EVALUATION OF PHYTOCHEMICALS, ANTIOXIDANT & ANTIMICROBIAL PROPERTIES OF *Cinnamomum verum* EXTRACTS

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by

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or currently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purpose.

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(Nurul Akmal binti Sabri)

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LIST OF SYMBOLS AND ABBREVIATIONS

°C	degree Celsius
%	percent
<	less than
>	greater than
±	plus-minus
=	equal
µL	microlitre
µm	micrometre
cm	centimetre
g	gram
L	litre
mg	milligram
mL	millilitre
mM	millimolar
nm	nanometre
AA	ascorbic acid
Aβ	amyloid-beta
ABTS	2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
AECV	aqueous extract *Cinnamomum verum*
AMR	antimicrobial resistance
argC	n-acetyl glutamyl phosphate reductase.
ATCC	American type culture collection
BHA	butylated hydroxyanisole

CFU	colony forming unit
DNA	deoxynucleic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
EC_{50}	half maximal effective concentration
EECV	ethanolic extract *Cinnamomum verum*
EO	essential oil
EPS	extracellular polymeric substance
FC	Follin-Ciocalteu
$FeCl_3$	ferric chloride
FRAP	ferric reducing antioxidant power
GAE	gallic acid equivalent
H_2SO_4	sulphuric acid
HCl	hydrochloride
HPLC	high performance liquid chromatography
IC_{50}	half maximal inhibitory concentration
IQR	interquartile range
luxS	s-ribosyl homocysteine lyase
MBC	minimum inhibitory concentration
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton broth
MIC	minimum inhibitory concentration
MMP	matrix metalloproteinase
MRSA	*methicillin-resistance Staphylococcus aureus*
Na_2CO_3	sodium carbonate
NaOH	sodium hydroxide

NF-kB	nuclear factor kappa B
OMP	outer membrane proteins
QE	quercetin equivalent
ROS	reactive oxygen species
SOD	superoxide dismutase
SPSS	statistical package for the social sciences
TE	Trolox equivalent
TFC	total flavonoid content
TM	traditional medicine
TPC	total phenolic content
UV	ultraviolet
UV-Vis	ultraviolet visible
w/v	weight per volume
WHO	World Health Organization

PENILAIAN SIFAT FITOKIMIA, ANTIOKSIDAN & ANTIMIKROB EKSTRAK *Cinnamomum verum*

ABSTRAK

Perubatan tradisional telah lama bergantung pada ubat berasaskan tumbuhan untuk menangani pelbagai isu kesihatan, dengan kulit kayu manis diiktiraf secara meluas kerana sifat terapeutiknya. Kajian ini bertujuan untuk menyiasat sifat fitokimia, antioksidan dan antimikrob ekstrak kayu manis, menekankan potensinya sebagai agen teraputik semula jadi. Ekstrak air *C. verum* (AECV) dan ekstrak etanolik *C. verum* (EECV) telah dianalisis untuk sebatian bioaktifnya, menunjukkan kehadiran alkaloid, fenol, flavonoid, tanin, glikosida, dan terpenoid, manakala saponin hanya dikesan dalam AECV. Aktiviti antioksidan dinilai menggunakan ujian pembersihan radikal DPPH dan jumlah kandungan fenolik (TPC). AECV menunjukkan potensi antioksidan yang lebih tinggi (IC_{50} = 0.233 mg/mL) daripada EECV (IC_{50} = 0.418 mg/mL), walaupun kedua-duanya memiliki aktiviti yang lebih rendah berbanding asid askorbik (AA) (IC50 = 0.00673 mg/mL). Analisis TPC mendedahkan bahawa EECV (0.1001 [0.236] mg GAE/g) mengandungi kandungan fenolik yang jauh lebih tinggi daripada AECV (0.0227 [0.036] mg GAE/g), menunjukkan keberkesanan etanol dalam mengekstrak fenolik. Aktiviti antimikrob terhadap *S. aureus* dan *E. coli* telah dinilai menggunakan ujian kepekatan perencatan minimum (MIC) dan kepekatan bakterisid minimum (MBC), di mana AECV menunjukkan aktiviti antibakteria terhad terhadap *S. aureus* (MIC = 20 mg/mL), manakala EECV tidak menunjukkan kesan yang ketara. Kedua-dua ekstrak tidak menghalang *E. coli*, berkemungkinan disebabkan oleh kepekatan ekstrak yang tidak mencukupi. Penemuan ini menunjukkan bahawa kulit kayu manis berpotensi sebagai

sumber antioksidan semula jadi, sementara penyelidikan lanjut diperlukan untuk meneroka potensi antimikrobnya.

EVALUATION OF PHYTOCHEMICAL, ANTIOXIDANT & ANTIMICROBIAL PROPERTIES OF *Cinnamomum verum* EXTRACTS

ABSTRACT

Traditional medicine has long relied on plant-based remedies to address various health issues, with cinnamon bark being widely recognized for its therapeutic properties. This study aims to investigate the phytochemical, antioxidant and antimicrobial properties of cinnamon extracts, emphasizing its potential as a natural therapeutic agent. Aqueous extract of *C. verum* (AECV) and ethanolic extract of *C. verum* (EECV) were analyzed for their bioactive compounds, revealing the presence of alkaloids, phenols, flavonoids, tannins, glycosides, and terpenoids, while saponins were detected only in AECV. Antioxidant activity was assessed using DPPH radical scavenging and total phenolic content (TPC) assays. AECV demonstrated higher antioxidant potential (IC_{50} = 0.233 mg/mL) than EECV (IC_{50} = 0.418 mg/mL), although both exhibited lower activity compared to ascorbic acid (AA) (IC50 = 0.00673 mg/mL). TPC analysis revealed that EECV (0.1001 [0.236] mg GAE/g) showed higher phenolic content than AECV (0.0227 [0.036] mg GAE/g), indicating ethanol's effectiveness in extracting phenolics. Antimicrobial activity against *S. aureus* and *E. coli* was evaluated using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays, where AECV demonstrated limited antibacterial activity against *S. aureus* (MIC = 20 mg/mL), while EECV exhibited no significant effects. Neither extract inhibited *E. coli*, likely due to insufficient extract concentrations. These findings indicate that cinnamon bark showed potential as a natural source of antioxidant, while further investigation is needed to explore its antimicrobial potential.

