A comparative study on yield of extract, chemical marker content and antioxidant activity of *Zingiber zerumbet* and *Zingiber officinale*

R. Hasham[1, a], N. A. Azelan[1,b], M. A. Awang[1,c] and R. Aziz[1,d]

[1]Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310

[a]rosnani@ibd.utm.my, [b]amirahazelan@yahoo.com, [c]azrieishar@gmail.com, [d]ramlan@ibd.utm.my

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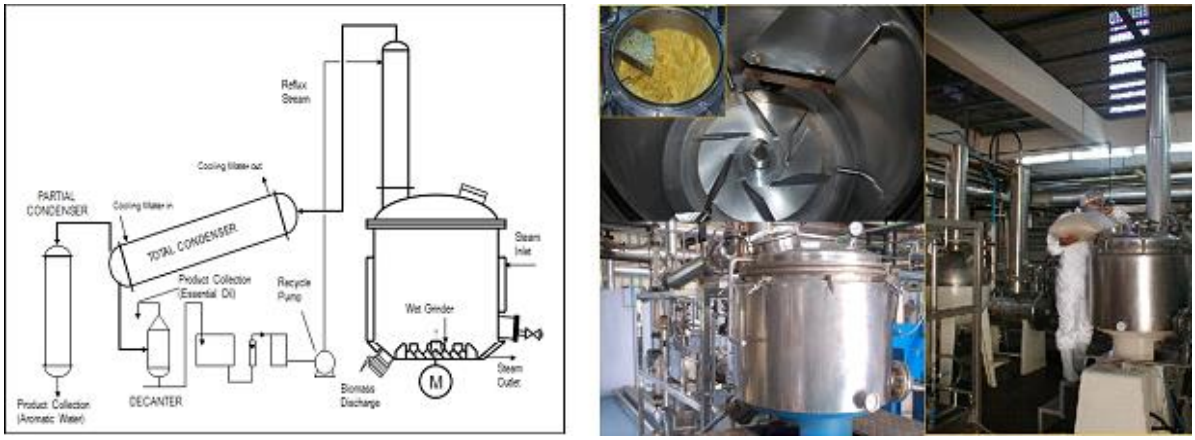
Abstract. *Zingiber officinale* (ZO) and *Zingiber zerumbet* (ZZ) are widely found in Southeast Asia and commonly used in herbal medicine practice for treating various diseases. This study was aimed to evaluate the yield of extract, chemical marker content and antioxidant activity of these Zingeberaceae species. Essential oils and hydrosols were obtained using turbo-extraction-distillation (TED). The highest yield of essential oil was obtained by the ZZ sample, which was 0.35 ± 0.09%, while the ZO showed the lowest yield (0.17 ± 0.02%). In hydrosol extracts the highest yield was obtained by the water extract of ZZ (2.50 ± 0.78%), while the ethanol/water (30:70) extract of ZO showed the lowest yield (0.90 ± 0.15%). The analysis of chemical marker content of ZO and ZZ showed 6-gingerol and zerumbone as the major component, respectively. The DPPH method showed the highest antioxidant activity for ZO essential oil (129.4 ± 14.47%), followed by ZZ essential oil (78.88 ± 9.35%) and hydrosol extracts. Collectively, these findings suggest that both ZO and ZZ can be used as potential sources of natural antioxidant in foods and herbal medicines.

Introduction

Zingiberaceae plant species have been widely used in traditional medicine in the treatment of various diseases. Essential oil from these plants possess diverse biological activities such as antioxidant, antimicrobial, antiulcer and antitumor (1-4). *Zingiber zerumbet* (ZZ) and *Zingiber officinale* (ZO) are among the taxonomical genus in Zingeberacea that mainly distributed in tropical and subtropical Asia. The major compound presented in the ZZ essential oils is zerumbone has been reported to exhibit antioxidant, antiproliferative and antiplatelet activities (3). Whilst, the gingerol derivative products in ZO essential oils have shown good antioxidants and anti-inflammatory effects (4).

Antioxidant is an extremely significant activity which can be used as a preventive agent against a number of diseases (1-2). Synthetic phenols, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisol (BHA) are the most commonly used antioxidants in food products. However, their safety has still remained uncertain. Recently, most of the researches are more focused on investigating the natural antioxidant that can be found in plant materials. The rhizomes of the Zingiberaceae plant, which their medicinal properties have been widely discussed and accepted worldwide are known to contain high antioxidant activity.

