

Original article

Comparative Study on Antioxidant Activities and Phytochemical Components of Steamed and Non-Steamed Ginger Extracts

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Abstract

Introduction: Ginger (*Zingiber officinale* Roscoe, Zingiberaceae), is a widely-used plant in cooking and medicine in Thailand, listed in the National List of Essential Medicines 2011. In the past, Thai traditional practitioners always steamed the ginger rhizome before drug preparation, but there is no report comparing the biological activity and composition of extracts of ginger prepared from steamed and non-steamed rhizomes. The objectives of this research were to compare antioxidant activity and chemical composition of steamed and non-steamed ginger extracts.

Method: Ginger rhizomes (steamed and non-steamed) were dried and reduced to crude powders. They were macerated in 95% ethanol and boiled in water to produce 4 different extracts, following a 2 x 2 factorial design, with codes: AZOE (steamed ethanol extract), HZOE (non-steamed ethanol extract), AZOW (steamed water extract), HZOW (non-steamed water extract). The DPPH radical scavenging assay and total phenolic content (TPC) by Folin-Ciocalteu's reagent were used to evaluate and compare all extracts. For determination of phytochemical components, gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC) were used to identify and quantify the phytochemical compounds.

Results: GC-MS analysis of essential oil and oleoresin of ginger rhizomes showed the major components were zingiberene followed by 10-shogaol, 6-shogaol and gingerol, especially in ethanol extracts. 6-gingerol content was 65.42 ± 4.23 mg/g of extract and 6-shogaol equal to 48.12 ± 2.54 mg/g of extract in the steamed ethanol extract (AZOE) analysed by HPLC. AZOE, extracted according to local wisdom of Thai traditional practitioners showed higher antioxidant activity than HZOE and BHT as positive control with EC_{50} of 11.69 ± 0.16 , 14.10 ± 0.61 and 12.34 ± 0.03 μ g/mL, respectively. In addition, AZOE also showed higher TPC values than HZOE with values of 108.60 ± 1.14 and 95.67 ± 0.99 GAE mg/g, respectively. For non-steamed and steamed ginger water extracts (HZOW and AZOW) the result showed low antioxidant activity via DPPH free radical scavenging assay and total phenolic content (TPC) values.

Discussion and Conclusion: These results support the traditional practice of steaming ginger before use. Maceration in 95% ethanol increases both anti-oxidant potency and TPC, and shows the potential for development of a more effective Thai traditional drug from steamed ginger.

Key words: Antioxidant, Phytochemical compound, Steamed ginger extract, Non-steamed ginger extract.

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Introduction

Medicinal plants are important due to their abilities to reduce free radical-mediated degradation of cells, tissues and organs in humans. Antioxidant compounds found in medicinal plants can inhibit oxidant formation, intercept oxidants and repair oxidant-induced injury¹. Therefore, a natural antioxidant is a chemical compound that delays or prevents oxidative stress². There are many plant sources of natural antioxidants that help decrease oxidative stress.

In Thailand, there are medicinal plants which have long been used for treatment of degenerative disease. Ginger (*Zingiber officinale* Roscoe, Zingiberaceae), is a plant widely used as an anti-oxidant and as a condiment in a variety of food and beverages³. Ginger is a Thai traditional medicine and has long been used to treat flatulence, relief of nausea and vomiting, an elixir and is included in National List of Essential Medicines 2011⁴. The rhizome of ginger contains both the flavor and pungency of the spice or oleoresin together with the essential oil⁵. The pungent principles of ginger (gingerol and shogaols)⁵ are used in medicine as a carminative and aromatic stimulant to the gastrointestinal tract⁶. Gingerols are a series of homologues with varied unbranched alkyl chain length, whereas shogaols are a series of homologues derived from gingerols with dehydration at the C-5 and C-4 during long-term storage or thermal processing⁷. Other active compounds from the oleoresin portion of ginger have also been reported, such as [6]-paradol; [6]- and [10]-dehydrogingerdione; [6]- and [10]-gingerdione; [4]-, [6]-, [8]-, and [10]-gingerdiol; [6]-methylgingerdiol; zingerone; [6]-hydroxyshogaol; [6]-, [8]-, and [10]-dehydroshogaol; and diarylheptanoids⁸. Gingerols are the major components in fresh ginger rhizomes.

