UNIVERSITI PUTRA MALAYSIA

EFFECTS OF DRYING METHODS ON CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF TORCH GINGER (Etlingera elatior Jack) FLOWER EXTRACTS AND ESSENTIAL OIL

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

EFFECTS OF DRYING METHODS ON CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF TORCH GINGER (*Etlingera elatior* Jack) FLOWER EXTRACTS AND ESSENTIAL OIL

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April 2018

Chairman : Associate Professor Anis Shobirin Meor Hussin, PhD
Faculty : Food Science and Technology

Torch ginger (*Etlingera elatior* Jack) flower is a well-known spice for local delicacies and natural remedies that has been reported to possess significant antioxidant and antibacterial activities. However, the maturity stages, drying conditions and extraction methods are still questionable in preserving bioactive compounds of torch ginger flower. Therefore, the aims of this study were to profile the bioactive compounds in different maturity stages (tight bud and full bloom) of torch ginger flowers using Gas Chromatography-Mass spectrometry (GC-MS). Then, the effect of drying methods; freeze, oven and sun drying on extraction yield and antioxidant activities of selected maturity stage of torch ginger flowers were studied. Torch ginger flower's essential oil extracted using subcritical carbon dioxide (SubC-CO_2) were evaluated for antioxidant and antibacterial activities against selected foodborne pathogens, namely *Bacillus cereus* ATCC10876, *Escherichia coli* ATCC11229, *Klebsiella pneumoniae* ATCC13773, *Listeria monocytogenes* ATCC19111, *Salmonella Typhimurium* ATCC13311, and *Staphylococcus aureus* ATCC29213. Maturity stage had significant effect on percentage area of bioactive compounds concentrations (caryophyllene and 1-dodecanol) in torch ginger flower extract. Oven drying was identified as optimum extraction yield of 37.8±2.48% with high level of scavenging activity of 62.4±1.24% and IC_{50} value of 9.68 mg/mL, ferric reducing assay power of 2340.7±65.53 mg Fe^{2+}/100 g, total phenols content of 331.6±2.85 mg GAE/100 g and total flavonoids of 465.8±2.08 mg QE/100 g. The antioxidant activities of torch ginger flower's essential oil through DPPH assay (90.0±0.25%), FRAP assay= 8876.5±36.35, ABTS scavenging activity= 81.8±2.10%, TPC= 1590.2±3.74 mg GAE/100 g and TFC= 1686.2±17.34 mg QE/100 g. Minimal Inhibitory Concentration (MIC)= 1.56 mg/mL and Minimal Bactericidal Concentration (MBC)= 3.13 mg/mL of torch ginger flower's essential oil were able to inhibit the growth of *B. cereus* and *K. pneumoniae*. This study clearly

indicated that torch ginger flower could be potentially used as natural antioxidant and antibacterial agent in the food and pharmaceutical fields.

Keywords: Antioxidant, antibacterial, torch ginger, drying methods

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN KAEDAH PENGERINGAN TERHADAP KOMPOSISI KIMIA, AKTIVITI ANTIOKSIDAN DAN ANTIBAKTERIA EKSTRAK DAN MINYAK PATI BUNGA KANTAN (*Etlingera elatior* Jack)

Oleh

ALIAA BINTI ANZIAN

April 2018

Pengerusi : Professor Madya Anis Shobirin Meor Hussin, PhD
Fakulti : Sains dan Teknologi Makanan

