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Supercritical Fluid Extraction (SFE) of Malaysian Wild Ginger *Zingiber puberulum* Inflorescence

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ABSTRACT

Study on bioactive compounds in essential oil of Malaysian wild ginger species, *Zingiber puberulum* was carried out. The essential oil was extracted from the inflorescence of *Z. puberulum* using SFE at 48 Mpa and 40°C of extracting pressure and temperature respectively. The oil was then analysed using gas chromatography-mass spectrometry (GC-MS) instrument. Twenty-one compounds were found in the SFE extract of *Z. puberulum* with fatty acids; i.e. palmitic acid (65.7%) and oleic acid (15.9%) were the major constituents and small percentages of various terpenic compounds. The sesquiterpene compounds identified in the SFE extract of *Z. puberulum* were α-bisabolol, β-elemene and caryophyllene. It was found that SFE could be used successfully to extract a wide variety of important bioactive compounds in *Z. puberulum* but that the conditions may need to be optimized to ensure the optimum yield of the volatile compounds.

Key words: SFE, volatile constituents, *Zingiber puberulum.*

INTRODUCTION

Malaysian natural product plants have been studied extensively. These include medicinal plant species from Andrographis[1,2,3,4], Musa[5], Plumeria[6,7], Citrus[8,9], Cymbopogon[10], Garcinia[11,12], Artocarpus[13], Kaempferia[14] and Alpinia[15]. Besides medicinal plants, fish products particularly ω-fatty acids have been studied extensively[16,17]. However, plant species of Zingiberaceae continue to attract much phytochemical interests due to their culinary uses, besides their biological and pharmaceutical activities. Recently, researcher from Universiti Putra Malaysia (UPM) has found a natural substance from *Z. zerumbet* to treat and prevent cervical cancer[18]. With the discovery of the new medicine, the cost of cancer treatment would be lower than the cost of using imported drugs. Unfortunately, of the *Zingiberaceae* family, *Z. puberulum* is less known and studied compared to the common ginger, *Z. officinale*. Only work by Sirat and co-workers[19] found that the extract of *Z. puberulum* contained the labdane diterpenic constituents. Meanwhile, Theilade[20] documented the existence of *Z. puberulum* in Thailand ginger.

The supercritical fluid extraction (SFE) is a technology of interest to the food, cosmetics and pharmaceuticals industries, as an alternative to conventional methods, i.e. solvent extraction and solid-phase microextraction (SPME)[21]. SFE employs the use of supercritical fluid as the extracting solvent replacing the use of conventional organic solvents. Many researchers have employed SFE to extract essential oils and oleoresins from ginger species. Nik Norulaini et al[22] extracted zerumbone from *Z. zerumbet* using SFE-CO_2 while Chen et al[23] have used Taguchi method in extracting *Z. officinale* extract with SFE-CO_2. Based on these approaches, the present study aims to extract bioactive constituents from *Z. puberulum* using the SFE technique.

EXPERIMENTAL

Materials

Samples of *Z. puberulum* inflorescences were taken from Lojing, near Cameron Highland, Pahang, Malaysia and stored at - 4°C in the freezer for preservation purposes. The samples were washed, and chopped horizontally into smaller pieces. Samples used for SFE were dried using a vacuum oven at 40°C for 48 hours to remove the water content and then ground into powder using a blender.

Extraction Of Volatile Components

Supercritical Fluid Extraction (SFE)

Supercritical CO_2 was carried out using SFT-150 Supercritical Fluid Extraction/Reaction System (Supercritical Fluid Technologies Inc. Newark, Delaware, USA) comprising of a carbon dioxide cylinder, stainless steel vessel, 100 ml hand-tight sample vessel, air regulator, PID temperature controller and variable restrictor valve (back pressure regulator).

Approximately 10g of blended *Z. puberulum* sample was placed in the sample vessel. The sample vessel was placed in the stainless steel vessel and allowed to equilibrate to the preset extraction temperature of 40°C. The high pressure pump compressed the CO_2 to the desired 48 Mpa pressure. The sample was subjected to 30 minutes static extraction (by closing the restrictor valve) followed by every 30 minutes dynamic extraction (by opening the restrictor valve). The essential oil collected in a pre weighted container was weighted after 3 hours of extraction.