A wide variety of extraction method has been used for the isolation of the essential oil and hydrosol extracts from plant materials. Common techniques for the isolation and extraction of the essential oils includes hydrodistillation, turbo-extraction-distillation (TED), solid-phase extraction and supercritical fluid extraction. TED is a simple and cost effective method for extracting volatile compounds (5). There are various parameters such as extraction time, solvent used and sample mass that affecting the extraction efficiency and product quality of this method. Therefore, the aim of the present study was to evaluate the yield of extract, chemical marker content and antioxidant activity of ZZ and ZO rhizome extract by using TED.



a b

Fig. 1: Schematic Diagram (a) and 200 L Pilot Scale (b) of Turbo-Extraction-Distillation (TED) located at IBD, UTM.

Materials and Methods

Preparation of sample. The plant materials (ZO and ZZ) were procured from local suppliers, Johor, Malaysia and identified by the Herbarium, Universiti Putra Malaysia. The mature rhizomes were washed thoroughly with tap water before being rinsed with distilled water and wiped with a clean cloth. The cleaned rhizomes were cut into thin layers of cross section (of 1-2 mm thick) and stored at 4ºC for further experiment.

Extraction of ZO and ZZ using TED. The technical process for extracting essential oil using TED was illustrated in Fig. 1. 10 kg fresh rhizomes, ZO and ZZ were extracted by water or 30% ethanol in water in a 200 L vessel under the stipulated processing parameters. Isolated oil was collected, dried with anhydrous sodium sulfate, and stored at 4 ºC for further synthesis and/or analysis. The water from TED was recovered and filtered to obtain hydrosol extracts. Each hydrosol was spray dried using a pilot spray dryer (Niro A/S, GEA Group, Soeborg Denmark)

HPLC Analysis. The targeted marker compounds for ZO and ZZ extracts were 6-gingerol and zerumbone, respectively. HPLC analysis of ZO and ZZ extracts were carried out on a C18 column (5 µ, 4.6 x 250 mm) using a Waters 2695 USA. The purity of 6-gingerol and zerumbone was checked with HPLC and detected at 230 nm. Flow rate was 1 mL/min. The mobile phase system used in this study for 6-gingerol and zerumbone were composed of acetonitrile:1% acetic acid (65:35, v/v) and 0.01 potassium dihydrogen phosphate: acetonitrile:methanol (20:25:55, v/v), respectively. The whole chromatogram took 30 minutes. The ratio of peak area of 6-gingerol and zerumbone over that of total peaks is a purity reference of 6-gingerol and zerumbone, respectively.

Antioxidant Analysis. The antioxidant activity was evaluated using the 1,1-diphenyl-2-picryhydrazil radical scavenging activity. The free radical scavenging capacity of ZO and ZZ extracts were determined using DPPH according to the method of Blois [6].

Results and Discussions

Extraction yield and time The values obtained for essential oil yield from the two types of Zingiberaceae plants (ZZ and ZO) are 0.35 ± 0.09% and 0.17 ± 0.02%, respectively (Table 1-2). TED is a technique which causes extraction to be accelerated (5). It is generally used on hard matter

such as wood or seeds. In this study, ZZ shows a higher yield of essential oils, probably because ZZ rhizomes are harder and less fragile compared to ZO rhizomes.

Fig 2. Shows the variety of extraction yield according to the extraction time for ZZ an ZO rhizomes and two phases can be observed. The first phase is represented by an increase in yield which characterizes the first quantities extracted, located at the surface of rhizome particles. This phase is followed by a second increasing line which represents the diffusion of the essential oil from the midst of the particles towards the external medium caused by the internal warming of the water located in the plant cells. The last phase corresponds to the plateau, which marks the end of the extraction process. ZZ and ZO rhizomes reach maximal yields of 0.35 ± 0.09% and 0.17 ± 0.02%, respectively, in 5-6 h extraction time. These results are in comparison with the results reported in other studies, where the essential oil content of fresh ZZ and ZO rhizomes for most of the varieties are around 0.1 to 0.5% depending upon the variety, climatic variation and locality.

