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Antioxidant and antimicrobial activities of essential oil and oleoresins extracted from Vietnamese ginger

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Abstract
The biofunctional properties of ginger essential oil and oleoresins including antioxidant and antimicrobial activities were investigated in this study. The essential oil was extracted using the hydro-distillation method and oleoresins were extracted using solvent extraction method (methanol and ethanol). The antioxidant activities of these samples were determined by DPPH scavenging assay, while the antimicrobial activities were determined by the dilution and diffusion methods against several foodborne pathogens. The yield of ginger essential oil was 1.5%, while the yields of methanol oleoresin and ethanol oleoresin were 10.70% and 6.66%, respectively. Although the extraction yield and antimicrobial activities of methanol oleoresin were higher than ethanol oleoresin, the antioxidant activities were not significantly different. Ginger oleoresins showed higher antioxidant and antimicrobial activities than ginger essential oil. All samples were found to possess the antimicrobial activities on tested bacteria except *B. cereus*. Among these samples, methanol oleoresin showed the highest antimicrobial activity against *P. aeruginosa* (14.0 ± 0.2 mm) and *S. aureus* (7.50 ± 0.5 mm), followed by ethanol oleoresin and essential oil. As a result, both ginger essential oil and oleoresins are considered to be natural sources of antioxidants and antimicrobial compounds.

Keywords: antimicrobial activity, antioxidant activity, essential oil, hydro-distillation, oleoresins, solvent extract

Introduction
Ginger, the rhizome of *Zingiber officinale* Roscoe, one of the most widely used species of the family Zingiberaceae, is a common condiment for various foods and beverages. While the origin of ginger is uncertain, it is grown extensively in many tropical countries. According to the United Nations Food and Agriculture Organization (FAO), global production of ginger in 2013 was over 2.1 million of tonnes, with major production in India, China, Indonesia, Nepal and Nigeria. The world trade in ginger was estimated at 190 million USD per year. The ginger root or rhizome is harvested because almost valuable compositions occurred here. In Vietnam, ginger is grown extensively for all year round from the North such as Bac Can and Thai Nguyen provinces; from the Middle such as Lam Dong province and especially from the South such as An Giang and Hau Giang provinces with more than 500 ha. Traditionally, ginger has been used to aid digestion and to treat stomach upset and diarrhea. In China and Japan, it was used as medicines to treat headaches, nausea, stomach ache and colds. Ginger rhizome was reported to have many biological and physiological activities such as antioxidant effect, anti-inflammatory activities and antimicrobial effect, etc.

The general composition of ginger rhizome contains starch which is the most abundant component, steam volatile oil, fatty oil, pungent compounds, resin, proteins, cellulose, pentosans and mineral elements [1]. Essential oils, also known as volatile oils are usually derived from plants (leaves, root and stem) and generally extracted by steam distillation. Ginger essential oil, obtained by steam distillation of the rhizome of *Z. officinale* Roscoe, is used in the beverage and fragrance industries [1]. It possesses the aroma and flavour of the spice but lack of the pungency [3]. Ginger essential oils are comprised mainly of sesquiterpene hydrocarbons (50-66%) including zingiberene, ar-curcumene, beta-bisabolene, beta-sesquiphellandrene; monoterpene hydrocarbons (17 – 33%) and oxygenated monoterpenes (up to 17%) which determined the odour and flavour of ginger. The monoterpenes contribute to the aroma of ginger. Oxygenated sesquiterpenes contribute to its flavour properties [3]. In contrast to essential oils, oleoresins are less volatile. It is the total soluble extract in a specified solvent such as ethanol, methanol or liquid carbon dioxide [4]. The components responsible for the pungent characteristic include gingerols (10.43%) as a major active component, shogaols (8%), paradols (0.3%) and zingerone (14.56%) that produce a 'hot' sensation in the mouth [5]. Both ginger essential oil and oleoresins are used as the secondary or derived products that could be applied in the food industries as flavoring, preservative and also in medicinal fields [6].

