# EXTRACTION, CHARACTERISATION AND BIOACTIVITY ASSESSMENT OF PECTIC-POLYSACCHARIDE FROM *CINNAMOMUM VERUM* USING DEEP EUTECTIC SOLVENT–MICROWAVE ASSISTED APPROACH

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# EXTRACTION, CHARACTERISATION AND BIOACTIVITY ASSESSMENT OF PECTIC-POLYSACCHARIDE FROM *CINNAMOMUM VERUM* USING DEEP EUTECTIC SOLVENT–MICROWAVE ASSISTED APPROACH

**by**

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## ABSTRAK

Pasaran kosmetik menunjukkan peningkatan permintaan terhadap produk berasaskan semula jadi dengan kesan anti-hiperpigmentasi yang ketara. Sementara itu, komuniti penyelidikan sedang meneroka pelarut pengekstrakan yang lebih mesra alam dan mampan. Walaupun minat terhadap Pelarut Eutektik Dalam (DES) semakin meningkat, sejenis DES yang khusus untuk pengekstrakan polisakarida masih belum dicipta. Bagi mengatasi kekurangan ini, satu DES berasaskan asid telah disintesis menggunakan komponen yang mudah diperoleh dan kos efektif bagi menyokong aplikasi yang lebih meluas. Kajian ini melaporkan sintesis pelarut eutektik dalam (DES) baharu daripada asid sitrik monohidrat dan L-asid glutamik dalam nisbah molar 1:1 melalui pemanasan gelombang mikro. Kaedah ini, yang dioptimumkan pada 10 minit dan kuasa 360 W, menawarkan pendekatan yang pantas dan menjimatkan berbanding teknik konvensional. DES yang terhasil mempamerkan warna jingga, yang dikaitkan dengan tindakbalas Maillard, menunjukkan interaksi sinergistik antara komponen asal. Pencirian menyeluruh menunjukkan bahawa DES ini bersifat hidrofilik dan berasid serta boleh bercampur sepenuhnya dengan air. Penilaian fisikokimia merangkumi pengukuran pH, ketumpatan, kelarutan, kelakuan reologi, dan spektroskopi FTIR, selain penilaian tindak balas Maillard. Analisis dok molekul turut menyokong kewujudan rangkaian ikatan hidrogen yang kukuh antara kumpulan amina dan karboksil dalam asid glutamik,

kumpulan karboksil dalam asid sitrik, serta molekul air, yang menstabilkan struktur supramolekul. DES baharu ini menunjukkan potensi untuk penyelidikan di peringkat makmal dan penskalaan untuk pengekstrakan polisakarida. Dalam usaha mencari sebatian bioaktif baharu dengan kesan anti-hiperpigmentasi yang setanding dengan rakan kimianya, potensi penjagaan kulit polisakarida daripada kulit kayu *Cinnamomum verum* telah dikaji menggunakan DES yang disintesis melalui pengekstrakan gelombang mikro. Pengoptimuman menggunakan reka bentuk Box-Behnken telah mencapai pengekstrakan optimum pada 4% (w/v) DES, 4 minit, dan kuasa gelombang mikro 360 W, yang menghasilkan: (i) hasil polisakarida 7.00±0.78%; (ii) 97.22±0.27% aktiviti perencatan monofenolase; (iii) 86.81±2.64% aktiviti perencatan difenolase; (iv) 60.47±4.23% kuasa penyingkiran DPPH (2,2-difenil-1-pikrilhidrazil); dan (v) faktor perlindungan matahari (SPF) sebanyak 5.919±0.60. Analisis biokimia menunjukkan bahawa polisakarida yang diekstrak mengandungi 39.18±0.28% asid uronik, 39.49±4.35% karbohidrat, dan <2% kandungan protein dan polifenol. Profil monosakarida mengenal pasti glukosa, galaktosa, ribosa, dan asid glukuronik sebagai unit gula dominan, mencadangkan ia merupakan heteropolisakarida jenis galaktan atau glukuronan. Polisakarida dengan jisim molekul 31.3±0.5 kDa ini menunjukkan nilai $IC_{50}$ sebanyak 1.59 mg/mL terhadap monofenolase dan 1.84 mg/mL terhadap difenolase, mengikuti mekanisme perencatan mod campuran seperti yang dicadangkan oleh analisis kinetik Lineweaver-Burk. Selain itu, keupayaan pengkelatan kuprum (74.1% pada 5 mg/mL) menunjukkan satu lagi mekanisme perencatan enzim. Ujian selular seterusnya mengesahkan keberkesanannya dalam mengurangkan aktiviti tirosinase intraselular dan kandungan melanin, memodulasi tahap cAMP intraselular, serta mengawal ekspresi gen *Mitf*, *Tyr*, *Trp-1*, dan *Trp-2* dalam sel melanoma B16F10. Penemuan ini membuktikan potensi DES baharu dan polisakarida

