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LE THI PHUONG

RESEARCH ON CHEMICAL COMPOSITION AND CANCER CELL TOXIC ACTIVITY OF TWO SPECIES OF CINNAMON *Cinnamomum bejolghota* (Buch.- Ham. ex Nees) Sweet and *Cryptocarya concinna* Hance BELONGING TO THE LAURACEAE FAMILY (LAURACEAE)

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Phản biện 1:.....

Phản biện 2:.....

Phản biện 3:.....

The dissertation is examined by Examination Board of Graduate University of Science and Technology, Vietnam Academy of Science and Technology at……..

LIST OF PUBLISHED ARTICLES RELATED TO THE THESIS

1. **Thi Phuong Le**, Bich Ngan Truong, Marc Litaudon, Thuy Linh Nguyen, Thi Mai Huong Doan, Van Cuong Pham, 2023, New alkaloids from the stem bark of *Cinnamomum bejolghota*, *Phytochemistry Letters*, 55, pp. 164-168.
2. **Thi Phuong Le**, Thuy Linh Nguyen, Bich Ngan Truong, Marc Litaudon, Thi Mai Huong Doan, Van Cuong Pham, 2025, New alkaloids and sesquiterpenoid from the leaves of *Cinnamomum bejolghota*, *Chemistry and Biodiversity,* e202502118
3. **Le Thi Phuong,** Trinh Thi Thanh Van, Nguyen Thuy Linh, Pham Van Cuong, Doan Thi Mai Huong, Truong Bich Ngan, 2024, Isolation, structural determination, and cytotoxicity of compounds from the non-alkaloid extract of *Cinnamomum bejolghota*, *Journal of Medicinal Materials*, 29, 87-92.

THESIS INTRODUCTION

1. Introduction

Plants, animals, microorganisms, terrestrial and marine organisms are an extremely rich treasure trove of natural compounds. Hundreds of thousands of natural compounds have been discovered and applied to many areas of life, especially in medicine. In the early 90s, combinatorial chemistry was chosen by the world's major pharmaceutical companies as the key tool for research to find new drugs, at which time the role of natural compounds was more or less underestimated. As a result, the investment efficiency to find active substances with new structures has decreased significantly. After that, the research direction was re-planned and natural compounds continued to play a key role in the search for new drugs to fight the deadly diseases that are taking the lives of many people every day.

Vietnam has a tropical monsoon climate with a typical rainy season in the South and a more temperate climate in the North. In terms of biogeography, Vietnam is the intersection of India, South China and Malaysia. Therefore, this is a region with very high biodiversity. In terms of flora alone, Vietnam has about 12,000 species of higher plants, 2,200 species of fungi and 481 species of moss. More than 5,000 species of plants have been used as food, medicine, wood, essential oils, construction materials... This is an extremely valuable source of natural compounds that need to be studied chemically and surveyed for biological activity to find active ingredients that can be used as medicine to serve the care and protection of people's health.

Within the framework of French-Vietnamese cooperation, we tested the cytotoxic activity with the KB cancer cell line and conducted a preliminary survey of the chemical composition of some species belonging to the two genera *Cinnamomum* and *Cryptocarya* of Vietnam. The results showed that the ethyl acetate extract of the leaves of *Cinnamomum bejolghota* (Buch.-Ham. ex Nees) Sweet inhibited 61.0% of the KB cancer cell line at a

concentration of 1 µg/ml, the ethyl acetate extract from the leaves of *Cryptocarya concinna* Hance inhibited 60.0% of the KB cancer cell line at a concentration of 1 µg/ml, the leaves and bark of *Cinnamomum bejolghota* (Buch.- Ham. ex Nees) Sweet and the leaves of *Cryptocarya concinna* Hance both contained alkaloid compounds.

From the above screening results, we have chosen the topic: "Research on the chemical composition and cytotoxic activity of two species of *Cinnamomum bejolghota* (Buch.- Ham. ex Nees) Sweet and *Cryptocarya concinna* Hance of the Lauraceae family (Lauraceae)".

2. Thesis objectives

- Determination of chemical composition from two species *Cinnamomum bejolghota* (Buch.- Ham. ex Nees) Sweet and *Cryptocarya concinna* Hance belonging to the family Lauraceae (Lauraceae).

- Evaluation of the cancer cell toxicity activity of the isolated compounds serves as a scientific basis for further studies to create products to protect and care for community health and contribute to the statistics of valuable plant species in the Vietnamese flora.

3. Contents of the thesis

- Isolation of compounds from two species *Cinnamomum bejolghota* (Buch.- Ham. ex Nees) Sweet and *Cryptocarya concinna* Hance by chromatographic methods.

- Determine the chemical structure of isolated compounds by physical and chemical methods.

- Evaluation of in vitro cancer cell cytotoxic activity of isolated compounds on some human cancer cell lines: KB, HepG2, MCF-7, SK-LU-1

MAIN CONTENT OF THE THESIS

CHAPTER 1. OVERVIEW

1.1. Introduction to the genus *Cinnamomum*

1.2.2.5. Terpenoid compounds

1.2.3. Research status on genus Cryptocarya and species Cryptocarya concinna Hance in Vietnam

CHAPTER 2. RESEARCH METHODS - EXPERIMENTS AND RESULTS

2.1. Research subjects

2.1.1. Sweet Cinnamon Cinnamomum bejolghota (Buch.- Ham. ex Nees)

2.1.2. Golden Fruit Sniffer Cryptocarya concinna Hance

2.2. Research methods

2.2.1. Methods of isolating compounds

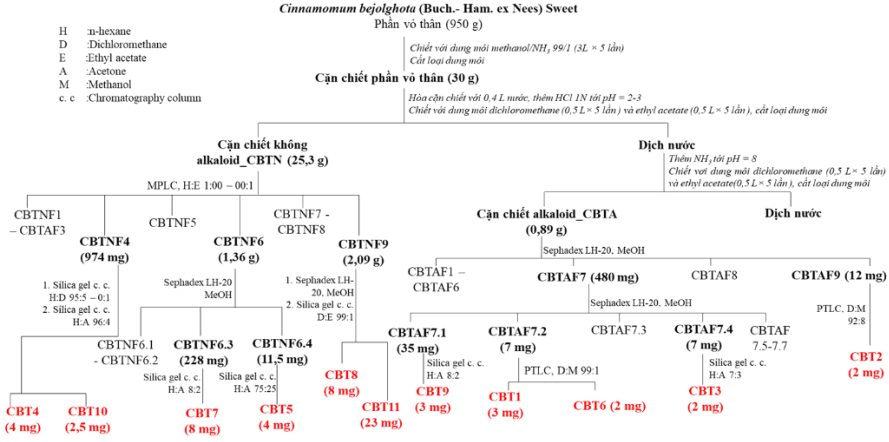
2.2.2. Methods for determining the chemical structure of compounds

