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**HA NOI**

**ANALYSIS OF OLEORESIN EXTRACTED FROM GINGER RHIZOMES IN NORTH CENTRAL OF VIETNAM BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-ESI-MS)**

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**Le Thi My Chau<sup>1,2</sup>, Nguyen Thi Minh Tu<sup>1</sup>, Tran Dinh Thang<sup>2</sup>, Le The Tam<sup>2</sup>**

*<sup>1</sup>Hanoi University of Science and Technology*

*<sup>2</sup>Vinh University*

**SUMMARY**

*The ginger are being used by consumers, and clinical trials using ginger have been carried out to evaluate their anti-inflammatory or anti-emetic properties with consistent results. Chemical standardization of these products is needed for quality control and to facilitate the design of clinical trials and the evaluation of data from these studies. To address this issue, methods based on liquid chromatography-mass spectrometry (LC-ESI-MS) were developed for the detection, characterization and quantitative analysis of gingerol-related compounds in ginger roots/rhizomes. This study of the chemical composition of the oleoresins extracted from *Zingiber cochinchinensis*, *Zingiber gramineum*, *Zingiber zerumbet* and *Zingiber rufopilosium*, collected from north central of Vietnam by LC-ESI-MS. Rhizomes of ginger samples were extracted in ethanol in order to obtain ginger oleoresin. Phenolic compounds, gingerol-related compounds ([4], [6], [8]-gingerol, [6]-shogaol...), are responsible for taste and aroma of ginger were determined by LC-MS method.*

**Keywords:** *oleoresin, (6)-gingerol, (6)-shogaol, LC-MS, Z. cochinchinensis, Z. gramineum, Z. zerumbet, Z. rufopilosium.*

**1. INTRODUCTION**

The genus *Zingiber* (*Zingiberaceae* family), which has about 150 species distributed in tropical forest (much of the Southeast Asia, China, India...) and throughout the Islands in the Pacific. In Vietnam, the genus is diverse with about

10 endemic species. They contained essential oils and oleoresins which are used as medicinal drugs, popular spices and raw materials in food, pharmaceutical industry etc...<sup>[9, 4, 3]</sup>. The ginger rhizomes are being used by consumers, and clinical trials using ginger have been carried out

to evaluate their anti-inflammatory or anti-emetic properties with inconsistent results. Chemical standardization of these products is needed for quality control and to facilitate the design of clinical trials and the evaluation of data from these studies. To address this issue, methods based on liquid chromatography-mass spectrometry (LC-ESI-MS) were developed for the detection, characterization and quantitative analysis of gingerol-related compounds in ginger rhizomes.

The ginger rhizomes or its extracts have been commonly used in medicine, because of their wide scope of biological effects-confirmed both in various *in vitro* models and in clinical trials. The plant has been found to show strong antiemetic activity and is now used to treat motion sickness, morning sickness, and post-chemotherapy nausea<sup>[2]</sup>.

Moreover, its analgesic and painkilling properties have been applied in pharmacotherapeutical strategies in the treatment of osteoarthritis due to the marked anti-inflammatory properties of the plant. The confirmed antimicrobial action of the rhizomes (including antibacterial, antiviral, antifungal, and antiparasitic activity) justify its traditional use in cold treatment [8, 2]. The active compounds from ginger rhizomes inhibit platelet aggregation and have a strong vasodilatory effect, which decreases blood pressure and improves blood circulation. Some studies suggest that ginger may be a potential drug in the treatment of diabetes and hypercholesterolemia because of its hypoglycemic and hypolipidemic

properties. Many reports provide information on ginger antineoplastic properties in the treatment of skin, breast, brain, or liver cancer. The two major groups of active compounds from ginger, which are responsible for most of the biological actions of this plant, are polyphenols (gingerols, shogaols, and paradols) and volatiles such as zingiberole, zingiberone, and zingiberene<sup>[5, 7, 12]</sup>.