The amount of shogaols is increased in dried ginger, as evidenced by the reduction of the ratio of 6-gingerol to 6-shogaol in fresh ginger compared to dried ginger⁷. Ginger extracts contain various components, and it is important to identify which compounds are responsible for their pharmacological activities. It has been demonstrated that [6]-, [8]-, and [10]-gingerols and 6-shogaol showed efficacy in antioxidant, anti-inflammatory and antibacterial activity^{9,10,11}. In addition, [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol were shown to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH) with IC₅₀ values of 26.3, 19.47, 10.47 and 8.05 μ M, respectively⁹. The ethanolic extract of ginger displayed the highest antioxidant activity by DPPH assay, with EC₅₀ of 8.84 ± 0.49 μ g/mL. It also displayed the highest antioxidant activity by FRAP assay, with FRAP value of 623.24 ± 9.65 Fe²⁺/g and the highest total phenolic content of 47.17 ± 5.38 mg GAE/g by Folin-Ciocalteu's method¹². Finally, the ethanolic extract of ginger showed anti-oxidant properties by DPPH radical scavenging assay with IC₅₀ value of 52.5 ± 3.3 μ g/mL and total phenolic content 11.0 ± 1.5 mg GAE/g¹³.

The Thai traditional method of ginger preparation is by steaming before using the drug. Past and present researchers have not prepared the ginger following the Thai traditional method and there is no report comparing the chemical content and biological activity of extracts from steamed and non-steamed ginger. In order to test the validity of the traditional practice this study pre-processed the ginger by steaming the raw material before preparation as a medicine, and compared the chemical content and biological activity of extracts from steamed and non-steamed ginger. It is hoped that the results will support the Thai traditional practice and the use of effective medicine.

Method

2.1 Plant materials

Fresh ginger rhizomes used in this study were derived from plants grown by Good Agricultural Practices (GAP) at Nam Nao District, Phetchabun Province.

2.2 Preparation of extracts

One hundred kilograms ginger rhizomes were cleaned, washed and air dried. All dried rhizomes were divided into two parts. Part one was steamed with autoclave at 121 °C (similar to Thai traditional method) at 15 psi for 15 minutes, then dried in a hot air oven and ground to powder. Part two was oven-dried at temperature of 50 °C and ground to powder (extract method normally used). Both parts one and two powders were macerated in 95% ethanol and decocted in distilled water, resulting in 4 extracts from these macerations and decoctions. The principles of maceration and decoction can be described as follows: maceration in 95% ethanol was for 3 days then filtered through a Whatman No.1 filter paper, and filtrate was dried by rotary evaporator. Maceration was repeated twice more on each residue. The three filtrates of macerated extraction were all combined together and the percentage yield calculated. For decoction method, the other two crude powders were boiled in distilled water for 15 minutes and filtered. This was repeated twice more on each residue and the filtrates combined together then reduced $\frac{2}{3}$ by boiling, then dried by lyophilizer. Percentage yields of all the aqueous extracts were calculated.

2.3 DPPH radical scavenging activity

A DPPH solution in absolute ethanol (6×10^{-5} M) was freshly prepared and kept at temperature 4 °C and protected from light before use. Butylated hydroxytoluene (BHT) was used as reference standard, prepared in absolute ethanol to 1 mg/ml

concentration. Samples (ginger extracts) for testing were prepared in absolute ethanol or distilled water and diluted to 100, 50, 10 and 1 µg/ml, respectively. A portion of the sample solution (100 µl) was mixed with an equal volume of DPPH solution in 96-well plate and was allowed to stand in the dark for 30 minutes at room temperature. The absorbance was measured at 520 nm¹⁴. The scavenging activity of the samples was calculated as a percent inhibition in the formula below:

$$\text{Percentage of inhibition} = \frac{(\text{OD control} - \text{OD sample}) \times 100}{\text{OD control}}$$

The effective concentration of sample required to scavenge DPPH radicals by 50%, EC₅₀, was calculated by linear regression analysis of dose response curve plotting of percent inhibition against concentrations.