CHAPTER 1

INTRODUCTION

1.1 Background of study

In past centuries, humans have been seeking healing and treating various illnesses from nature due to their significant values to human health. These approaches have established the cornerstone of traditional medicine (TM) in which they are passed down through generations in several countries. Until now, plant-based medicines are still crucial, with more than 65% of the global population depending on them since most plants used in these methods are abundant in natural bioactive compounds that can provide health benefits with little adverse effects (Nabavi et al., 2015). According to the World Health Organization (WHO), traditional medicine can be referred to as a collection of knowledge, skills and practices focused primarily on health and well-being. As for its importance benefits, the WHO has collaborated with communities and healthcare systems globally to integrate safe and effective TM practices, aiming to enhance overall health and well-being worldwide (Hoenders et al., 2024).

Cinnamomum verum, typically known as Ceylon cinnamon or "Kayu Manis" is an evergreen tree that is widely used in traditional medicine for its therapeutic properties. Cinnamon that is derived from the interior bark of *C. verum* has garnered worldwide attention due to its unique flavour and potential health benefits such as antioxidant, anti-inflammatory, antidiabetic and antimicrobial (Błaszczyk et al., 2021). According to Nabavi et al. (2015), cinnamon is employed for treating a range of ailments, including inflammation, gastrointestinal disorders and urinary infections. Besides of its pharmacological properties, cinnamon is also one of the aromatic spices in Malaysia that is often used in culinary dishes to add sweet-spicy flavour and a comforting aroma in dishes like curries, desserts and stews. There have been a lot of studies regarding the therapeutic properties of cinnamon globally, but in Malaysia, the research is still limited.

The increasing prevalence of chronic illnesses in Malaysia is profoundly alarming and cannot be overlooked. These conditions are adversely affecting public health, economic burden and healthcare system. A primary component contributing to this problem is oxidative stress, which occurs when there is an imbalance of equilibrium between oxidants and free radicals, leading to tissue damage and necrosis. Some studies have reported that oxidative stress could also influence chronic age-related diseases such as dementia, diabetes, cancer, atherosclerosis, obesity, osteoporosis, vascular diseases and metabolic disorders (Tan et al., 2018). Besides focusing on lifestyle modifications, antioxidant supplementation can also mitigate the oxidative stress and its associated health risks. Cinnamon can exhibit as a powerful antioxidant agent that can minimize tissue damage by neutralizing ROS, inhibiting their synthesis and facilitating their breakdown (Lobo et al., 2010).

Furthermore, antibacterial properties of cinnamon have also developed interest as a promising candidate to natural antimicrobial agents in order to combat the rising prevalence of antibiotic resistance. In fact, antibiotic resistance is a natural occurrence in which microorganisms, especially bacteria, acquire the capability to withstand antibiotics that would typically eradicate them or hinder their growth. This process is mostly influences by genetic alterations in bacteria, which may occur over time due to exposure to certain medications either misuse or overuse of antibiotics. Therefore, this study provides as one of the alternatives that may aid in developing new antimicrobial agents in the future. The bioactive compounds of *C. verum,* particularly cinnamaldehyde are responsible for its antimicrobial properties in which has the capacity to rupture microbial cell membranes and impede the proliferation of various pathogenic microorganisms such as bacteria, fungi, and viruses (Al-Garadi et al., 2023).

1.2 Problem statement

The generation of free radicals significantly poses a danger to public health since it can contribute to a variety of chronic diseases such as cancer, diabetes, Alzheimer's and cardiovascular disease (Al-Mijalli et al., 2023). Although synthetic antioxidants have been effectively employed in pharmaceuticals, cosmetics, and the food industry due to their high efficacy, inexpensive cost and stability to minimize free radicals in the human body, the pronounced side effects and health concerns have recently diminished their widespread application (Stoia & Oancea, 2022). This further emphasizes the rising demand for natural plant-based antioxidants as safer alternatives.

On top of that, antimicrobial resistance (AMR) has also arisen as a global health crisis, with an alarming 1.27 million fatalities directly attributable to AMR in 2019 and an additional 4.95 million deaths worldwide (World Health Organization, 2023). AMR compromises the advancement of modern medicine by making infections more difficult to treat and increasing the risk of other medical procedures and treatments. This concerning situation requires the immediate development of antibacterial drugs that are both efficacious and sustainable.

TM is considered as one of the natural alternatives that are directly derived from nature. *Cinnamomum verum*, an ancient spice, is extensively used as traditional medicine due to its numerous health benefits, including antioxidant and antimicrobial properties (Asyikin et al., 2021). Despite its potential, there is a lack of comprehensive studies in Malaysia that compare and evaluate the biological activities of Cinnamomum verum extracts. This research gap constrains the determination of optimal extraction processes and their therapeutic uses.