2.2.3. Method for evaluating in vitro cancer cell cytotoxic activity

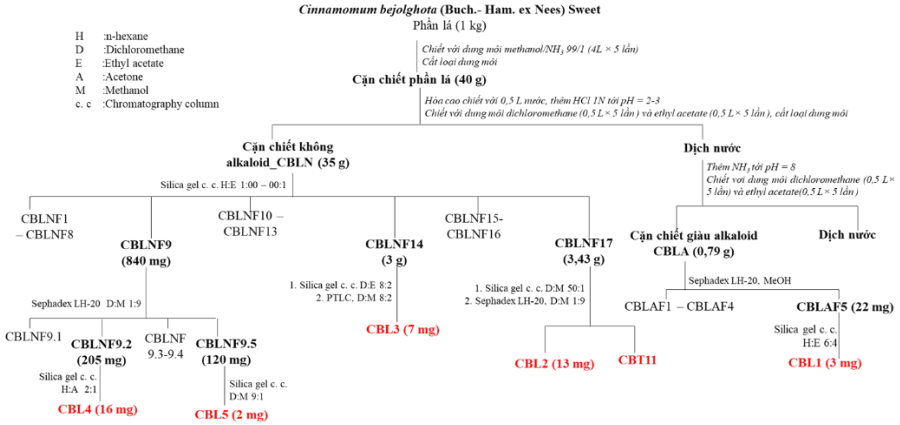
2.3. Isolation of compounds

2.3.1. Isolation of compounds from Cinnamomum bejolghota (Buch.- Ham. ex Nees) Sweet cinnamon

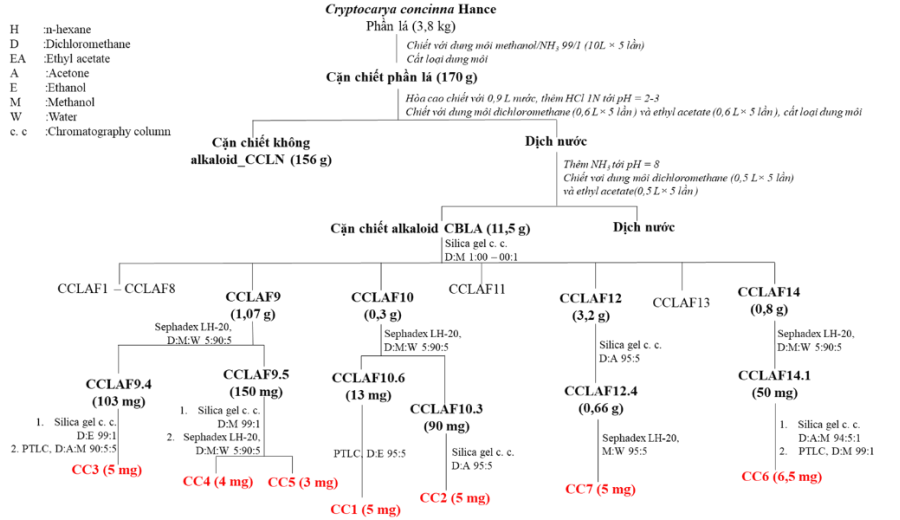
2.3.1.1. Isolation of compounds from the bark of C. bejolghota



2.3.1.2. Isolation of compounds from the leaves of C. bejolghota



2.3.2. Isolation of compounds from the leaf part of Cryptocarya concinna Hance



2.4. Physical parameters and spectral data of the isolated compounds

2.4.1. Physical parameters and spectral data of compounds isolated from the bark of Cinnamomum bejolghota (Buch.- Ham. ex Nees) Sweet

*2.4.1.1. Compound **CBT1**: 3,4-bis(3,4-dimethoxyphenyl) pyridine (new substance)*

Pale yellow solid.

Melting point: 189-190°C

Molecular formula: $C_{21}H_{21}NO_4$

Molecular formula: 351

IRν_{max} spectrum (KBr): 1603,1583, 1511, 1465, 1395 cm^{-1} (Figure 3.3 page 56)

HR-ESI-MS spectrum *m/z* 352,1547 $[M + H]^+$ (theoretical calculation for molecular formula $[C_{21}H_{22}NO_4]^+$ *m/z* 352,1549) (Figure 3.2 page 55).

Spectrum data ^1H NMR (CD_3OD, 500 MHz) và ^{13}C NMR (CD_3OD, 125 MHz) see table 3.1 (page 60).

*2.4.1.2. Compound **CBT2**: 1-(4-hydroxybenzyl)-6-hydroxyisoquinoline (new substance)*

Yellow solid

Melting point: 270-271°C

Molecular formula: $C_{16}H_{13}NO_2$

Molecular mass: 251

HR-ESI-MS spectrum *m/z* 252,1023 $[M + H]^+$ (theoretical calculation for the formula $[C_{16}H_{14}NO_2]^+$ *m/z* 252,1025) (Figure 3.12 page 61).

Spectrum data ^1H NMR (CD_3OD, 500 MHz) và ^{13}C NMR (CD_3OD, 125 MHz) see table 3.2 (page 64).

*2.4.1.3. Compound **CBT3**: 4-(3,4-dimethoxyphenyl)-2-methyl pyridine (new substance)*

Yellow solid

Melting point: 230-231°C

Molecular formula: $C_{14}H_{15}NO_2$

Molecular mass: 229

HR-ESI-MS spectrum *m/z* 230,1179 $[M + H]^+$ (theoretical calculation for the formula $[C_{14}H_{16}NO_2]^+$ *m/z* 230,1181) (Figure 3.19 page 65).

Spectrum data ^{1}H NMR (CD_3OD, 500 MHz) và ^{13}C NMR (CD_3OD, 125 MHz) see table 3.3 (page 68).

*2.4.1.4. Compound **CBT4**: Spathulenol*

Oil, colorless

Molecular formula: $C_{15}H_{24}O$

Molecular mass: 220

Extreme rotation $[\alpha]_D^{28}$ +1,14 (*c* 0,1, MeOH)

Spectrum data ^{1}H NMR ($CDCl_3$, 500 MHz) và ^{13}C NMR ($CDCl_3$, 125 MHz) see table 3.4 (page 69)

*2.4.1.5. Compound **CBT5**: 5,7-di-O-methyl-3',4'-methylenedioxyflavan-3-ol*

Yellow, amorphous powder

Molecular formula: $C_{18}H_{18}O_6$

Molecular mass: 330

Spectrum data ^{1}H NMR ($CDCl_3$, 500 MHz) và ^{13}C NMR ($CDCl_3$, 125 MHz) see table 3.5 (page 71)

*2.4.1.6. Compound **CBT6**: 3,4-dimethoxycinnamyl alcohol*

White, amorphous powder

Molecular formula: $C_{11}H_{14}O_3$

Molecular mass: 194

Spectrum data ^{1}H NMR ($CDCl_3$, 500 MHz) và ^{13}C NMR ($CDCl_3$, 125 MHz) see table 3.6 (page 72).

*2.4.1.7. Compound **CBT7**: 3,4-dimethoxycinnamaldehyde*

Yellow, amorphous powder

Molecular formula: $C_{11}H_{12}O_3$

Molecular mass: 192

Spectrum data ^{1}H NMR ($CDCl_3$, 500 MHz) và ^{13}C NMR ($CDCl_3$, 125 MHz) see table 3.7 (page 73).

*2.4.1.8. Compound **CBT8**: Veratric acid*

White, amorphous powder

Molecular formula: $C_9H_{10}O_4$

Molecular mass: 182

Spectrum data ^{1}H NMR ($CDCl_3$, 500 MHz) và ^{13}C NMR ($CDCl_3$, 125 MHz) see table 3.8 (page 74).