daripada kulit kayu *Cinnamomum verum* sebagai calon yang berpotensi untuk digunakan dalam formulasi penjagaan kulit anti-pigmentasi, antioksidan, dan perlindungan matahari.

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## ABSTRACT

The cosmetics market exhibited increasing demand in natural-based product with excellent anti-hyperpigmentation effects. Meanwhile, the research community is venturing on extraction solvents that is eco-sustainable. Despite increasing interest, a Deep Eutectic Solvent (DES) specifically tailored for polysaccharide extraction remained undeveloped. In response, a novel acidic DES was synthesized from cost-effective, readily available components: citric acid monohydrate and L-glutamic acid in a 1:1 molar ratio, using microwave-assisted heating (10 min, 360 W). The resulting DES exhibited an orange hue, attributed to the involvement of the Maillard reaction during synthesis, indicating a synergistic interaction between the parent compounds. Characterization revealed that the DES is hydrophilic and acidic, with full water miscibility. Physicochemical evaluations included pH, density, solubility, rheological behaviour, and FTIR spectroscopy, alongside Maillard reaction assessments. Molecular docking analysis further supported the presence of a robust hydrogen-bond network between the amine and carboxyl groups of glutamic acid, carboxyl groups of citric acid, and water molecules, stabilizing the supramolecular structure. This newly synthesized DES demonstrated strong potential for both laboratory-based research and scale-up for polysaccharide extraction. In search of a novel bioactive compound with anti-hyperpigmentation effects comparable to its chemical counterparts, the skincare potential of *Cinnamomum verum* bark polysaccharides was investigated using the synthesized DES under microwave-assisted

extraction. Extraction optimization using Box-Behnken design has achieved optimal extraction at 4% (w/v) DES, 4 min and 360 W microwave power that resulted in (i) 7.00±0.78% polysaccharide yield; (b) 97.22±0.27% monophenolase inhibitory activity; (c) 86.81±2.64% diphenolase inhibitory activity; (d) 60.47±4.23% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging power and (e) 5.919±0.60 sun protection factor (SPF). Biochemical analysis revealed that the extracted polysaccharide consisted of 39.18 ± 0.28% uronic acids, 39.49 ± 4.35% carbohydrates, and <2% protein and polyphenolic content. Monosaccharide profiling identified glucose, galactose, ribose, and glucuronic acid as the dominant sugar units, suggesting a galactan or glucuronan-type heteropolysaccharide. The 31.3 ± 0.5 kDa polysaccharide exhibited $IC_{50}$ values of 1.59 mg/mL and 1.84 mg/mL against monophenolase and diphenolase, respectively, following a mixed-mode inhibition mechanism as suggested by Lineweaver-Burk kinetics analysis. Additionally, copper chelation capacity (74.1% at 5 mg/mL) indicated an additional mode of enzyme inhibition. Cellular assays further confirmed its efficacy in reducing intracellular tyrosinase activity and melanin content, modulating intracellular cAMP levels, and regulating melanogenesis-associated gene expressions including *Mitf*, *Tyr*, *Trp-1*, and *Trp-2* in B16F10 melanoma cells. These findings demonstrate the potential of the novel DES and the *Cinnamomum verum* bark-derived polysaccharide as promising candidates for incorporation into antioxidant, anti-pigmentation, and sun-protective skincare formulations.

