

Article

Quantification of Phytochemicals in *Cephalotaxus harringtonia*: Insights into Plant Tissue-Specific Allocation

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Abstract: *Cephalotaxus harringtonia* has garnered recent attention for its promising medicinal properties attributed to its alkaloid composition, including harringtonine and homoharringtonine known for their radical scavenging activities. High-performance liquid chromatography was used to assess the distribution of harringtonine, homoharringtonine, and ginkgetin in different plant parts of *C. harringtonia*. Additionally, DPPH and ABTS⁺ assays were conducted to evaluate the radical scavenging activity of *C. harringtonia* extracts. These results revealed that bud extracts from *C. harringtonia* exhibited the highest levels of polyphenols, along with elevated concentrations of harringtonine and homoharringtonine; nevertheless, this phenomenon only marginally influenced their antioxidant potential. These results suggest that, although a high concentration of compounds was detected in the buds of *C. harringtonia*, the detected compounds and their correlation with radical scavenging activity appears to be weak. While harringtonine and homoharringtonine are synthesized and maintained at elevated levels within buds to fulfill various physiological functions, including modulation of signal transduction pathways and reinforcement of defense mechanisms, the involvement of other constituents and the potential synergistic interactions among compounds cannot be overlooked in mediating the observed radical scavenging activity. Moreover, the significant concentrations of harringtonine and homoharringtonine in bud extracts highlight the potential applications of *C. harringtonia* in the pharmaceutical industry and other similar fields. This study emphasizes the imperativeness of further exploring the medicinal applications of *C. harringtonia* and underscores its prospective implications in pharmaceutical and functional materials development.

Keywords: *Cephalotaxus harringtonia*; colorimetry; high-performance liquid chromatography; harringtonine; homoharringtonine; radical scavenging activity



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1. Introduction

Cephalotaxus harringtonia, a member of the Cephalotaxaceae family, has garnered recent attention due to its rich composition of bioactive compounds, encompassing lignans, alkaloids, flavonoids, and terpenoids [1]. *C. harringtonia* is a small, evergreen coniferous tree or shrub that thrives in the mountainous regions of China and the Republic of Korea [2]. *Cephalotaxus* species have been widely used in traditional medicine to treat several diseases, such as dyspepsia [3], ascariasis [4], and inflammation [5]. Lignans, alkaloids, flavonoids, and terpenoids are abundant in *C. harringtonia*. These compounds have neuroprotective, anti-inflammatory, antioxidant, and anticancer activities [6,7].

Numerous studies have extensively investigated *Cephalotaxus* for its potential medicinal applications, leading to the identification of various alkaloids, including harringtonine, homoharringtonine, and ginkgetin [8]. The two former compounds are renowned for their

biological activities. Ginkgetin modulates biochemical processes within the body, including the regulation of neurotransmitter levels and the mitigation of oxidative stress [9,10]. In particular, these three compounds have attracted considerable research attention due to their potent anticancer activities [11–13].

Harringtonine and homoharringtonine, two plant-derived compounds, are important compounds in the treatment of acute myeloid leukemia and chronic myeloid leukemia, as well as other forms of these diseases [14]. The primary route of administering harringtonine and homoharringtonine is via intravenous injection, which has demonstrated efficacy in the treatment of malignant tumors and various types of cancer [15]. Ongoing research seeks to enhance the therapeutic efficacy and mitigate the toxicity associated with these agents, thereby fostering their evolution as potential anticancer medications [16]. Beyond their anticancer activities, harringtonine and homoharringtonine exhibit anti-inflammatory and antioxidant activities, showing promise in the realms of depression treatment and cognitive enhancement [17]. Furthermore, ginkgetin has been implicated in augmenting cerebral blood circulation and vascular integrity, suggesting its potential utility in the therapeutic management of Alzheimer's disease [18]. Nevertheless, further investigation is imperative to comprehensively explore these compounds' industrial applications.

To comprehensively evaluate the health-promoting properties and industrial application potential of *C. harringtonia* and its various compounds, previous studies have investigated the content and antioxidant activity of harringtonine, homoharringtonine, and ginkgetin [19–21].