2.4 Determination of total phenolic content

Total phenolic content was determined by using the Folin-Ciocalteu method¹⁵. Standard gallic acid was dissolved in absolute ethanol and a calibration curve of absorbance against concentration obtained. The dilutions had standard concentration of 2.5, 5, 10, 20, 40, 80 and 100 µg/ml, respectively. From each sample, or blank (distilled water and absolute ethanol) 20 µl were placed into a 96-well plate and then 100 µl. Folin-Ciocalteu's reagent was added, mixed thoroughly by pipetting then incubated 1 to 8 min. 80 µl of sodium carbonate solution was added to the well. The plate was mixed and incubated for 30 min. at room temperature. The absorbance was measured at 765 nm using a microplate reader spectrophotometer. The amount of total phenolic compounds in the extracts was determined as milligram gallic acid equivalents (mg of GAE/g dry extract) using an equation that was obtained from a standard calibration graph. All measurements for each sample were done in triplicate.

2.5 Phytochemical components of steamed ginger extracts and non-steamed ginger extracts by using gas chromatography-mass spectrometry (GC-MS)

The steamed ginger extracts and non-steamed ginger extracts were analyzed by using a Thermo focus GC Gas Chromatography-Mass Spectrometry with capillary column TG-5 slim (30 m x 0.25 mm x 0.25 μ m), (Thermo Fisher Scientific). The ionization energy was achieved by electron impact at 70 eV. Helium (He) gas was the carrier with flow rate 1.0 mL/min. The initial temperature of column oven was programed for 60 °C, and then heated to 300 °C with a rate of 5 °C/min and kept constant at 300 °C for 10 min. The mass spectrum of each peak was recorded in the positive ion current mode of mass spectrometer within a mass range of 35 to 400. Identification of oil constituents was achieved using the Central Scientific Instrument Center (CSIC) TU Science.

2.6 Determination of active constituents in extracts using HPLC

The study on chemical fingerprint was carried out by HPLC system (Agilent® 1200) composed of a solvent degasser (G1322A), a quaternary solvent pump (G1311A), an autosampler (G1329A), a column oven (G1316A) and a photodiode array detector (G1315D). with a diodearray detector (G1315D), and automatic injector (G1329A). Data were analyzed with Chemstation® software. A reversed-phase column, Phenomenex® Luna C18 (150 mm x 4.6 mm, 5 μ m) analytical column connected with a guard column of the same material was used for isolation. 6-gingerol (Sigma, USA), 6-shogaol (Sigma, USA) were used as standards for quantitative analysis. Using 40 minutes run time with a flow rate of 1.0 mL/min, gradient mobile phase composed of water (A) and acetonitrile (B) were set as follows: 0 - 25 min, 40%B - 50%B; 25 - 30 min, 50%B - 95%B; 30 - 35 min, 95%B to 100%B; 35.1 - 40 min, 40%B. Samples were injected into the HPLC system and detected with diode array detector using wavelength of 227 nm. The content of 6-gingerol and 6-shogaol in the sample were determined by using standard curve constructed by using seven concentrations by serial dilution of standard solutions (Figure1).