# CHAPTER 1

# INTRODUCTION

## 1.1 Research background

Hyperpigmentation is a significant skin concern that arises with aging due to increased tyrosinase activity. Although common, some individuals perceive hyperpigmentation as undesirable and seek ways to maintain a fairer skin tone. According to Sanchez-Ferrer (1995), tyrosinase is the key enzyme regulating pigmentation; thus, inhibiting its activity can prevent hyperpigmentation. However, commercially available tyrosinase inhibitors, such as kojic acid, arbutin, and hydroquinone, are associated with adverse side effects, including contact dermatitis, irritation, genotoxicity, and even carcinogenicity (Nohynek et al., 2004; Rendon & Gaviria, 2005; Westerhof & Kooyers, 2005). These concerns have led to increased interest in natural alternatives that can effectively inhibit tyrosinase without the associated risks.

In recent years, research on natural tyrosinase inhibitors has been growing, with varying degrees of effectiveness (Zolghadri et al., 2019). While natural extract-based inhibitors have shown potential, their efficacy remains inconsistent, and the search for a highly effective natural alternative continues. Market studies indicate that consumers are increasingly open to natural cosmetics. While some prefer entirely natural formulations, most base their purchasing decisions on product efficacy (Amberg & Fogarassy, 2019). This project aims to introduce the skincare industry to polysaccharides derived from Ceylon cinnamon (*Cinnamomum verum*) as a potential natural tyrosinase inhibitor. These polysaccharides offer a promising alternative by effectively reducing hyperpigmentation while minimizing risks compared to synthetic agents. Furthermore, their natural origin

enhances consumer confidence in the product, making it an appealing choice for individuals who prioritize both efficacy and safety in skincare products.

Ceylon cinnamon (*Cinnamomum verum*) is a widely accessible and commonly used type of cinnamon, particularly in East and Southeast Asia. This evergreen aromatic plant has been recognized for its pharmacological properties, including antioxidant, antidiabetic, neuroprotective, and anti-cholesterol effects (AlMohaimeed et al., 2021; Nwanade et al., 2021). In northern Malaysia, a small community has traditionally used Ceylon cinnamon to treat skin issues, inspiring further exploration of its potential in skincare. The traditional application of this plant in treating dermatological conditions suggests the presence of bioactive compounds that could be harnessed for cosmetic and medical purposes.

As with most natural product research, this project begins with the extraction of desired compounds, in this case, polysaccharides from Ceylon cinnamon. However, background research indicates that existing polysaccharide extraction methods require improvement. Commonly used techniques include hot or cold-water extraction, enzymatic extraction, and acid or alkaline extractions (Tang & Huang, 2022; Xue et al., 2022). These conventional methods, while effective to some extent, they do have limitations which would result in negative impacts towards the extracted products. Some instances of extraction methods and their respective limitations, are discussed below. As reviewed by Huang et al. (2021), water-based extraction are simple to operate, but it could be time consuming and yields low product purity; acid-base extraction are highly specific to the target polysaccharides; enzyme extraction usually comes with high cost and byproducts degraded by the enzymes; ultrasonic extraction which could alter the polysaccharide

structure and its bioactive compounds, and lastly, supercritical fluid extraction which is complex to operate and does not contribute to the concept of green and sustainable extraction procedures.

A recent advancement, deep eutectic solvent (DES) extraction, offers significant benefits over conventional methods. DES is a solvent formed by mixing two or more Lewis acids and bases under specific conditions, creating a new compound with lower melting and boiling points than its constituents (Abbott et al., 2001). Formation occurs at a specific molar ratio and eutectic point, where stable intermolecular forces, including hydrogen bonds, contribute to its unique properties (Fernandes et al., 2023; Smith et al., 2014). DES is considered an environmentally friendly alternative to traditional organic solvents due to its biodegradability, low volatility, and non-toxic nature. The reduced toxicity of DES makes it particularly suitable for applications in the food, pharmaceutical, and cosmetic industries, where consumer safety is a top priority. Despite the versatility of DES, no reports exist on its synthesis using citric acid monohydrate and L-glutamic acid. This project proposed using citric acid monohydrate and glutamic acid as parent components to create a novel DES.