1.3 Rationale of study

First of all, the primary concern about synthetic antioxidants is their potential health concerns when ingested or exposed to excessive amounts. For example, Butylated Hydroxyanisole (BHA) has been associated with carcinogenic repercussions due to its capacity to produce tumours in research on animals (Xu et al., 2021). Hence, this study seeks to examine natural compounds as natural antioxidants, serving as sustainable alternatives. Besides, the outcomes of this study may contribute to the advancement of prospective antimicrobial drugs to combat AMR strains and reduce mortality rates. The study could also strengthen public health initiatives by advocating the use of plant-based substances with proven efficacy and safety. Cinnamon is an excellent option chosen in this study due to its widespread application in Malaysian dishes and ease of accessibility. Thus, the outcome of this study could address the dual challenges of AMR and the demand for potent antioxidants by evaluating the phytochemical profiles of cinnamon extracts, antioxidant activity and antimicrobial properties of *C. verum* through aqueous and ethanolic extraction.

1.4 Objectives of study

1.4.1 General objective

To evaluate the phytochemical composition, antioxidant and the antimicrobial properties of *Cinnamomum verum* extracts (aqueous and ethanolic)

1.4.2 Specific objectives

1. To screen the phytochemical composition of *Cinnamomum verum* extracts
2. To determine the antioxidant activity of *Cinnamomum verum* extracts
3. To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Cinnamomum verum* extracts against bacterial strains

CHAPTER 2

LITERATURE REVIEW

2.1 Traditional medicine

Traditional medicine (TM) has a longstanding history in the treatment of diseases. They have served as a fundamental aspect of healthcare in several cultures for many centuries, providing alternative remedies for numerous diseases. These approaches are highly linked to indigenous knowledge in using plants, minerals and other natural resources for their therapeutic benefits. Herbal medicine is a significant element of traditional medicinal systems such as Ayurveda, Traditional Chinese Medicine and African Traditional Medicine. TM is often referred to as complementary and alternative medicine or ethnic medicine, which continues to be significant in many nations today (Yuan et al., 2016).

Despite the development of modern medicine, TM is still used as a complementary therapy especially in underdeveloped nations. According to the WHO (2023), about 80% of the population worldwide receive their primary healthcare through TM. The incorporation of TM into healthcare systems has accelerated, highlighting the need for scientific evidence of these treatments. This parameter is an importance aspect to guarantee that TM practices satisfy the criteria of quality, safety and effectiveness, which are crucial in delivering holistic treatment that respects cultural norms while safeguarding patient safety. Fortunately, the isolation and characterization of bioactive molecules from natural resources has become achievable by recent developments in pharmacology and biotechnology, which have bridged the gap between classical knowledge and contemporary treatment methods (Nasim et al., 2022).

2.2 Cinnamon species

Cinnamon is an ancient spice acquired from the inner bark of numerous species of the genus *Cinnamomum*, which belongs to the Lauraceae family. For generations, it has been esteemed for its unique flavour and widespread use in culinary traditions and TM. The pharmacological properties of cinnamon, along with its distinctive flavour and fragrance, have made it a significant commodity globally. Over 250 species have been identified within the cinnamon genus that are found throughout the world. Among these, only four species possess significant commercial value, which is being used as a spice, including *Cinnamomum verum* (Sri Lanka cinnamon), *Cinnamon loureiroi* (Vietnamese cinnamon), *Cinnamon burmanni* (Indonesian cinnamon) and *Cinnamon cassia* (Chinese cinnamon) (Ullah & Hassan, 2022). However, *C. verum* is often considered the most favourable, owing to its exceptional antioxidant properties and extensive array of medical benefits. Moreover, some studies have reported that *C. verum* has significantly lower coumarin (toxic substance) concentrations than *C. cassia*, making it a safer and preferable option for the food, beverage and pharmaceutical sectors (Madhushika et al., 2024).

2.2.1 *Cinnamomum verum*

Cinnamomum verum J. Presl, typically known as true cinnamon or Ceylon cinnamon, is an evergreen tree that is native to Sri Lanka and southern India. It is an evergreen tree that often attains a height of 8 to 17 meters (Figure 2.1). The *C. verum* leaves are oval to elliptic in shape, ranging from 5 to 18 cm in length, with a glossy dark green shade with distinct veins. The *C. verum* produces are little and greenish yellow of flowers that are organized in panicles and have a distinctive fragrance. Meanwhile, the fruit of *C. verum* is a purple drupe with a solitary seed that is approximately around 1 inch in length (Petruzzello, 2019).

The *C. verum* species is widely distributed in China, Indonesia, Madagascar, Southeast Asia, Myanmar, Australia, the Caribbean and Africa (Andrade-Hoyos et al., 2023). This plant survives in warm, humid environments with well-drained soils. Nonetheless, it needs regular rainfall and is intolerant of extended dryness or saturated soil conditions (Pasiecznik, 2017).

Figure 1.1 *Cinnamomum verum* tree

(Adapted from: National Parks Flora and Fauna Web, 2023)

2.3 Medicinal properties of cinnamon

Cinnamomum verum has considerable therapeutic advantages from a pharmacological perspective. These attributes include antibacterial, antifungal and antiviral efficacy. The active substance, like cinnamaldehyde has shown the ability to suppress the proliferation of numerous infections such as *S. aureus, B. cereus, E. coli* and *C. albicans* (Ranasinghe et al., 2013). This substance is a significant contributor to antioxidant properties of cinnamon since they are able to stabilize the free radical compounds and stimulate the activity of the antioxidant enzymes such as superoxide dismutase (SOD) and catalase (Pagliari et al., 2023). Some studies also demonstrated that *C. verum* may assist in regulating blood glucose levels in patients with type 2 diabetes by improving insulin sensitivity and glucose metabolism (Sharifi-Rad et al., 2021). The spice is also believed to assist in alleviating inflammation-related disorders, including arthritis. This is due to its capacity to inhibit the production of nuclear factor kappa B (NF-kB), a crucial regulator of inflammatory reactions (Davoudi & Ramazani, 2024). Other studies also discovered that the compound in cinnamon may prevent the accumulation of amyloid-beta (Aβ) plaques, which is a distinctive hallmark of Alzheimer’s disease. This condition may aid in preserving cognitive function and slowing the development of Alzheimer's disease (Lim et al., 2024).

