UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF EXTRACTION METHOD AND IMPROVEMENT OF GROWTH, PHYSIOLOGY, YIELD AND ANTIOXIDANTS PROPERTIES AS AFFECTED BY CHITOSAN IN Ocimum basilicum L.

QAZIZADAH AHMAD ZUBAIR

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By

QAZIZADAH AHMAD ZUBAIR

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

March 2022

DEDICATION

To my beloved mother who always prays for me day and night to achieve my goal

To my family members:

and

To all my friends who supported me all these years

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

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Chair : Juju Nakasha Jaafar, PhD
Faculty : Agriculture

Sweet basil (*Ocimum basilicum* L.) is one of aromatic herbs belonging to Lamiaceae family and is widely used in pharmaceutical, culinary and traditional medicine industries. Sweet basil holds high potential and demand in mentioned industries, but biomass production and phytochemical content are still not sufficient to cater the demand. The quantity of the extraction of phytochemical content is depends on the extraction technique. It is therefore, important to increase biomass production and the content of phytochemicals at field level and establish suitable extraction technique, in order to achieve high amount of extraction. Two experiments were carried out with the objectives of (i) to establish a suitable combination of ethanol concentration and temperature in order to obtain high amount of selected antioxidant constituents from sweet basil leaves, (ii) to identify the suitable concentration of chitosan and time of application for improvement of the growth, herbal yield and antioxidant contents in sweet basil at field. In the first experiment, three different concentrations of ethanol (60, 75 and 90% v/v) were combined with three different temperatures (40, 60 and 80˚C) for the extraction of sweet basil leaves. The experiment was arranged in 3 X 3 factorial Complete Randomized Design (CRD) with four replications and three samples per replicate. The result showed that 90% ethanol at 80℃ had the highest percentage of extraction yield (11.56%) compared to other treatments. However, extracting dried sweet basil leaves with 60% ethanol at 80℃ temperature recorded the highest extraction of total phenolic content (67.02 mg of GAE/g of DE), total phenolic yield (693.5 mg of GAE/100 g of DW), total flavonoid content (44.7 mg of QUE/g of DE), total flavonoid yield (462.52 mg of QUE/100 g of DW) and antioxidant activity (66.8%). Results from the correlation analysis showed a positive relationship between phenolic, flavonoid and antioxidant activity of the extract. Therefore, it is recommended to extract sweet basil leaves using 60% ethanol under 80°C. This combination was applied in the second experiment. In the second experiment, chitosan at four different concentrations (0, 2, 4 and 6 ml/L) were applied at three different application times of (20, 40 and 20 + 40 days after transplanting, DAT) at the field. The experiment was organized in 4 X 3 factorial Randomized Complete Block Design (RCBD) with four replications and five plants per replicate. The results showed an interaction between chitosan concentrations and times of applying on growth, herbal yield and secondary metabolites performance of sweet basil plants. From the results, application of chitosan at concentration of 4 ml/L on 20 DAT gave the highest biomass yield components such as leaf fresh weight and leaf dry weight by 43.45 and 59.71% as well as total content of flavonoid and antioxidant activity by 40.21 and 22.81%, respectively, when compared with the control group. In addition, the same treatment resulted in the highest value of plant height, stem diameter, dry weight of stem per plant, number of leaves per plant, total leaf area per plant, average chlorophyll-a, chlorophyll-b, total chlorophyll, actual chlorophyll and dry weight of root per plant by 40.73, 37.47, 40.38, 25.71, 37.19, 9.90, 37.36, 18.75, 18.86 and 143.97%, respectively compare to the control groups. Besides, correlation analysis showed positive relationship among variables as well as dry weight of leaves with total leaf area (r = 0.96) and total chlorophyll (r = 0.76), the AA with TPC (r = 0.80) and TFC (r = 0.57). Most of the growth and physiology variables positively correlated with biomass yield components. In conclusion, it is recommended that the application of 60% ethanol at 80℃ extraction temperature showed greater strength in extracting phenolic, flavonoid and antioxidant activity from sweet basil's leaf. Meanwhile, application of 90% ethanol at 80℃ temperature showed greater percentage of extraction yield in comparison to other treatments. Besides, the performance of sweet basil plants at field level could be improved by the application of 4 ml/L chitosan at 20 DAT.

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

PENGOPTIMUMAN KAEDAH PENGEKSTRAKAN DAN PENAMBAHBAIKAN PERTUMBUHAN, FISIOLOGI, HASIL DAN BAHAN ANTIOKSIDA TERPILIH YANG DIPENGARUHI OLEH KITOSAN KE ATAS *Ocimum basilicum* L.