These inexpensive and widely available components could significantly reduce extraction costs. Moreover, citric acid and glutamic acid, being environmentally friendly, contribute to a greener alternative for large-scale extraction processes. Citric acid, widely used in the food and pharmaceutical industries, is known for its antioxidant properties and ability to chelate metal ions. Glutamic acid, an amino acid with numerous biological functions, enhances the biocompatibility of the DES, making it an ideal candidate for cosmetic applications. The synthesized DES will then be applied in polysaccharide

extraction from Ceylon cinnamon, offering a novel and sustainable approach to obtaining bioactive compounds. By optimizing this extraction process, the study aims to improve the yield and quality of polysaccharides obtained, ensuring that they retain their bioactive properties for effective use in skincare formulations.

While DES is relatively simple to prepare, developing a more efficient synthesis method is necessary. A microwave-assisted approach is proposed as an alternative, offering even heat distribution and radiation to induce compound interactions in a shorter time than conventional methods. Microwave-assisted synthesis has gained attention in recent years for its ability to enhance reaction rates, reduce energy consumption, and improve product yield. It is hypothesized that DES can be successfully synthesized using citric acid monohydrate and L-glutamic acid. Microwave-assisted synthesis will improve efficiency. Citric acid, acting as a weak base relative to glutamic acid, will facilitate DES formation. The Maillard reaction may occur due to glutamic acid's amino group, lowering DES pH via Amadori rearrangement (Cui et al., 2021). Citric acid also exhibits anti-browning properties and serves as an effective antioxidant (Lim et al., 2010; Peeters et al., 2018). Given its presence in DES, it is expected to retain antioxidant properties, minimizing oxidative degradation during polysaccharide extraction.

This study aimed to optimize DES synthesis using microwave-assisted methods by determining appropriate molar ratios of L-glutamic acid and citric acid monohydrate. It also seeks to identify optimal synthesis parameters, including time and microwave power. The physicochemical properties of synthesized DES will be characterized through microscopy analysis, Fourier transform infrared spectroscopy (FTIR), density and viscosity measurements, pH and solubility testing, and Maillard reaction analysis.

Additionally, the efficacy of DES as a polysaccharide extraction buffer will be evaluated. The study will assess the impact of extraction conditions on polysaccharide yield and bioactivity, ensuring that the final product retains its functional properties.

Microwave-assisted extraction conditions significantly impact the properties of the extracted polysaccharides. Harsh conditions (e.g., high temperature, prolonged exposure) may yield higher extraction efficiency but risk structural damage and loss of bioactivity. Conversely, milder conditions preserve bioactivity but may reduce yield. Optimizing extraction parameters is therefore crucial. The extracted polysaccharides will be assessed for their ability to inhibit tyrosinase activity, their antioxidant potential, and their UV-protective effects. These bioactivities will provide insight into their potential as cosmetic ingredients.

The mechanism underlying polysaccharide-mediated tyrosinase inhibition, antioxidant activity, and UV protection remains largely unexplored, particularly regarding the melanogenesis pathway. This study aims to provide fundamental insights into these mechanisms. To further assess bioactivity, the extracted polysaccharide will be administered to B16F10 melanoma cells to determine cytotoxicity, measure intracellular tyrosinase activity and melanin levels, and analyze effects on intracellular cAMP levels and gene expression. Additionally, kinetic studies on tyrosinase inhibition and copper chelation will be conducted to elucidate the underlying mechanisms.

By integrating green chemistry principles with advancements in extraction technology, this research not only aims to develop a novel, eco-friendly DES for polysaccharide extraction from Ceylon cinnamon but also contributes to the broader field of sustainable cosmetic ingredient development. As consumer preferences shift towards safer and more

sustainable skincare products, the findings of this study will be highly relevant for both academic and industrial applications.

## 1.2 Problem Statements

Prior to designing this research project, it is noticed that the potential of Ceylon cinnamon, specifically, on its polysaccharides towards anti-hyperpigmentation properties, remained unexplored. However, by keeping the concept of eco-sustainability in mind, it is necessary that the approaches that are applied in the analysis of the Ceylon cinnamon polysaccharides should be as eco-friendly as possible, therefore, pointing the researchers to consider the creation of an extract solvent which fulfils the conditions. To facilitate the research, the issue was segregated into multiple parts for the researchers to progressively tackle them.

