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Research on Extraction and Determination of the Chemical compositions from Rhizomes of three species of *Curcuma*: *Curcuma longa* Linn., *Curcuma aeruginosa* Roxb. and *Curcuma mangga* Valeton & Zijp. collected in Champasack province, Laos

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SUMMARY OF CHEMISTRY DOCTORAL THESIS

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INTRODUCTION

1. Introduction

Curcumin is a major chemical composition of turmeric, which has long been known as a plant-based active ingredient, it plays an important role in the food, cosmetic and pharmaceutical industries. Curcuma is one of the very popular plants that were used nearly 4000 years ago, derived from the AyerVeda culture in India, it is added to most dishes whether it is meat or vegetables. Today, Curcuma is a valuable source of high economic value. Therefore, the research on extraction, determination of the chemical composition and the compound's formulation extracted from Curcuma is extremely important and necessary. Many researches in countries around the world have shown that curcumin has high biological activity such as protect the liver, pain relief, anti-cancer, anti-ulcer, anti-fungal, anti-bacterial, antioxidant. Due to the valuable biological activity of curcumin, so the research on extraction, determination of chemical composition and structure and use of curcumin are being deeply studied in many countries. Due to the importance and application of curcumin in many aspects, the research, isolation and determination of the curcumin's structure in Curcuma has important scientific significance as well as the practical application of Curcuma in Laos. So i chose the topic "Research on Extraction and Determination of the Chemical compositions from the Rhizomes of three species of Curcuma : Curcuma longa Linn., Curcuma aeruginosa Roxb. and Curcuma mangga Valeton & Zijp. collected in Champasack province, Laos" to carry out the contents of doctoral thesis.  

2. Research purposes

- Development of the process of extraction, determination of chemical composition of Curcuma in Laos by different methods; - To determine the
curcumin content in some *Curcuma* in Laos; - Isolation, determination of the formula of curcumin by spectral method.

3. **Research object and scope**

* **Research object:** Rhizome of *Curcuma* longa Linn., Rhizome of *Curcuma aeruginosa* Roxb. and Rhizome of *Curcuma mangga* Valeton & Zijp. collected in Champasack province, Laos.

* **Research scope:** Determine some physical and chemical properties of the material such as moisture, ash content, composition and content of heavy metals; Extract the essential oil of Rhizome of *Curcuma longa* Linn., *Curcuma aeruginosa* Roxb. and *Curcuma mangga* Valeton & Zijp. in Laos by means of steam distillation; Determine the physical constants and chemical indices of the essential oil of *Curcuma longa* Linn., *Curcuma aeruginosa* Roxb. and *Curcuma mangga* Valeton & Zijp. in Laos; extract the organic constituents in the dry rhizome of *Curcuma longa* Linn., *Curcuma aeruginosa* Roxb. and *Curcuma mangga* Valeton & Zijp. with solvent of n- hexane, dichloromethane, ethyl acetate and methanol; extract the curcumin in the dry rhizomes of *Curcuma longa* Linn. with KOH solution; isolation, determine the chemical structure of curcumin of *Curcuma longa* Linn.

4. **Research Method**

* **Theoretical method:** Collect, synthesize documents and data about the source of raw materials, research methods on natural compounds, chemical composition and application of *Curcuma*; Understand the method of sampling, extraction and determination of chemical compositions of plant substances; Learn about the most effective extraction methods for *Curcuma*, determine the structure of curcumin.

* **Experimental method:** Method of collecting raw materials, processing and preservation of samples. Apply weight methods, decomposing sample to investigate moisture content, ash content, heavy metal content. Extract
the essential oil by steam distillation. Determine the physical constants and chemical indices of *Curcuma* essential oil in Laos. Study and investigate the extraction process and the sample extraction of the dry rhizome of *Curcuma longa* Linn., *Curcuma aeruginosa* Roxb. and *Curcuma mangga* Valeton & Zijp. by soxhlet method with solvent of n-hexane, dichloromethane, ethyl acetate and methanol. Study on the extraction of curcumin with KOH solution. Quantify coloring from *Curcuma longa* Linn. by using molecular absorption spectroscopy (UV-vis) method and spectral method of high performance liquid chromatography (HPLC). Isolate curcumin by thin layer chromatography and column chromatography. Solution spectrometry for determination of structure by method: infrared spectrum (IR), (MS) mass spectrometry, one-way nuclear magnetic resonance spectroscopy (\(^1\text{H. NMR, }^{13}\text{C-NRM, DEPT}\)) and two-way (HSQC, HMBC, \(^1\text{H}-^{1}\text{H COSY}\)).