Ginger has been reported tohave potential antimicrobial activities against different microbial pathogens and exhibited a strong antioxidative activity [7, 8]. However, essential oil and oleoresins extracted from Vietnamese gingers have not been extensively studied while the production of ginger has been increasing recently. In this study, the biological functions of ginger rhizomes such as antimicrobial, antioxidant activities of essential oil (extracted by hydrodistillation method) and oleoresins

(extracted by using ethanol and methanol) were investigated.

Materials and Methods

Materials

Fresh ginger rhizomes, originated from Lam Dong province, were used in this study. Fresh rhizomes were collected and washed to remove dirt and soil particles. Peeling and washing were done consecutively. Then the rhizomes were cut into small 1 to 3 mm thick slices and dehydrated in hot air oven (ThermoStable™ SOF-W105 – Wisd) at 40°C to achieve moisture content of 12%. The ginger rhizomes were ground into fine particles of 250 µm and stored in the air-tight bag at 4°C before going to further analysis [4].

Hydro-distillation method

The essential oil of ginger rhizomes were extracted by using the Clevenger-type apparatus. To extract the essential oil, 30g of the powder was placed in a 1-litre conical flask and connected to the Clevenger-type apparatus. An amount of distilled water (500 mL) was added to the flask and heated to the boiling point. The steaming water vapour combining with the essential oil were distilled into a graduated cylinder for 4 h and the oil was kept in the refrigerated until required for further analysis [9]. The yield of extracted essential oil was calculated by the following formula:

$$\text{Oil yield}(\%) = \frac{Weight\ of\ oil\ extracted\,(g)}{Weight\ of\ dried\ ginger\ rhizome\ powder\,(g)}\ x\ 100$$

Solvent extraction using Methanol and Ethanol

Ginger oleoresins were extracted by Soxhlet extractor using methanol (≥99% purity) and ethanol (≥99% purity) as solvents according to the method of Said *et al*. [4]. According to the method of solvent extraction, 25g of dried ginger rhizome powder was weighed into cellulose thimble and kept in a Soxhlet extractor for 6 h. It was carried out above the boiling point of methanol (64.7°C) and ethanol (78.2°C). The extraction yield was calculated by the following formula:

$$\text{Oil yield}(\%) = \frac{Weight\ of\ oleoresin\ extracted\,(g)}{Weight\ of\ dried\ ginger\ rhizome\ powder\,(g)}\ x\ 100$$

DPPH scavenging assay

Antioxidant activity of the ginger essential oil and oleoresin was investigated based on the scavenging capacity of these compounds against the stable radical of 2,2 diphenyl-1-picrylhydrazyl (DPPH). The solutions (0.5 ml) of ginger oleoresins with different concentrations (20, 40, 60 and 80 µg/mL) and ginger essential oil (40, 60, 80 and 100 mg/ml) were mixed with equal volume of methanolic DPPH solution (100 µM) (0.5 ml). The blank samples were made by adding 0.5 ml of methanol to 0.5 ml DPPH solution. The mixtures were stood for 30 min in dark at room temperature. The absorbance was measured at 517 nm using a spectrophotometer (UVD-3500, Labomed, Inc). The DPPH inhibition (%) was calculated by the following equation:

$$\text{Inhibition}(\%) = \frac{Acontrol - Asample}{Asample} \times 100$$

Where $A_{control}$ was the optical density of the blank and A_{sample} was the optical density of the sample. The concentration causing 50% inhibition (IC_{50}) was calculated from the graph-plotted scavenging percentage against ginger essential oil and oleoresins concentration.

Microbial strains

Four bacteria were used to determine the antimicrobial activities of ginger essential oil and oleoresins. These microorganisms included two Gram-positive bacteria: *Staphylococcus aureus* supplied by the Institute of Drug Quality Control - HoChiMinh City and *Bacillus cereus* supplied from the Institute of Microbiology and Biotechnology, Vietnam National University and two Gram-negative bacteria: *Salmonella typhi* and *Pseudomonas aeruginosa* supplied from the Institute of Drug Quality Control - HoChiMinh City. The concentration of bacteria was prepared to match 0.5 McFarland standard (1.5×10^8 CFU/ml).