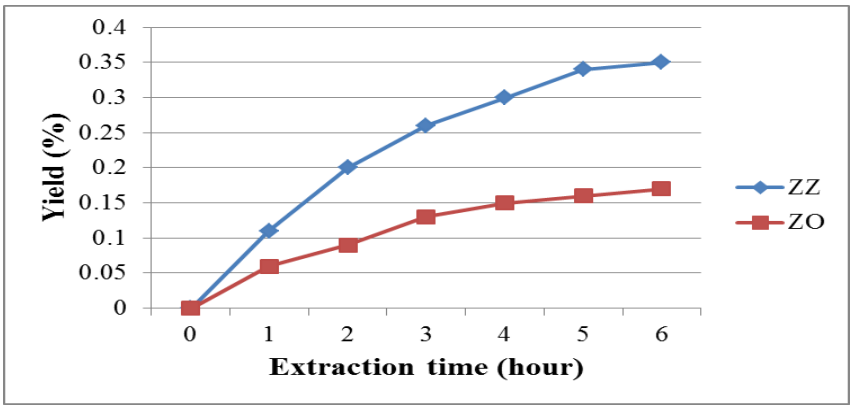


Fig. 2: Cumulative yields (%) of the ZZ and ZO essential oils obtained by TED

Essential oil and hydrosol. Table 1 and 2 present the yield of extracts, the total chemical marker content and antioxidant activities of ZZ and ZO, respectively. The essential oils and hydrosols extracted from ZZ showed higher yields than ZO. Results also found with ethanol/water (30:70) extraction solvent is unsuitable to isolate essential oil from both rhizomes by TED. Similarly, the yield of ZZ hydrosols (aromatic water) by the water extract (2.50 ± 0.78%) was found higher than the ethanol/water (30:70) extract (1.80 ± 0.37%). However, the yield of hydrosol extracts obtained from ZO was not much different by both solvent (Table 2). Hydrosols contain a part of the essential oil compound dissolved in and remain in the distillation water. It has a very pleasant aroma and is recognized as having commercial applications.

Chemical marker content. The chemical marker of ZZ and ZO extracts are zerumbone and 6-gingerol, respectively. These compounds were identified using HPLC. The analyses of essential oils and hydrosol extracts revealed the difference of chemical marker content in the samples. If we compare ZZ and ZO, we noticed that the chemical marker content in ZZ (zerumbone) is higher than ZO (6-gingerol); 126.54 ± 34 mg/ml against 16.00 ± 5.21 µg/ml, respectively. On the other hand, zerumbone content is higher in ZZ essential oils than hydrosol extracts of both solvent systems. Whilst, 6-gingerol content is higher in ZO hydrosol extract with 100% water than essential oils. Generally, the essential oil fraction are present in hydrosol, its give strong aroma, but, compositions are often different from the primary essential oil (7-8). Yet, the major components are generally the same of those present in oxygenated fraction of corresponding essential oils (9).

Antioxidant activity. The essential oils and hydrosol extracts of ZZ and ZO were subjected to screening for their potential antioxidant activity by DPPH free radical scavenging assay. The

weakest radical-scavenging activity in the extracts of ZZ was exhibited by the ZZ hydrosol water extract, whereas the strongest activity was exhibited by the ZZ essential oil extract (78.88 ± 9.35%). The antioxidant activity of essential oils and hydrosol extracts in ZO showed that all samples expressed strong antioxidant effects. The radical-scavenging activity in ZO essential oils (129.4 ± 14.47%) higher than that of the hydrosol extracts with water and 30% ethanol/water more than one fold.

Table 1. Yields, total biomarker content and antioxidant activity of the ZZ extracts obtained by TED

Extraction solvent	**Extract**	**ZZ (%)**	**Total zerumbone content (µg/ml)**	**Antioxidant activity (%)**
Water (100%)	Essential oil	0.35 ± 0.09	(126.54 ± 34) x 10^3	78.88 ± 9.35
	Hydrosol	2.50 ± 0.78	14.84 ± 2.33	29.18 ± 5.37
Ethanol:Water (30:70)	Hydrosol	1.80 ± 0.37	46.89 ± 7.07	61.29 ± 2.28

Table 2. Yields, total biomarker content and antioxidant activity of the ZO extracts obtained by TED

Extraction solvent	**Extract**	**ZO (%)**	**Total 6-gingerol content (µg/ml)**	**Antioxidant activity (%)**
Water (100%)	Essential oil	0.17 ± 0.02	16.00 ± 5.21	129.4 ± 14.47
	Hydrosol	1.00 ± 0.21	24.81 ± 2.17	60.64 ± 5.26
Ethanol:Water (30:70)	Hydrosol	0.90 ± 0.15	4.71± 0.42	58.31 ± 4.69

Summary

In conclusion, ZZ and ZO could be a valuable aromatic material for not only essential oil production, but also for hydrosol production with higher yield and quality. The use of 30% ethanol/water is not a good solvent to extract ZZ and ZO essential oils by using TED. The overall results on antioxidant activity obtained for essential oils were better that that obtained for hydrosol extracts. Data showed that high antioxidant activity was related to the high chemical marker content in the extracts. The essential oils from both rhizomes (ZZ and ZO) are potentially used as source of natural antioxidants. Whereas, hydrosol of ZZ and ZO should not be discarded as usually done with commercial distillation of aromatic crops. It could be redistilled or reused for distillation of fresh herb to minimize the loss of valuable components of the essential oil. Alternatively, its excellent quality and the natural odour to the essential oil (stronger than the essential oil) make them useful for food and cosmetic industries. Taken together, the results could potentially be beneficial, since essential oil producers need alternative essential oil crops producing marketable aromatic products, and this study might open new opportunity to the exploitation of ZZ and ZO distillation and extraction products and process with high quality.

Acknowledgements

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