Notably, homoharringtonine inhibits translation elongation by blocking peptide bond formation and aminoacyl tRNA binding [14]. In addition, homoharringtonine has been approved by the U.S. Food and Drug Administration for the treatment of chronic myeloid leukemia [22]. However, there are some differences between these studies in terms of the plant parts used and the amount and radical scavenging activity of these compounds in different parts of *C. harringtonia*, such as the leaves, stems, and buds, have not been fully elucidated. By comparing the levels of harringtonine, homoharringtonine, and ginkgetin in different parts of *C. harringtonia* and investigating their effects on the total phenolic content and radical scavenging activities in different parts of the plant, this study aimed to address the aforementioned gaps and provide a thorough evaluation.

2. Materials and Methods

2.1. Plant Materials

A *C. harringtonia* (Knight) K. Koch (CHK) sample was obtained from the Department of Forest Bioresources, National Institute of Forest Science, Suwon, Republic of Korea (37.2512528° N, 126.9591645° E). Samples were collected from the leaves, stems, and buds of a 10-year-old CHK plant (Figure 1). All voucher specimens (NIFS24-001) were deposited at the herbarium at the Department of Forest Bioresources, National Institute of Forest Science, Suwon, Republic of Korea.



Figure 1. The *C. harringtonia* samples used in the study.

2.2. Instruments and Reagents

High-performance liquid chromatography (HPLC) analysis was performed using an HPLC instrument (Agilent 1260 Infinity II Quat Pump, Santa Clara, CA, USA) equipped

with a variable wavelength detector (Agilent VW Detector, Santa Clara, CA, USA), a pump, and an autosampler with a YMC Pack Pro C18 column (4.6×250 mm, $5 \mu\text{m}$). HPLC-grade solvents, including water, acetonitrile, and methanol (MeOH), were purchased from J.T. Baker (Philipsburg, PA, USA). Ethanol (EtOH) and trifluoroacetic acid (TFA) were purchased from Samchun Chemicals (Pyeongtaek, Republic of Korea). The reagents for the colorimetric method, the 2N Folin–Ciocalteu phenol reagent, 2,2-diphenyl-1-picrylhydrazyl, and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, were purchased from Sigma–Aldrich (St. Louis, MO, USA). An Epoch microplate spectrophotometer (BioTek, Winooski, VT, USA) was used for all the assays, along with a microplate reader. Harringtonine (1), homoharringtonine (2), and ginkgetin (3) were obtained from the Natural Product Institute of Science and Technology (www.nist.re.kr; accessed on 7 July 2024) in Anseong, Republic of Korea. Their chemical structures are shown in Figure 2.

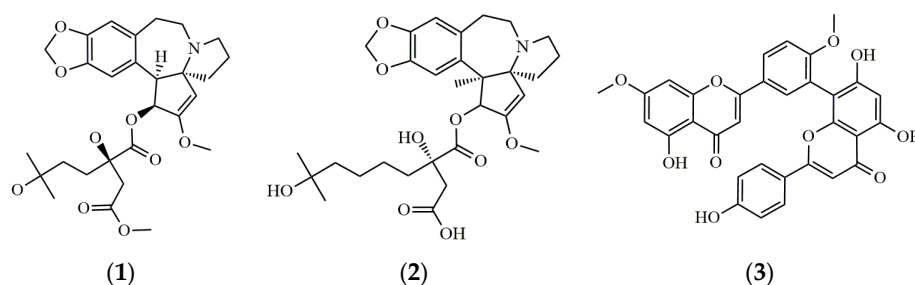


Figure 2. Chemical structures of harringtonine (1), homoharringtonine (2), and ginkgetin (3).

2.3. Sample Extraction and Preparation

The CHK (10 g), dried in a well-ventilated shade, was refluxed three times with EtOH (300 mL) for 3 h. The samples were dried using a rotary evaporator (55°C) to obtain the extracts for HPLC/UV analysis. In preparing the samples for HPLC analysis, 10 mg of each CHK extract was dissolved in 1 mL of MeOH to generate a stock solution with a concentration of 10 mg/mL. Afterward, the solutions were sonicated for 20 min and filtered through a $0.45 \mu\text{m}$ polyvinylidene fluoride (PVDF) membrane. In addition, stock solutions were prepared, and calibration curves were used to determine the content of all compounds in the samples.

2.4. Analysis of Total Phenolic Content (TPC)

The TPC of the CHK samples was determined using a previously described method [20]. Initially, $60 \mu\text{L}$ of the extract was mixed with $40 \mu\text{L}$ of the 2N Folin–Ciocalteu phenol reagent. Afterward, $100 \mu\text{L}$ of 7.5% sodium carbonate solution was added to the mixture and incubated for 30 min in the dark. After the reaction had occurred, absorbance was measured at 760 nm using a microplate reader (BioTek, Winooski, VT, USA). A calibration curve was constructed using tannic acid as the standard, with concentrations ranging from 0.00625 to 0.1 mg/mL. The TPC was expressed as milligrams of tannic acid equivalents (TAE) per gram of extract. Each analysis was performed in triplicate, and a blank containing all reagents except the sample was used to correct for background absorbance. The final TPC values were calculated using the linear regression equation derived from the calibration curve.