*2.4.1.9. Compound **CBT9**: Veratraldehyde*

White, amorphous powder

Molecular formula: $C_9H_{10}O_3$

Molecular mass: 166

Spectrum data ^{1}H NMR (CD_3OD, 500 MHz) và ^{13}C NMR (CD_3OD, 125 MHz) see table 3.9 (page 74).

*2.4.1.10. Compound **CBT10**: Ergosta-4,6,8(14), 22-tetraen-3-one*

Yellow, amorphous powder

Molecular formula: $C_{28}H_{40}O$

Molecular mass: 392

Spectrum data ^{1}H NMR (CD_3OD, 500 MHz) và ^{13}C NMR (CD_3OD, 125 MHz) see table 3.10 (page 76).

*2.4.1.11. Compound **CBT11**: Afzelin*

Yellow, amorphous powder

Molecular formula: $C_{21}H_{20}O_{10}$

Molecular mass: 432

Spectrum data ^{1}H NMR (CD_3OD, 500 MHz) và ^{13}C NMR (CD_3OD, 125 MHz) see table 3.11 (page 77).

2.4.2. Physical parameters and spectral data of compounds isolated from the leaves of Cinnamomum bejolghota (Buch.- Ham. ex Nees) Sweet

*2.4.3.2.1. Compound **CBL1**: 3,6-dimethoxy-9H-pyrido[3,4-b]indole (new substance)*

Yellow solid

Melting point: 142-143°C

Molecular formula: $C_{13}H_{12}N_2O_2$

Molecular mass: 228

HR-ESI-MS spectrum *m/z* 229,0972 $[M + H]^+$ (theoretical calculation for the formula $[C_{13}H_{13}N_2O_2]^+$ *m/z* 229,0977) (Figure 3.37 page 79).

Spectrum data ^1H NMR ($CDCl_3$, 500 MHz) và ^{13}C NMR ($CDCl_3$, 125 MHz) see table 3.12 (page 81).

*2.4.3.2.2. Compound **CBL2**: 4α,10α-dihydroxyaromadendrane-13-oic acid (new substance)*

Oil, colorless

Molecular formula: $C_{15}H_{24}O_4$

Molecular mass: 268

Extreme rotation $[\alpha]_D^{32}$: +97,68° (*c* 0,51, MeOH)

HR-ESI-MS spectrum *m/z* 286,2011 $[M + NH_4]^+$ (theoretical calculation for the formula $[C_{15}H_{28}NO_4]^+$ *m/z* 286,2018) (Figure 3.45 page 84).

Spectrum data ^1H NMR ($(CD_3)_2SO$, 600 MHz) và ^{13}C NMR $(CD_3)_2SO$, 150 MHz) see table 3.13 (page 88).

*2.4.3.2.3. Compound **CBL3**: 4β,10α-dihydroxyaromadendrane*

White, amorphous powder

Molecular formula: $C_{15}H_{26}O_2$

Molecular mass: 238

Extreme rotation $[\alpha]_D^{28}$: -1,63 (*c* 0,2, MeOH)

Spectrum data ^1H NMR ($CDCl_3$, 500 MHz) và ^{13}C NMR ($CDCl_3$, 125 MHz) see table 3.14 (page 89).

*2.4.3.2.4. Compound **CBL4**: Litseachromolaevane A*

White, amorphous powder

Molecular formula: $C_{15}H_{22}O_2$

Molecular mass: 234

Spectrum data ^1H NMR ($CDCl_3$, 500 MHz) và ^{13}C NMR ($CDCl_3$, 125 MHz) see table 3.15 (page 90).

*2.4.3.2.5. Compound **CBL5**: Curcumin*

Dark yellow, amorphous powder

Molecular formula: $C_{21}H_{20}O_6$

Molecular mass: 368

Spectrum data ^{1}H NMR ($CDCl_3$, 500 MHz) và ^{13}C NMR ($CDCl_3$, 125 MHz) see table 3.16 (page 91).

2.4.3. Physical parameters and spectral data of compounds isolated from the leaves of Cryptocarya concinna Hance

*2.4.3.1. Compound **CC1**: Atheroline*

Dark yellow, amorphous powder

Molecular formula: $C_{19}H_{15}NO_5$

Molecular mass: 337

Spectrum data ^{1}H NMR $(CD_3)_2SO$, 600 MHz) và ^{13}C NMR $(CD_3)_2SO$, 150 MHz) see table 3.17 (page 94).

*2.4.3.2. Compound **CC2**: 3- methoxynuciferine*

Yellow, amorphous powder

Molecular formula: $C_{20}H_{23}NO_3$

Molecular mass: 325

Spectrum data ^{1}H NMR ($CDCl_3$, 600 MHz) và ^{13}C NMR ($CDCl_3$, 150 MHz) see table 3.18 (page 96).

*2.4.3.3. Compound **CC3**: O-methylmoschatoline*

Chất bột màu vàng, vô định hình

Molecular formula: $C_{19}H_{15}NO_4$

Molecular mass: 321

Spectrum data ^{1}H NMR ($CDCl_3$, 600 MHz) và ^{13}C NMR ($CDCl_3$, 150 MHz) see table 3.19 (page 97).

*2.4.3.4. Compound **CC4**: Lysicamine*

Dark yellow, amorphous powder

Molecular formula: $C_{18}H_{13}NO_3$

Molecular mass: 291

Spectrum data ^{1}H NMR ($CDCl_3$, 600 MHz) và ^{13}C NMR ($CDCl_3$, 150 MHz) see table 3.20 (page 98).

*2.4.3.5. Compound **CC5**: Oxodiscoguattine*

Yellow, amorphous powder

Molecular formula: $C_{19}H_{13}NO_5$

Molecular mass: 335

Spectrum data ^{1}H NMR ($(CD_3)_2SO$, 600 MHz) và ^{13}C NMR ($(CD_3)_2SO$, 150 MHz) refer to document number [97].

*2.4.3.6. Compound **CC6**: N-trans-feruloyl-3′-0-methyldopamine*

White, amorphous powder

Molecular formula: $C_{19}H_{21}NO_5$

Molecular mass: 343

Spectrum data ^{1}H NMR (CD_3OD, 600 MHz) và ^{13}C NMR (CD_3OD, 150 MHz) see table 3.21 (page 101).

*2.4.3.7. Compound **CC7**: Goniothalesdiol A*

White, amorphous powder

Molecular formula: $C_{14}H_{18}O_5$

Molecular mass: 266

Spectrum data ^{1}H NMR (CD_3OD, 600 MHz) và ^{13}C NMR (CD_3OD, 150 MHz) see table 3.22 (page 102).