Analysis Of Volatile Components

Gas Chromatography-Mass Spectrometry Analysis (GC-MS)

1 µl of extract in dichloromethane was analyzed using PerkinElmer AutoSystem XL Gas Chromatograph (PerkinElmer, Shelton, CT, USA). The temperature was initially kept at 40°C for 1 minute, then programmed at 4°C per minute until it reaches 250°C, and then kept at the final temperature for 5 minute. Helium gas was used as a carrier gas with a flow rate of 1 ml/min. For identification of the chemical constituents in the samples, the PerkinElmer TurboMass Gold Mass Spectrometer was used. The compounds are identified by comparing the MS spectrum obtained with the standard library (NIST 2000)

RESULTS AND DISCUSSION

Extraction

The supercritical fluid extraction (SFE) of the inflorescences of *Z. puberulum* gave dark yellowish viscous oil in 1.2% yield (w/w). Previous report found that by using simultaneous distillation extraction (SDE), a colourless non-viscous oil of *Z. puberulum* in 0.15% yield was produced[24]. Similarly, the result showed that the higher yield and the more concentrated essential oils were extracted using SFE than SDE.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Table 1 shows the volatile constituents identified in *Z. puberulum* extract by GC-MS technique. It was found that the main constituents of *Z. puberulum* extracted using SFE were palmitic acid (C16:0) (65.7%), followed by 1-heptatriacotanol (16.9%) and oleic acid (C18:1) (15.%). This result verified that in this study SFE extracted the non volatile fatty acids along with the volatile compounds. According to Diaz-Morato et al.[25], using high pressure CO_2 densities may allow terpenes and oxygenated terpenes to be completely miscible in supercritical CO_2, but other non volatile compounds such as fatty acids, waxes and paraffins can also appear in the extract. Given that high pressure was used in SFE (48 Mpa), this explains why SFE extracts of *Z. puberulum* had high amount of fatty acids in it. It was also proved that high pressure is not recommended for complex matrices owing to the higher solubility of solutes when the pressure is elevated, resulting in complex extracts and difficult analysis as is in this case[26].

In this study, many of the volatiles identified in *Z. puberulum* oil were similar to the volatiles in *Z. spectabile* cultivated in Amazon[27]. However, the percentages were quite different: β-phellandrene, α-pinene and β-pinene were not the major components of the *Zingiber spectabile* oil extracted by SFE, and neither were dodecanal, tetradecanol and α-pinene in SFE extracted oils of *Zingiber puberulum*. The reason could be attributed to difference in cultivation, geographical location and method of extraction.[28]

Table 1: Volatiles identified in the essential oils of *Zingiber puberulum* extracted using SFE

Peak	Compound	% relative
1	β-pinene	0.001
2	Undecanal	0.01
3	α-Terpineol	0.02
4	Decanal	0.01
5	*cis*-Geraniol	0.03
6	Isopinocarveol	0.01
7	Caryophyllene	0.07
8	α- Caryophyllene	0.05
9	Phellandral	0.03
10	β-Bisabolene	0.01
11	β -Farnesene	0.02
12	β-Sesquiphellandrene	0.03
13	Caryophyllene oxide	0.04
14	α-Farnesene	0.01
15	β-Elemene	0.14
16	α-Bisabolol	0.57
17	Tetradecanal	0.07
18	Palmitic acid	65.7
19	Phytol	0.43
20	Oleic acid	15.9
21	1-Heptatriacotanol	16.9

Comparison of the compounds in the essential oils of *Zingiber spectabile* and *Zingiber puberulum* showed the presence of 7 similar compounds; undecanal, α-terpineol, phellandral, zingiberene, farnesene, caryophyllene oxide, 1-heptatriacotanol and phytol. This showed the connection between the different species in the same Zingiberaceae family.

Many of the bioactive compounds identified in the ginger flower had been known to have certain properties. β-sesquiphellandrene and ar-curcumene are the prime contributors to the characteristic ginger attribute while α-terpineol, neral, and geranial contribute to the lemony aroma of ginger oil, and may therefore be desirable additives to whole ginger oil to intensify its lemony or citrus character. β-elemene, a sesquiterpene hydrocarbon found in abundance in *Z. puberulum,* is a novel anticancer drug, which has also been found in the *Zingiber officinalis* species[29]. Thus, the results of the present investigation clearly indicate that the flower of wild ginger species possess important bioactivities which could be manipulated commercially.

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