleum ether, ethyl acetate, n-butanol, and water were 5.007, 1.613, 2.441, and 4.874 mg/ml, respectively. This result suggests that the HGA and HGB extracts in this study possess higher antioxidative effects. Moreover, HGB shows more potential and can be considered for further research.

Effect of the extract on the viability of RAW 264.7: The cytotoxicity of HGA and HGB extracts on RAW 264.7 cells was evaluated using the MTT assay. The results (Fig. 1) indicated that the extract in HGA exhibited cytotoxicity at the investigated concentrations (ranging from 10 to 50 µg/ml). In contrast, the extract of *D. cambodiana* in HGB showed lower cytotoxicity at the investigated concentrations, with cells remaining viable at concentrations from 10 to 20 µg/ml. Previously, a research by W.L. Mei, et al. (2013) [12] showed that a newly isolated flavonoid compound (*Cambodianin* G) could be toxic to K562 and SGC-7901 cell lines, with IC_{50} values of 9.5 and 16.2 g/ml, respectively.

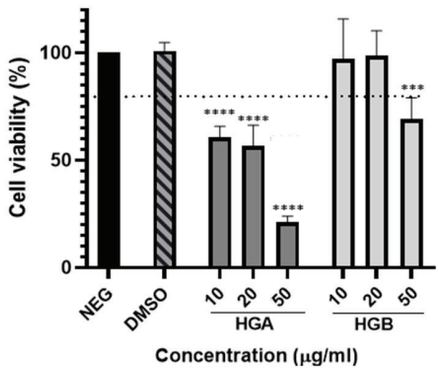

Fig. 1. Effects of dichloromethane and methanol extracts on the cell viability of RAW 264.7. NEG: untreated control; DMSO at 0.5%. Data are expressed as means ± standard deviation of the three independent experiments (***p<0.001; ****p<0.0001) vs. untreated control.

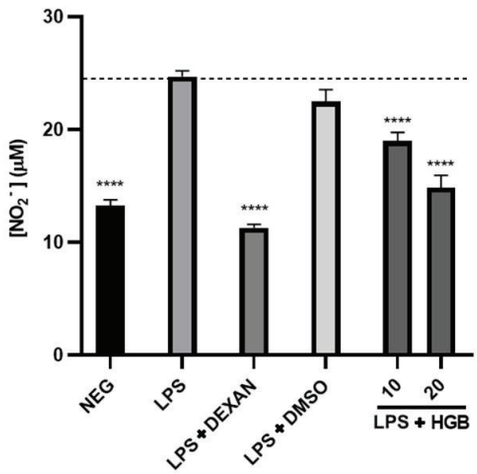

Fig. 2. Inhibition of nitric oxide by methanol in lipopolysaccharide-stimulated RAW 264.7. NEG: untreated control. DEXAN: dexamethasone 15 µM. Data are expressed as means ± standard deviation of the three independent experiments (****p<0.0001) vs. LPS-induced cell.

Attenuation of LPS-induced nitric oxide by the methanol extract in RAW 264.7: The anti-inflammatory activity of the extract was assessed by its ability to inhibit NO secretion in inflammatory RAW 264.7 cells induced by LPS at a concentration of 1 µg/ml. To evaluate the cytotoxicity of HGA and HGB extracts on RAW 264.7 cell line, two concentrations of 10 and 20 µg/ml of the extract with HGB were prepared, followed by an assessment of their ability to inhibit NO secretion. The positive control was dexamethasone at 15 µM. The results are shown in Fig. 2. The NO concentration in inflammatory cells induced by LPS significantly increased (1.8 times higher than in untreated cells). RAW 264.7 cells treated with dexamethasone showed a significant inhibitory effect on NO secretion compared to untreated cells. DMSO at a concentration of 0.2% had no significant effect on NO secretion. The HGB extract at all three concentrations also exhibited marked inhibition of NO secretion, especially at concentrations of 20 µg/ml (more than a 40% reduction compared to the LPS control). Therefore, the anti-inflammatory potential of the HGB extract can be observed through its ability to inhibit NO secretion. Previous studies have reported that certain types of inflammatory tissue injury are mediated by reactive oxygen metabolites, and administering specific antioxidants has shown an effective reduction of tissue inflammation and injury [13, 14]. Furthermore, polyphenols are known to possess anti-inflammatory properties through their ability to modulate MAPK, Akt, and NF-κB signalling pathways, suppress several cytokines related to inflammation, as well as hinder the activity of COX and iNOS [15]. Considering the above, we believe that as the HGB extract carries a high total polyphenol content and has significant antioxidant capacity, it is capable of reducing the inflammatory response. This led us to conduct further experiments to demonstrate the HGB extract's ability to attenuate inducible nitric oxide synthase (*iNOS*) expression.