2.5. Results of testing the activity of some isolated compounds

2.5.1. Activity test results of some compounds isolated from Cinnamomum bejolghota (Buch.- Ham. ex Nees) Sweet

The cytotoxic activity test was performed according to the method described in section 2.2.3. The compounds **CBT1 – CBT5**, **CBT10**, **CBT11**, **CBL1 – CBL4** isolated from the stem bark and leaves of *C. bejolghota* were tested for in vitro cytotoxic activity on four human cancer cell lines: KB, SK-LU-1, MCF-7, HepG2. The activity test results are shown in table 3.23 page 104

2.5.2. Activity test results of isolated compounds from leaves of Cryptocarya concinna Hance

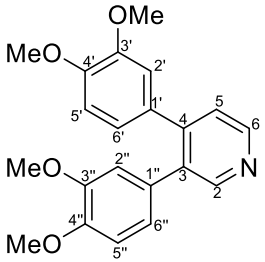
The cytotoxic activity test was performed according to the method described in section 2.2.3. Compounds **CC1 – CC7** isolated from the leaves of *C. concinna* were tested for in vitro cytotoxic activity on four human cancer cell lines: KB, A-549, MCF-7, HepG2. The activity test results are shown in table 3.24 page 105.

CHAPTER 3. DISCUSSION OF RESULTS

3.1. Determine the chemical structures of the isolated compounds

3.1.1. Structure determination of compounds isolated from the bark of Cinnamomum bejolghota (Buch.- Ham. ex Nees) Sweet

*3.1.1.1. Compound **CBT1**: 3,4-bis(3,4-dimethoxyphenyl) pyridine (new substance)*



Compound **CBT1** was obtained as a pale yellow powder with a melting point of 189-190°C. On the high resolution mass spectrum HR-ESI-MS of the compound **CBT1**, a pseudomolecular ion peak at *m/z* 352,1547 $[M + H]^+$, combined with ^{13}C-NMR spectral data consistent with the molecular formula $C_{21}H_{21}NO_4$ (theoretical calculation gave the molecular formula $[C_{21}H_{22}NO_4]^+$ *m/z* 352,1549). The IR spectrum showed the absorption peaks of the aromatic C=C ring at 1511 và 1465 cm^{-1}.

On the ^{1}H-NMR spectrum of **CBT1** signals of six aromatic protons of two aromatic rings of the ABX system appeared at δ_H 6,98 (1H, d, J = 8,0 Hz, H-5″), 6,85 (1H, dd, J = 2,0; 8,5 Hz, H-6″) và 6,68 (1H, d, J = 2,0 Hz, H-2″), 6,96 (1H, d, J = 8,0 Hz, H-5′), 6,91 (1H, dd, J = 2,0; 8,5 Hz, H-6′) and 6,69 (1H, d, J = 2,0 Hz, H-2′) demonstrating the presence of two substituted phenyl groups at position 1,3,4. Three proton signals of the pyridine ring at

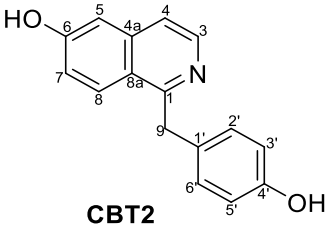
δ_H 7,50 (1H, d, J = 5,0 Hz, H-5), 8,52 (1H, d, J = 5,0 Hz, H-6) and 8,54 (1H, s, H-2) allowed to determine the presence of a substituted pyridine ring at position 3,4. In addition, signals of four methoxy groups at δ_H 3,57 (3H, s, OMe-3″), 3,60 (3H, s, OMe-3′), 3,84 (3H, s, OMe-4′), 3,85 (3H, s, OMe-4″) were also observed in the ^{1}H-NMR spectrum.

^{13}C-NMR spectrum analysis of **CBT1** combined with HSQC spectrum allowed to identify 21 carbon atoms including 9 carbon methine sp^2 ở δ_C 150,9 (C-2); 126,0 (C-5); 148,7 (C-6); 114,6 (C-2′); 112,8 (C-5′); 123,2 (C-6′); 115,1 (C-2″); 113,0 (C-5″); 123,4 (C-6″), eight sp^2 carbon atoms not directly bonded to hydrogen at δ_C 150,3 (C-4); 150,3(C-3′); 150,7 (C-4′); 150,1 (C-3″); 149,9 (4″); 137,6 (C-3); 131,7 (C-1″); 132,4 (C-1′) and four methoxy groups at δ_C 56,3; 56,4; 56,4; 56,5 The chemical shifts of the two methine groups CH-2 (δ_H 8,54; δ_C 150,9) and CH-6 (δ_H 8,52; δ_C 148,7) allow to identify them attached to the nitrogen atom. The COSY spectrum of **CBT1** shows the presence of three spin-spin interaction systems H-5′/H-6′, H-5″/H-6″ và H-5/H-6.

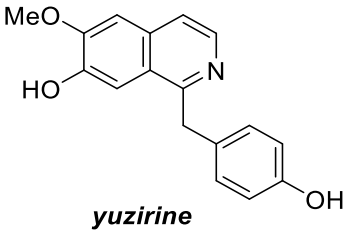
HMBC spectrum analysis of **CBT1** showed long-range interactions between H-2 with C-3/C-4/C-6, H-5 with C-3/C-6 and H-6 with C-2/C-4/C-5 confirming the pyridine ring at position 3,4. The first phenyl group attached to position C-3 of the pyridine ring was confirmed through interactions in the HMBC spectrum between H-2″ with C-1″/C-3″/C-4″/C-6″/C-3 and between H-6″ with C-2″/C-4″/C-3 The second phenyl group is attached to the C-4 position of the pyridine ring through HMBC interactions between H-2′ and C-1′/C-3′/C-4′/C-6′/C-4 and between H-6′ and C-2′/C-4′/C-4. Finally, four methoxy groups at δ_H 3,60 (OMe-3′), 3,84 (OMe-4′), 3,57 (OMe-3″), 3,85 (OMe-4″) were identified as being attached at C-3′, C-4′, C-3″ and C-4″ positions, respectively, based on the proton interactions with the carbons at C-3′, C-4′, C-3″ and C-4″ positions in the HMBC spectrum. This is also consistent with the NOESY interactions between H-2′ and 3′-OMe, between

H-5′ and 4′-OMe, between H-2″ and 3″-OMe and between H-5″ and 4″-OMe in the NOESY spectrum. From the HR-ESI-MS, 1D-NMR, 2D-NMR spectral data, the structure of compound **CBT1** was determined to be 3,4-bis(3,4-dimethoxyphenyl) pyridine and is a new compound.

*3.1.1.2. Compound **CBT2**: 1-(4-hydroxybenzyl)-6-hydroxyisoquinoline (new substance)*