5. **New contributions of the thesis**

According to lookup of references:

a. For the first time, the results of a relatively comprehensive study of three species Curcuma (*Curcuma longa* Linn., *Curcuma aeruginosa* Roxb. and *Curcuma mangga* Valeton & Zijp.) were announced.  

b. For the first time, useful scientific information on the chemical composition of the rhizomes of the Curcuma in Laos has been identified: 199 chemical compounds have been identified in 3 kinds of Curcuma, of which *Curcuma longa* Linn. is 97 compounds, *Curcuma aeruginosa* Roxb. is 111 compounds and *Curcuma mangga* Valeton & Zijp. is 117 compounds.  

c. Compared the chemical constituents essential oil of Lao *Curcuma* with the chemical constituents essential oil of East Asian countries *Curcuma* have been announced. In general, the percentage of identified constituents is not much different only varies in the number of constituents.
d. A process has been developed with appropriate parameters to obtain pure colorants by alkalisation with 7.26% efficiency KOH solution, compared to dry powder of *Curcuma longa* Linn.

6. **The scientific and practical significance of the topic**

- Provide scientific information on the process and composition of some compounds in the rhizome of *Curcuma longa* Linn., *Curcuma aeruginosa* Roxb. and *Curcuma mangga* Valeton & Zijp. in Laos. Orient the scientific application of *Curcuma* species in Laos to industry and life. Develop a curcumin extraction process in the rhizome of *Curcuma* in Laos with industrial scale, create healthy curcumin products in traditional medicine and modern medicine. Curcumin product helps local businesses in Laos actively prepare the source of raw materials as well as restrict imports from abroad. Raise income and create more jobs for employees, shift the plant structure, develop economy in rural areas of Laos.

7. **The Thesis's structure**

The thesis consists of 129 pages, consisting of parts:

Introduction: 4 pages

Chapter 1: Overview, 32 pages

Chapter 2: Materials and research methods, 13 pages

Chapter 3: Results and discussion, 68 pages.

Conclusion and Recommendations 12 pages

**CHAPTER 1. OVERVIEW**

**1. Introduction to the plant belonging to Curcuma genus, ginger family**

1.1. **Learn about the Curcuma genus, ginger family**

The ginger (zingiberaceae) family includes 47 genera and about 1300 species, in Vietnam and Indochinese countries, *Curcuma* genus includes 19 species, in Bangladesh there are 16-20 species, in India, China and Indochina, there are 20-25 species, in Malaysia, there are 16-20 species, in Nepal, there are 10-15 species, in Philippines, there are 12-15 species, in
Thailand, there are 30-40 species. Therefore, there is generally no consensus among the literature on the number of species in the *Curcuma* genus.

1.2. **Plant characteristics, distribution and chemical composition of some Curcuma species and summary of Curcuma research situation**

Turmeric (*Curcuma*) belonging to Ginger family (zingiberaceae) is not only a perennial herb, it can reach a height of more than 1 meter. The plant creates branch highly, with cylindrical form, strong rhizome, fleshy and has many branches, rhizome, fragrant smell, grows into tuber. Leaves are flat with spearhead or oval shape, grow with flowers or grow after flowers, the leaves have ochrea at root and are distinct from each other by leaf-blade in the middle. The flowers usually have the cylindrical shape with some flowers, characteristic colors, sometimes it only has some flowers with egg shape and hairless. Turmeric has a bitter and spicy taste, acridity. *Curcuma* origins from the tropics of Tamil Nadu in the southeastern part of India. Today, *Curcuma* is a common plant in tropical countries: India, Vietnam, Laos, China, Indonesia, Cumpuchia, Thailand ...

1.2.1. *Curcuma aromatica* Salisb.
1.2.2. *Curcuma longa* Linn.
1.2.3. *Curcumina zedoaria* Roscoe.
1.2.4. *Curcuma xanthorhiza* Roxb.
1.2.5. *Curcuma aeruginosa* Roxb.
1.2.6. *Curcuma elata* Roxb.
1.2.7. *Curcuma pierreana* Gagnep.
1.2.8. *Curcuma cochinchinnenis* Gagnep.
1.2.9. *Curcuma sp. aff. Rubescens*.

1.3. Some Curcuma species are found in Laos
In Laos, the tropical climate is monsoon, with lots of light, high rainfall, high humidity, so plants grow very strong. The *Curcuma* genus in Laos has 4 species of *curcuma* species including:

1.3.1. *Curcuma longa* Linn.
1.3.2. *Curcuma aeruginosa* Roxb.
1.3.3. *Curcuma mangga* Valeton & Zijp.
1.3.4. *Curcuma aromatica*.

These species have not been studied in Laos.

1.4. The situation of researching on *Curcuma longa* Linn. *Curcuma aeruginosa* Roxb. and *Curcuma mangga* Valeton & Zijp. domestic and foreign

1.4.1. *Curcuma longa* Linn.
1.4.2. *Curcuma aeruginosa* Roxb.
1.4.3. *Curcuma mangga* Valeton & Zijp.

1.5. Use of some species of turmeric belonging to *Curcuma* genus

Turmeric has many applications such as coloring, spice, treatment for diseases such as stomach pain, arthritis, wounds, abdominal pain, cough, irregular menstruation, tumor treatment, liver detoxification....

