SUMMARY OF DISSERTATION

1. INTRODUCTION

Name of Ph.D. candidate: Nguyen Thi Thu

Dissertation title: Study on chemical constituents and *in vitro* anticancer activity of *Curcuma zedoaroides* Chaveer. & Tanee, Zingiberaceae.

Specialty: Medicinal Materials - Traditional Pharmacy **Code number**: 9720206

Scientific supervisors:

1. Assoc. Prof. Dr. Do Thi Ha
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2. SUMMARY

2.1. Objectives

+ Determine the chemical composition and content of essential oils and isolated chemical components in extracts of *C. zedoaroides* with *in vitro* anticancer activity.
+ Screen *in vitro* the cytotoxic activity of essential oils, extracts, and isolated compounds on cancer cell lines and evaluate the effects of potential compounds on the expression of relevant proteins.

2.2. Methods

2.2.1. Phytochemical study

+ Essential oils in medicinal materials were quantified using the hydrodistillation method according to Appendix 12.7 of the Vietnamese Pharmacopoeia V.
+ The composition of essential oils was determined by GC-MS.
+ The total extract of the plant materials was obtained by maceration with 70% ethanol, and liquid-liquid extraction was used to yield fractionated extracts.
+ Compounds were isolated using column chromatography, and the fractions were monitored with thin-layer chromatography. Detection of compounds was performed by spraying a 10% H_2SO_4 in 96% ethanol, heating, and observing under UV light at wavelengths of 254 nm and 365 nm.
+ The structures of the compounds were elucidated using spectral methods, including mass spectrometry (ESI-MS), nuclear magnetic resonance (NMR), and circular dichroism (CD), along with comparisons to reference data.
+ The volatile components in the *n*-hexane extract were determined by GC-MS.
+ Quantification of the main and potential compounds in the rhizome and aerial parts was performed using HPLC-DAD.

2.2.2. Anticancer activity evaluation

+ Screening for cytotoxicity against cancer cell lines *in vitro* using the MTT and SRB methods.
+ Investigating the protein expression of potential compounds using the Western blot method.
+ Studying the relationship between structure and anticancer activity through molecular docking method.

2.3. Results and Conclusion

2.3.1. Chemical constituents

- The content and chemical components in the essential oils of the rhizome (**EOR**), pseudostem (**EOPS**), and leaf (**EOL**) of *C. zedoaroides* were elucidated:
  + The rhizome essential oil (0.84%) contained 46 components, predominantly oxygenated sesquiterpenes. Components with content greater than 4% included curdion, widdrol, and *trans*-*β*-elemenone.
  + The pseudostem essential oil (0.10%) comprised 47 components, primarily consisting of oxygenated sesquiterpenes. Compounds present at levels greater than 5% included curzerene, *trans*-*β*-elemenone, curdion, *β*-elemen, (*E*)-*β*-farnesene epoxide, and humulene.
  + The leaf essential oil (0.38%) contained 48 components, with oxygenated sesquiterpenes as the dominant group. Constituents present at levels higher than 4% included 1,8-cineole, camphor, *trans*-*β*-elemenone, (*E*)-*β*-farnesene epoxide, and curzerene.
- Fourteen compounds were isolated and structurally determined from *C. zedoaroides*, including: phaeocaulisin E (**R1**), (1*R*,4*S*,5*S*,10*R*)-zedoarondiol (**R2**), (1*S*,4*S*,5*S*,10*R*)-zedoarondiol (**R3**), isoprocurcumenol (**R4**), neoprocurcumenol (**R5**), procurcumenol (**R6**), 1-*epi*-procurcumenol (**R7**), aerugidiol (**R8**), curcumenol (**R9**), curcumenon (**R10**), curcuminol E (**R11**), zerumin A (**R12**), curdion (**AP1**), and *β*-sitosterol (**AP2**).
- The volatile components in the rhizome *n*-hexane extract (**RH**) and the aerial part (**APH**) of *C. zedoaroides* were analyzed:
  + In the **RH** extract, 16 components were identified, with curdion, ambrial, curcumenon, procurcumenol, and (*E*)-labda-8(17),12-dien-15,16-dial found in high contents.

+ In the **APH** extract, 11 components were identified, with curcudion and curcumenon present in significant amounts.

– The rhizome and aerial parts of *C. zedoaroides* contained curdion (**AP1**) at concentrations ranging from 0.322 to 0.502% and (1*R*,4*S*,5*S*,10*R*)-zedarondiol (**R2**) from 0.017 to 0.071%.

2.3.2. Anticancer activity

+ *In vitro*, the essential oils of *C. zedoaroides*' rhizomes (**EOR**, IC_{50}: 23.14 – 83.67 µg/mL) and leaves (**EOL**, IC_{50}: 43.88 – 81.32 µg/mL) showed weak cytotoxic activity against cancer cell lines.

+ The rhizome of *C. zedoaroides* exhibited the greatest *in vitro* cytotoxic activity against cancer cell lines (**RH**, IC_{50}: 5.43 – 11.96 µg/mL), whereas the EtOAc (**RE**, IC_{50}: 7.61 – 11.96 µg/mL) and water extracts (**RW**, IC_{50}: 7.53 – 11.88 µg/mL) displayed comparable activity. On the other hand, the aerial parts' *n*-hexane extract had a lower effect (**APH**, IC_{50}: 49.76 – 86.30 µg/mL).

+ Ten compounds (**R1**–**R9**, **R11**, and **R12**) showed the highest *in vitro* cytotoxic effect on the A549 cell line (IC_{50}: 3.13 – 13.54 µM). Among them, **R2** (IC_{50}: 3.64 – 11.91 µM), **R8** (IC_{50}: 7.22 – 12.03 µM), and **R11** (IC_{50}: 3.13 – 10.98 µM) showed greater activity.

+ Compound **R8** (aerugidiol, 0.3 – 1 µM) enhanced the expression of p53 and p21 proteins, and the effect on p53 increased alongside the tested concentration. It also demonstrated high binding affinities for HER2 (ΔG = -8.613 kcal/mol) and EGFR (ΔG = -7.209 kcal/mol).

Hanoi, Octctober 29, 2024

THE SCIENTIFIC SUPERVISORS **Ph.D. CANDIDATE**

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