CBT2



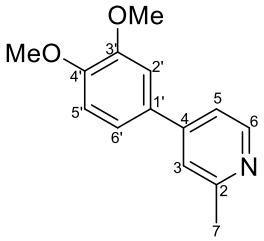
yuzirine

Compound **CBT2** was obtained as a yellow powder, melting point 270-271°C. On the high-resolution HR-ESI-MS mass spectrum of compound **CBT2**, a pseudomolecular ion peak appeared at *m/z* 252,1023 $[M + H]^+$, combined with ^{13}C-NMR spectral data consistent with the molecular formula of $C_{16}H_{13}NO_2$ (theoretical calculation for the formula $[C_{16}H_{14}NO_2]^+$ *m/z* 252,1025). On the ^{1}H-NMR spectrum of compound **CBT2**, signals of 5 protons belonging to the isoquinoline ring substituted at position 1,6 appeared at δ_H 8,17 (1H, d, J = 5,5 Hz, H-3); 7,88 (1H, d, J = 5,5 Hz, H-4); 7,12 (1H, dd, J = 2,5, 8,5 Hz, H-7); 7,51 (1H, d, J = 2,0 Hz, H-5); 7,44 (1H, d, J = 8,5 Hz, H-8) and signals of 4 protons belonging to the A_2B_2 system of 1,4-substituted phenyl groups at δ_H 7,13 (2H, d, J = 9,0 Hz, H-2′,6′); 6,70 (2H, d, J = 9,0 Hz, H-3′,5′). The ^{1}H-NMR spectrum also showed the signal of a methylene group in singlet form at δ_H 4,39 (2H, s, CH_2-9). ^{13}C-NMR spectrum and HSQC spectrum of compound **CBT2** showed signals of 16 carbon atoms including one sp^3 methylene group at δ_C 39.6; nine sp^2 methine groups at δ_C 137,2 (C-3); 114,4 (C-4); 106,6 (C-5); 117,6 (C-7); 113,5 (C-8); 130,6 (C-2′,6′); 116,3 (C-3′,5′) and six carbons not directly bonded to hydrogen at δ_C 145,8 (C-1); 123,0 (C-4a); 152,3 (C-6); 137,0 (C-8a); 130,8 (C-1′); 157,0 (C-4′). Analysis of ^{1}H-NMR and ^{13}C-NMR data revealed that the structure of **CBT2** is an isoquinoline alkaloid, similar to that of yuzirine, except for the absence of the methoxy group and the different position of the hydroxy group on the isoquinoline ring.

HMBC spectrum analysis of **CBT2** revealed interactions between H-4 with C-3/C-4a/C-8a, H-3 with C-4a/C-1, H-5 with C-6/C-7/C-8a, H-7 with C-5/C-8a and H-8 with C-4a/C-6 of the 1,6-substituted isoquinoline

ring. The methylene group was determined to be attached to the C-1 position of the isoquinoline ring and C-1′ of the phenyl group based on the HMBC interactions of H-9 with C-1/C-8a/C-1′/C-2′,6′. Combining the spectral data, compound **CBT2** was identified as 1-(4-hydroxybenzyl)-6-hydroxyisoquinoline and a new compound.

*3.1.1.3. Compound **CBT3**: 4-(3,4-dimethoxyphenyl)-2-methyl pyridine (new substance)*



Compound **CBT3** was isolated as a yellow powder, melting point 230-231°C. On the high-resolution HR-ESI-MS mass spectrum of compound **CBT3**, a pseudomolecular ion peak appeared at *m/z* 230,1179 $[M + H]^+$, combined with ^{13}C-NMR spectral data consistent with the molecular formula of $C_{14}H_{15}NO_2$ (theoretical calculation for the formula $[C_{14}H_{16}NO_2]^+$ *m/z* 230,1181). The ^{1}H-NMR spectrum shows the signals of three protons belonging to the 2,4-substituted pyridine ring at δ_H 7,50 (1H, dd, J = 1,0; 5,0 Hz, H-5); 8,41 (1H, d, J = 5,0 Hz, H-6); 7,58 (1H, s, H-3) and three aromatic protons of the 1,3,4-substituted benzene ring with δ_H 7,10 (1H, d, J = 8,5 Hz, H-5′); 7,36 (1H, dd, J = 2,5; 8,5 Hz, H-6′); 7,33 (1H, d, J = 2,5 Hz, H-2′). In addition, signals of two methoxy groups at δ_H 3,95 (3H, s); 3,91 (3H, s) and a methyl group at δ_H 2,60 (3H, s, H-7). ^{13}C-NMR spectrum analysis combined with HSQC spectrum showed signals of 14 carbon atoms, including a methyl group at δ_C 23,8 (C-7), two methoxy groups at δ_C 56,7 (3′-OMe); 56,5 (4′-OMe), six sp^2 methine groups at δ_C 122,2 (C-3); 119,9 (C-5); 149,7 (C-6); 111,7 (C-2′); 113,3 (C-5′); 121,1 (C-6′), five carbons do not interact directly with hydrogen at δ_C 131,8 (C-1′); 150,8 (C-4); 151,1 (C-3′); 151,9 (C-4′); 159,6 (C-2). The COSY spectrum of **CBT3** shows two spin-spin interactions between H-5/H-6 and H-5′/H-6′.

The 2,4-substituted pyridine ring and the 1,3,4-substituted benzene ring are linked via C-4 and C-1′, based on the HMBC interactions between H-3 with C-2/C-5/C-1′, H-5 with C-3/C-6/C-1′, H-6 with C-2/C-4/C-5, H-2′ with C-3′/C-6′/C-4, H-6′ with C-2′/C-4′/C-4, and H-5′ with C-1′/C-3′. Additionally, interactions between the methyl protons with C-2/C-3 suggest that this group is attached at C-2. Two methoxy groups were attached at C-

3′ and C-4′ based on the interactions of methoxy protons at δ_H 3,95 with C-3′, δ_H 3,91 with C-4′. From the 1D-NMR, 2D-NMR, HR-ESI-MS spectral data, compound **CBT3** was identified as 4-(3,3-dimethoxyphenyl)-2-methyl pyridine. This compound has been synthesized previously, however, this is the first report of **CBT3** from natural origin.

*3.1.1.4. Compound **CBT4**: Spathulenol*

*3.1.1.5. Compound **CBT5**: 5,7-di-O-methyl-3',4'-methylenedioxyflavan-3-ol*

*3.1.1.6. Compound **CBT6**: 3,4-dimethoxycinnamyl alcohol*

*3.1.1.7. Compound **CBT7**: 3,4-dimethoxycinnamaldehyde*

*3.1.1.8. Compound **CBT8**: veratric acid*

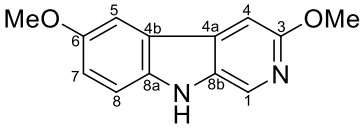
*3.1.1.9. Compound **CBT9**: veratraldehyde*

*3.1.1.10. Compound **CBT10**: Ergosta-4,6,8(14),22-tetraen-3-one*

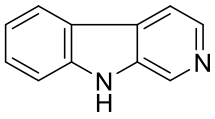
*3.1.1.11. Compound **CBT11**: Afzelin*

3.1.2. Structure determination of compounds isolated from the leaves of Cinnamomum bejolghota

*3.1.2.1. Compound **CBL1**: 3,6-dimethoxy-9H-pyrido[3,4-b]indole (new substance)*



CBL1



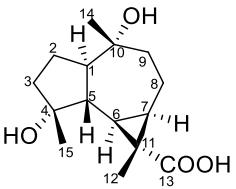
norharmane

Compound **CBL1** was obtained as a yellow powder, melting point 142 – 143°C. On the high resolution HR-ESI-MS mass spectrum of compound **CBT1**, a pseudomolecular ion peak appeared at *m/z* 229,0972 $[M + H]^+$, combined with ^{13}C-NMR spectral data consistent with the molecular formula $C_{13}H_{12}N_2O_2$ (theoretical calculation for the formula $[C_{13}H_{13}N_2O_2]^+$ *m/z* 229,0972). On the ^{1}H-NMR spectrum of compound **CBL1**, signals of three aromatic protons of the ABX system appeared at δ_H 7,50 (1H, d, J = 2,0 Hz, H-5); 7,17 (1H, dd, J = 2,0, 8,0 Hz, H-7) and 7,34 (1H, d, J = 8,0 Hz, H-8) and two protons in singlet form at δ_H 8,40 (1H, s, H-1); 7,30 (1H, s, H-4). In addition, there are signals of two methoxy groups at δ_H 3,92 (3H, s, 6-