1.6. Research history on the structure of curcumin

- In 1953, Srinivasan K.R demonstrated by chromatography on a silic column that curcumin is a mixture, of which curcumin I accounts for 60%, curcumin II accounts for 24% and curcumin III accounts for 14%.

1.6.1. Structure of Curcumin

- In nature curcumin exists in four forms as:
  - Curcumin I: Molecular formula: C_{21}H_{20}O_{6}

  Structural formula:
IUPAC Name: 1.7-bis (4-hydroxy-3-methoxyphenyl) - 1.6-heptadiene-3.5-dione. Molecular mass: 368 g/mol, melting point: 183°C.

- Curcumin II: Molecular formula: C_{20}H_{18}O_{5}

Structural formula:

IUPAC Name: 1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1.6-diene-3.5-dione. Molecular mass: 338 g/mol, melting point: 168°C.

- Curcumin III: Molecular formula: C_{19}H_{16}O_{4}

Structural formula:

IUPAC Name: 1.7-bis-(4-hydroxyphenyl)-hepta-1.6-diene-3.5-dione. Molecular mass: 308 g/mol, melting point: 224°C.

- Curcumin IV: Curcumin IV exists in two types of equilibrium: keton and enol.
In addition, a new compound found is cyclocurcum accounting for 1%.

![Cyclocurcumin](image)

1.6.2. Physical properties of curcumin
1.6.3. Chemical properties of curcumin
1.6.4. Biological activities

1.7. Curcumin application
1.7.1. In the medical profession
1.7.2. In industry
1.7.3. Some folk remedies used in life
1.7.4. Nano curcumin

1.8. General comment

CHAPTER 2: MATERIALS AND RESEARCH METHODS

2.1. Materials

2.2. Research methods

2.2.1. Mass analysis method
  a. Determination of moisture content of raw materials; b. Determination of ash content

2.2.2. Atomic absorption spectroscopy method AAS

2.2.3. Identification Method of Chemical Composition GC-MS

2.2.4. Methods of researching essential oil

2.2.5. Research methods of organic extract

2.2.6. Curcumin extraction with KOH solution
2.2.7. Isolation to determine the formula of curcumin

CHAPTER 3: RESULTS AND DISCUSSION

3.1. Survey results of some physico-chemical indicators

3.1.1. Determine the moisture, ash content of the material
- Results obtained: average moisture content of fresh *Curcuma longa* Linn. is 91.810%; fresh *Curcuma aeruginosa* Roxb. is 90.521% and fresh *Curcuma mangga* Valeton & Zijp. is 74.543%. The results show that this moisture is very high so after harvest should be dried to not damage the material. - Results obtained: average moisture content of dry *Curcuma longa* Linn. is 11.596%; dry *Curcuma aeruginosa* Roxb. is 5.976% and dry *Curcuma mangga* Valeton & Zijp. is 5.636%. The results show that this moisture is within the allowable moisture content of medicinal herbs (10-12%) according to Pharmacopoeia IV of the Ministry of Health. - Ash content of dry turmeric powder of *Curcuma* species in Laos obtained: *Curcuma longa* Linn. is 8.883%, *Curcuma aeruginosa* Roxb. is 7.671% and *Curcuma mangga* Valeton & Zijp. is 4.663%, it is low so the content of inorganic substances and heavy metals in the material is low, turmeric has the high use value.

3.1.2. Determination of heavy metal content
The results of determination of heavy metal content (mg/kg) obtained in dry turmeric powder of *Curcuma* species in Laos: As (*Curcuma longa* Linn.: 0.152; *Curcuma aeruginosa* Roxb.: 0.215; *Curcuma mangga* Valeton & Zijp.: 0.106), Hg (*Curcuma longa* Linn.: 0.168; *Curcuma aeruginosa* Roxb.: 0.167; *Curcuma mangga* Valeton & Zijp.: 0.093), Pb (*Curcuma longa* Linn.: 0.512; *Curcuma aeruginosa* Roxb.: 0.545; *Curcuma mangga* Valeton & Zijp.: 0.160) and Cu (*Curcuma longa* Linn.:
7,201; Curcuma aeruginosa Roxb.: 16,325; Curcuma mangga Valeton & Zijp.: 14,656). The content of these metals is lower than permitted level in Vietnam.

3.2. Research results of essential oil of Curcuma in Laos

3.2.1. Steam distillation

Results obtained: average essential oil content of Curcuma longa Linn. is 0.237%; Curcuma aeruginosa Roxb. is 0.355% and Curcuma mangga Valeton & Zijp. is 0.235%.

3.2.2. Sensory evaluation of rhizome essential oil of Curcuma in Laos

Essential oil of Curcuma longa Linn. has pale yellow, characteristic aroma and spicy taste. Essential oil of Curcuma aeruginosa Roxb. has pale black-yellow, characteristic aroma, spicy and bitter taste. Essential oil of Curcuma mangga Valeton & Zijp. has pale yellow, characteristic aroma and pungent taste.

