INVESTIGATION OF ANTIMICROBIAL, ANTIOXIDANT AND TOXICITY ANALYSES OF *Centella asiatica* AS POTENTIAL FRESH-CUT PRODUCES PRESERVATIVE

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SULTAN IDRIS EDUCATION UNIVERSITY

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ABSTRACT

Investigation of antimicrobial, antioxidant and toxicity analyses of *Centella asiatica* as potential fresh-cut produces preservative were conducted. The aims of this study are antimicrobial and antioxidant activities of *C. asiatica* extracts; to determine the toxicity study of *C. asiatica* extract using the brine shrimp lethality assay (BSLA) and to determine the effectiveness of *C. asiatica* extract as a potential natural food additive on different types of fresh-cut fruits and vegetables (FCFV). The antimicrobial, antioxidant and toxicity assessments of *C. asiatica* extracts were then assayed based on the solvents (ethanol, methanol, water and hexane) and investigating of total phenolic content (TPC) and total flavonoid content (TFC). Results of objective-1 showed ethanolic extract recorded the highest extraction yield (14.7%), TPC (69.54 mg GAE/g) and TFC (13.90 mg QE/g). The ethanolic extract also exhibited the highest readings in disc diffusion assay (13.50 mm inhibition zone), minimum inhibition concentration (MIC; 7.81-125 mg/mℓ), minimum bactericidal concentration (MBC; 5.63-62.50 mg/mℓ), and minimum fungicidal concentration (MFC; 7.81-250 mg/mℓ). Objective-2 result showed the ethanolic extract recorded the highest inhibition concentration (IC_{50}: 68.12 µg/mℓ). Based on BSLA, the result of objective-3 indicated that ethanolic extract exhibited non-toxic properties with LC_{50} of 597.72 mg/mℓ. The effectiveness of the ethanolic extract in inhibition of microbial growth and browning of FCFV was evaluated under room temperature displayed (RTD) (4 h, 22 ± 1°C) and chilled storage (CS) (4 days, 4 ± 0.5°C). Objective-4 results showed the range of microbial population reduction were 4.44–7.87 log CFU/g (RTD-FCFV) and 3.00–4.92 log CFU/g (CS-FCFV), respectively. Browning-indexes were ranged 4.72–9.26 (RTD-FCFV) and 3.96–16.39 (CS-FCFV), respectively. In conclusion, the results had answered all objectives and successful. As an implication, the ethanolic extract is having potential to become alternative candidate in synthetic preservative.

PENYIASATAN ANTIMIKROB, ANTIOKSIDAN DAN TOKSISITI ANALISIS *Centella asiatica* SEBAGAI POTENSI PENGAWET POTONGAN SEGAR

ABSTRAK

Penyiasatan antimikrob, antioksidan dan toksisiti analisis *Centella asiatica* sebagai potensi pengawet potong segar telah dijalankan. Objektif untuk kajian ini mengandungi penyiasatan antimikrob dan antioksidan aktivit esktrak Centella asiatica; penyiasatan toksisiti ekstrak *C. asiatica* dengan ujian kematian udang laut (BSLA); penyiasatan keberkesanan esktrak bagi penghalangan mikorb dan perperangan dalam potongan buah-buahan dan sayur-sayuran (FCFV) sebegai potensi untuk pengawet semula-jadi. Penilaian antimikrob, antioksidan dan ketoksisiti ekstrak *C. asiatica* kemudiannya diuji dengan pelarut (etanol, metanol, air, dan heksana), penyaisiatan jumlah kandungan fenolik (TPC) dan jumlah kandungan flavonoid (TFC). Keputusan objektif-1 menujukkan ekstrak etanol mencatatkan hasil pengekstrakan tertinggi (14.7%), TPC (69.54 mg GAE/g) dan TFC (13.90 mg QE/g) yang paling tinggi. Ekstrak etanol juga menunjukkan keputusan yang terbaik dalam kaedah penyebaran cakera agar (13.50 mm zon perencatan), penghalangan pertumbuhan (MIC; 7.81– 125 mg/mℓ), pembunuhan bakteria (MBC; 5.63–62.50 mg/mℓ) dan pembunuhan kulat (MFC; 7.81–250 mg/mℓ). Keputusan objektif-2 menujukkan ekstrak etanol menunjukkan nilai kepekatan perencatan (IC_{50}) yang tertinggi (89.54%; 68.12 µg/mℓ). Berdasarkan keputusan onjektif-3 dalam BSLA, ekstrak etanol menunjukkan tiada toksik dengan LC_{50} sebanyak 597.72 mg/mℓ. Keberkesanan ekstrak etanol untuk menghalang pertumbuhan mikrob dan perperangan FCFV dinilai di bawah keadaan suhu bilik (RTD) (4 jam, 22 ± 1°C) dan penyimpanan sejuk (CS) (4 hari, 4 ± 0.5°C). Keputusan objektif-4 menujukkan julat perencatan populasi mikrob sebanyak 4.44-7.87 log CFU/g (RTD-FCFV) dan 3.00–4.92 log CFU/g (CS-FCFV). Indeks peperangan berjulat sebanyak 4.72–9.26 (RTD-FCFV) dan 3.96–16.39 (CS-FCFV). Kesimpulannya, keputusan telah menjawab semua objektif dan berjaya. Sebagai implikasinya, ekstrak etanol berpotensi untuk menjadi sumber alternatif dalam pengawet sintetik