Oleh

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Selasih (*Ocimum basilicum* L.) adalah salah satu herba aromatik di bawah keluarga Lamiaceae dan digunakan secara meluas di dalam industri farmaseutikal, masakan dan perubatan tradisional. Selasih mempunyai potensi dan permintaan yang tinggi di dalam industri, namun, pengeluaran biojisim serta kandungan fitokimianya masih rendah berbanding permintaan. Kuantiti pengekstrakan kandungan fitokimia terletak kepada teknik pengekstrakan. Oleh itu, adalah penting untuk meningkatkan pengeluaran biojisim serta kandungan fitokimia semasa di peringkat ladang dan juga teknik pengekstrakan yang sesuai, bagi mencapai jumlah pengekstrakan yang tinggi. Dua eksperimen telah dijalankan dengan objektif, (i) untuk mengukur kepekatan etanol dan suhu yang diperlukan bagi mendapatkan kuantiti bahan antioksida t erpiih yang tinggi daripada daun selasih, (ii) untuk mengenal pasti kepekatan kitosan dan masa penggunaan yang sesuai untuk meningkatkan pertumbuhan, hasil herba dan antioksida tanaman selasih di ladang. Di dalam eksperimen pertama, tiga kepekatan etanol yang berbeza (60, 75 dan 90% v/v) telah digabungkan dengan tiga suhu berbeza (40, 60 dan 80˚C) untuk pengekstrakan daun selasih. Eksperimen telah disusun dalam bentuk 3 X 3 faktorial Reka Bentuk Rawak Lengkap (CRD). Keputusan menunjukkan bahawa kepekatan etanol pada 90% dan pada suhu 80°C menghasilkan peratusan hasil pengekstrakan yang paling tinggi (11.56%) berbanding rawatan lain. Walau bagaimanapun, pengekstrakan daun selasih dengan 60% etanol pada suhu 80°C mencatatkan pengekstrakan tertinggi bagi jumlah kandungan fenolik (67.02 mg of GAE/g of DE), jumlah hasil fenolik (693.5 mg of GAE/100 g of DW), jumlah kandungan flavonoid (44.7). mg QUE/g DE), jumlah hasil flavonoid (462.52 mg QUE/100 g DW) dan aktiviti antioksida (66.8%). Keputusan daripada analisis korelasi menunjukkan terdapatnya hubungan positive di antara fenolik, flavonoid dan aktiviti antioksida di dalam ekstrak tersebut. Oleh itu, adalah disyorkan untuk mengekstrak daun selasih dengan menggunakan etanol pada 60% dengan suhu 80°C. Keputusan ini seterusnya digunakan di dalam eksperimen kedua. Di dalam eksperimen kedua, empat kepekatan kitosan yang berbeza (0, 2, 4 dan 6 ml/L) telah digunakan pada tiga masa penggunaan yang berbeza (20, 40 dan 20 + 40 hari selepas pemindahan anak pokok, DAT) di lapangan. Eksperimen ini disusun dalam Reka Bentuk Rawak Blok Lengkap (RCBD) 4 X 3 faktorial. Keputusan menunjukkan terdapatnya interaksi di antara kepekatan kitosan dan masa penggunaannya terhadap hasil herba dan prestasi metabolit sekunder tumbuhan selasih. Daripada keputusan, data dengan hasil tertinggi seperti berat segar daun (54.26 g/pokok), berat kering daun (8.8 g/pokok) serta kandungan flavonoid (33.23 mg QUE/g DE) dan aktiviti antioksidan (92.34%) adalah diperolehi daripada penggunaan 4 ml/L kitosan pada 20 DAT, jika dibandingkan dengan kawalan (37.84 g/pokok, 5.51 g/pokok, 23.70 mg QUE/g DE dan 75.18%), masing-masing. Selain itu, rawatan yang sama ini telah menghasilkan ketinggian tumbuhan (55.08 cm), diameter batang (11.08 mm), bilangan daun (296.57), jumlah luas daun (2234.31 cm^2), purata klorofil-a (4.33). mg/cm^2), klorofil-b (2.5 mg/cm^2), jumlah klorofil (6.84 mg/cm^2), klorofil sebenar (4.79 mg/cm^2), berat kering batang (11.09 g/pokok) dan berat kering akar (2.83 g/pokok). Selain itu, analisis korelasi juga turut menunjukkan hubungan positif antara pembolehubah serta berat kering daun dengan jumlah luas daun (r = 0.96) dan jumlah klorofil (r = 0.76), AA dengan TPC (r = 0.80) dan TFC (r = 0.57). Kesimpulannya, adalah disyorkan bahawa penggunaan 60% etanol pada suhu pengekstrakan 80℃ mampu mengekstrak fenolik, flavonoid dan aktiviti antioksidan yang paling tinggi daripada daun selasih. Sementara itu, penggunaan 90% etanol pada suhu 80℃ menunjukkan peratusan hasil pengekstrakan yang lebih besar berbanding rawatan lain. Di samping itu, prestasi tumbuhan selasih di peringkat ladang boleh dipertingkatkan dengan penggunaan 4 ml/L kitosan pada 20 DAT.