Fig. 3.1. Essential oil of Curcuma in Laos

3.2.3. Results of the determination of physicochemical parameters of rhizome's essential oil of Curcuma longa Linn., Curcuma aeruginosa Roxb. and Curcuma mangga Valeton & Zijp. in Laos.

- Proportion result of essential oil of Curcuma longa Linn. is 0.965, Curcuma aeruginosa Roxb. is 0.963 and Curcuma mangga Valeton & Zijp. is 0.966. This value is equivalent to turmeric essential oil in the world and predictions of the chemical composition of essential oil are mainly hydrocarbons and alcohol. - The results of the refractive index obtained through the measurement of rhizome's essential oil of Curcuma longa Linn.1.511, Curcuma aeruginosa Roxb.1.512 and Curcuma mangga
Valeton & Zijp. 1,472. This index corresponds to the average value of refractive index reported by many publications (1,555-1,520). - Result of determination of acid index of rhizome's essential oil of Curcuma longa Linn. 2,60, Curcuma aeruginosa Roxb. 2,42 and Curcuma mangga Valeton & Zijp. 2,39. This is a low acid index, essential oil has a good quality, less oxidized during storage and use. - The result determines the average ester index of the rhizome's essential oil of Curcuma longa Linn. 21,75; Curcuma aeruginosa Roxb. 26,6; Curcuma mangga Valeton & Zijp. 18,52. This mean value indicates that in the essential oil of Curcuma in Laos has less ester constituent, creates a characteristic aroma. - The results showed that the saponification index of Curcuma longa Linn. was 32,87; Curcuma aeruginosa Roxb. is 28,99 and Curcuma mangga Valeton & Zijp. is 20,92.

3.3. Chemical composition of extract of Curcuma in Laos

3.3.1. Chemical composition of essential oil of Curcuma longa Linn.

The identification results of the chemical composition of essential oil of Curcuma longa Linn. in Laos by GC-MS have identified 30 constituents, of which the major constituents of zingiberene are (22,98%); ar-turmerone with (17,45%); eucalyptol with (15,99%); the remaining constituents accounted for 11,16% - 0,04%.

3.3.2. Chemical composition of essential oil of Curcuma aeruginosa Roxb.

The identification results of the chemical composition of essential oil of Curcuma aeruginosa Roxb. in Laos by GC-MS have identified 25 constituents. The highest percentage content was curenzen with (37,69%), followed by δ-elemene with (13,45%), the remaining constituents accounts for 8,85% -0,02%

3.3.3. Chemical composition of essential oil of Curcuma mangga Valeton & Zijp.
The identification results of the chemical composition of essential oil of *Curcuma mangga* Valeton & Zijp. in Laos by GC-MS have identified 30 constituents. The constituent with highest percentage is zingiberene (10.72%); followed by β-myrcene (10.70%); eucalyptol (9.71%). The remaining constituents accounts for 0.03% -3.34%. - The summary result of the chemical compositions identified in the essential oil of *Curcuma* in Laos has 42 constituents, including 30 constituents in the essential oil of *Curcuma longa* Linn.; 25 constituents in the essential oil of *Curcuma aeruginosa* Roxb.; 30 constituents in the essential oil of *Curcuma mangga* Valeton & Zijp. There are 16 constituents available in all three types of turmeric essential oil. Among the identified ingredients, we mention compounds with strong biological activity such as ar-turmerone, caryophyllene, eucalyptol, curzerene.

### 3.3.4. Comparing the chemical composition of Lao turmeric essential oil with the same turmeric essential oil in countries

- There are 30 constituents in Champasack essential oil of *Curcuma longa* Linn. have identified, higher than Kon Tum essential oil of *Curcuma longa* Linn. with 10 constituents. There are 9 constituents available in all two types of turmeric essential oil. The constituent with highest percentage is zingiberene (22.98%), higher than 10 times compared with percentage of zingiberene (7.16%) in Kon Tum essential oil of *Curcuma longa* Linn.

- There are 25 constituents in the Champasack essential oil of *Curcuma aeruginosa* Roxb. have identified, higher than Huong Hoa -Quang tri essential oil of *Curcuma aeruginosa* Roxb. with 14 constituents; 9 constituents in the Soc Son -Ha Noi essential oil of *Curcuma aeruginosa* Roxb.; 6 constituents in the Malaysian essential oil of *Curcuma aeruginosa* Roxb.; 6 constituents in the Indonesian essential oil of *Curcuma aeruginosa* Roxb. and 3 constituents in the Thai essential oil of *Curcuma aeruginosa* Roxb. The constituent with highest percentage is curzerene.
(37.69%) in the Champasack essential oil of *Curcuma aeruginosa* Roxb.; germacrone (6.69%) in the Huong Hoa -Quang tri essential oil of *Curcuma aeruginosa* Roxb.; curdione (15.30%) in the Soc Son -Hà Noi essential oil of *Curcuma aeruginosa* Roxb.; dehydro-curdione (24.60%) in the Malaysian essential oil of *Curcuma aeruginosa* Roxb.; curcumanol A và B (11.40%) in the Indonesian essential oil of *Curcuma aeruginosa* Roxb. and curcumenol (41.63%) in the Thai essential oil of *Curcuma aeruginosa* Roxb.