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
AMP	Amphotericin B
ANOVA	Analysis of one-way variance
BI	Browning indexes
BSLA	Brine shrimp lethality assay
C. asiatica	*Centella asiatica*
CFU	Colony forming units
log CFU/mℓ or g	Logarithm colony forming units per millilitre or gram
CHX	Chlorhexidine
CS	Chilled storage
CS-FCFV	Fresh cut samples kept in chilled storage
DPPH	2,2-diphenyl-1-picrylhydrazyl
ED	Extract-treated/Extract-dipped FCFV
FCFV	Fresh cut fruits and vegetables
FDA	Food and Drug Administration of United States
GAE	Gallic acid equivalent
GRAS	Generally recognized as safe
IC	Inhibitory concentration
LC	Lethality concentration
MBC	Minimum bactericidal concentration
MFC	Minimum fungicidal concentration

MHA	Mueller Hinton agar
MHB	Mueller Hinton broth
MIC	Minimum inhibitory concentration
ND	No detection
OD	Optical density
PDA	Potato Dextrose agar
PDB	Potato Dextrose broth
PPO	Polyphenol oxidase
QE	Quercetin equivalent
RPM	Revolutions per minute
RTD	Room temperature displayed
RTD-FCFV	Fresh cut samples displayed under room temperature
RTE	Ready-to-eat
SDW	Sterile distilled water
SDW-D	Dipping treatment with sterile distilled water
spp.	Species
SPSS	Statistical Package for the Social Science
TFC	Total flavonoids content
TP	Total Plate Count
TPC	Total phenolic content
USDA	United States Department of Agriculture

CHAPTER 1

INTRODUCTION

1.1 Background Research

Fruits and vegetables are essential for humans as the main source of micronutrients that can prevent chronic diseases (Vivek et al., 2019). In recent years, the global industrial fresh-cut fruits and vegetables (FCFV) market has rapidly expanded and evolved to meet the increasing demand for convenient, fresh, and ready-to-eat (RTE) products (FDA, 2018; Moreira et al., 2015). FCFV is defined as any whole fresh horticultural produces (fruits or vegetables) or the combination of fruits and vegetables that have been physically altered from their natural form without impairing their freshness (Qadri et al., 2015). FCFV is abundantly available in supermarkets and sold by street vendors and hawkers in open-air markets across many Southeast Asian countries, such as Thailand and Malaysia (James & Ngamsark, 2010).

FCFV is not subjected to further food washing processes and preservation treatments to the extent of altering their quality (colour, nutritional contents, smell, appearance, texture and etc.) and shelf life so that their natural flavour and nutrient contents are retained. Such handling practices would also compromise public health in excessive use of chemical preservative, which is the main problem in using synthetic additives in the food industry. Overly consuming artificial additives will cause carcinogenic and mutagenic effect (FDA, 2018; Murray et al., 2017). *Staphylococcus aureus*, *Salmonella enterica*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Bacillus cereus* are well-recognised microbial contaminants that result in foodborne illnesses linked to FCFV (Mir et al., 2018; Prakash et al., 2018; Choi et al., 2015). Besides the harmful effects of foodborne pathogens, 25% of food spoilage microbes have been accounted for post-harvest spoiling loss. Despite that spoilage microorganisms are non-pathogenic to humans, they could negatively impact the organoleptic aspects of the food (Bondi et al., 2017). The release of available nutrients (sugars, minerals, vitamins, and water) from the cellular components of FCFV provides an ideal environment for microbial proliferation, especially when the food is stored above 4°C (Agriopoulou et al., 2020).

Additionally, natural food colouration is a critical component of the FCFV quality that affects consumers' acceptance of the product. Over half of the marketed food in the current food industry are susceptible to discolouration and browning due to unfavourable chemical reactions by endogenous enzymes (Shrestha et al., 2020). Since FCFV are processed into smaller pieces, the exposed surfaces are prone to free radical attacks, which could degrade the cellular compartmentalisation of FCFV and alter their appearances. Furthermore, enzymatic browning reaction is frequently

observed in degraded FCFV due to the phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) oxidised with polyphenol compounds (Kim, 2020; Sommano et al., 2019). Eventually, the melanin formation in FCFV induces the alteration of sensory aspects of the food (Al-Amrani et al., 2020). Therefore, a more natural-based additive that could effectively preserve the quality of FCFV without harming the consumer should be studied to replace the unhealthy synthetic additives that currently dominate the market.