Bunga kantan (*Etlingera elatior* Jack) adalah rempah yang terkenal dalam makanan tempatan dan sebagai remedi semula jadi yang dilaporkan memiliki aktiviti antioksidan dan antibakteria yang penting. Walau bagaimanapun, peringkat kematangan, kaedah pengeringan dan pengekstrakan masih boleh dipersoalkan dalam mengekalkan sebatian bioaktif yang terkandung di dalam ekstrak dan minyak pati bunga kantan. Oleh itu, matlamat kajian ini adalah untuk mengenalpasti sebatian bioaktif di dalam bunga kantan pada tahap kematangan yang berbeza (kuntum ketat dan mekar penuh) menggunakan kromatografi gas-spektrometri massa (GC-MS). Seterusnya, kesan kaedah pengeringan; sejukbeku, ketuhar dan cahaya matahari terhadap hasil ekstrasi dan aktiviti antioksidan daripada peringkat kematangan bunga kantan yang dipilih telah dikaji. minyak pati bunga kantan yang diekstrak menggunakan karbon dioksida separa kritikal (SubC-CO_2) telah dinilai untuk aktiviti antioksidan dan menentang bakteria bawaan makanan yang terpilih iaitu *Bacillus cereus* ATCC10876, *Escherichia coli* ATCC11229, *Pneumoniae Klebsiella* ATCC13773, *Listeria monocytogenes* ATCC19111, *Salmonella Typhimurium* ATCC13311 dan *Staphylococcus aureus* ATCC29213.Tahap kematangan mempunyai kesan ketara terhadap peratusan keluasan konsentrasi sebatian bioaktif (caryophyllene dan 1-dodecanol) dalam ekstrak bunga kantan. Pengeringan ketuhar telah dikenalpasti sebagai hasil ekstrasi yang optima sebanyak 37.8±2.48% dengan peratus aktiviti perencatan radikal (DPPH) yang tinggi (62.4±1.24% dan IC_{50}= 9.68 mg/mL), kuasa penurunan asai antioksidan ferik (2340.7±65.53 mg Fe^{2+}/100 g), jumlah kandungan fenolik (331.6±2.85 mg GAE/100 g) dan flavonoid (465.8±2.08 mg QE/100 g). Aktiviti antioksidan minyak pati bunga kantan dikenalpasti melalui DPPH (90.0±0.25%), FRAP= 8876.5±36.35, ABTS= 81.8±2.10%, TPC= 1590.2±3.74 mg GAE/100 g dan TFC= 1686.2±17.34 mg QE/100 g. Kepekatan perencatan minimum (MIC)= 1.56 mg/mL dan kepekatan bakteria minimum (MBC)= 3.13 mg/mL oleh minyak pati bunga kantan dapat merencatkan ketumbuhan *B. cereus* dan *K. pneumoniae*. Kajian ini jelas menunjukkan bahawa

minyak pati yang diperolehi dari bunga kantan berpotensi digunakan sebagai ejen antioksidan dan antibakteria semula jadi di dalam bidang makanan dan farmaseutikal.

Kata kunci: Antioksidan, antibakteria, bunga kantan, kaedah pengeringan,

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I certify that a Thesis Examination Committee has met on 11 April 2018 to conduct the final examination of Aliaa binti Anzian on her thesis entitled "Effects of Drying Methods on Chemical Composition, Antioxidant and Antibacterial Activities of Torch Ginger (*Etlingera elatior* Jack) Flower Extracts and Essential Oil" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Name of Member of Supervisory Committee: Prof. Dr. Nazamid Saari

Signature: _____
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AA	Ascorbic acid
$ABTS^+$	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
ATCC	American Type Collection Culture
CLSI	Clinical and Laboratory Standard Institute
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
FRAP	Ferric reducing antioxidant power assay
GC-MS	Gas chromatography mass spectrometry
GRAS	Generally recognized as safe
HS-SPME	Headspace-solid phase micro extraction
IC_{50}	Inhibition concentration
MBC	Minimum bactericidal concentration
MHA	Muller-Hinton agar
MHB	Muller-Hinton broth
MIC	Minimum inhibitory concentration
mg GAE/ 100 g DW	Milligram gallic acid equivalent per 100 g dry weight
mg QE/ 100 g DW	Milligram quercetin equivalent per 100 g dry weight
NA	Nutrient agar
NIST-MS	National Institute of Standards and Technology Mass Spectral
ROS	reactive oxygen species
RNS	reactive nitrogen species
SubC-CO_2	Subcritical carbon dioxide extraction
SFE	Supercritical fluid extraction
TFC	Total flavonoid content
TPC	Total phenolic content
TPTZ	2,4,6- Tris (2-pyridyl)-s- triazine

CHAPTER 1

INTRODUCTION

Phytochemicals are secondary metabolites of plants that have been reported to possess a broad range of biological activities, such as anti-allergic, anti-carcinogenic, anti-oxidant, anti-microbial, anti-cancer, anti-viral and anti-inflammatory (Barros et al., 2008). Previous studies have proven that phytochemicals play imperative role as therapeutic agents in reducing or preventing chronic diseases including arteriosclerosis, cancer, cardiovascular disease (CVD), hypertension, Alzheimer's and age related disorders (Patel et al., 2016; Wijekoon et al., 2013). Due to new interest, researchers nowadays are struggling to exploit every part of the plants (bark, flower, leaves, rhizomes, roots and stem) as natural alternative antioxidant and antibacterial agent in food and nutraceutical applications (Tao and Liu, 2012; Yao et al., 2011).

Antioxidants are compound that capable of delaying or inhibiting the oxidation of other molecules. Oxidation occurs when an electron was transferred from a substance to an oxidizing agent in a chemical reaction. In brief, antioxidants remove free radical intermediates and inhibit oxidation reactions by oxidizing themselves thus lead to the termination of the chemical chain reaction. Nowadays, synthetic antioxidants which are obtained from chemical process are being incorporated in food to inhibit or slow down oxidation of food when exposed to environmental factors (air, light and temperature). Butylate hydroxyanisole (BHA), butylate hydroxytoluene (BHT), propyl gallate (PG) and tertbutylhydroxyhydroquinone (TBHQ) are commonly used as synthetic antioxidants in the food industry. Nevertheless, these synthetic antioxidants have several harmful effects toward human health (Bulbul et al., 2012). For instance, BHA was proven to be carcinogenic in animal experiments; meanwhile, high doses of BHT might cause death in some strain of guinea pigs and mice due to internal and external hemorrhaging (Phoopuritham et al., 2006).