2.5. Analysis of Total Flavonoid Content (TFC)

The TFC of the CHK samples was analyzed using a previously described method, with some modifications [23,24]. Briefly, $100 \mu\text{L}$ of each sample was mixed with $100 \mu\text{L}$ of 2% AlCl_3 . The solution was then incubated for 10 min and the absorbance was measured at 430 nm using a microplate reader (BioTek Co.). A calibration curve was constructed using quercetin as the standard, with concentrations ranging from 0.00625 to 0.1 mg/mL. The TFC was expressed as milligrams of quercetin equivalents (QE) per gram of extract. Each analysis was performed in triplicate, and a blank containing all reagents except the sample

was used to correct for background absorbance. The final TPC values were calculated using the linear regression equation derived from the calibration curve.

2.6. DPPH Radical Scavenging Assay

DPPH radical scavenging activity was evaluated according to a previously described method [25–27]. Ascorbic acid was diluted to prepare a standard solution to six different concentrations (0–0.2 mg/mL), while the test solutions were prepared at three concentrations of 10, 5, and 2.5 mg/mL. Subsequently, 10 µL each of the standard solution, test solution, and blank solution (pure EtOH) were added to a 96-well plate, followed by 200 µL of the DPPH working solution. A microplate shaker was used to thoroughly mix the sample, and the mixture was incubated for 30 min at room temperature in the dark. After the mixture had reacted, absorbance was measured at 514 nm using a microplate reader (BioTek Co.). The DPPH radical scavenging rate was calculated using the example 1 of an equation:

$$\text{DPPH radical scavenging activity (\%)} = (\text{Blank O.D} - \text{Sample O.D}) / \text{Blank O.D} \times 100 \quad (1)$$

The assay was performed in triplicate, and the results were expressed as IC₅₀ values (mg/mL), which represent the concentration of the sample required to scavenge 50% of the DPPH radicals. The IC₅₀ values were calculated by plotting the scavenging activity (%) against three concentrations of the sample using nonlinear regression analysis. A blank containing only DPPH and pure EtOH were included to correct for background absorbance.

2.7. ABTS⁺ Radical Scavenging Activity

The radical scavenging activity of the CHK samples was analyzed using the ABTS⁺ assay, using a method similar to that used in a previous study [28–30]. The ABTS⁺ and potassium persulfate solutions were mixed and diluted in distilled water to obtain an absorbance value of 1.0 mg/mL. Ascorbic acid was diluted to prepare a standard solution at six different concentrations (0–0.2 mg/mL), while the test solutions were prepared at three concentrations of 10, 5, and 2.5 mg/mL. Ten microliters of the standard, test solutions, and blank solution (pure EtOH) were added separately to a 96-well plate. Then, 200 µL of the ABTS⁺ working solution was added. The samples were incubated for 30 min in the dark and the absorbance was measured at 734 nm using a microplate reader (BioTek Co.). The ABTS⁺ radical scavenging rate was calculated using the example 2 of an equation:

$$\text{ABTS}^+ \text{ radical scavenging activity (\%)} = (\text{Blank O.D} - \text{Sample O.D}) / \text{Blank O.D} \times 100 \quad (2)$$

The assay was performed in triplicate, and the results were expressed as IC₅₀ values (mg/mL), representing the concentration of the sample required to scavenge 50% of ABTS⁺ radicals. The IC₅₀ values were calculated by plotting the scavenging activity (%) against the sample concentration using nonlinear regression analysis. A blank containing only ABTS⁺ and pure EtOH was included to correct for background absorbance.

2.8. HPLC Conditions

Quantitative analysis of the chemical composition of CHK was performed using a reversed-phase HPLC system (Agilent 1260 Infinity II) equipped with a variable wavelength UV detector (VW Detector, Agilent, Santa Clara, CA, USA) and an autosampler injector. Chromatography was performed using a YMC Pack Pro C18 column (4.6 × 250 mm, 5 µm) and the gradient method with 0.1% TFA in water (A) and acetonitrile (B). The elution system was as follows: 0–10 min, 7% B; 40 min, 60% B; 45 min, 100% B; 50 min, 7% B; and 60 min, 7% B. The temperature of the column was maintained at 30 °C. The injection volume was 10 µL, the flow rate was set to 1.0 mL/min, and UV detection was performed at 270 nm. The samples were prepared by dissolving the extracts in HPLC-grade methanol at an initial concentration of 10 mg/mL. All analyses were carried out in triplicate to ensure reproducibility.