- There are 30 constituents in the Champasack essential oil of *Curcuma mangga* Valeton & Zijp. have identified. lower than Malaysian essential oil of *Curcuma mangga* Valeton & Zijp. with 44 constituents. There are 6 constituents available in all two types of turmeric essential oil. The constituent with highest percentage is myrcene (78.6%) in the Malaysian essential oil of *Curcuma mangga* Valeton & Zijp. and zingiberene (10.72%) in the Champasack essential oil of *Curcuma mangga* Valeton & Zijp.

- The difference in percentage content, quantify and content of constituent identification in the Champasack essential oil of turmeric´s rhizome with other countries has proved that quality of plant essential oils depends entirely on natural conditions in which the plants survival and develop.

3.4. Chemical composition of n-hexane extract from *Curcuma* in Laos

3.4.1. Chemical composition of n-hexane extract from *Curcuma longa* Linn.

a. Effect of time to effective collection of extracts : The results obtained for the appropriate extraction time were 10 hours with a percentage of extracted volume of 4,100%.

b. Chemical composition of *Curcuma longa* Linn. in n-hexane extract

The identification results of the chemical composition of hexane extract from *Curcuma longa* Linn. by GC-MS, have identified 14 constituents. The
constituent with highest percentage is ar-turmerone (22,65%). The remaining constituents accounts for 6,66% - 0,10%.

3.4.2. Chemical composition of n-hexane extract from *Curcuma aeruginosa* Roxb.

*Effect of time to effective collection of extracts*: The results obtained for the appropriate extraction time were 10 hours with a percentage of extracted volume of 10,333%.

*b. Chemical composition of Curcuma aeruginosa Roxb. in n-hexane extract*: The identification results of the chemical composition of n-hexane extract from *Curcuma aeruginosa* Roxb. by GC-MS, have identified 24 constituents. The constituent with highest content was curzerene with (44,02%), followed by δ-elemene with (15,47%), the remaining constituent accounts for 0,01% -3,85%.

3.4.3. Chemical composition of n-hexane extract from *Curcuma mangga* Valeton & Zijp.

*a. Effect of time to effective collection of extracts*: The results obtained for the appropriate extraction time were 8 hours with a percentage of extracted volume of 6,709%.

*b. Chemical composition of Curcuma mangga Valeton & Zijp in n-hexane extract*: The identification results of the chemical composition of n-hexane extract from *Curcuma mangga* Valeton & Zijp. by GC-MS, have identified 27 constituents. The constituent with highest content was curzerene with (19,79%), the remaining constituent accounts for 0,02% - 4,99%. The summary result of the chemical compositions identified in the n-hexane extract of *Curcuma* in Laos has 41 constituents, including 14 constituents in the extract of *Curcuma longa* Linn.; 24 constituents in the extract of *Curcuma aeruginosa* Roxb.; 27 constituents in the extract of *Curcuma mangga* Valeton & Zijp. There are 4 constituents available in all three types of turmeric extract. Among the identified ingredients, we
mention compounds with strong biological activity such as ar-turmerone, caryophyllene, eucalyptol, isoborneol, camphene.

3.5. **Chemical composition of dichloromethane extract from Curcuma in Laos**

3.5.1. **Chemical composition of dichloromethane extract from Curcuma longa Linn.**

*Effect of time to effective collection of extracts*: The results obtained for the appropriate extraction time were 10 hours with a percentage of extracted volume of 3.395%.

*b. Chemical composition of Curcuma longa Linn. in dichloromethane extract*: The identification results of the chemical composition of dichloromethane extract from *Curcuma longa* Linn. in Laos by GC-MS, have identified 22 constituents. The constituent with highest content was ar-turmerone (15.92%), the remaining constituent accounts for 5.10% đến 0.10%.

3.5.2. **Chemical composition of dichloromethane extract from Curcuma aeruginosa Roxb.**

*a. Effect of time to effective collection of extracts*: The results obtained for the appropriate extraction time were 10 hours with a percentage of extracted volume of 5.717%.

*b. Chemical composition of Curcuma aeruginosa Roxb. in dichloromethane extract*: The identification results of the chemical composition of dichloromethane extract from *Curcuma aeruginosa* Roxb. in Laos by GC-MS, have identified 22 constituents. The constituent with highest content was curzerene with (42.26%), followed by δ-elemene with (15.37%), the remaining constituent accounts for 0.01% 3.57%.