In food industry, benzoic acid, sodium benzoate potassium sorbate and sodium nitrite are also antimicrobial agents, which are normally used as preservatives to extend shelf life of food products. However, these chemical preservatives have potential toxicity (Bulbul et al., 2012). Consequently, food manufacturers present a growing interest on the potential of plant containing essential oil because essential oil have been known to exhibit antibacterial properties and inhibit foodborne pathogens causing food poisoning (Susanti et al., 2013). Many studies have demonstrated the efficiency of essential oil even at low doses, which are able to inhibit bacterial pathogens encountered in food industry and meat product (Oussalah et al., 2006; Oussalah et al., 2007).Thus, there has been considerable interest in finding natural antioxidant and antibacterial agent from plant sources to replace the synthetic antioxidant and antibiotics in market.

Torch ginger is an edible aromatic plant, and works on extracting its extract and essential oil from different parts of plant (flower, leaf, rhizome and inflorescence) in order to determine its phytochemical and pharmacological properties have been extensively reported (Abdelmageed et al. 2011; Abdelwahab et al. 2010; Chan et al, 2007, 2009(b); Jackie et al. 2011; Lachumy et al. 2010; Mohamad et al. 2005). Recently, the studies on chemical composition and antimicrobial activity of torch ginger flower were conducted by Nurain et al. (2013), Susanti et al. (2013) and Wijekoon et al. (2013). The results proved that torch ginger flower has a high probability to become a natural alternative of antibacterial agents and can provide a strong base for utilization of torch ginger flower in food packaging to reduce the detrimental effects associated with foodborne pathogens to be applied in food system.

Torch ginger flowers have very short shelf life as it is highly perishable in nature. Usually, these flowers deteriorate rapidly after harvesting which leads to loss of aroma, flavour and quality. Therefore, drying is an important technology in preserving the product's quality and to prevent the spoilage of the product during storage. Drying decreases the moisture content and water activity, which eventually minimize microbial growth, helps to maintain desirable qualities and reduces storage volume (Juhari et al. 2012). According to Que et al. (2008), freeze drying and spray drying are known to be costly and required complicated preparation processes, which are not suitable to be applied in a production with a limited budget. In this present study, oven drying might be a more suitable technique for the processing and production of torch ginger flower powder, particularly for local companies.

Varieties of extraction techniques have been employed for the separation of active medicinal portions of plants from inactive or inert compounds using various solvents and procedures (Wang and Weller, 2006). Conventionally, bioactive compounds from plants especially torch ginger flower are extracted by hydro distillation, maceration, solvent extraction, soxhlet extraction, steam distillation and heating reflux extraction (Vairappan et al., 2012; Susanti et al., 2013). In the last decades has leads to the advanced extraction methods in extracting natural bioactive compounds which are enable to reduce extraction time and energy consumption, improve extract and essential oil's qualities yet increase the extraction yields (Khoddami et al., 2013; El Asbahani et al., 2015).

For example, subcritical carbon dioxide (SubC-CO_2) extraction is an effective technique to preserve bioactive compounds under low temperature and pressure. This method avoids degradation or entrainment by vapour due to high temperature and the presence of water (El Asbahani et al., 2015). In addition, the previous studies done by Khajeh et al. (2004) and Hamdan et al. (2008) proved that better qualities of the extracts obtained by subcritical carbon dioxide (SubC-CO_2) extraction in oil composition of ajwain (*Carum copticum*) and cardamom (*Elettaria cardamomum* Maton). However, there was no research done on the extraction of torch ginger flowers by subcritical carbon dioxide (SubC-CO_2) extraction.

Therefore, it is important to evaluate the maturity stage, effect of drying methods and extraction techniques, which could maintain or increase the chemical composition of torch ginger flower to act as natural antioxidant and antibacterial. To the best of author's knowledge, this study is the first to report the chemical composition, antioxidant and antibacterial activities of torch ginger flower's essential oil extracted from subcritical carbon dioxide (SubC-CO_2) extraction.

The objectives of this study were:

I. To profile the bioactive compounds in different maturity stages (tight bud and full bloom) of torch ginger (*E. elatior* Jack) flowers
II. To study the effect of drying methods (freeze, oven and sun drying) on extraction yield and antioxidant activities of selected maturity stage of torch ginger flowers
III. To characterise the chemical constituents, antioxidant and antibacterial activities of torch ginger essential oil extracted with subcritical carbon dioxide (SubC-CO_2)

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