2.9. Calibration Curve

Standard solutions were prepared by dissolving compounds 1–3 in MeOH (1 mg/mL). The working solution used to construct the calibration curve was prepared by serial dilution of the standard solution to the desired concentrations. CHK samples were dissolved in MeOH (10 mg/mL). Subsequently, both standard and sample solutions were filtered through a 0.45 µm PVDF filter (Whatman, Kent, UK), and the concentration of CHK was determined using the calibration curve. The calibration curve for the five compounds was established using peak area (Y) versus concentration (X, µg/10 µL), with values presented as the mean ± standard deviation (SD) (Table 1).

Table 1. Calibration curve equations for harringtonine (1), homoharringtonine (2), and ginkgetin (3).

Compound	t _R	Calibration Equation	Correlation Factor, r ²
harringtonine (1)	23.3	Y = 1.0541X – 34.697	0.9999
homoharringtonine (2)	24.8	Y = 1.0266X – 3.4901	0.9999
ginkgetin (3)	43.7	Y = 18.055X – 69.013	0.9998

t_R = retention time; Y = peak area; X = concentration of the standard (mg/mL); r² = correlation coefficient for the five data points in the calibration curve.

2.10. Statistical Analysis

Results are expressed as the mean ± SD derived from three independent experiments. Statistical analysis was performed using a one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. All statistical analyses and graphs were generated using GraphPad Prism 8.0.2 statistical software (GraphPad Software, Boston, MA, USA). NS, *, **, ***, and **** mean not significant and significant at $p < 0.05$, 0.01, 0.001, and 0.0001, respectively. A correlation coefficient network was generated using the MetScape (Version 3.1.3) plugin for Cytoscape (Version 3.10.2; <https://cytoscape.org/>, accessed on 30 October 2024).

3. Results

3.1. TPC and TFC Assays

The TPC and TFC in the different extracts of CHK are shown in Figure 3. The results of this investigation showed that the bud extract contained more TPC than TFC, with the highest TPC value being 78.9 mg TAE/g ext. and the lowest TFC value being 11.6 mg QE/g ext. In contrast, the TPC values of the leaf and stem extracts were lower than those of the buds; however, the TFC values of the buds were lower than those of the other extracts.