2.4 Phytochemicals in medicinal plants

Phytochemicals are bioactive molecules derived from plants for their defence mechanism against environmental risks such as fungus, bacteria, viruses and herbivores (Thiede & Zidenberg-Cherr, 2016). To date, over a thousand phytochemicals have been identified that may be obtained from several sources, including whole grains, fruits, vegetables, nuts and herbs. These substances are essential to the immunity of the plant and contribute to their overall health and survival. In plants, these secondary metabolites are responsible in response to environmental stress like UV radiation, drought or nutritional deficits by functioning as an antioxidant to the plant (A. Kumar et al., 2023). Despite this, they also can act as an ecological interaction in which they aid in the creation of aromas and pigments that may attract pollinators and promote reproduction. They can also serve as allelopathy to plants in which a few plants emit their phytochemicals into the soil that prevent the establishment of rival species (Petrén et al., 2024).

2.4.1 Classes of phytochemicals

The abundance of phytochemicals present in plants is often classified into two distinct groups, which are primary and secondary metabolites. This classification is depending on their functions in various metabolic processes. The primary metabolites usually participate in essential functions like respiration, growth, photosynthesis, nutrient storage and cell division. Since they are essential reactants and intermediates in the metabolism of carbon, nitrogen and related pathways, biomolecules such as carbohydrates, amino acids and lipids have been designated as primary metabolites. Conversely, secondary metabolites are produced in minimal quantities from primary metabolites, which commonly occurs during a specific development phase or to fulfil a particular purpose. There are several secondary metabolites that are derived from plants, which are alkaloids, phenolics, terpenoids, saponins, tannins, glycosides and flavonoids. These compounds have the capacity to withstand the plant against biotic and abiotic stresses. The defensive mechanisms in plants are different based on their individual needs and are influenced by physiological variables, climatic fluctuations and environmental influences (Kaushik et al., 2021; Kawale, 2018).

2.4.2 Phytochemical composition of *Cinnamomum verum*

As the nutraceutical products, *C. verum* encompasses a diverse phytochemical profile that enhances its flavour, fragrances and therapeutic features. Cinnamaldehyde, cinnamic acid and coumarins are prevalent phenolic compounds that substantially increase the antioxidant and anti-inflammatory properties of the plant. The recent study from Aloraby et al. (2024) reported that *C. verum* is rich in alkaloids, glycosides, tannins, flavonoids, steroids, gums, resins, fixed oils, saponins and proteins. This study also highlighted that the presence of anthraquinone glycosides and terpenoid content were deemed inconclusive, requiring further confirmation tests. Another study also found that the *C. verum* are rich in phenolic, flavonoids, tannins, cinnamyl acetate and camphor. The essential oils of *C. verum* contains eugenol, cinnamaldehyde, linalool and β-caryophyllene (Błaszczyk et al., 2021). However, the phytochemical composition of *C. verum* varies according to variables such as plant maturity, soil composition, climate changes, specific plant part, extraction method and development stage, underscoring the importance of understanding their influence on the plant's pharmacological functions (Madhushika et al., 2024).