Disc diffusion testing

As recommended by the National Committee for Clinical Laboratory Standards [10], the antimicrobial activity of ginger essential oil and oleoresins were determined by the disc diffusion testing method. The Tryptone Soybean Agar (TSA, Himedia, India) plates were prepared in 90 mm petri dishes with 22 mL of agar medium with 3 mm in depth. A broth culture of bacteria (100 µL) was spread on TSA agar plates. Filter paper discs of 5 mm diameter were prepared and sterilized. These discs were impregnated with 20 µL of essential oil and oleoresins (20mg/ml) which were diluted in absolute ethanol. There were four impregnated filter paper discs placed on nutrient agar containing respectively microbial strains. The plates were incubated in an inverted position at 37°C for 24h [5]. The clear zone were measured (including filter paper discs of 5 mm diameter) to assess the microbial activity.

Determination of the minimum inhibitory concentration (MIC)

Diluted essential oil and oleoresins in absolute ethanol were prepared to obtain the concentration of 20 mg/L to the final concentration of 2.5 mg/L. The antimicrobial susceptibility test was performed with different concentrations [11]. The MIC was the lowest concentration that inhibited the microbes.

Results and Discussion

Extraction of ginger essential oil and oleoresins

The dried ginger powder (8.12% moisture content) was used to extract essential oil and oleoresin using the hydro-distillation and solvent extraction methods, respectively. The results of extraction yields of ginger essential oil and oleoresins are shown in Table 1.

Table 1: Yields (%, w/w) of essential oil and oleoresins of ginger rhizome

Sample	Essential oil yield	Oleoresins yield	
		Ethanol	Methanol
Dried ginger powder	1.5 ± 0.05	6.66 ± 0.06	10.70 ± 0.06

Data are expressed as the means of duplicate ± standard deviation.

The essential oil extracted from ginger using hydro-distillation method had a pale yellow color and was 1.5 % (w/w, db), which was higher than previously stated by Kamaliroosta *et al*. [9], who found that the essential oil of ginger was about 1.2%. The odour was spicy and warm. The ginger oleoresins extracted by solvents extraction method with methanol and ethanol solvents had different appearances and colors. The ethanol oleoresin was dark golden brown, while the methanol oleoresin was lighter. The higher extraction yield (10.70%) was obtained using methanol than by using ethanol (6.66%). The previous study reported that the yield of ethanol oleoresin and methanol oleoresin were 5.8% and 7.3%, respectively [4]. Therefore, the yields of oleoresins extracted from this study were 14.0 – 32.0% higher as the previous reported. The higher extraction yield using methanol to extract ginger oleoresin than using ethanol might be due to the different polarity of solvents. Although the extraction yields of oleoresins were high, the major problem of solvent extraction method was residual solvents and need to be analyzed after the extracting process. Thus, the extraction yields of both ginger essential oil using hydro-distillation method and ginger oleoresins using solvent extraction method varied depending on the varieties, country of origin, the state of samples and experimental conditions.

DPPH scavenging assay

DPPH radical-scavenging activities of ginger essential oil and oleoresins, expressed in term of 50% inhibition concentrations (IC_{50}), are given in **Table 2**. The results showed that methanol oleoresin had the lowest IC_{50} value (66.73 mg/L), followed by ethanol oleoresin (67.17 mg/L) and essential oil (51234.76 mg/L). It indicated that DPPH scavenging capacity of methanol oleoresin was the highest, followed by ethanol oleoresin and essential oil. It was clear from this study that the higher samples concentration, the stronger inhibition capacity to radical scavenging. The ginger essential oil had a lower antioxidant capacity than the ginger oleoresins. The previous study also reported that ginger extracts from solvents had a higher antioxidant capacity than ginger essential oil [12]. It might be correlated to the major bioactive compounds of these samples.