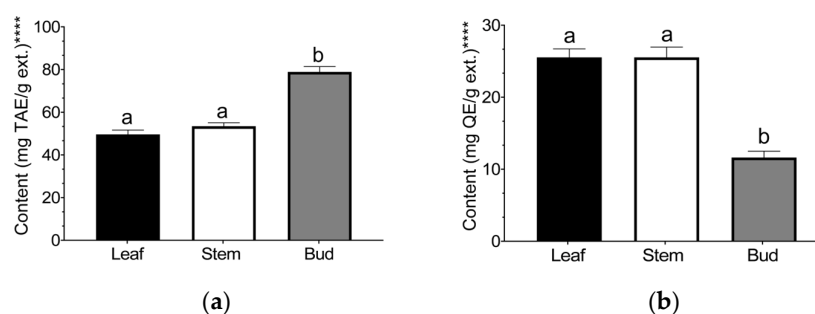


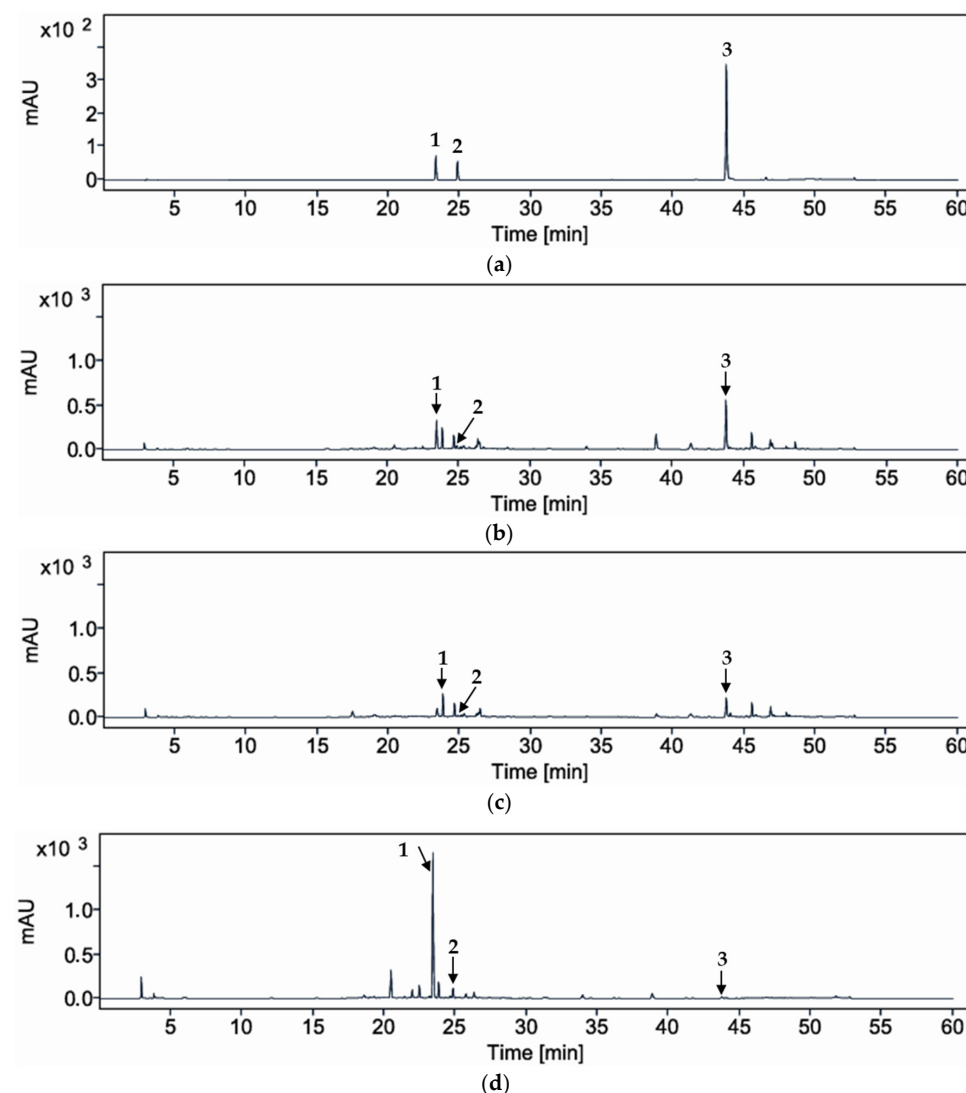
Figure 3. TPC (a) and TFC (b) in different parts of *C. harringtonia*. Bars represent the mean ± SD. Lowercase letters indicate significant differences. **** means significant at $p < 0.0001$.

3.2. HPLC Analysis

The HPLC data showed the distribution of harringtonine (1), homoharringtonine (2), and ginkgetin (3) in the three parts of CHK (Table 2). These bioactive compounds are associated with various pharmacological functions in different plant parts. Compared to those in the leaf and stem extracts, the bud extract contained the highest amount of harringtonine (1) and homoharringtonine (2) (Figure 4).

Table 2. Quantitative analysis of harringtonine (1), homoharringtonine (2), and ginkgetin (3) in different parts of *C. harringtonia*.

Compound	Contents (mg/g) ****		
	Leaf	Stem	Bud
harringtonine (1)	25.3 ± 0.2 ^b	9.6 ± 0.1 ^b	240.7 ± 0.3 ^a
homoharringtonine (2)	3.2 ± 0.1 ^b	1.3 ± 0.1 ^c	16.6 ± 0.5 ^a
ginkgetin (3)	2.8 ± 0.1 ^a	1.0 ± 0.1 ^b	tr
Total	31.4 ± 0.2 ^b	12.0 ± 0.1 ^c	257.4 ± 0.8 ^a

tr: trace; ^{a-c} Different letters indicate significant differences; **** means significant at 0.0001.**Figure 4.** HPLC/UV chromatograms of standard compounds (a), leaf (b), stem (c), and bud (d) of *C. harringtonia*. (1: harringtonine, 2: homoharringtonine, 3: ginkgetin).