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

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research and the writing of this thesis were done under our supervision;
- supervisory responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2015-2016) are adhered to.

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ROS	Reactive oxygen species
EY	Extraction yield (%)
TPC	Total phenolic content
mg of GAE/g of DE	Milligram gallic acid equivalent per gram of dry extract
TPY	Total phenolic yield (mg of GAE/100 g of DW)
mg GAE/100 g DW	Milligram gallic acid equivalent per 100 gram of dry weight
TFC	Total flavonoid content (mg of QUE/g of WE)
mg of QUE/g of WE	Milligram of quercetin equivalent per gram of dry extract
TFY	Total flavonoid yield (mg of QUE/100 g of DW)
mg of QUE/100 g of DW	Milligram of quercetin equivalent per 100 gram of dry weight
AA	Antioxidant activity (%)
LSD	Least significant difference
CHI	Chitosan
TA	Times of application
DAT	Days after transplanting

CHAPTER 1

INTRODUCTION

1.1 Introduction

Sweet basil (*Ocimum basilicum* L.) is a common herb which is extensively consumed in pharmaceutical, cosmetic, nutraceutical and beverage industries. Because of its strong aroma and a wide range of beneficial properties, sweet basil is also known as "King of Aromatic Herbs" (Filip, 2017). Sweet basil consists of more than 200 secondary metabolite bioactive compounds (Ghasemzadeh et al., 2016). However, phenolics and flavonoids are the most important groups of antioxidant compounds, involving health beneficial properties in sweet basil (Filip, 2017; Ghasemzadeh et al., 2016; Koca and Karaman, 2015).

Although sweet basil contains various types of phytochemicals, however, good extraction method is the key to extracting high phytochemicals content from the leaves. According to Dorta et al. (2012) the solvent concentration and temperature used during the extraction are two essential factors in achieving high extraction yield. Khoddami et al. (2013) stated that phenolic compounds could be easily extracted using organic solvents. Among the organic solvents, it was said that ethanol is the most inexpensive solvent to be used in extraction (Chiaramonti et al., 2012), nontoxic to human body (Casagrande et al., 2018) and effective in extracting the phytochemicals from plant materials (Truong et al., 2019; Chigayo et al., 2016; Dent et al., 2013). It was reported that ethanol at 62.7% concentration was able to extract high phenolic compounds (7.21 g of GAE/g) with 80% of antioxidant activity at 49.7°C in *Feronia limonia* fruit (Ilaiyaraja et al., 2015). This is supported by Hassan et al. (2021) where low concentration of ethanol (60%) recorded to extract the highest phenolic compounds (807.20 mg of GAE/g) from *Padina australis*. However, another researcher proved that high concentration of ethanol (90%) yielded better result in extracting those antioxidants in *Toona sinensis* (Maulana et al., 2019). Due to high variations in concentration that should be used, it is then important to test the concentration on a specific plant.

Apart from this, earlier report by Su et al. (2017) and Dent et al. (2013) revealed that temperature plays a vital role during the extraction of phytochemical. The use of higher temperature during the extraction can help in enhancing the solvent to enter the cells and stimulate the solubility of bioactive compounds which lead to the increase in extraction efficiency (Najafabadi et al., 2020; Su et al., 2017; Setford et al., 2017). However, the optimum extraction temperature is not same for all plants. This is proven when Juntachote et al. (2006) tested different extraction temperatures on different crops. They found that suitable temperature for extracting phenolic compounds from *Cymbopogon citratus* was 25℃, while from *Alpinia galanga* plants was 75℃. Similar finding was also obtained by

Oreopoulou et al, (2019) and Milevskaya et al. (2019) where different crops required different temperatures to extract the same compound. Thus, it is important to test different temperatures on different plant species. Besides, in order to increase the efficiency in the extract, Ilaiyaraja et al. (2015) suggested to combine the temperature with different percentage of ethanol. This is agreed by Dorta et al. (2012) who stated that the efficiency of extraction process depends on solvent concentration and level of extraction temperature.

Furthermore, as sweet basil is rich in phytochemical compounds, there is an increase in the number of products that use sweet basil as part of the ingredients. There were several attempts from previous researchers on methods to increase the number of leaves in plants other than sweet basil, which were by using auxin, fertilization and managing the light intensity (Jebapriya and Somasundaram, 2021; Uzun, 2006; Dieleman and Heuvelink, 1992). However, these methods are costly, time consuming and hard to maintain.