The chemical composition of *Z. cochinchinensis*, *Z. gramineum*, *Z. zerumbet*, *Z. rufopilosium* rhizomes cultivated on ecological plantations in north central region of Viet Nam. The wide-ranging pharmacological applications of ginger and the increasing consumption of the spice encouraged the authors to perform these studies on the chemical composition of ginger rhizomes, as there are no data in the literature on the active components or element content of ginger grown on North Central region of Vietnam.

## 2. MATERIALS AND METHODS

### 2.1. Plant materials and Sample preparation

Fresh ginger rhizomes (*Z. cochinchinensis*, *Z. gramineum*, *Z. zerumbet*, *Z. rufopilosium*) were collected from north central region of Viet Nam and identified by Dr. Do Ngoc Dai, Nghe an college of economics. The voucher specimens were deposited at the Botany Museum, Institute of Ecology and Biological Resource, Viet Nam Academy of Science and Technology.

100 g ginger rhizomes dried was extracted with ethanol 96% (500 ml x 3) under the following conditions: 16 hours, at normal

temperature and pressure.

## 2.2. LC-ESI-MS analysis

The LC-MS system, 1200 series (Agilent Technology, Santa Clara, CA, USA) equipped with an autosampler Syringe (kdScientifit, USA), a degasser, a ion multichannel detector, and a binary pump was used for chromatographic separation. A microQTOF-QII (broker Daltonic, Germany) mass spectrometer was applied for the identification and determination of gingerols and shogaols in the obtained extracts. The injection volume was 200  $\mu$ L/hour of each standard and extract. The analytes were separated on a ACE3-C<sub>18</sub> column from Agilent Technology (dimension: 150 mm x 4.6 mm, dp = 3.5  $\mu$ m) in a flow rate of 0.3 mL/min for 40 min. The mobile phase consisted of a combination of solvent A (0.1% formic acid) and solvent B (acetonitrile + 0.1% formic acid). The gradient elution was as follows: t = 0 min, 10% B; t = 15 min, 100% B; t = 30 min, 100% B; t = 31 min, 10% B; t = 40 min, 10% B. The photodiode detector continuously recorded the chromatograms in the range of absorbance from 190 to 500 nm. Mass spectra were simultaneously acquired using ESI in positive ionization modes with a capillary voltage of 4500 V. The mass spectra were recorded in the *m/z* range of 50 to 3000 *m/z*. The gas temperature and drying gas flow were 200°C and 9.0 L/min. The skimmer and fragmentation voltages were set at -500 and 4500 V, respectively. The nebulization pressure was 1.5 bar. The MS/MS spectra were recorded for the two most intensive peaks each time.

Three selected gingerols, two shogaols and five gingerdiols present in the extracts were determined qualitatively in the ethanolic rhizome extract based on their fragmentation spectra, the scientific literature, and retention times. A quantitative analysis of the selected phenolics was performed in accordance with the calibration curves of two standards<sup>[12]</sup>.