3.3. DPPH and ABTS⁺ Radical Scavenging Assays

The ability of analytes to scavenge free radicals is one of the methods used to assess their antioxidant potential [31]. Hence, antioxidant activity should not be measured using a single approach. In this study, the DPPH and ABTS⁺ assays were used in conjunction as a way to examine the radical scavenging activity of extracts. Previously, it was reported that the inhibitory concentration 50% (IC₅₀) value of the DPPH radical scavenging activity of the EtOH extract of CHK was almost identical to that of ascorbic acid, the positive standard [32,33]. In the DPPH assay performed in this study (Figure 5a), the IC₅₀ values

of the CHK leaf, stem, and bud samples were 5.2 mg/mL, 4.7 mg/mL, and 5.3 mg/mL, respectively, which were not significantly different from each other.

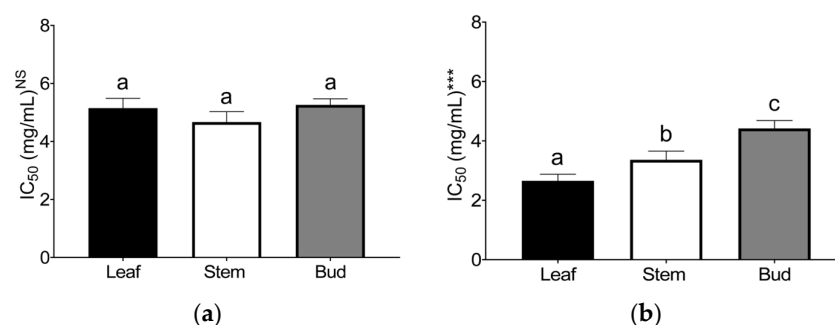


Figure 5. DPPH (a) and ABTS⁺ (b) in different parts of *C. harringtonia*. Bars represent the mean ± SD. Lowercase letters indicate significant differences. NS and *** mean not significant and significant at $p < 0.001$, respectively.

Compared to the CHK samples, the ascorbic acid control had a significantly lower IC₅₀ value of 0.2 mg/mL, demonstrating its strong radical scavenging activity. In the ABTS⁺ assay (Figure 5b), the CHK leaf, stem, and bud samples showed IC₅₀ values of 2.7 mg/mL, 3.4 mg/mL, and 4.4 mg/mL, respectively. Meanwhile, the ascorbic acid control outperformed the CHK samples in terms of radical scavenging activity, as evidenced by its significantly lower IC₅₀ value of 0.1 mg/mL.

3.4. Pearson's Correlation Analysis

The Pearson's correlation analysis revealed several significant relationships between the tested compounds (Figure 6). In contrast, TFC showed a strong inverse correlation with ABTS⁺, emphasizing the inverse relationship between the two compounds. DPPH radical scavenging activity showed a moderate positive correlation with harringtonine and homoharringtonine, whereas ABTS⁺ radical scavenging activity showed a moderate positive correlation with harringtonine and homoharringtonine, suggesting potential antioxidant properties in these alkaloids. Interestingly, ginkgetin (3) showed negative correlations with most compounds, including ABTS⁺ and homoharringtonine, suggesting that it may have antagonistic effects.

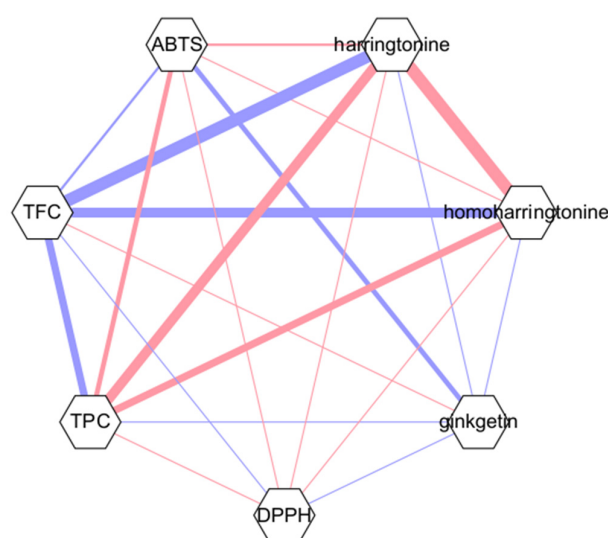


Figure 6. Pearson's correlation coefficient network ($r \geq |1.00|$) of the response variables' phytochemical concentrations and radical scavenging activity measures in different parts of *C. harringtonia*. The red and blue lines indicate positive and negative correlation coefficients between variables, respectively.