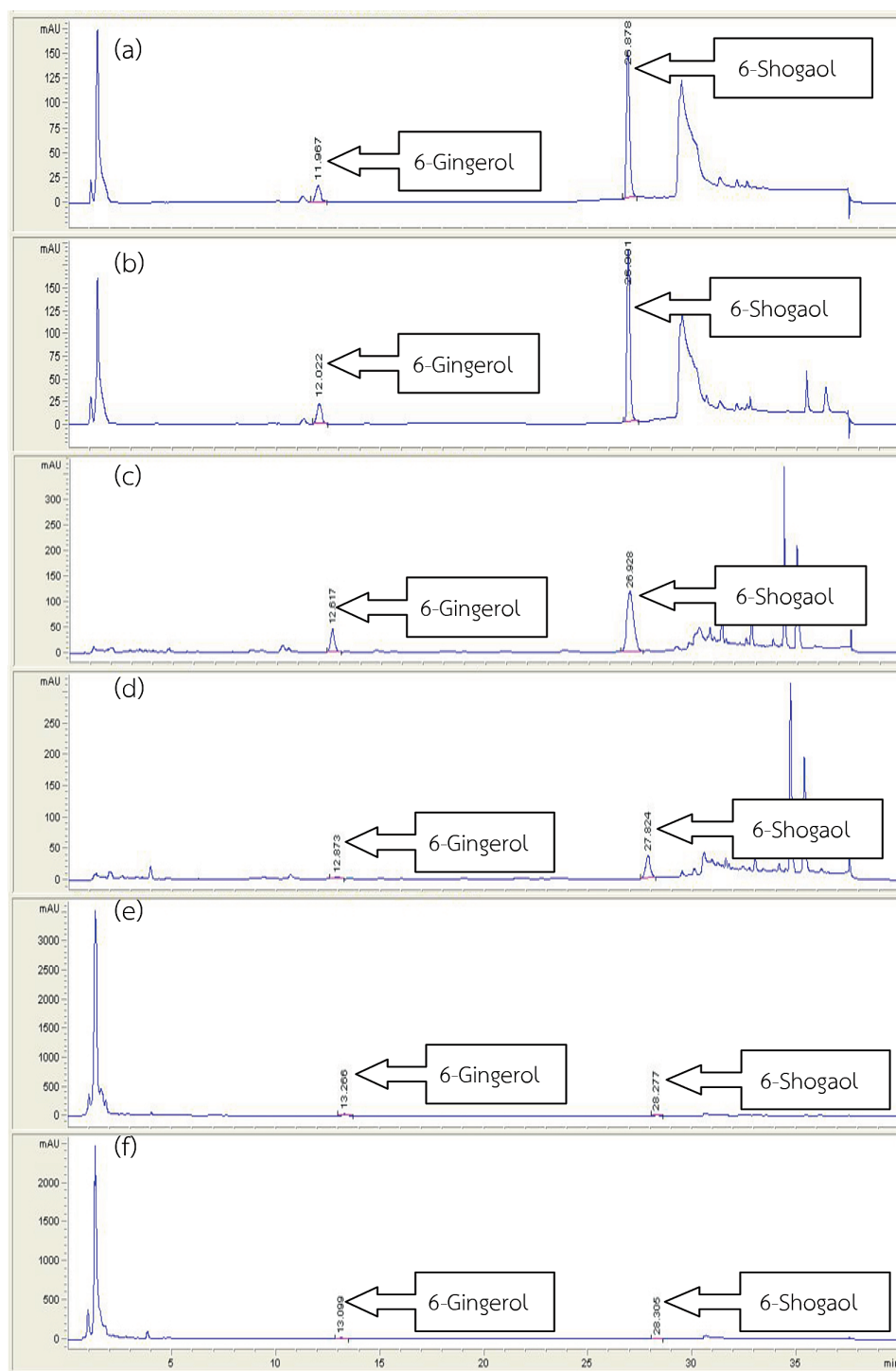


Figure 1 (a) Standard 6-Gingerol (25 µg/mL) and 6-Shogaol (25 µg/mL) (b) AZOE spiked with Standard 6-Gingerol (25 µg/mL) and 6-Shogaol (25 µg/mL) (c) AZOE (d) HZOE (e) AZOW (f) HZOW