Alternatively, one of the growth regulators that is gaining more attention in agriculture is chitosan. Chitosan is one of the plant regulators that is safe, easily available and low-cost (Khan, et al., 2018; Emami-Bistgani, et al., 2017). It is said that chitosan increases growth performances in several medicinal herbs such as *Carum copticum* L. (Razavizadeh et al., 2020), *Pisum sativum* (Khan et al., 2018) and *Salvia officinalis* L. (Vosoughi et al., 2018). However, the effectiveness of chitosan was reported to be dependent on its concentration (Irawati et al., 2019; El-Miniawy et al., 2013; Abdel-Mawgoud, 2010) and growing stage of the plants (Rasheed et al., 2020; Pichyangkura and Chadchawan, 2015). Lei et al. (2011) stated that 100 mg/L of chitosan produced higher biosynthesis of phytochemicals in *Artemisia annua* herbs, while maintaining plant growth and development. However, Singh (2016) stated that low concentration at 10 mg/L was sufficient to obtain the highest accumulation of phytochemicals in *Spinacia oleracea*.

In terms of plant's growing stage, Ali et al. (1997) suggested that it is recommended to apply chitosan at later growing stage which is approximately 42 days after planting compared to early growing stage (28 days after planting) in *Glycine max* Merr. Nevertheless, this is disagreed by Cuibu and Shiayama (2001) who recommended to applying chitosan at the early growing stage of *Glycine max, Solanum lycopersicum* and *Oryza sativa* where the plant height, leaf surface area, chlorophyll contents and dry plant materials are higher. This is also supported by Mondal et al. (2016) who stated applying chitosan at 25 days after transplanting was more suitable compared to 35 DAT in *Solanum lycopersicum*. Besides, Ohta et al. (2004) stated that higher accumulation of phytochemicals was found when applying chitosan at early growing stage in *Torenia fournieri*, *Calceolaria herbeohybrida* and *Campanula fragilis* L. plants. Other than affecting the plant's growth and development, chitosan has also been described to increase the biosynthesis of phytochemicals in several plants (Silva et al., 2020; Jiao et al., 2018 and Talón et al., 2017).

1.2 Problem statement

Today providing antioxidants as well as in natural form is very challenging for the researchers (Mairapetyan and Mamikonyan, 2016). Medicinal herbs are the most important source of natural antioxidant compounds. Due to this, the demand for this crops in global market will be increased from $6.2 billion to $5 trillion till 2050 (Govindaraju and Arulselvi, 2018; Rao et al., 2017; Khanna, 2015; Kumar and Janagam, 2011). Particularly, sweet basil as a common mostly consumed herb is highly contributing to this demand (Rahman et al., 2021; Dou et al., 2018; Liaros et al., 2016). This demand is including both biomass (as spice) and phytochemicals as well as antioxidant compounds. As king of medicinal herbs, it is needed to increase leaf yield and phytochemicals in sweet basil in order to cater the demand. In literature, attempts were introduced to increase leaf yield, however, they were not economic, easy to maintain and ecofriendly.

On the other hand, the quantity of phytochemicals lies on the efficiency extraction technique. In extraction process, concentration of solvent and level of temperature are key factors and needs to be optimized since they are very from crop to crop.

Therefore, the problem is how to improve biomass and phytochemical production of sweet basil at field, and establish suitable extraction technique in order to obtain optimum phytochemicals with higher antioxidant property from sweet basil leaves.

1.3 Research objectives

The main objective of this study was to improve herbal yield and phytochemical production of sweet basil at field, and optimize extraction of its phytochemicals. Hence, the specific objectives of this study were as follows:

1) To establish a suitable combination of ethanol concentration and temperature in order to obtain high amount of selected antioxidant constituents from sweet basil leaves.
2) To identify the suitable concentration of chitosan and time of application for improvement of the growth, herbal yield and antioxidant contents in sweet basil at field.

1.4 Significance of the study

This study provides new insights in to the easy, economic and ecofriendly attempt to improve production of biomass and antioxidant phytochemicals of sweet basil at filed. Besides of that, as a result of this study suitable extraction technique will be established. While the first attempt will benefit farmers who are seeking for easy way to increase quality and quantity of sweet basil's production as well as in Malaysia. The second attempt will contribute to medicinal and food industries who extract and process antioxidant phytochemicals of sweet basil in all over the world. As a result, practical application of findings from this study will be a great contribution to meet the increasing demand for antioxidant resources. Moreover, the analysis that presented in this study will convey valuable information for future research that will explore various phytochemicals from sweet basil.

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