4. Discussion

The TPC and TFC of the extracts were first evaluated to determine if there are bioactive components present in the extracts (Figure 3). The results showed an inverse relationship, wherein the stems and leaves were low in TPC while the buds had the highest TPC, and TFC was lowest in the buds but highest in the stems and leaves. As expected, TFC would be higher in the stems and leaves because these are plant parts more exposed to the sun's UV rays [34], the presence of these flavonoids acts as a protection against the oxidation brought upon by these UV rays [35]. On the other hand, the buds having low TFC might be due to the fact that these tissues are still developing and not yet as exposed to the sun compared to their counterparts. A previous study evaluated the TPC and TFC of the same plant extracted with different solvents [36]. However, the plant part used was the stem bark, and no comparisons were made with the other plant tissues. Their results showed that the TPC and TFC levels in the three extracts ranged from 72.5 ± 5.1 mg to 609.6 ± 10.1 mg gallic acid equivalents (GAE)/g of dried extract and 7.6 ± 0.6 mg to 19 ± 0.6 mg quercetin equivalents (QE)/g of dried extract, respectively, which is in a similar range with the TFC, but not the TPC, of the present study.

The distribution of compounds in different parts of the CHK plant was investigated using HPLC/UV (Table 2). Interestingly, the results showed that the buds were reservoirs for high concentrations of harringtonine (1) and homoharringtonine (2), with concentrations exceeding those found in leaves and stems. In contrast, only trace amounts of ginkgetin (3) were detected in the buds. This is probably due to the physiological function of buds as storage sites for vital metabolites required for growth and development [37]. Harringtonine (1) and homoharringtonine (2), as important pharmacological agents, may be produced and maintained at higher concentrations in buds to support a variety of biological processes, including signaling pathways and defense mechanisms [38]. However, the lower concentrations of ginkgetin (3) in buds than those in leaves and stems may indicate that ginkgetin is preferentially distributed to other parts of the plant or that its production is restricted to buds. These results suggest the tissue-specific distribution of these compounds in plants [39]. Furthermore, they support further investigation into the medicinal applications of *C. harringtonia* and its potential in pharmaceutical and functional materials development. Industrially, choosing buds for extraction would be beneficial.

Previous research has shown that polyphenols and alkaloids are significant antioxidants in natural goods [40]. The detection of these compounds warrants investigation of the extracts' radical scavenging activity. The results showed that the stem had the highest inhibitory activity, followed by the leaf and the bud, respectively (Figure 5). Despite this, the differences observed are not significant. On the other hand, the leaf extracts had the highest inhibitory activity in the ABTS⁺ assay, followed by the stem and the bud, respectively. Similarly, the positive control still had a lower IC₅₀ value compared to the extracts. The same levels of radical scavenging activity were found across the different parts of the plant despite differences in the amounts of compounds, suggesting that radical scavenging activities depend on complex interactions between several elements [5]. The high TPC in buds suggests that polyphenols may be largely responsible for radical scavenging by contributing hydrogen atoms or electrons to neutralize free radicals. While harringtonine (1) and homoharringtonine (2) were present in large concentrations, their exact involvement in radical scavenging activity is unknown, necessitating molecular docking or enzyme inhibition investigations to investigate their interactions with reactive oxygen species (ROS). Synergistic effects could explain this observation, as interactions between flavonoids, alkaloids, and polyphenols frequently result in increased radical scavenging activity [41]. Future fractionation or reconstitution experiments may help elucidate these relationships and evaluate whether compound combinations outperform individual effects.

High levels of certain molecules, such as harringtonine (1) and homoharringtonine (2), may be partially responsible for the radical scavenging activity, although other components and interactions between different compounds may also be important. In addition, the higher TPC values of the bud extracts may be a factor in their higher radical scavenging

activity. Similarly, the differences in the concentrations of flavonoids and polyphenolic compounds in different parts of the plant, especially in the buds, indicate different sources of various bioactive compounds with radical scavenging activities [42,43]. These results suggest that the presence of harringtonine (1), homoharringtonine (2), and ginkgetin (3) in the extracts might not correlate with the radical scavenging activity of the extracts because these compounds were detected in high amounts. Hence, high inhibitory activity against the radicals was expected. To answer this question, Pearson's correlation analysis was conducted (Figure 6). Differences in radical scavenging activity may also result from the extraction methods utilized. Solvent polarity, as well as temperature and time, all have a substantial impact on bioactive molecule yields [44]. Optimizing these parameters utilizing sophisticated technologies, like ultrasonic-assisted or supercritical fluid extraction, could boost yields while maintaining compound integrity [45].