3.5.3. **Chemical composition of dichloromethane extract from Curcuma mangga Valeton & Zijp.**
a. Effect of time to effective collection of extracts: The results obtained for the appropriate extraction time were 8 hours with a percentage of extracted volume of 6,709%.

b. Chemical composition of Curcuma mangga Valeton & Zijp. in dichloromethane extract: The identification results of the chemical composition of dichloromethane extract from Curcuma mangga Valeton & Zijp. in Laos by GC-MS, have identified 23 constituents. The constituent with highest content was curzerene with (24,48%), followed by δ-elemene with (7,74%), the remaining constituent accounts for 0,05% 2,72%. - The summary result of the chemical compositions identified in the dichloromethane extract of Curcuma in Laos has 44 constituents, including 22 constituents in the extract of Curcuma longa Linn.; 22 constituents in the extract of Curcuma aeruginosa Roxb.; 23 constituents in the extract of Curcuma mangga Valeton & Zijp. There are 3 constituents available in all three types of turmeric extract. Among the identified ingredients, we mention compounds with strong biological activity such as ar-turmerone, caryophyllene, eucalyptol, isoborneol, camphene, borneol, limonene, curzerene.

3.6. Chemical composition of ethyl acetate extract from Curcuma in Laos
3.6.1. Chemical composition of ethyl acetate extract from Curcuma longa Linn.

a. Effect of time to effective collection of extracts: The results obtained for the appropriate extraction time were 10 hours with a percentage of extracted volume of 5,717%.

b. Chemical composition of Curcuma longa Linn. in ethyl acetate extract: The identification results of the chemical composition of ethyl acetate extract by GC-MS, have identified 16 constituents. The constituent with highest content was ar-turmerone (17,19%), the remaining constituent accounts for 7,30% - 0,10%.
3.6.2. Chemical composition of ethyl acetate extract from *Curcuma aeruginosa* Roxb.

*a. Effect of time to effective collection of extracts*: The results obtained for the appropriate extraction time were 8 hours with a percentage of extracted volume of 4,545%.

*b. Chemical composition of Curcuma aeruginosa Roxb. in ethyl acetate extract*: The identification results of the chemical composition of ethyl acetate extract from *Curcuma aeruginosa* Roxb. in Laos by GC-MS, have identified 20 constituents. The constituent with highest content was curzerene with (36,72%), followed by δ-elemene with (13,18%), the remaining constituent accounts for 0,02% - 2,34%.

3.6.3. Chemical composition of ethyl acetate extract from *Curcuma mangga* Valeton & Zijp.

*a. Effect of time to effective collection of extracts*: The results obtained for the appropriate extraction time were 8 hours with a percentage of extracted volume of 5,334%.

*b. Chemical composition of Curcuma mangga Valeton & Zijp. in ethyl acetate extract*: The identification results of the chemical composition of ethyl acetate extract from *Curcuma mangga* Valeton & Zijp. in Laos by GC-MS, have identified 15 constituents. The constituent with highest content was β-elemenone with (7,16%), the remaining constituent accounts for 3,51% 0,15%. - The summary result of the chemical compositions identified in the ethyl acetate extract of *Curcuma* in Laos has 37 constituents, including 16 constituents in the extract of *Curcuma longa* Linn.; 20 constituents in the extract of *Curcuma aeruginosa* Roxb.; 15 constituents in the extract of *Curcuma mangga* Valeton & Zijp. There are 3 constituents available in all three types of turmeric extract. Among the identified ingredients, we mention compounds with strong biological
activity such as ar-turmerone, caryophyllene, eucalyptol, isoborneol, camphene, curzerene.

3.7. Chemical composition of methanol extract from Curcuma in Laos

3.7.1. Chemical composition of methanol extract from *Curcuma longa* Linn.

*a. Effect of time to effective collection of extracts* : The results obtained for the appropriate extraction time were 8 hours with a percentage of extracted volume of 2,087%.

*b. Chemical composition of Curcuma longa Linn. in methanol extract* : The identification results of the chemical composition of methanol extract from *Curcuma longa* Linn. in Laos by GC-MS, have identified 15 constituents of methanol extract from the roots of *Curcuma longa* Linn. The constituent with highest content was n-hexadecanoic axit with (15%), the remaining constituent accounts for 14%-0,21%.

3.7.2. Chemical composition of methanol extract from *Curcuma aeruginosa* Roxb.

*a. Effect of time to effective collection of extracts* : The results obtained for the appropriate extraction time were 8 hours with a percentage of extracted volume of 13,901%.

*b. Chemical composition of Curcuma aeruginosa Roxb. in methanol extract* : The identification results of the chemical composition of Methanol extract from *Curcuma aeruginosa* Roxb. in Laos by GC-MS, have identified 19 constituents. The constituent with highest content was curzerene with (41,17%), followed by δ-elemene with (14,03%), the remaining constituent accounts for 0,03% - 2,29%.