Results

Antioxidant activity, DPPH radical scavenging activity and total phenolic compounds of the four ginger powders are shown in Table 1. The result found that the standard control, BHT, had a mean EC_{50} value of 12.34 ± 0.03 $\mu\text{g/ml}$. The ethanolic extract of steamed ginger (AZOE) has higher antioxidant activity than the standard BHT with a mean EC_{50} value of 11.69 ± 0.16 $\mu\text{g/ml}$, whereas the ethanolic extract of non-steamed ginger (HZOE) was very close to the standard BHT with a mean EC_{50} value of 14.10 ± 0.61 $\mu\text{g/ml}$. On the other hand, both of the water extracts of steamed ginger (AZOW) and non-steamed ginger (HZOW) had low antioxidant activity via DPPH radical scavenging with a mean EC_{50} value of 88.03 ± 0.87 $\mu\text{g/ml}$ and >100 $\mu\text{g/ml}$, respectively.

Determination of total phenolic content, showed that the ethanolic extract of steamed ginger (AZOE) has the highest total phenolic content (108.60 ± 1.14 mg GAE/g), followed by the ethanolic extract of non-steamed ginger (HZOE), the water extract of steamed ginger (AZOW) and the water extract of non-steamed ginger (HZOW), recording total phenolic contents 95.67 ± 0.99 , 26.76 ± 0.99 and 10.79 ± 0.82 mg GAE/g, respectively.

For phytochemical compounds, studies on chemical composition of the steamed and non-steamed ginger extracts by using GC-MS, the area under the peaks in Table 2. The major compounds from the AZOE were zingiberene (5.33%), phenol (3.44%) and dihydrostilbene (2.09%). The major compounds from the AZOW were 6-shogaol (5.77%), n-hexadecanoic acid (5.65%), zingiberene (4.47%) and capsaicin (4.46%). The major compounds from the HZOE were zingiberene (7.57%) and 6-shogaol (4.38%). The major compounds from the HZOW were 10-shogaol (16.57%), 6-shogaol (3.22%) and zingiberene (2.21%). From these results, 10-shogaol from the water extract of non-steamed ginger (HZOW) showed the highest percentage content.

HPLC technique was used for determine of the chemical content of all ginger extraction methods. Comparison of the chemical content by using HPLC as shown in Figure 1, found 6-gingerol and 6-shogaol were marker compounds of all ginger extraction methods. (AZOE, HZOE, AZOW, HZOW), while AZOE (the ethanol extract of steamed ginger) had the highest content of 6-gingerol with 65.42 ± 4.23 mg/g of extract, followed by HZOE, AZOW, HZOW, respectively.

Table 1 Free radical scavenging activity and total content of phenolic compounds of various ginger extraction methods. (mean \pm SEM, n = 3)

| Plant | Part Used and Extraction method | Solvent | Code | DPPH ($EC_{50}, \mu\text{g/ml}$) ^a | Total content of phenolic compounds (mg GAE/g dry material) ^{a, b} |
|----------------------|---------------------------------------|----------|------|---|---|
| <i>Z. officinale</i> | Dry rhizomes Hot Oven and Maceration | 95% EtOH | HZOE | 14.10 ± 0.61 | 95.67 ± 0.99 |
| | Dry rhizomes Hot Oven and Decoction | Water | HZOW | > 100 | 10.79 ± 0.82 |
| | Dry rhizomes Autoclave and Maceration | 95% EtOH | AZOE | 11.69 ± 0.16 | 108.60 ± 1.14 |
| | Dry rhizomes Autoclave and Decoction | Water | AZOW | 88.03 ± 0.87 | 26.76 ± 0.99 |
| | Butylated hydroxytoluene | | BHT | 12.34 ± 0.03 | - |

^a Values are mean \pm S.E.M. (n = 3).