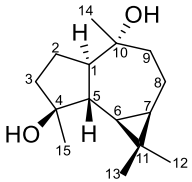
OMe); 4,02 (3H, s, 3-OMe). The ^{13}C-NMR spectrum combined with the HSQC spectrum of **CBL1** shows signals of 13 carbon atoms, including 5 sp^2 methine groups at δ_C 104,1 (C-5); 118,8 (C-7); 112,2 (C-8); 129,0 (C-1); 98,8 (C-4), two methoxy groups at δ_C 54,1 (3-OMe); 56,1 (6-OMe), six carbon signals not directly bonded to hydrogen. The shifts of the carbons at δ_C 133,7 (C-8b); 137,2 (C-8a), 158,0 (C-3); 153,9 (C-6) allow to determine that these carbons are directly bonded to oxygen and nitrogen. These data suggest the structure of a β-carboline alkaloid compound [109] containing two methoxy groups. In the COSY spectrum of compound **CBL1**, a spin-spin interaction system of the aromatic ring between H-7 and H-8 was observed

In the HMBC spectrum of **CBL1**, we see long-distance interactions between H-5 with C-7/C-8a/C-6, interaction between H-7 with C-5/C-8a and interaction between H-8 with C-4b/C-6 allowing to determine the substituted A ring at 3 positions 1, 3, 6. Interaction between H-1 with C-8b/C-3, interaction between H-4 with C-4a/C-4b/C-8b are also observed in the HMBC spectrum. In addition, HMBC interactions between two methoxy groups at δ_H 4,02 (3H, s, 3-OMe) and 3,92 (3H, s, 6-OMe) with C-3 and C-6 respectively allow to determine the positions of two methoxy groups attached to C-3 and C-6. From the 1D-NMR and 2D-NMR spectral data, the structure of compound **CBL1** was determined to be 3,6-dimethoxy-9H-pyrido[3,4-b]indole and was a new compound.

*3.1.2.2. Compound **CBL2**: 4α,10α-dihydroxyaromadendrane-13-oic acid (new substance)*



CBL2



4β,10α-dihydroxyaromadendrane

Compound **CBL2** was obtained as a colorless oil, polar rotation $[\alpha]_D^{32}$: +97,68 (c 0,51, MeOH). On the high-resolution mass spectrum HR-ESI-MS of compound **CBL2**, a pseudomolecular ion peak appeared at *m/z* 286,2011 $[M + NH_4]^+$ (Theoretical calculation for molecular formula $[C_{15}H_{28}NO_4]^+$, *m/z* 286,2018), combined with ^{13}C-NMR and HSQC spectral data consistent with the molecular formula $C_{15}H_{24}O_4$. On the ^{1}H-NMR spectrum of compound **CBL2**, signals of three methyl singlet groups appeared at δ_H 1,08 (3H, s, H-12); 1,06 (3H, s, H-15); 0,93 (3H, s, H-14), signals of four methylene groups at δ_H 0,81 (1H, m, H-8a); 1,70 (1H, m, H-8b); 1,56 (1H, m, H-2a); 1,73 (1H, m, H-2b); 1,31 (1H, m, H-3a); 1,54 (1H, m, H-3b); 1,44 (1H, m, H-9a); 1,59 (1H, m, H-9b), signals of four methine groups at δ_H 2,07 (1H, ddd, J = 4,2, 10,8, 10,8 Hz, H-1); 1,58 (1H, m, H-6); 1,36 (1H, m, H-7); 0,82 (1H, m, H-5). There are also signals of two protons belonging to two hydroxyl groups at δ_H 4,02 (1H, br. s); 4,05 (1H, br. s).

The ^{13}C-NMR spectrum combined with the HSQC spectrum of **CBL2** showed signals of 15 carbon atoms, including three methyl groups at δ_C 11,0 (C-12); 19,6 (C-14); 25,5 (C-15), four methylene groups at δ_C 19,4 (C-8); 23,6 (C-2); 40,2 (C-3); 43,4 (C-9), four methine groups at δ_C 26,3 (C-7); 26,4 (C-6); 46,0 (C-5); 53,6 (C-1), three carbon atoms not directly bonded to hydrogen at δ_C 73,4 (C-10); 78,0 (C-4); 27,1 (C-11), and a carbonyl group at δ_C 178,3 (C-13). The above spectral data allow to predict the structure of CBL2 as a sesquiterpene. The COSY spectrum of CBL2 shows a system of spin-spin interactions between H-3 with H-2/H-1/H-5/H-6/H-7/H-8/H-9. Observation on the HMBC spectrum, the interactions between H-12 with C-11/C-13/C-6/C-7 allow to determine the position of the CH_3-12 group and the -COOH group attached at position C-11. The interactions between H-14 with C-10/C-1/C-9 allow to determine the position of the CH_3-14 group attached at position C-10, the interactions between H-15 with C-4/C-3/C-5 allow to determine the position of the CH_3-15 group attached at position C-4. Thus, two hydroxyl groups were identified at positions C-4 and C-10. In

addition, the HMBC spectrum also showed interactions between H-1 and C-5/C-10/C-14, between H-5 and C-1/C-4/C-10/C-15, between H-6 and C-1/C-4/C-5, between H-2a and C-1/C-5/C-4, between H-3b and C-4/C-5/C-1, between H-8b and C-6/C-7/C-10, between H-9a and C-7/C-8/C-10/C-14, between H-9b and C-1/C-10.