Reduction of LPS-stimulated iNOS expression by HGB in RAW 264.7: The extract of *D. cambodiana* Pierre ex Gagnep with HGB at a concentration of 20 µg/ml exhibited the ability to inhibit NO secretion in LPS-stimulated RAW 264.7 cells without inducing cell death. NO is produced from the amino acid L-arginine by the enzymatic action of *iNOS*, which is induced by inflammatory stimuli such as bacterial lipopolysaccharide. Therefore, the HGB extract was further evaluated for its ability to inhibit the expression

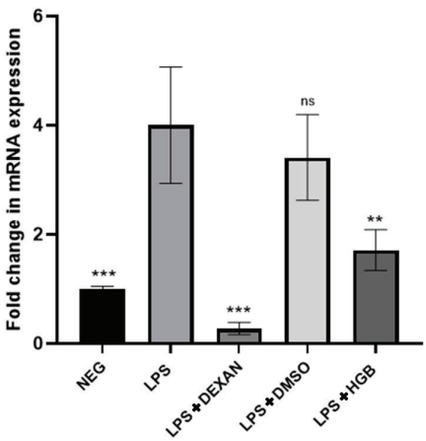

Fig. 3. Effects of methanol extract on the expression of *iNOS* in lipopolysaccharide-stimulated RAW 264.7 cells. Cells were stimulated with 1 µg/ml LPS after 1-hour treatment with HGB extract. GAPDH was used as an internal control for the RT-qPCR. NEG: untreated control; DEXAN: dexamethasone 15 µM; DMSO 0.2%. Data are represented as mean ± SD; number of RT-qPCR experiments (n)=3. **p<0.01; ***p<0.001 vs. control treated with lipopolysaccharide.

of mRNAs responsible for encoding proinflammatory cytokines such as *iNOS*. The results indicated that HGB extract significantly inhibits the expression of inflammatory genes (Fig. 3). Compared with the LPS-stimulated cells, the inhibitory activity on the expression of the *iNOS* gene was 1.8 times higher. However, the effect of reducing the expression of the HGB extract at 20 µg/ml was lower than that of dexamethasone at 15 µM, suggesting that the methanolic extract of *D. cambodiana* Pierre ex Gagnep

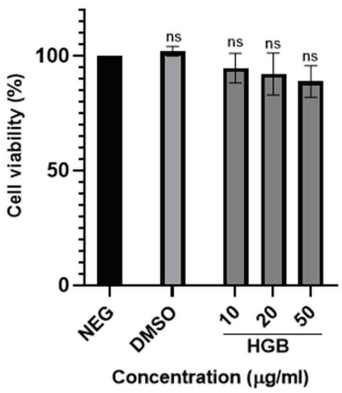

Fig. 4. Effects of methanol extract on cell viability of SW 1353 cell line. NEG: untreated control, DMSO at 0.5%. Data are expressed as mean ± SD of the three independent experiments (ns: no significant vs. untreated control).

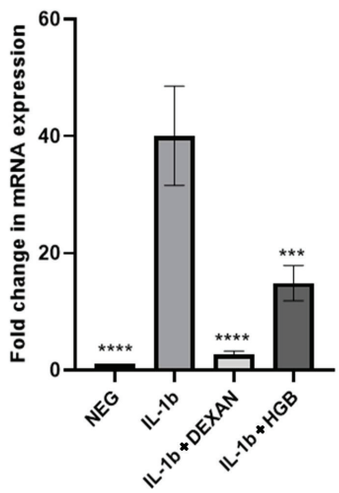

Fig. 5. Effects of methanol extract on the expression of *MMP13* in IL-1β-stimulated SW 1353 chondrocytes. Cells were stimulated with 10 ng/ml IL-1β after 1-hour treatment with HGB extract. GAPDH was used as internal controls for the RT-qPCR. NEG: untreated control; DEXAN: dexamethasone 15 µM; Data are represented as mean ± SD; number of RT-qPCR experiments (n)=3. ***p<0.001; ****p<0.0001 vs. control treated with IL-1β.

was effective but had a relatively lower potency. Further isolation of compounds in the solvent may help identify those with higher activity.

Effect of the HGB extract on the viability of SW 1353 cells: The cytotoxicity on SW 1353 cells of the methanol extract was evaluated by MTT assay (Fig. 4). The results showed that HGB extract did not induce cytotoxicity in SW 1353 cells at all three concentrations investigated. However, the HGB extract at a concentration of 50 µg/ml was toxic to RAW 264.7 cells (60% live cells compared with control). We aim to select a concentration that is effective on both cell lines while still conserving the amount of extract used. Therefore, the HGB extract at a concentration of 20 µg/ml was used to evaluate the ability to inhibit the expression of genes associated with osteoarthritis.