3.7.3. Chemical composition of methanol extract from *Curcuma mangga* Valeton & Zijp.
a. Effect of time to effective collection of extracts: The results obtained for the appropriate extraction time were 8 hours with a percentage of extracted volume of 7.927%.

b. Chemical composition of Curcuma mangga Valeton & Zijp. in methanol extract: The identification results of the chemical composition of methanol extract from *Curcuma mangga* Valeton & Zijp. in Laos by GC-MS, have identified 21 constituents. The constituent with highest content was curzerene with (19,63%), followed by δ-elemene with (6,04%), the remaining constituent accounts for 0,04% -3,14%. - The summary result of the chemical compositions identified in the methanol extract of *Curcuma* in Laos has 35 constituents, including 15 constituents in the extract of *Curcuma longa* Linn.; 19 constituents in the extract of *Curcuma aeruginosa* Roxb.; 21 constituents in the extract of *Curcuma mangga* Valeton & Zijp. There are 3 constituents available in all three types of turmeric extract. Among the identified ingredients, we mention compounds with strong biological activity such as ar-turmerone, caryophyllene, eucalyptol, isoborneol, camphene, ar-curcumene.

3.8. Results of extraction and determination of the Curcumin's structure by alkalisation method

3.8.1. Effect of some factors on the efficiency of curcumin extraction

a. The influence of time: The appropriate extraction time is 4 hours.

b. Effect of KOH solution concentration: The appropriate concentration is 0,025N.

c. Effect of solid/liquid ratio on the extraction process: The results of the solid/liquid ratio selected for the color extraction from *Curcuma longa* Linn. in Laos were 1/100 (1g of powder from Curcuma longa Linn with 100mL KOH solution 0,025N).

d. Effect of temperature on the extraction process: The selected temperature to extract the color from *Curcuma longa* Linn. in Laos is
110°C. The most suitable conditions for extracting curcumin from *Curcuma longa* Linn. in Laos with KOH solution are: Time: 4 hours; concentration: 0.025N; Solid/liquid ratio: 1/100; Temperature: 110°C.

3.9. Quantification, isolation and determination of Curcumin structure from Curcuma longa Linn. colorants

3.9.1. Crystallization for collection of colorants:
KOH solution is neutralized with HCl acid to pH = 7, after filtration, and is crystallized in absolute alcohol to obtain crystal of colorants Figure 3.2 has 7.26% content compared to dry powder of *Curcuma longa* Linn.

![Figure 3.2. Crystal of colorants from Curcuma longa Linn.](image)

3.9.2. Quantification of curcumin

![Fig. 3.3. UV-VIS spectra of curcumin from Curcuma longa Linn.](image)

(A) and standard curcumin (B)

The results determine that the curcumin contained in HPLC table are shown in Figure 3.4
The results showed that the curcumin content in the sample was 90.899%.

3.9.3. Isolatation and determination of the Curcumin formulation

Column chromatography using silicagel 60 F_{524} of Merck brand. The appropriate solvent system for the isolation of the substance is the n-hexane system: ethyl acetate with the rate ranging from (65:35) to (15:85). The collection phase is from containers no. 35 to 82 for a rounded chromatogram with a dark yellow color, symbol: M1 with R_f = 0.625. - MS Mass spectrometry of M1 substance for pseudo- molecular pic ion [M-H] has the mass number m/z = 337, the molecular mass of M1 is 338. - IR spectrum of M1 substance gives the characteristic signal at v (cm^{-1}): 3308; 1574; 1510; 1436; 1271; 1139; 967; 824. - ^1H-NMR spectrum of M1 substance showed the signal of 18 protons, ^13C-NMR spectrum showed that the signal of 20 C atoms combined with MS spectra allowed to predict molecular formula of M1 substance as C_{20}H_{18}O_5. Symmetric signals on ^1H-NMR spectrum and ^13C-NMR with the number of protons and C in M1 corresponds fully to the number of protons and C present in demethoxycurcumin molecule (C_{20}H_{18}O_5). In the high field, there is a proton signal, of which a single pic (s) at a displacement of 3.88 ppm is the signal of the group (-OCH_3), in the low field of 5.90-6.85 ppm there are seven proton signals. Two-dimensional NMR spectra appears the proton-proton interactions: H-4, H-3; H4' H3'; H-10, H-9; H-10', H-9'; H-6', H-7',
in accordance with the molecular interactions of Demethoxycurcumin substance.

The structure of M1 substance was confirmed by comparison with demethoxycurcumin substance (Fig. 3.6).

![Structural formulation of demethoxycurcumin (DMC)](image)

**Fig. 3.6. Structural formulation of demethoxycurcumin (DMC)**

\[(\text{C}_{20}\text{H}_{18}\text{O}_5)\]

**CONCLUSION AND RECOMMENDATIONS**

**I. CONCLUSION**

1. By mass analysis method have been investigated some physicochemical parameters of fresh rhizome and dry powder of *Curcuma* in Laos such as: moisture content, ash content, by atomic absorption spectroscopy (AAS) were investigated content of 4 heavy hazardous metals: (As, Hg, Pb and Cu). The content of these metals is lower than permitted level in Vietnam.