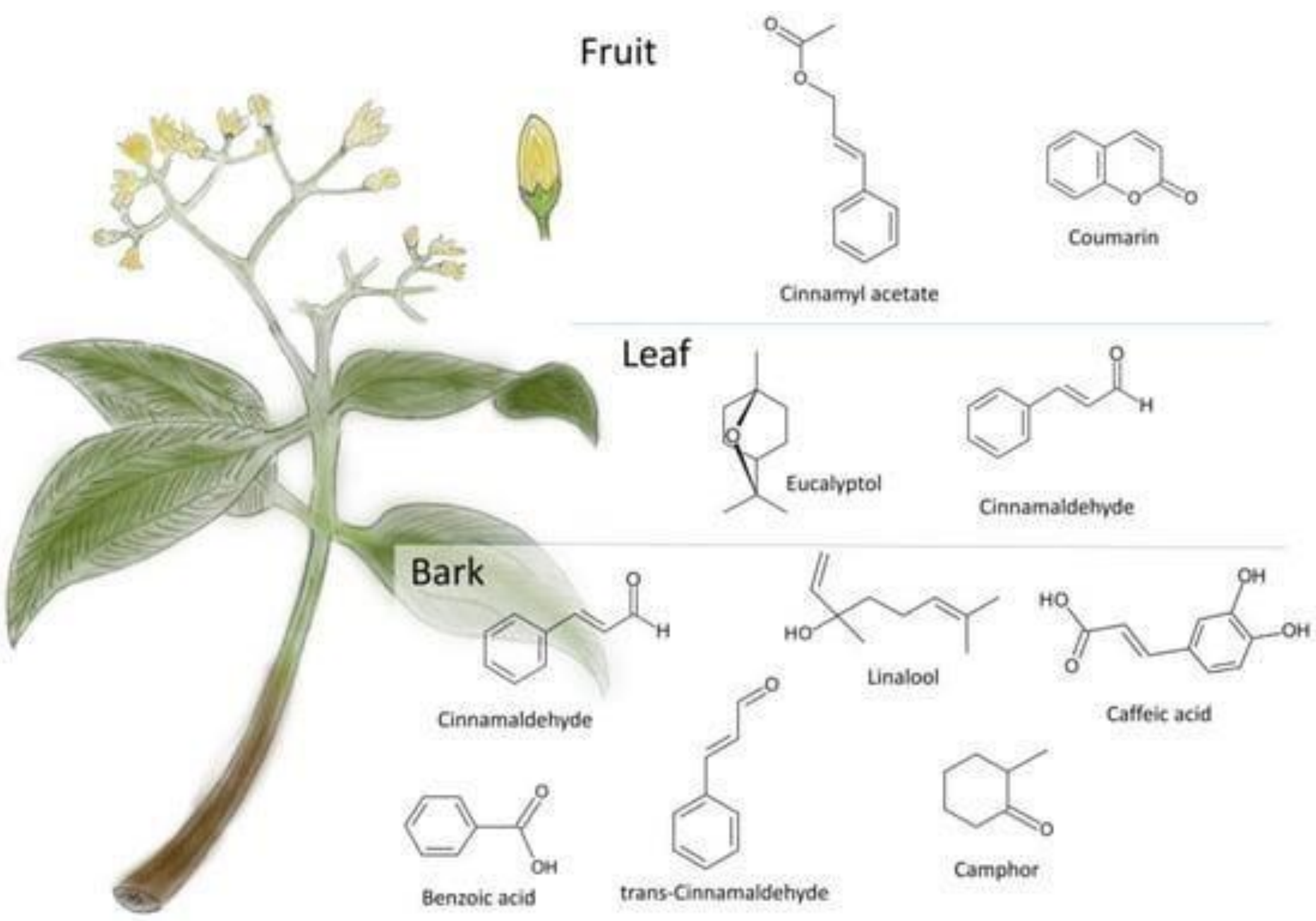

Figure 2.2 Bioactive constituents of cinnamon discovered across different plant sections

(Adapted from: Błaszczyk et al., 2021)

2.5 Antioxidants

Antioxidants are substances often known as secondary metabolites which responsible to lessen or avoid oxidative damage to a specific molecule in living organisms, including human body, fruits and vegetables. However, since human body does not have the ability to generate enough antioxidant to protect itself against persistent danger of ROS, plant-derived dietary antioxidants are considered essential for maintaining human health. This is because plants are known as one of the sources of antioxidants due to their capacity to produce wide range of antioxidants, including alkaloids, phenolics and vitamin C and E (Ayoka et al., 2022).

Oxidative damage represents the injury experienced by cells and tissues as a result of an overabundance free radicals. Free radicals are ROS that persistently travel inside the body and arise as byproducts of several biochemical events occurring in humans. Under atypical circumstances, they are eliminated from the body by antioxidant mechanisms. The disruption of these natural systems leads to an accumulation of radicals, which contributes to the onset of many illnesses (Chaudhary et al., 2023). These extremely reactive chemicals may cause the oxidation of lipids, proteins and nucleic acids, leading to cellular malfunction, inflammation and eventually contributing to illnesses that include cancer, cardiovascular problems and neurological conditions (Pizzino et al., 2017).

2.5.1 Importance of antioxidants in human health

Antioxidants play a crucial role in the human body as a prevention of chronic disease since they can neutralize free radicals through electron donation. Upon neutralization, these unstable molecules lose their ability to induce cellular damage to biological components, like DNA, lipids and proteins. Hence, they can minimize the fluctuations of intrinsic membrane-like fluidity and ion transport, along with enzyme function loss, protein crosslinking, suppression of protein synthesis, DNA damage and eventually cell death due to oxidative damage (Ayoka et al., 2022).

On the other hand, antioxidant agents derived from plants not only neutralize free radicals but also strengthen the skin's defence systems and regeneration abilities. To explain, antioxidants like Vitamin C may accelerate the production of fibroblasts, which are responsible for the generation of elastin and collagen synthesis. Besides, the development of matrix metalloproteinases (MMPs) is also being restricted by antioxidant substances that will maintain the firmness of the skin (Michalak, 2022).

Furthermore, oxidative stress may adversely affect oocyte maturation, endometrial growth and hormonal balance during the menstrual cycle. This disorder arises from an imbalance between free radicals and antioxidants in the body. The prevalent antioxidants that contribute to women's reproductive health include vitamin C and E, glutathione and coenzyme Q10. Apart from maintaining the integrity of ovarian follicles and mitochondrial activity, they are also crucial for improving the uterine environment by diminishing inflammation and enhancing cellular function, which is vital for successful implantation and conception (Vašková et al., 2023).

2.5.2 Antioxidants properties of *Cinnamomum verum*

The antioxidant properties of *C. verum* are mainly due to its abundant bioactive components, especially phenolic compounds and essential oils. These chemicals have considerable free radical scavenging capabilities and provide several medical benefits. Previous study done by Sudan et al. (2013) showed that the phenolic content of *C. verum* bark exhibited superior antioxidant activity compared to *C. tamala* leaf with 210 mg GAE/g and 161 mg GAE/g, respectively. The methanol extract of *C. verum* bark also exhibited the lowest IC_{50} (111.5 mg/mL) in the DPPH radical scavenging assay compared to *C. tamala* leaf (175 mg/mL).