Inhibitory effects of HGB extract on MMP13 expression in IL-1β-stimulated SW 1353 cell line: Osteoarthritis has been described as an inflammatory process of the joints. Previous research has indicated that low-grade systemic and local inflammation is involved in the progression of osteoarthritis. Several inflammatory mediators, such as interleukin (IL)-1β, nitric oxide (NO), prostaglandin E2 (PGE2), and fragments of cartilage proteins, including matrix metalloproteinases (MMPs), have been reported to be elevated in patients with osteoarthritis [16]. IL-1β is an important proinflammatory cytokine during the inflammatory response, which has been reported to serve critical roles in joint inflammation and cartilage destruction processes. IL-1β elevates NO production and upregulates the expression of MMPs, including MMP13, which is associated with extracellular matrix (ECM) degradation in cartilage. Based on the significant role of inflammation in osteoarthritis, anti-inflammatory analysis may have significant value in the treatment of osteoarthritis. In this study, the expression levels of the *MMP13* gene were evaluated in the SW 1353 chondrocytes stimulated by IL-1β. The results are shown in Fig. 5, HGB at 20 µg/ml inhibited the overexpression of the *MMP13* gene (reduced by more than 60% compared to IL-stimulated cells). Dexamethasone - a commercial drug - significantly reduced *MMP13* gene expression at 15 µM (93.22% reduction compared to IL-1β treated cells). In a rabbit osteoarthritis model, W.P. Chen, et al. (2011) [17] proved that chlorogenic acid (CGA), a polyphenol isolated from natural plants, had the ability to reduce *MMP13* expression at both mRNA and protein levels. Other polyphenols were also reported to be capable of downregulating *MMP13*, such as curcumin in human and bovine chondrocytes [18], or epigallocatechin-3-O-gallate (EGCG) in human OA chondrocytes [19]. Through antioxidative action, these polyphenols downregulated transcription factors including NF-κB, AP1, Sp1, β-catenin, ERK1/2, p38, and Hsp27, all of which were in control of MMP expression [20]. These findings suggest that as HGB

possesses a high total polyphenolic content, it holds the potential to reduce *MMP13* production, which, as a result, attenuates osteoarthritis.

4. Conclusions

D. cambodiana Pierre ex Gagnep extracted in HGA and HGB was reported to contain polyphenolic and flavonoid components. Both extracts exhibited antioxidant activity *in vitro*. Besides, methanol extracts from *D. cambodiana* also showed anti-inflammatory activity and inhibited joint degeneration without causing cell cytotoxicity at 20 µg/ml. These results suggest that *D. cambodiana* Pierre ex Gagnep, as a medicinal herb, is a potential candidate in the treatment of inflammation and osteoarthritis. Further research is needed to identify the biologically active compounds to provide more scientific evidence about the species and promote the potential usage of this medicinal plant.

CRediT author statement

Huynh Cong Duy: Writing - Original draft preparation, Methodology, Data analysis; Nguyen Trung Quynh Nhu: Formal analysis, Writing; Mai Kieu Tien, Nguyen Phuc Thinh: Methodology; Nguyen Thi Le Thuy: Support in the evaluation of expression of pro-inflammatory genes; Le Thi Truc Linh: Methodology, Writing - Reviewing and Editing; Tran Thi Van Anh: Preparing the extracts; Pham Thi Kim Tram: Project administration, Supervision, Investigation, Formal analysis, Writing - Reviewing and Editing.

ACKNOWLEDGEMENTS

The results of this article are part of a project carried out at the Biotechnology Center of Ho Chi Minh City, sponsored by the People's Committee of Ho Chi Minh City, Vietnam.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] L.T.H. Pham, T.Q. Lai, L.D. Mai, et al. (2014), "Prevalence of radiographic osteoarthritis of the knee and its relationship to self-reported pain", *PLOS ONE*, **9(4)**, DOI: 10.1371/journal.pone.0094563.

[2] J.L. Zhao, L. Zhang, S. Dayanandan, et al. (2013), "Tertiary origin and Pleistocene diversification of dragon blood tree (*Dracaena cambodiana* - Asparagaceae) populations in the Asian tropical forests", *PLOS ONE*, **8(4)**, DOI: 10.1371/journal.pone.0060102.

[3] T.L. Do (2004), *Vietnamese Medicinal Plants and Herbs*, Medical Publishing House, Hanoi, 1294pp (in Vietnamese).

[4] H.D. Nguyen, V.K. Phan, V.M. Chau, et al. (2008), "Antifungal constituents from the stems of *Dracaena cambodiana*", *Vietnam Journal of Chemistry*, **46(4)**, pp.503-508.

[5] Y. Luo, H. Wang, X. Xu, et al. (2010), "Antioxidant phenolic compounds of *Dracaena cambodiana*", *Molecules*, **15(12)**, pp.8904-8914, DOI: 10.3390/molecules15128904.