2. By steam distillation method was obtained essential oil from Rhizome of *Curcuma* in Laos with content % of fresh weight: *Curcuma longa* Linn.: 0,237, *Curcuma aeruginosa* Roxb.: 0,355 and *Curcuma mangga* Valeton & Zijp.: 0,235 and it has identified some important quality indicators of essential oil: density (*Curcuma longa* Linn.: 0,965, *Curcuma aeruginosa* Roxb: 0,963 and *Curcuma mangga* Valeton & Zijp.: 0,966); Refractive index (*Curcuma longa* Linn.: 1,511, *Curcuma aeruginosa* Roxb.: 1,512 and *Curcuma mangga* Valeton & Zijp.: 1,472); Acid index (*Curcuma longa* Linn.: 2,60, *Curcuma aeruginosa* Roxb.: 2,42 and *Curcuma mangga* Valeton & Zijp.: 2,39); Ester index (*Curcuma longa* Linn.: 21,75, *Curcuma aeruginosa* Roxb.: 26,61 and *Curcuma mangga* Valeton & Zijp.: 18,52);
Saponification index (Curcuma longa Linn.: 32.87, Curcuma aeruginosa Roxb.: 28.99 and Curcuma mangga Valeton & Zijp.: 20.92).

3. By gas chromatography mass spectrometry (GC-MS) have been identified in Champasack essential oil of Curcuma in Laos, of which 30 constituents are found in essential oil of Curcuma longa Linn.; 25 constituents in essential oil of Curcuma aeruginosa Roxb. and 30 constituents in essential oil of Curcuma mangga Valeton & Zijp. There are 16 constituents available in all three types of turmeric essential oil. Thereby comparing the chemical composition of Champasack essential oil of Curcuma in Laos with other countries, the percentage of constituents in general is not much different only varies the number of constituents.

4. By the Soxhlet method was determined the appropriate extraction time to obtain the highest n-hexane and dichloromethane extract for each of rhizome of Curcuma in Laos: Curcuma longa Linn.: 10 hours; Curcuma aeruginosa Roxb.: 10 hours and Curcuma mangga Valeton & Zijp.: 8 hours and identified constituents in the n-hexane extraction, of which 14 constituents in extract of Curcuma longa Linn., 24 constituents in extract of Curcuma aeruginosa Roxb., 27 constituents in extract of Curcuma mangga Valeton & Zijp. There are 4 constituents available in all three types of turmeric extract. and identified constituents in the dichloromethane extraction, of which 22 constituents in extract of Curcuma aeruginosa Roxb., 23 constituents in extract of Curcuma mangga Valeton & Zijp. There are 3 constituents available in all three types of turmeric extract.

- Determined the appropriate extraction time to obtain the highest amount of ethyl acetate extract for each type of rhizome of Curcuma in Laos: Curcuma longa Linn.: 10 hours; Curcuma aeruginosa Roxb.: 8 hours and Curcuma mangga Valeton & Zijp.: 8 hours and identified constituents in the ethyl acetate extraction, of which 16 constituents in extract of Curcuma longa Linn., 20 constituents in extract of Curcuma aeruginosa Roxb., 15
constituents in extract of *Curcuma mangga* Valeton & Zijp. There are 3 constituents available in all three types of turmeric extract. - Determined the appropriate extraction time to obtain the highest amount of methanol extract for each type of rhizome of *Curcuma* in Laos: *Curcuma longa* Linn.: 8 hours; *Curcuma aeruginosa* Roxb.: 8 hours and *Curcuma mangga* Valeton & Zijp.: 8 hours and identified constituents in the methanol extraction, of which 15 constituents in extract of *Curcuma longa* Linn., 19 constituents in extract of *Curcuma aeruginosa* Roxb., 21 constituents in extract of *Curcuma mangga* Valeton & Zijp. There are 3 constituents available in all three types of turmeric extract.

5. It has been developed the process of collecting colorants from rhizome of *Curcuma* in Laos by alkaline method and 0,025N KOH solution concentration, the ratio of dry powder with 0,025N KOH solution is 1/100, alkaline temperature 110°C. Pure colorants receiving capacity, it was 7,26% comparing with dry powder of *Curcuma longa* Linn.

6. By the method of infrared (IR) spectroscopy, mass spectrometry (MS), one-way nuclear magnetic resonance spectroscopy (\(^1\)H. NMR, \(^{13}\)C- NMR, DEPT) and two-way (HSQC, HMBC, \(^1\)H-\(^1\)H COSY) can prove demonstrate demethoxycurcumin (DMC) structure (C\(_{20}\)H\(_{18}\)O\(_5\)).

**II. RECOMMENDATIONS**

- Further research with larger scale to develop technological processes for the collection of essential oil, extraction and curcumin with pilot scale and industrial scale. - Research the applied technologies to create high value products (medicines, functional foods) for people's life, society and economic development. - Research the selection of *Curcuma* species with high-curcumin content and suitably make plan of places and area for planting to meet the demand for raw materials for domestic consumption and export.
LIST OF PUBLISHED SCIENTIFIC WORKS