Some studies also reported that the ethanolic extract of *C. verum* leaf possesses greater phenolic content than of *C. verum* bark, with the mean value of 44.57 ± 0.51 mg GAE/g and 33.43 ± 0.51 mg GAE/g, respectively. This higher antioxidant activity is also consistent with the greater flavonoid content in *C. verum* leaf compared to *C. verum* bark, with the average values of 12.00 ± 0.37 mg QE/g and 3.07 ± 0.24 mg QE/g, respectively. However, DPPH radical scavenging activity showed that the ethanolic extract of *C. verum* bark has higher scavenging activity compared to the ethanolic extract of *C. verum* leaf, which are 107.69 ± 2.01 mg TE/g and 33.96 ± 0.47 mg TE/g, respectively. The elevated total phenolic content (TPC) and total flavonoid content (TFC) in the *C. verum* leaf suggest a broader spectrum of phenolic and flavonoid substances in the extract, but they may exhibit inferior efficacy in scavenging DPPH radicals relative to the more potent antioxidant compounds such as cinnamaldehyde and eugenol found in the bark (Abeysekera et al., 2013). The present study also reported that cinnamon EO has the highest antioxidant activity compared to other EOs, such as thyme, clove, lavender and peppermint oils. This can be proven when cinnamon EO exhibited the lowest EC_{50} among those five EOs, which is 0.03 mg/mL, compared to the other EOs, which are 0.14, 0.05, 12.1, and 33.9 mg/mL. This higher antioxidant activity is observed due to the presence of the main bioactive compound of cinnamon, which is eugenol with a relative concentration of 547 ± 10.4 mg/mL (Chen et al., 2023). These findings suggest that the *C. verum* bark has a significant potential for human health as a natural antioxidant.

2.6 Antimicrobials

Antimicrobials are the substances that impede the proliferation of or eradication of microorganisms, including bacteria, viruses, fungi and parasites. This agent play an important role in the management and prophylaxis of infectious illnesses. Nonetheless, the rising of antimicrobial resistance has reduced the effectiveness of traditional antibiotics, requiring the exploration of natural alternatives. Plants inherently synthesize antimicrobials molecules, secondary metabolites, as a defence strategy against infections, pests and herbivores. These compounds, such as phenols, terpenes, alkaloids, cinnamaldehyde, cinnamate and cinnamic acid. Each metabolite possesses different modes of action against infections, thereby making them appealing candidates to synthetic antibiotics. However, the quantity and occurrence of each component vary based on the specific portion of the plant (Vasconcelos et al., 2018).

2.6.1 Antimicrobial properties of *Cinnamomum verum*

The antimicrobial activity of *C. verum* interacts through numerous pathways. Firstly, studies have reported that cinnamon extract can alter the cell membrane and lipid profile of bacteria. To elaborate, the presence of compounds like trans-cinnamaldehyde in essential oils or ethanolic extracts can compromise the bacterial cell membrane, which will affect their permeability and lipid content. This condition will lead to protein denaturation and depletion of metabolites and ions, eventually leading to bacterial cell death, which has been reported in bacterial strains like *E. coli* and *L. innocua* (Vasconcelos et al., 2018).

On top of that, the presence of phenolic compounds like eugenol in the cinnamon extract also demonstrated the antimicrobial action against *methicillin-resistant Staphylococcus aureus* (MRSA). Based on this research, the eugenol stimulated the activation of the argC gene, implying bacterial stress responses and the downregulation of the luxS gene, a gene involved in quorum sensing and disruption of biofilm formation (Buru et al., 2022). These combined impacts indicate that eugenol not only disrupts bacterial communication and biofilm stability but also induces bacteria to undergo energy-consuming stress adaptation processes, possibly undermining their overall defence systems.

Although eugenol and trans-cinnamaldehyde are the main bioactive substances that provide *C. verum* its antibacterial properties, other compounds such as flavonoids, tannins and cinnamic acid are also important contributors. To explain, cinnamic acid impaired bacterial membranes and altered energy metabolism, thereby increasing bacterial vulnerability to stress (Ruwizhi & Aderibigbe, 2020). Besides, tannins also showed antibacterial properties by precipitating microbial proteins and blocking essential enzymes, consequently affecting bacterial adherence and biofilm development. Also, flavonoids, particularly quercetin and kaempferol, can disrupt bacterial membranes and induce ROS, hence reducing bacterial viability. These chemicals often function synergistically with trans-cinnamaldehyde and eugenol, enhancing the bacterial efficacy of *C. verum.* Overall, this chemical variance underscores the broad-spectrum efficacy of *C. verum* against both gram-positive and gram-negative bacteria, rendering it a valuable natural antimicrobial agent (Vasconcelos et al., 2018).

2.6.2 Antimicrobial activity of *Cinnamomum verum*

In the study conducted by Al-Garadi et al. (2023), the antibacterial efficacy of 100% ethanol extract (C100EOH), 50% ethanolic extract (C50EOH) and *C. verum* water extract (CWA) were examined, exhibiting different levels of activity against selected bacteria. This research highlighted that the lowest MIC and MBC values were reported when C50EOH was evaluated against *S. Typhimurium*, which are 3.26 mg/mL and 6.51 mg/mL, suggesting that these two extracts may not be beneficial for targeting this bacterium. Conversely, the greatest MIC (62.50 mg/mL) and MBC (125 mg/mL) were observed with C50EOH and CWA against *L. monocytogenes*. This indicates that C50EOH has more efficacy against S. Typhimurium, underscoring its potential as a tailored antimicrobial drug.

Besides, another study showed that *C. verum* essential oil (EO) and its primary compound, cinnamaldehyde, demonstrated potent antimicrobial activity against *S. aureus* (ATCC 14458) as evidenced by their identical MIC values, which are 1.6 mg/mL. However, EO exhibited superior bactericidal effects, comparable to the lowest MBC values (1.6 mg/mL) compared to the cinnamaldehyde compound (3.2 mg/mL). This suggests that *C. verum* EO may be more effective in combating *S. aureus* at lower doses (Franciscato et al., 2022).

CHAPTER 3

METHODOLOGY

3.1 Study Design

This study aimed to evaluate the phytochemical composition, antioxidant and the antimicrobial properties of aqueous and ethanolic *Cinnamomum verum* extracts. The overall study design is shown in Figure 3.1. In the first phase of this study, aqueous and ethanolic extracts of cinnamon were prepared using the maceration technique. The phytochemical composition of both extracts was analyzed through qualitative screening to detect the presence of alkaloids, phenols, flavonoids, tannins, glycosides, terpenoids, and saponins.