[6] Y. Luo, H. Wang, Y.X. Zhao, et al. (2011), "Cytotoxic and antibacterial flavonoids from dragon's blood of *Dracaena cambodiana*", *Planta Medica.*, **77(18)**, pp.2053-2056, DOI: 10.1055/s-0031-1280086.

[7] L.T.T. Nguyen, T.H.D. Doan, T.N.Y. Ngo, et al. (2018), "Investigation of acute toxicity and anticoagulant effects of hemoglobin (*Dracaena cambodiana* Pierre ex *Gagnep, Dracaenaceae*)", *Journal of Pharmacy*, **58(6)**, pp.50-53 (in Vietnamese).

[8] G. Yang, K. Lee, M. Lee, et al. (2012), "Inhibition of lipopolysaccharide-induced nitric oxide and prostaglandin E_2 production by chloroform fraction of *Cudrania tricuspidata* in RAW 264.7 macrophages", *BMC Complement. Altern. Med.*, **12**, DOI: 10.1186/1472-6882-12-250.

[9] S. Bose, H. Kim (2013), "Evaluation of *in vitro* anti-inflammatory activities and protective effect of fermented preparations of Rhizoma Atractylodis Macrocephalae on intestinal barrier function against lipopolysaccharide insult", *Evidence-Based Complementary and Alternative Medicine*, **2013**, DOI: 10.1155/2013/363076.

[10] J.E. Huh, P.S. Koh, B.K. Seo, et al. (2014), "Mangiferin reduces the inhibition of chondrogenic differentiation by IL-1β in mesenchymal stem cells from subchondral bone and targets multiple aspects of the SMAD and SOX9 pathways", *International Journal of Molecular Sciences*, **15(9)**, pp.16025-16042, DOI: 10.3390/ijms150916025.

[11] K.J. Livak, T.D. Schmittgen (2001), "Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method", *Methods*, **25(4)**, pp.402-408, DOI: 10.1006/meth.2001.1262.

[12] W.L. Mei, Y. Luo, H. Wang, et al. (2013), "Two new flavonoids from dragon's blood of *Dracaena cambodiana*", *Bulletin of the Korean Chemical Society*, **34(6)**, pp.1791-1794, DOI: 10.5012/bkcs.2013.34.6.1791.

[13] U. Skaleric, J.B. Allen, P.D. Smith, et al. (1991), "Inhibitors of reactive oxygen intermediates suppress bacterial cell wall-induced arthritis", *J. Immunol.*, **147(8)**, pp.2559-2564.

[14] D.A. Clark, D.M. Fomabaio, H. McNeill, et al. (1988), "Contribution of oxygen-derived free radicals to experimental necrotizing enterocolitis", *Am. J. Pathol.,* **130(3)**, pp.537-542.

[15] B. Veres (2012), "Anti-inflammatory role of natural polyphenols and their degradation products", *Severe Sepsis and Septic Shock - Understanding a Serious Killer*, pp.379-410, DOI: 10.5772/27889.

[16] C.C. Liu, Y. Zhang, B.L. Dai, et al. (2017), "Chlorogenic acid prevents inflammatory responses in IL-1β-stimulated human SW-1353 chondrocytes, a model for osteoarthritis", *Mol. Med. Rep.*, **16(2)**, pp.1369-1375, DOI: 10.3892/mmr.2017.6698.

[17] W.P. Chen, J.L. Tang, J.P. Bao, et al. (2011), "Anti-arthritic effects of chlorogenic acid in interleukin-1β-induced rabbit chondrocytes and a rabbit osteoarthritis model", *Int. Immunopharmacol*., **11(1)**, pp.23-28, DOI: 10.1016/j.intimp.2010.09.021.

[18] A. Liacini, J. Sylvester, W.Q. Li, et al. (2002), "Inhibition of interleukin-1-stimulated MAP kinases, activating protein-1 (AP-1) and nuclear factor kappa B (NF-kappa B) transcription factors down-regulates matrix metalloproteinase gene expression in articular chondrocytes", *Matrix Biol.*, **21(3)**, pp.251-262, DOI: 10.1016/s0945-053x(02)00007-0.

[19] Z. Rasheed, A.N. Anbazhagan, N. Akhtar, et al. (2009), "Green tea polyphenol epigallocatechin-3-gallate inhibits advanced glycation end product-induced expression of tumor necrosis factor-alpha and matrix metalloproteinase-13 in human chondrocytes", *Arthritis Res. Ther*., **11(3)**, DOI: 10.1186/ar2700.

[20] T. Suzuki, T. Ohishi, H. Tanabe, et al. (2023), "Anti-inflammatory effects of dietary polyphenols through inhibitory activity against metalloproteinases", *Molecules*, **28(14)**, DOI: 10.3390/molecules28145426.