Table 2: Antioxidant capacity (IC_{50}) of ginger essential oil and oleoresins using DPPH scavenging method

Samples	IC_{50} (mg/L)
Ginger oil	51,234.76
EtOH oleoresin	67.17
MeOH oleoresin	66.73

Disc diffusion assay

The inhibition zone of ginger essential oil and oleoresins are given in **Table 3**. Overall, ginger oleoresins exhibited higher antimicrobial activity than ginger essential oil. All samples were found to have antimicrobial activity against all tested bacteria except *B. cereus*. Among these samples, methanol oleoresin showed the highest antimicrobial activity against *P. aeruginosa* (14.0 ± 0.2 mm) and *S. aureus* (7.50 ± 0.5 mm), followed by ethanol oleoresin and essential oil. The ethanol oleoresin had the most impact on *S. typhi* (17.0 ± 0.6 mm), whereas it did not show powerful activity against *S. aureus* (6.50 ± 0.1 mm).

Table 3: Inhibition zone (mm) of ginger essential oil and oleoresins against microorganisms

Sample	Zone Of Inhibition (mm)[a]			
	Bacillus cereus	***Pseudomonas aeruginosa***	***Samonella typhi***	***Staphylococcus aureus***
Ginger oil	-	11.9 ± 0.5	7.0 ± 0.2	7.0 ± 0.2
EtOH ole.	-	13.5 ± 0.4	17.0 ± 0.6	6.50 ± 0.1
MeOH ole.	-	14.0 ± 0.2	12.50 ± 0.2	7.50 ± 0.5

- No inhibition was observed.

[a]Data are expressed as the means of duplicate ± standard deviation

Minimum inhibitory concentration

The minimum inhibitory concentrations (MIC) of the ginger essential oil and oleoresins against tested bacteria are given in **Table 4**. The MIC values were in the range of 5.0 – 20.0 mg/L. *B. cereus* was found to be resistant to all samples. The minimum concentration to inhibit the growth of *S. aureus* was the highest (20 mg/L), followed by *P. aeruginosa* (10.0 mg/L) and *S. typhi* (5.0 mg/L). Among tested bacteria strains, the two Gram negative bacteria: *P. aeruginosa* and *S. typhi* were more sensitive to the essential oil and oleoresins than the two Gram positive: *B. cereus* and *S. aureus.* The previous study reported that all essential oil and oleoresin also showed a strong inhibitory effect on the two Gram negative bacteria: *P. vulgaris* and *K. pneumoniae* but they had no effect to the Gram positive bacteria: *S. aureus* [5]. It meant that using ginger oil and oleoresins are considered to have more antimicrobial effect on these Gram negative bacteria than Gram positive bacteria. The antimicrobial activity of ginger oleoresins was found to be more effective than essential oil. It seemed to be the fact that ginger oleoresins contain the major pungency active components such as: gingerols, shogaols, paradols and zingerone [5], whereas ginger essential oil possessed the aroma and the flavour of the spice but lack of the pungency.

Table 4: Minimum inhibition concentration (MIC) of ginger oil and oleoresins in agar disc diffusion assay

Sample	MIC (mg/L) in agar disc diffusion method			
	Bacillus cereus	***Pseudomonas aeruginosa***	***Samonella typhi***	***Staphylococcus aureus***
Ginger oil	-	10.0	5.0	20.0
EthOH oleo.	-	10.0	5.0	20.0
MeOH oleo.	-	10.0	5.0	20.0

- No inhibition was observed.

Conclusion

Antimicrobial and antioxidant activities of ginger essential oil and oleoresins were investigated in this study. Overall, ginger oleoresins had a higher antimicrobial and antioxidant activities than ginger essential oil. All of samples were found to possess antimicrobial activities on tested bacteria except *B. cereus.* To be specific, the tested Gram negative bacteria were found to be more sensitive to all samples than the Gram positive bacteria. Using methanol to extract oleoresin showed a higher extraction yield and antimicrobial activities than using ethanol, while the antioxidant activity was not significantly different. Although ginger essential oil were found to have lower biofunctional properties than

oleoresins, it is considered could be applied in the food industries and natural fragrance.

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