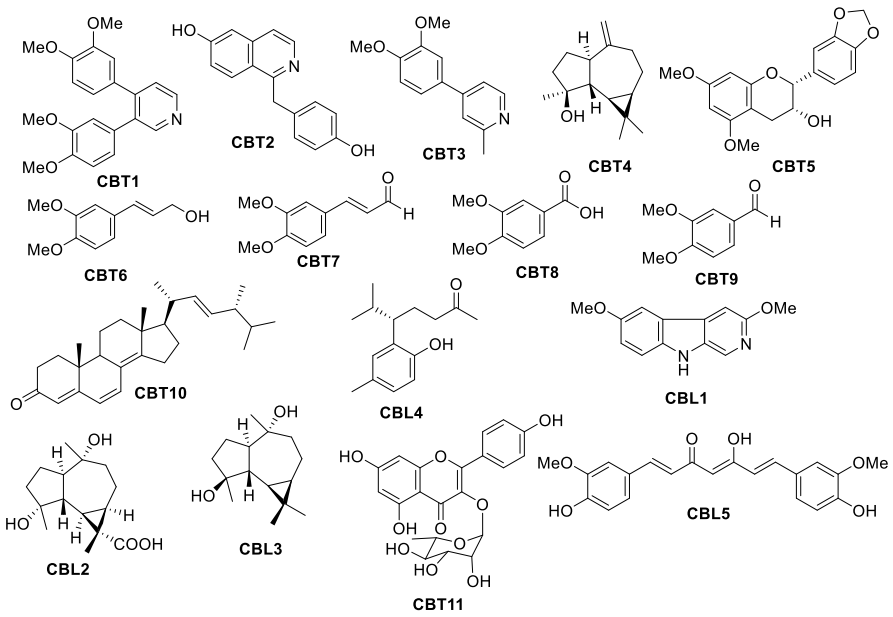
The relative configuration of compound **CBL2** was determined based on comparison with reference NMR spectral data, coupling constant values and analysis of interactions on the NOESY spectrum of compound **CBL2**. The coupling constant J = 10,8 between the H-5 proton and the H-1/H-6 proton demonstrates that the H-5 proton has a trans configuration with the H-1 proton and the H-6 proton. This is also demonstrated by the absence of observed NOESY interactions of the H-5 proton with the H-1 proton and the H-6 proton. The NOESY interactions of the H-5 proton (β-oriented) with the CH_3-14 proton and the CH_3-15 proton showing that the CH_3-14 and CH_3-15 groups are β-oriented. In addition, comparison of the ^{13}C-NMR chemical shifts of the methyl group CH_3-12 (δ_C 11.0) and the carboxylic acid group C-13 (δ_C 178.3) of compound **CBL2** with the reference compounds 10β,14α-dihydroxy-alloaromandendran-12-oic acid (C-12 (δ_C 23.7); C-13 (δ_C 174.5)) [110] and soltorvum D (C-12 (δ_C 11.7), C-13 (δ_C 179.5)) [111] helped to determine the relative configuration of the methyl group CH_3-12 là β and the carboxylic acid group C-13 as α. Thus, compound **CBL2** was identified as 4α,10α-dihydroxyaromadendrane-13-oic acid and is a new compound.

*3.1.2.3. Compound **CBL3**: 4β,10α-dihydroxyaromadendrane*

*3.1.2.4. Compound **CBL4**: Litseachromolaevane A*

*3.1.2.5. Compound **CBL5**: Curcumin*

3.1.3. Synthesis of compounds isolated from Cinnamomum bejolghota (Buch.- Ham. ex Nees) Sweet



3.1.4. Structure determination of compounds isolated from Cryptocarya concinna species

*3.1.4.1. Compound **CC1**: Atheroline*

*3.1.4.2. Compound **CC2**: 3- methoxynuciferine*

*3.1.4.3. Compound **CC3**: O-methylmoschatoline*

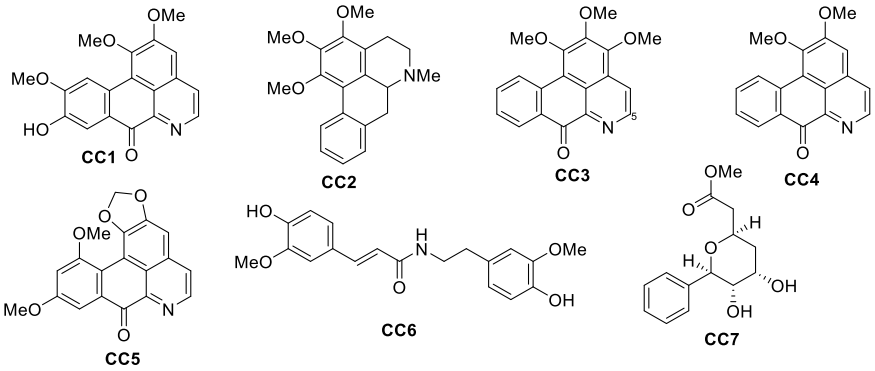
*3.1.4.4. Compound **CC4**: Lysicamine*

*3.1.4.5. Compound **CC5**: Oxodiscoguattine*

*3.1.4.6. Compound **CC6**: N-trans-Feruloyl 3'-O-methyldopamine*

*3.1.4.7. Compound **CC7**: Goniothalesdiol A*

3.1.5. Synthesis of compounds isolated from the golden fruit fly Cryptocarya concinna Hance



3.2. Cytotoxic activity of some isolated compounds

3.2.1. Cytotoxic activity of some compounds isolated from Cinnamomum bejolghota (Buch.- Ham. ex Nees) Sweet

Compounds **CBT1-CBT5**, **CBT10-CBT11**, **CBL1-CBL4** were evaluated for cytotoxic activity against four human cancer cell lines: KB, SK-LU-1, MCF-7, HepG2 (Table 3.23)

Among the tested compounds, only three compounds **CBT1**, **CBT5**, **CBT10** showed cytotoxic activity against four tested cancer cell lines. Among them, the new compound 3,4-bis(3,4-dimethoxyphenyl) pyridine (**CBT1**) showed activity with IC_{50} values ranging from 31,1 to 48,8 µM. Compounds 5,7-di-*O*-methyl-3',4'-methylenedioxyflavan-3-ol (**CBT5**) and 22-tetraen-3-one (**CBT10**) showed weak activity with IC_{50} values ranging from 72,3 to 118,3 µM, while the remaining compounds showed no activity against the tested cell lines. Comparison of IC_{50} values of compounds **CBT1** and **CBT3** on four cancer lines showed that the presence of 3,4-dimethoxyphenyl group on the pyridine ring resulted in better activity. However, as demonstrated by Zheng et al., the position of methoxy and hydroxy groups on aromatic rings and the position of phenyl groups on the pyridine ring are decisive for cytotoxic activity. In addition, according to literature review, compound **CBT5** has antioxidant activity with IC_{50} value of 0,07 mM. In the report of Wi and colleagues, compound **CBT10** was

tested for cytotoxic activity against 4 human cancer cell lines: HT-29 (colon cancer), HeLa 229 (cervical cancer), Hep3B (liver cancer) and AGS (stomach cancer). The results showed that **CBT10** inhibited the growth of all 4 cancer cell lines with IC_{50} values of 5,0; 7,2; 26,3 and 22,0 µg/mL, respectively.

Table 3.23. Results of cytotoxic activity testing of some compounds isolated from Cinnamomum bejolghota (Buch.- Ham. ex Nees) Sweet

Compound	IC_{50} (µM)			
	KB	MCF7	HepG2	SK-LU-1
CBT1	48,8±1,8	45,9±2,7	37,0±1,9	31,1±2,1
CBT2	>296	> 296	> 296	> 296
CBT3	>296	> 296	> 296	> 296
CBT4	>296	> 296	> 296	> 296
CBT5	118,3±8,0	110,7±10,4	90,2±3,8	95,8±7,8
CBT10	117,3±3,3	107,8±3,0	72,3±0,1	> 296
CBT11	>296	> 296	> 296	> 296
CBL1	>296	> 296	> 296	> 296
CBL2	>296	> 296	> 296	> 296
CBL3	>296	> 296	> 296	> 296
CBL4	>296	> 296	> 296	> 296
Ellipticine	1,66±0,2	1,33±0,16	1,50±0,16	1,54±0,16

3.2.2. Cytotoxic activity of compounds isolated from Cryptocarya concinna Hance

Compounds **CC1 – CC7** were evaluated for cytotoxic activity against four human cancer cell lines: KB, A-549, MCF-7, HepG2 (Table 3.24).