A similar study investigated the radical scavenging activity of the in vitro shooting and callus cultures in the same plant using the DPPH assay [5]. The IC_{50} values of their extracts were lower compared to those in the present study. Another study investigated the radical scavenging activities of 140 botanicals, including CHK, collected from regions in Korea using oxygen radical absorbance capacity and DPPH assays [33]. These studies emphasize the variety of bioactive compounds found in CHK, as well as their radical scavenging activities, highlighting CHK's potential use in medicine and the healthcare industry. Beyond antioxidants, CHK extracts have the potential for larger uses in food science, nutraceuticals, and cosmetics. For example, their radical scavenging properties can be applied to food preservation or as active ingredients in cosmetics compositions [46]. The pharmacological characteristics of harringtonine (1) and homoharringtonine (2) suggest potential applications in drug development.

Although CHK belongs to the Cephalotaxaceae family and is mainly valued for its ecological and decorative properties, there is currently little data to support the potential industrial or agricultural applications of the species, although its wood may have some uses [47]. This plant is one of many understudied plants in Korea, hence another reason why the present work was conducted. Nonetheless, there is ongoing research and development in South Korea for the strategic use of pharmaceutical bioresources, including possible uses for CHK [48]. In addition to maintaining biodiversity in the ecosystem in Korea, CHK may have other benefits that are currently unknown; therefore, further research is required. According to our results, the CHK bud extract demonstrated substantial radical scavenging activity in the ABTS⁺ assay. Furthermore, significant differences were found in the levels of flavonoids and polyphenolic compounds, as well as in the TPC, in different parts of the plant. Future research should investigate CHK's anti-inflammatory and antibacterial properties using cell-based and pathogen-specific assays. In-depth research into biosynthetic routes for important molecules could help improve production efficiency, paving the road for scalable industrial applications [49].

Finally, the unique biochemical profiles in the different parts of CHK, especially the high amounts of chemical compounds in the bud, provide insights into its potential therapeutic uses. Further research using a variety of assays is needed to investigate the biological activity of CHK beyond antioxidation, which may lead to the discovery of new therapeutic uses for CHK in the healthcare and pharmaceutical industries.

5. Conclusions

The various analyses of CHK revealed novel findings regarding its pharmacological potential and molecular complexity. Analysis of the various botanical constituents in CHK revealed different distributions of bioactive compounds in different parts of the plant. In particular, the buds appeared to be stores of harringtonine and homoharringtonine, with concentrations higher than those in the leaves and stems. Although ginkgetin levels in the buds were low, CHK extracts of all three plant parts exhibited strong radical scavenging activity. However, DPPH assays showed no difference in activity across the plant parts, while ABTS⁺ assays revealed moderate significance. The significant concentrations of

harringtonine and homoharringtonine in the CHK bud extracts highlight its potential as a valuable resource for the pharmaceutical industry and other fields. Furthermore, the presence of phenolic compounds, particularly in the buds, makes them suitable for use in antioxidant-rich nutraceutical and cosmetic compositions. For example, CHK extracts could be used as dietary supplements to reduce oxidative stress or as active ingredients in anti-aging cosmetics. The tissue-specific distribution of ginkgetin in stems and leaves provides possibilities for investigating its antibacterial and anti-inflammatory capabilities in topical formulations or as a preservative in the food and beverage industry. Future studies should prioritize the assessment of bioactivities other than antioxidation, such as anti-inflammatory, antibacterial, and anticancer characteristics, employing both in vitro and in vivo models. Advanced extraction techniques, such as ultrasound-assisted or supercritical fluid extraction, could be investigated to improve the recovery of bioactive chemicals while maintaining their integrity. Furthermore, the biosynthetic routes of important chemicals, such as harringtonine and ginkgetin, should be studied to ease large-scale manufacturing in the future. These implications are significant and highlight a wealth of therapeutic opportunities that are yet to be explored. Therefore, a part-by-part analysis of CHK is a prospective pharmaceutical biological resource and requires more thorough investigations and novel approaches than an always-on assay. For example, its prospective applications in cancer therapy, oxidative stress management, and skin health highlight its adaptability in a variety of sectors. The antioxidant-rich buds, in particular, are a viable target for future pharmacological and nutraceutical development. With further research and tactical applications, CHK has the potential to advance our understanding of therapeutic flora while also significantly benefitting the healthcare and industrial fields.

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