## 3. RESULTS AND DISCUSSION

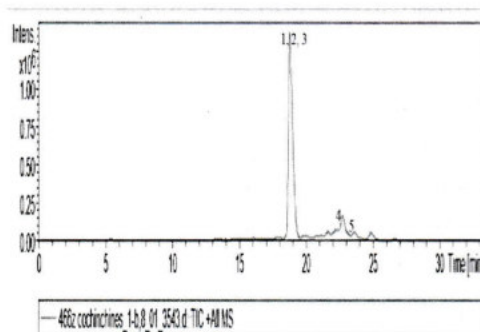


Figure 1. LC/MS analysis of *Z. cochinchinensis* rhizomes extract

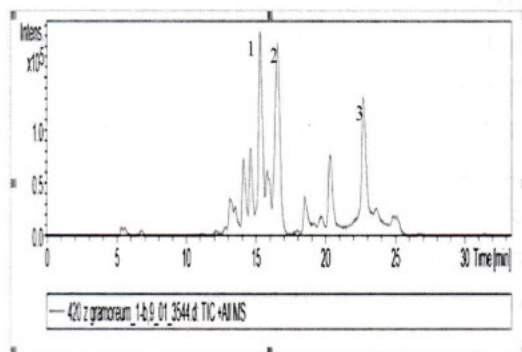


Figure 2. LC/MS analysis of *Z. gramineum* rhizomes extract

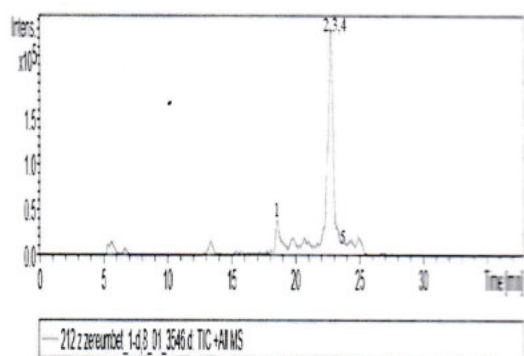


Figure 3. LC/MS analysis of *Z. zerumbet* rhizomes extract

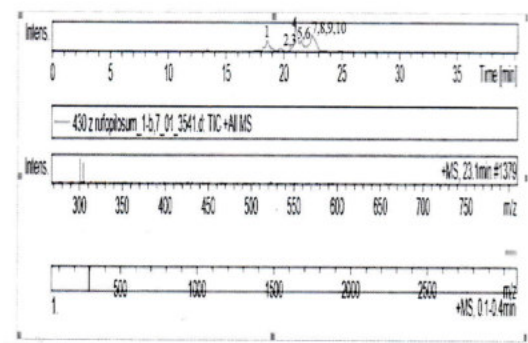


Figure 4. LC/MS analysis of *Z. rufopilosum* rhizomes extract

Crude ethanol extracts, produced from fresh-dried ginger rhizomes (*Z. cochinchinensis*, *Z. gramineum*, *Z. zerumbet*, *Z. rufopilosum*), were used directly for LC/ESI-MS/MS analyses. As shown in Figure 1, positive ionization ESI-MS was used to detect gingerol-related compounds. (Figure 1, 2, 3, 4 and Table 1)

Table 1. Chromatographic and mass spectral characteristics of gingerol-related compounds detected by LC-ESI-MS in extracts from ginger rhizomes

| Sample | tR (min) | Positive ESI(+) ESI-MS (m/z)     | Compound name             | Area Frac. % |
|--------|----------|----------------------------------|---------------------------|--------------|
| (1)    | 18,7     | 365,1025 ([M + H] <sup>+</sup> ) | Me-[10]-Gingerdiol        | 5,2          |
|        | 18,8     | 343,1215 ([M + H] <sup>+</sup> ) | Undetermined              | 45,7         |
|        | 18,8     | 707,2101 ([M + H] <sup>+</sup> ) | Undetermined              | 26,3         |
|        | 22,5     | 317,2116 ([M + H] <sup>+</sup> ) | 1-Dehydro-[8]-Gingerdione | 2,1          |
|        | 22,7     | 301,1470 ([M + H] <sup>+</sup> ) | OAc-[4]-Gingerol          | 6,2          |