1. The synthesis efficiency and quality of the novel deep eutectic solvent (DES) are influenced by variations in the ratio of its constituent compounds and processing parameters, including microwave power and heating time. These factors remained unknown for a new DES synthesis using L-glutamic acid and citric acid monohydrate.
2. The anti-hyperpigmentation properties (tyrosinase inhibition, antioxidant activity, and UV protection) of the polysaccharide could be affected by extraction conditions, specifically microwave power, extraction time, and the concentration of the extraction buffer. Therefore, investigation and optimization of the extraction parameters are necessary.

3. The structural and molecular mechanisms underlying the Ceylon cinnamon polysaccharide's anti-hyperpigmentation, antioxidant, and UV-protective effects need to be elucidated, particularly regarding binding interactions, conformational changes, and self-assembly processes that influence tyrosinase inhibition and other functional properties are still unexplored.
4. The polysaccharide's impact on the melanogenesis pathway must be investigated by assessing its effects on tyrosinase activity, melanin production, cyclic adenosine monophosphate (cAMP) levels, and the expression of pigmentation-related proteins (*Mitf, Tyr, Trp-1, Trp-2*) in melanoma cells as the effect of anti-hyperpigmentation.

## 1.3 Hypothesis

1. A distinct ratio of compounds and parameters, such as microwave power and heating time will affect the synthesis and quality of the novel DES.
2. The microwave-assisted extraction parameters (i.e. microwave power, extraction time, concentration of extraction buffer) will affect the anti-hyperpigmentation properties (i.e., tyrosinase inhibitory, antioxidant and UV protection activities) of the polysaccharide. A revised microwave-assisted extraction approach will be required to maximize the anti-hyperpigmentation properties of the polysaccharide.
3. The mechanism of the anti-hyperpigmentation, anti-oxidant and UV protection properties will also be well understood by investigating their structural properties, binding sites, binding energy and the mode of inhibition. Specific contacts within interactive residues at the inhibition sites are proposed as the strategy for the inhibitory polysaccharides on tyrosinase. Conformation transition due to the presence of polysaccharides might induce a self-assembled structure, which could promote the interaction, as well as addressing the effects of subtle changes in the polysaccharides composition by studying the structure-function relationships of the polysaccharides.
4. The melanogenesis pathway will be revealed based on the tyrosinase activity as well as melanin, cyclic adenosine monophosphate (cAMP) and pigmentation-related proteins (*Mitf, Tyr, Trp–1, Trp-2*) levels in melanoma cells.

## 1.4 Research Questions

1. What are the appropriate parameters to synthesize a novel DES for polysaccharide extraction from cinnamon?
2. How do the extraction parameters, such as microwave power, extraction time and concentration of extraction buffer affect the anti-hyperpigmentation properties (i.e., tyrosinase inhibitory, antioxidant and UV protection activities) of the polysaccharide?
3. How do the structures of the polysaccharide affect the mechanism of the anti-hyperpigmentation, anti-oxidant and UV protection properties and how is the conformation of tyrosinase affected by the presence of polysaccharide?
4. How do the polysaccharides play the role as the inhibitor in the melanogenesis pathway?

## 1.5 Research Objective

The aim of this study is to investigate the anti-tyrosinase, anti-oxidant and UV protection effects of the bioactive polysaccharides that derived from Ceylon cinnamon that can be potentially used as a skincare product. The parameters of microwave-assisted approach extraction, in addition with characterization of the novel DES as a highly specific extraction solvent that targets the Ceylon cinnamon polysaccharide, should be explored as well.