Among the tested compounds, only three compounds **CC2**, **CC3**, **CC4** showed cytotoxic activity against the tested cancer cell lines. Of which, lysicamine compound (**CC4**) showed activity against three cell lines KB,

HepG2, A-549 with IC_{50} values ranging from 23,7–36,9 µM, *O*-methylmoschatoline compound (**CC3**) showed activity against A-549 cell line with IC_{50} value of 31,4 µM better than the other three cancer cell lines. Compound **CC2** showed weak cytotoxic activity against all four cancer cell lines with IC_{50} values ranging from 81,7 to 127,2 µM. The remaining compounds did not show activity against the tested cell lines. According to literature review, compounds **CC3** and **CC4** showed cytotoxic activity against Vero cell line with IC_{50} values of 7 and 8 µg/mL [123]. In addition to cancer cell cytotoxic activity, compound **CC3** also showed other activities such as antiparasitic activity against Trypanosoma cruzi strain in Trypomastigote form with EC_{50} value of 3,8 µg/mL [124], antibacterial activity against Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus cereus strains [125]. Compound **CC4** was also reported to have strong antibacterial activity against Bacillus subtilis, S. aureus and Staphylococus epidermidis strains with inhibition zones of 15,5, 13,3 and 12,0 mm, respectively [126]. And this is also the first report on the cytotoxic activity of compound **CC2** against the four tested cell lines KB, A-549, MCF-7, HepG2.

Table 3.24. Results of cytotoxic activity testing of compounds isolated from Cryptocarya concinna Hance

Compound	IC_{50} (µM)			
	KB	**MCF7**	**HepG2**	**A-549**
CC1	>296	> 296	> 296	> 296
CC2	127,2±2,4	81,7±1,1	116,4±2,7	86,1±0,9
CC3	85,6±2,1	> 296	128,1±3,2	31,4±0,1
CC4	36,1±0,1	83,5±0,8	36,9±1,4	23,7±0,3
CC5	>296	> 296	> 296	> 296
CC6	>296	> 296	> 296	> 296
CC7	>296	> 296	> 296	> 296

Compound	IC_{50} (μM)			
	KB	MCF7	HepG2	A-549
Ellipticine	1,75±0,08	1,75±0,08	1,75±0,08	1,79±0,08

CONCLUDE

By combined chromatographic methods and modern spectroscopic methods, with comparison with spectral data of similar compounds in reference documents, from two species *Cinnamomum bejolghota* (Buch.-Ham. ex Nees) Sweet and *Cryptocarya concinna* Hance graduate student isolated and determined the structure of 23 compounds and evaluated the cytotoxic activity of these compounds. Specifically:

- ***About chemical composition:***

1. From *C. bejolghota* species, 16 compounds (**CBT1-CBT11, CBL1-CBL5**) were isolated and determined the chemical structure, including 5 new compounds and 11 known compounds. The five new compounds identified were: 3,4-bis(3,4-dimethoxyphenyl) pyridine (**CBT1**); 1-(4-hydroxybenzyl)-6-hydroxyisoquinoline (**CBT2**); 4-(3,4-dimethoxyphenyl)-2-methyl pyridine (**CBT3**); 3,6-dimethoxy-9H-pyrido[3,4-b]indole (**CBL1**); 4*α*,10*α*-dihydroxyaromadendrane-13-oic acid (**CBL2**) and 11 known compounds including: spathulenol (**CBT4**); 4*β*,10*α*-dihydroxyaromadendrane (**CBL3**); (3,4-dimethoxycinnamyl alcohol (**CBT6**); 3,4-dimethoxycinnamaldehyde (**CBT7**); veratric acid (**CBT8**); veratraldehyde (**CBT9**); curcumin (**CBL5**); 5,7-di-*O*-methyl-3',4'-methylenedioxyflavan-3-ol (**CBT5**); ergosta-4,6,8(14), 22-tetraen-3-one (**CBT10**); afzelin (**CBT11**); litseachromolaevanes A (**CBL4**).

2. From *C. concinna* species, 7 compounds were isolated and determined in structure: atheroline (**CC1**); 3-methoxynuciferine (**CC2**); *O*-methylmoschatoline (**CC3**); lysicamine (**CC4**); oxodiscoguattine (**CC5**); N-*trans*-feruloyl-3′-*0*-methyldopamine (**CC6**); goniothalesdiol A (**CC7**)

- ***On biological activity:***

The cytotoxic activity of 18 compounds isolated from two species *C. bejolghota* and *C. concinna* was studied. The results showed that compound 3,4-bis(3,4-dimethoxyphenyl) pyridine (**CBT1**) showed activity against KB, MCF-7, HepG-2 and SK-LU-1 cell lines with IC_{50} values ranging from 31,1-48,8 µM, compounds *O*-methylmoschatoline (**CC3**) và lysicamine (**CC4**) showed activity against KB, HepG-2 and A-549 cell lines with IC_{50} values ranging from 23,7 – 36,9 µM, while compounds **CC2**, **CBT5**, **CBT10** had very weak cytotoxic activity and the remaining compounds did not show activity against the four tested cell lines.

PROPOSAL

Compounds isolated from 2 species *Cinnamomum bejolghota* (Buch.- Ham. ex Nees) Sweet and *Cryptocarya concinna* Hance should continue to conduct research on other biological activities, such as anti-inflammatory, antioxidant activities, ... aiming at the possibility of practical application in the future. Especially alkaloid and terpenoid compounds are compounds with unique chemical structures..

NEW CONTRIBUTIONS OF THE THESIS

1. From two species *Cinnamomum bejolghota* (Buch.- Ham. ex Nees) Sweet and *Cryptocarya concinna* Hance isolated and determined the chemical structures of 23 compounds, including five new compounds and two compounds published for the first time from *Cinnamomum bejolghota.*

- The five new identified compounds are 3,4-bis(3,4-dimethoxyphenyl) pyridine (**CBT1**); 1-(4-hydroxybenzyl)-6-hydroxyisoquinoline (**CBT2**); 4-(3,4-dimethoxyphenyl)-2-methyl pyridine (**CBT3**); 3,6-dimethoxy-9H-pyrido[3,4-b]indole (**CBL1**); 4*α*,10*α*-dihydroxyaromadendrane-13-oic acid (**CBL2**).
- The two compounds first reported from *C. bejolghota* were ergosta-4,6,8(14), 22-tetraen-3-one (**CBT10**) và afzelin (**CBT11**).

2. Five new compounds **CBT1**, **CBT2**, **CBT3**, **CBL1** and **CBL2** isolated from *C. bejolghota* were first tested for their cytotoxic activity against four cancer cell lines KB, SK-LU-1, MCF-7 and HepG2. The results showed that the new compound 3,4-bis(3,4-dimethoxyphenyl) pyridine (**CBT1**) exhibited cytotoxic activity against all four tested cancer cell lines KB, MCF-7, HepG-2 and SK-LU-1 with IC_{50} values ranging from 31,1 to 48,8 μM.
3. Compound **CC3** isolated from *C. concinna* exhibited the best activity against the lung cancer cell line A549 (IC_{50} = 31.42 μM) compared with three cancer cell lines KB, MCF-7, HepG-2. Compound **CC4** exhibited less activity against the breast cancer cell line MCF-7 (IC_{50} = 83,51 μM) compared with three cancer cell lines KB, HepG-2 and SK-LU-1 (IC_{50} ranged from 23,77-36,93 μM).