In the second phase, the antioxidant activity of these extracts was evaluated using the DPPH radical scavenging assay. Serial dilutions of each extract were prepared within the range of 0.008 to 0.5 mg/mL, and the DPPH reagent was added to each well in a 96-well microplate. After incubation, absorbance was measured at 517 nm, and the IC_{50} values were calculated for each extract based on the dose-response curve.

In the next phase, the total phenolic content (TPC) of both extracts was determined using the Folin-Ciocalteu method. The extracts were prepared in a two-fold dilution series, ranging from 0.016 to 2 mg/mL. Then, the absorbance was measured at 765 nm after 2 hours of incubation. The TPC values were quantified using a gallic acid standard curve, and the results were expressed as mg GAE per gram of dry sample.

Lastly, the antimicrobial activity of the cinnamon extracts was assessed using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923). Two-fold microdilution of the cinnamon extracts was prepared in the Mueller-Hinton broth in the concentration range of 0.078 to 20 mg/mL and tested against the bacterial inoculum. The bacterial growth was monitored after incubation at 37°C for 18-24 hours. The MIC values were recorded as the lowest concentration that inhibited visible bacterial growth, and the MBC values were determined by sub-culturing onto fresh agar plates. The findings from this study could contribute to the development of natural antioxidants and antimicrobial agents, addressing the challenges of oxidative stress and antimicrobial resistance.

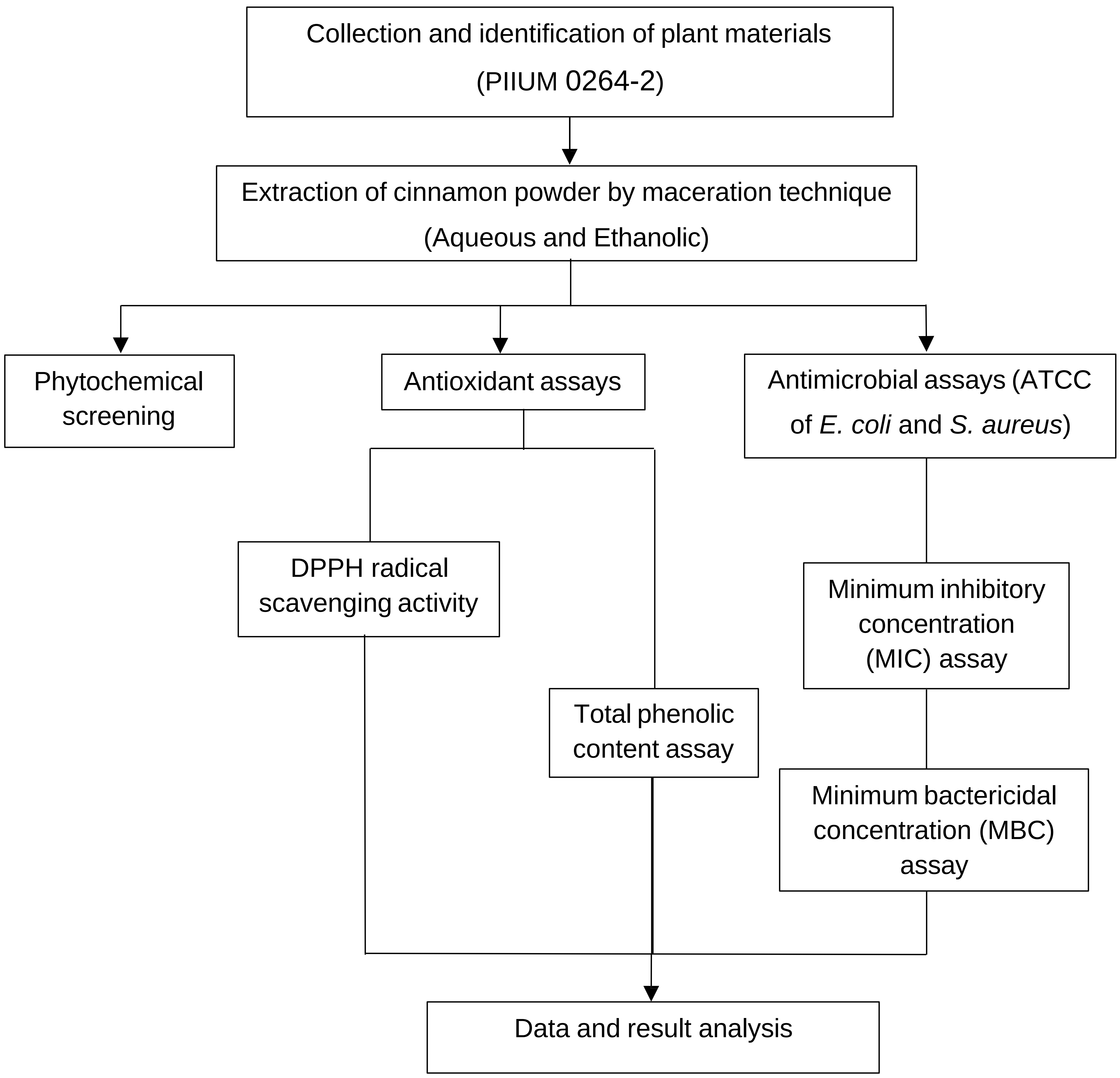

Figure 3.1 The flowchart of this study

3.2 Materials

3.2.1 List of chemicals, reagents, apparatus, equipment, consumables and other materials

The chemicals, reagents, equipment, consumables and other materials used in this study were listed in Table 3.1, Table 3.2 and Table 3.3, respectively.