^b Gallic acid equivalents are expressed as mg GAE/g dry material

Table 2 Chemical composition of the steamed and non-steamed ginger extracts by GC-MS

| Compounds | AZOE | | HZOE | | AZOW | | HZOW | |
|------------------------|----------|--------|----------|--------|----------|--------|----------|--------|
| | RT (min) | % Area | RT (min) | % Area | RT (min) | % Area | RT (min) | % Area |
| 1 Zingiberene | 27.402 | 5.33 | 27.283 | 7.57 | 26.838 | 4.47 | 26.982 | 2.21 |
| 2 Benzene | 32.747 | 0.37 | 37.467 | 0.67 | 26.534 | 0.70 | 26.668 | 1.14 |
| 3 n-Hexadecanoic acid | 37.587 | 0.43 | 37.594 | 0.78 | 37.409 | 5.65 | 37.440 | 0.93 |
| 4 10-shogaol | 39.956 | 0.53 | - | - | - | - | 40.757 | 16.57 |
| 5 Capsaicin | 40.159 | 1.16 | - | - | 36.842 | 4.46 | - | - |
| 6 (-)-Nortrachelogenin | - | - | 42.772 | 0.64 | 42.601 | 1.97 | 28.215 | 1.55 |
| 7 Dihydrostilbene | 42.901 | 2.09 | 40.590 | 0.78 | - | - | - | - |
| 8 Phenol | 44.378 | 3.44 | 21.051 | 0.04 | - | - | - | - |
| 9 6-shogaol | 44.654 | 1.85 | 44.241 | 4.38 | 43.870 | 5.77 | 43.872 | 3.22 |
| 10 Gingerol | 45.735 | 1.02 | 50.770 | 0.33 | 45.881 | 1.97 | - | - |

Discussion and Conclusion

In summary, the results indicate that the Thai traditional practice of ginger preparation by steam before using the medicine resulted in strong anti-oxidative activity via DPPH radical scavenging assay and high total phenolic content. When compared with related research, this result shows that the steamed ginger macerated in 95% ethanol has stronger activity than the result of Klinthong where the ethanolic extract of ginger by DPPH radical scavenging assay with IC_{50} value of $52.5 \pm 3.3 \mu\text{g/ml}$. and total phenolic content is $11.0 \pm 1.5 \text{ mg GAE/g}^{13}$. Comparing the chemical content by HPLC method of ethanol and water extracts from steamed and non-steamed ginger found the highest content of 6-gingerol and 6-shogaol was in AZOE (ethanolic steamed) and should be used as marker for standardization because 6-gingerol had potent antioxidant activity ($EC_{50} = 11.69 \pm 0.16 \mu\text{g/ml}$). Surprisingly, 6-gingerol level found in AZOE which corresponds to the Thai traditional method of steaming the ginger rhizome before drug preparation. Rather, the result found the highest content of AZOE was zingiberene, and showed low 6-gingerol and 6-shogaol content by GC-MS method. This agrees with the result of Charoenchai¹⁶ who found that the main compo-

nent found in volatile oil of fresh ginger rhizomes and ginger capsules was zingiberene (11.9 - 39.5% of the area under the peaks) with a small amount of gingerol and shogaol via GC-MS. A suggested reason is that gingerol and shogaol are easily decomposed by GC-MS method, therefore shows low content by comparison with HPLC method which showed the highest. In summary, this finding supports the steaming of ginger rhizomes before maceration in ethanol.

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บทคัดย่อ

การศึกษาเปรียบเทียบฤทธิ์ต้านอนุมูลอิสระและส่วนประกอบทางพฤกษเคมีของสารสกัดซึ่งที่ผ่านการนึ่งและไม่ผ่านการนึ่ง นักสรีรุษณ์ ฤกษ์เรืองฤทธิ์*, นวลจันทร์ ใจอารีย์**, อรุณพร อธิรัตน์**, ***, อติวุธ กมฺพมาศ****, ปรีชา วาณิชยเศรษฐกุล****, วีระชัย พิพัฒน์รัตนเสรี*****