Centella asiatica is a traditional herb with a long history in medicinal practice for centuries in ancient Indian (Ayurveda) and Chinese medicinal systems (Byakodi et al., 2018). The herbal plant is a perennial herbaceous creeper belonging to the Apiaceae family (Purkait et al., 2018; Taemchuay et al., 2009) that is known to effectively treat different diseases, such as blood pressure, eczema, asthma, ulcers, leprosy, kidney disease, and wound healing (Ramli et al., 2020a; Pittella et al., 2009). *C. asiatica* thrives abundantly on damp, wet, and moist soil surfaces throughout many tropical and sub-tropical countries, mainly in Southeast Asia and certain Western regions (Dash et al., 2011; Kesornbuakao et al., 2018). In Malaysia, *C. asiatica* is indigenously known as '*pegaga*' and traditionally consumed as fresh '*ulam*' (salad) (Hashim, 2011). The plant is an essential source of triterpenoids, volatile oils, and phenolic constituents, especially flavonoids, which crucially contribute to its antioxidant and antimicrobial capabilities (Wong & Ramli, 2021; Sieberi et al., 2020; Yasurin et al., 2016; Edgar et al., 2014; Pitinidhipat & Yasurin, 2012).

As researchers are more interested in utilising plant extract as potential candidates for alternative coating packaging or preservatives to improve the

organoleptic properties and shelf life of food products (Pitinidhipat & Yasurin, 2012; Kalita & Saika, 2012; Mamtha et al., 2004), the application of natural resources that are labelled Generally Recognised as Safe (GRAS) would help reduce the reliance on synthetic additives and promote natural-based options. Given the increasing awareness and demand for natural- and plant-based food products, it is necessary to gain further insights on the future application of *C. asiatica* extract as a natural additive against different foodborne pathogenic microbes. The incorporation of *C. asiatica* into several types of food models would provide a valuable evaluation of the potential inhibition effects against foodborne-related microorganisms in extract-treated FCFV that reflects the risks associated with RTE food under long-term storage practices in retail or home environments. The effect of the extracted material on the natural colouring of FCFV is also an important factor that needs to be thoroughly evaluated to improve its overall performance.

1.2 Problem Statement

C. asiatica is considered a popular Malaysian traditional herb and it is claimed to possess strong antioxidant and antimicrobial properties (Ramli et al., 2020a; Wong & Ramli, 2021). Phenolic contents and flavonoids are primary sources that contribute to the antioxidant and antimicrobial properties of *C. asiatica* extract. According to previous literature studies, *C. asiatica* extract demonstrates effective pathogenic growth inhibition, such as *L. monocytogenes*, *Bacillus subtilis*, and *Salmonella enterica* Serovar Enteritidis (Rattanakom & Yasurin, 2015; Pitinidhipat & Yasurin, 2012; Sandhar et al., 2011). However, these preliminary data are limited without any

supporting investigation on the incorporation of *C. asiatica* extract in the application of food models. Instead, most of the past studies were more focused on pharmaceuticals and cosmetics product applications (Sieberi et al., 2020; Yasurin et al., 2016; Edgar, 2014; Pitinidhipat & Yasurin, 2012).

In addition, consumers are becoming more aware of the negative impacts arising from the use of synthetic additives in food and pharmaceutical products. However, there is lacking information on the potential candidates from natural- and plant-based sources that can offer an alternative option to replace commercialised artificial synthetic additives. Realising the increasing trend by consumers for more healthy and organic-based products, the food industry has cooperated with the scientific community in search of suitable natural additives derived from animals or plants (Silva & Lidon, 2016; Silva-Weiss et al., 2013). Although synthetic additives, such as benzoates and nitrates, have been widely used to inhibit the growth of pathogenic microorganisms and extend the shelf life of food products, the overuse and over-consumption of artificial additives would result in undesirable effects to the food products as well as to the consumers, such as the alteration of the food taste, development of antimicrobial resistance strains in different microorganisms, and carcinogenic to the public health (Mir et al., 2018; Ramli et al., 2017).

The food industry is currently facing a greater challenge in terms of the shorter shelf life of FCFV products due to the minimal processing operations, such as peeling, chopping, or shredding that damages the natural cellular structure of the fruits and vegetables. As a result, fresh-cut products would be more susceptible to rapid microbial contamination, oxidation, and enzymatic degradation during long-term

storage, which could drastically alter the natural qualities of FCFV, including the colour, texture, flavour, and nutritional value (Kim et al., 2020; Shrestha et al., 2020; Murray et al., 2017). Besides the health concern, the stability of plant-based materials is a major issue that needs to be examined without affecting the sensory properties of the food products (Ziegler et al., 2018; Alvarez et al., 2015).

1.3 Objective of Research

The main aim of this study is to determine the potential application of *C. asiatica* extract as a natural additive in FCFV. The objectives of this study are as follows:

1. To determine the antimicrobial activities of *C. asiatica* extract towards different types of food spoilage and foodborne pathogenic microorganisms.
2. To determine the antioxidant activities of *C. asiatica* extract using the free radical scavenging activity assay.
3. To determine the toxicity study of *C. asiatica* extract using the brine shrimp lethality assay (BSLA).
4. To determine the effectiveness of *C. asiatica* extract as a potential natural food additive on different types of FCFV.