Table 3.1 List of chemical and reagents

Chemical and reagent	Manufacturer
Ethanol	Merck (Germany)
Sulphuric acid	Merck (Germany)
Ferric chloride	Sigma-Aldrich (USA)
Sodium hydroxide	Bio Basic (Canada)
Bromine water	Merck (Germany)
Acetic acid	Sigma-Aldrich (USA)
Chloroform	Merck (Germany)
Sodium carbonate	Bendosen (Turkey)
2,2-diphenyl-1-picrylhydrazyl	Sigma-Aldrich (USA)
Ascorbic acid	Bendosen (Turkey)
Gallic acid	Bendosen (Turkey)
Dimethyl sulfoxide	Sigma-Aldrich (USA)
Folin-Ciocalteu reagent	Sigma-Aldrich (USA)
Tetracycline hydroxide	Biobasic (Canada)
Gram crystal violet	Remel (USA)
Gram iodine	Remel (USA)
Gram decolorizer	Remel (USA)

Gram safranin	Remel (USA)
Mueller-Hinton agar	Oxoid (UK)
Mueller-Hinton broth	Oxoid (UK)
Nutrient agar	Oxoid (UK)

Table 3.2 List of apparatus and equipment

Apparatus and equipment	**Manufacturer**
Beaker (500, 1000 mL)	Schott Duran (Germany)
Schott bottle (250, 500, 1000 mL)	Bomex (Turkey)
Glass rod	-
Filter funnel	-
Magnetic stirrer	-
Measuring cylinder (100 mL)	Bomex (Turkey)
Petri dish glass	-
Spatula	-
Test tube (15 mL)	Pyrex Iwaki (Japan)
Hot plate	Stuart (UK)
Vortex	ERLA (Malaysia)
Analytical balance	Metler Toledo Dragon 204 (Switzerland)
Oven	Binder (Germany)
Water bath	Memmert (Germany)
Pipette (10, 100, 1000 microL)	Eppendorf (Germany)
Microplate reader	NanoQuant Infinite M200 (Austria)
Light microscope	Olympus (Japan)

Table 3.3 List of consumables and other materials used

Consumables and other materials	Manufacturer
Filter paper	Smith (USA)
Muslin cloth	-
Sterile urine container	MyMedic (Malaysia)
Pipette tips (10, 100, 1000 microL)	Axygen (USA)
Aluminium foil	-
96-well microplate without lid	Greiner Bio-one (Germany)
96-well microplate with lid	Greiner Bio-one (Germany)
Microcentrifuge tube (2 mL)	Axygen (USA)
Falcon tube (15 mL, 50 mL)	SPL Life Sciences (South Korea)
Syringe	Ciringe (Malaysia)
Syringe-driven filter unit 0.22 µm	Millex Milipore (USA)
Parafilm	Bemis (USA)
Sterile collection swab	Premier diagnostics (Malaysia)

3.3 Preparation of samples

3.3.1 Plant collection and authentication

The cinnamon powder and barks (Appendix A) were purchased from Fruit and Vegetables Store of Rural Transformation Centre (RTC) at Tunjong, Kelantan, Malaysia with latitude of 6.0773 and longitude of 102.2348. Cinnamon barks were sent to Herbarium Kulliyah of Pharmacy, International Islamic University of Malaysia (IIUM) in Kuantan for plants authentication and verification. The plant samples were authenticated by the botanist, Dr. Shamsul Khamis. The specimen number for *Cinnamomum verum* is PIIUM 0264-2, with the pressed voucher and verification letter were shown in Appendix B and Appendix C.

3.3.2 Plant extraction

The extraction method used in this study was the maceration technique as described by Asyikin et al., (2021). 80 g of cinnamon powder was weighed and incorporated into 800 mL of distilled water at a 1:10 (w/v) ratio, then stirred with a magnetic stirrer on a hot plate for 72 hours. Concurrently, an ethanolic extract was prepared by immersing 80 g of cinnamon powder in 800 mL of 70% ethanol. Then, each extract was filtered using muslin cloth and subsequently using filter paper. The aqueous extract was subjected to freeze-drying, while the ethanolic extract was dried in an oven at 50 °C for 48 hours. The resulting powder was preserved at 4 °C for further use. The percentage of yield extraction was calculated using the following formula and was shown in Appendix D.

$$\text{Percentage of yield extraction} = \frac{\text{Final weight (g)}}{\text{Initial weight (g)}} \times 100\%$$

Where the final weight is the weight of AECV or EECV and the initial weight is the original weight of the powder form of *C. verum* barks.

3.4 Phytochemical analysis

The qualitative phytochemical screening was conducted using standard protocols with slight modification to determine the presence of phytochemicals such as alkaloids, on each extract of *C. verum* (Al-Mijalli et al., 2023; Kancherla et al., 2019; Wan Abdul Wahab et al., 2022).

3.4.1 Test for alkaloids

Dragendorff's test was carried out by mixing 1 mL of Dragendorff's reagent (Potassium bismuth iodide) to 2 mL of extract in the test tube. The formation of red orange precipitate indicated the presence of alkaloids.

3.4.2 Test for phenols

The presence of phenols was conducted using Ferric Chloride test where 2 mL of 3% ferric chloride ($FeCl_3$) was incorporated with 2 mL of pre-warmed extract in test tube. The development of dark green or blue colour demonstrates the presence of phenols.

3.4.3 Test for flavonoids

Alkaline reagent test was performed to detect the presence of flavonoids in the extract sample. 2 mL of 2% sodium hydroxide (NaOH) was mixed with 2 mL of extract sample in test tube. The appearance of yellow coloration indicates the presence of flavonoids.

3.4.4 Test for tannins

Bromine water test was carried out by mixing 1 mL of bromine water with 2 mL of extract sample in a test tube. The presence of tannins is shown by the decolourization of bromine water.