|     |      |                                  |   |      |
|-----|------|----------------------------------|---|------|
| (2) | 15,3 | 287,0636 ([M + H] <sup>+</sup> ) | Undetermined                                    | 15,8 |
|     | 16,6 | 517,1389 ([M + H] <sup>+</sup> ) | Undetermined                                    | 9,4  |
|     | 22,7 | 301,1489 ([M + H] <sup>+</sup> ) | OAc-[4]-Gingerol                                | 12,3 |
| (3) | 18,5 | 274,2808 ([M + H] <sup>+</sup> ) | 6-Shogaol                                       | 7,6  |
|     | 22,5 | 241,1646 ([M + H] <sup>+</sup> ) | Undetermined                                    | 5,6  |
|     | 22,5 | 219,1837 ([M + H] <sup>+</sup> ) | Undetermined                                    | 15,6 |
|     | 22,7 | 301,1502 ([M + H] <sup>+</sup> ) | OAc-[4]-Gingerol                                | 43,4 |
|     | 23,3 | 425,2193 ([M + H] <sup>+</sup> ) | Undetermined                                    | 2,1  |
| (4) | 18,5 | 274,2764 ([M + H] <sup>+</sup> ) | 6-Shogaol                                       | 2,2  |
|     | 20,7 | 688,3993 ([M + H] <sup>+</sup> ) | Undetermined                                    | 2,2  |
|     | 20,9 | 375,2125 ([M + H] <sup>+</sup> ) | 1-Dehydro-[12]-gingerdione                      | 3,3  |
|     | 21,0 | 277,1829 ([M + H] <sup>+</sup> ) | [6]-Shogaol                                     | 9,4  |
|     | 21,1 | 299,1638 ([M + H] <sup>+</sup> ) | Undetermined                                    | 12,8 |
|     | 21,5 | 403,2074 ([M + H] <sup>+</sup> ) | 1-(4-hydroxy-3-methoxyphenyl)-2-nonadecen-1-one | 8,1  |
|     | 21,9 | 291,1620 ([M + H] <sup>+</sup> ) | 1-Dehydro-[6]-Gingerdione                       | 3,4  |
|     | 22,3 | 304,3011 ([M + H] <sup>+</sup> ) | Undetermined                                    | 9,8  |
|     | 22,4 | 417,2226 ([M + H] <sup>+</sup> ) | 1-(3,4-dimethoxyphenyl)-2-Nonadecen-1-one       | 7,0  |
|     | 22,7 | 301,1428 ([M + H] <sup>+</sup> ) | OAc-[4]-Gingerol                                | 6,9  |

Note: (1)-*Z. cochinchinensis*, (2)-*Z. gramineum*, (3)-*Z. zerumbet*, (4)-*Z. rufopilosium*

Spectrometric analysis of the obtained ethanol extracts revealed the presence of the five major phenolic compounds in ginger rhizomes OAc-[4]-gingerol, 8-gingerol, 6-gingerdiol, 6-shogaol, 7-gingerdiol. In addition, another the CID (collision induced dissociation). The ginger spices have different compounds (Table 1).

The application of an optimized LC method, all major phenolic constituents were well separated on a chromatographic column. The fragmentation patterns of all the phenolic compounds listed above were carefully compared with those presented in the scientific literature and are presented in the Figure 1 under application of higher collision energies in the LC-ESI-Q-TOF-MS analysis provided a lower intensity of MS/MS fragments and the loss of the molecular ion peak, which made the identification of compounds more difficult.

The major compound present in the highest amount was OAc-[4]-gingerol in both samples *Z. zerumbet* and *Z. gramineum*. The content of (6)-gingerol was higher compared to that of different cultivars of fresh Australian ginger determined by Wohlmuth et al<sup>[11]</sup> and in fresh Japanese ginger<sup>[12]</sup>. The large concentration of [6]-gingerdiol and [6]-shogaol may also show important health benefits, as this compound has been shown to have strong anticancer properties than fresh plant material<sup>[12]</sup>.

The results of the analysis revealed that the components of incense and gingival composition contained in ginger oleoresin were suited to the research results of other authors. Besides that, different ginger species, there is a relative difference in some components and amounts, possibly due to differences in study species, growth conditions, growth habitats, and the time of sample collection.

#### 4. CONCLUSIONS

LC-ESI-MS analysis of the obtained ethanolic extract revealed that the main

phenolic compound was OAc-[4]-gingerol, [6]-gingerol, [6]-gingerdiol and [6]-shogaol which is characteristic of fresh rhizomes and is responsible for their taste and aroma. Moreover, high amounts of (6)-shogaol were determined, which is interesting because this phenolic compound usually occurs in old or processed material.

Gingerol-related compounds, were identified in ethanol crude extracts from fresh-dried ginger rhizomes by LC/ESI-MS/MS coupled to diode array detection. Interestingly, many of the identified compounds were only detected by the MS detector, therefore, suggesting that the LC/MS analysis is not only more specific, but also more sensitive than diode array analysis for this group of compounds [1, 10].

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