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บทนำ: ชิง (Zingiber officinale Roscoe, Zingiberaceae) เป็นพืชที่นิยมใช้ในการทำอาหารและยา อยู่ในบัญชียาหลักแห่งชาติ พ.ศ. ๒๕๕๔ วิธีการเตรียมตัวแบบไทยดั้งเดิมคือการนึ่งก่อนเพื่อใช้ทำยา ไม่พบงานวิจัยที่เปรียบเทียบส่วนประกอบทางเคมีและฤทธิ์ทางชีวภาพของสารสกัดจากชิงที่ผ่านการนึ่งและไม่นึ่ง เพื่อศึกษาเปรียบเทียบฤทธิ์ต้านอนุมูลอิสระของสารสกัดซึ่งที่ผ่านการนึ่งและไม่นึ่ง

วิธีการศึกษา: เตรียมชิงสดด้วยสองวิธีที่ต่างกันคือ วิธีการนึ่งชิงด้วย autoclave (AZO) และวิธีการอบร้อนด้วยตู้อบลมร้อน (Hot oven) อุณหภูมิ ๕๐ องศาเซลเซียส (HZO) นำชิงทั้งหมดมาทำให้แห้งและบดผงหยาบ แล้วนำมาสกัดด้วยวิธีการหมักเอทานอล 95% ได้สารสกัดคือ (AZOE) และ (HZOE) และนำมาสกัดด้วยวิธีการต้มน้ำได้สารสกัดคือ (AZOW) และ (HZOW) ตามลำดับ หลังจากนั้นจะนำสารสกัดทั้ง ๔ สารตัวอย่างมาทดสอบฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH radical scavenging assay และปริมาณสารประกอบฟีนอลรวมด้วยวิธี Folin-Ciocalteu's reagent แล้วนำสารสกัดทั้งหมดมาเปรียบเทียบสารสำคัญโดยใช้เทคนิคแก๊สโครมาโตกราฟีแมสสเปกโตรเมตรี (Gas chromatography-mass spectrometry)

ผลการศึกษา: การทดสอบฤทธิ์ต้านอนุมูลอิสระพบว่าสารสกัดซึ่งขึ้นเอทานอลที่ผ่านการนึ่ง (AZOE) มีฤทธิ์ต้านอนุมูลอิสระสูงกว่าสารสกัดซึ่งอบด้วยตู้อบลมร้อน (HZOE) และ สารมาตรฐาน BHT โดยมีค่า EC_{50} เท่ากับ 11.69 ± 0.16 , 14.10 ± 0.16 และ 12.34 ± 0.03 ไมโครกรัมต่อมิลลิกรัม ตามลำดับ นอกจากนี้พบว่า AZOE มีปริมาณสารประกอบฟีนอลรวมสูงกว่า HZOE โดยมีค่า TPC เท่ากับ 108.60 ± 1.14 และ 95.67 ± 0.99 GAE mg/g ทั้งสองวิธีสารสกัดซึ่งขึ้นน้ำมีฤทธิ์ต้านอนุมูลอิสระน้อยและปริมาณสารประกอบฟีนอลรวมน้อย

วิจารณ์ และสรุปผลการศึกษา: ผลการวิจัยนี้สนับสนุนการทำยาแบบดั้งเดิมด้วยวิธีการนึ่งก่อนหมักด้วย 95% เอทานอลมีฤทธิ์ต้านอนุมูลอิสระ และปริมาณสารประกอบฟีนอลรวมดีที่สุด แสดงให้เห็นถึงประสิทธิภาพในการพัฒนายาไทยของวัตถุดิบซึ่งที่ผ่านการนึ่ง

คำสำคัญ: ฤทธิ์ต้านอนุมูลอิสระ, ปริมาณสารประกอบฟีนอลรวม, สารสกัดซึ่งที่ผ่านการนึ่ง, สารสกัดซึ่งที่ไม่ผ่านการนึ่ง