This study has 4 specific objectives:

1. To synthesize a novel DES to extract polysaccharides from Ceylon cinnamon, and to characterise the synthesised DES.
2. To examine the effects of the extraction buffer concentration, microwave power and extraction time that influence the anti-hyperpigmentation properties of cinnamon pectic-polysaccharide.
3. To relate the structural properties of the extracted pectic-polysaccharide and their anti-hyperpigmentation properties, which includes the study of mechanism and mode of tyrosinase inhibition, DPPH scavenging power and SPF values as quantified readings to evaluate anti-hyperpigmentation properties of the pectic-polysaccharide.
4. To investigate inhibitory action of the extracted polysaccharides in the melanogenesis (pigmentation) pathway using B16F10 melanoma cells.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 The human skin

The human skin is the largest organ of the body, serving as a protective barrier and playing crucial roles in thermoregulation, immune defence, and sensory perception. It consists of three primary layers: the epidermis, dermis, and hypodermis, each with distinct structural and functional attributes. The skin, comprising approximately 16% of total body weight, is an essential organ for homeostasis and survival (Proksch et al., 2008). Understanding its complex structure and function is essential for dermatological and medical research.

The outermost layer, the epidermis, is composed mainly of keratinocytes arranged in stratified layers (Slominski et al., 1991). It lacks blood vessels and relies on diffusion from the underlying dermis for nutrient supply. The epidermis contains five sublayers: the stratum corneum, composed of dead, flattened keratinocytes forming a waterproof barrier; the stratum lucidum, a thin, transparent layer present only in thick skin of the palms and soles; the stratum granulosum, involved in keratinization and lipid barrier formation; the stratum spinosum, which contains desmosomes that provide mechanical strength; and the stratum basale, the deepest layer housing melanocytes responsible for pigmentation and basal cells essential for epidermal regeneration (Fuchs & Raghavan, 2002). Being the outermost layer of the skin, the epidermis is also ascribed with the development of skin colour, due to the production and deposition of melanin (dark pigment) in melanocytes and keratinocytes, the cells that made up 95% of the epidermis (Costin & Hearing, 2007; Wang, 2017). Melanocytes are dendritic cells found in the stratum basale of epidermis and

contain melanosomes, the site of melanin production or melanogenesis. Once filled with melanin, the membrane-bound melanosomes are trafficked to the surrounding keratinocytes where they will be deposited near the nucleus through the processes of release, uptake, and dispersion as proposed by Ando et al. (2012). Melanin is a light-absorbing pigment that scatters UVR and scavenges UVR-induced free radicals including the reactive oxidative species (ROS) to protect damages on DNA (Ortonne, 2002; Park et al., 2009).

Beneath the epidermis lies the dermis, which provides structural support through collagen and elastin fibers. It consists of two layers: the papillary dermis, a thin upper layer rich in capillaries and sensory neurons, and the reticular dermis, a thicker, deeper layer composed of dense connective tissue, sweat glands, sebaceous glands, and hair follicles (Lodish et al., 2000). The deepest layer of the skin is the hypodermis, also known as subcutaneous tissue, which consists of adipose tissue that provides insulation and cushioning. It contains larger blood vessels and lymphatics essential for thermoregulation and systemic metabolism (Schaefer, Redelmeier & Lademann, 2010).

The skin serves several critical functions (Madison, 2003). It acts as a barrier to prevent microbial invasion and minimize water loss. It plays a role in thermoregulation by maintaining body temperature through sweat gland activity and vasodilation (Elias, 2005). Sensory perception is another key function, as specialized receptors detect stimuli such as touch, temperature, and pain (Purves et al., 2001). Additionally, the skin is an integral part of the immune response, hosting Langerhans cells and dermal immune components to combat pathogens (Nestle et al., 2009). Finally, it facilitates the production of vitamin D when exposed to UV radiation (Holick, 2007).

Hyperpigmentation is a common dermatological condition characterized by the overproduction of melanin, leading to darker patches of skin (**Figure 2.1**). This phenomenon occurs due to various factors, including prolonged UV exposure, hormonal changes, inflammation, and genetic predisposition (Passeron et al., 2021). Melanocytes, located in the basal layer of the epidermis, synthesize melanin, which provides pigmentation and protects against UV radiation. However, excessive stimulation of melanocytes, often triggered by sun exposure, leads to uneven pigmentation and conditions such as melasma, post-inflammatory hyperpigmentation, and solar lentigines (Ortonne & Bissett, 2008).

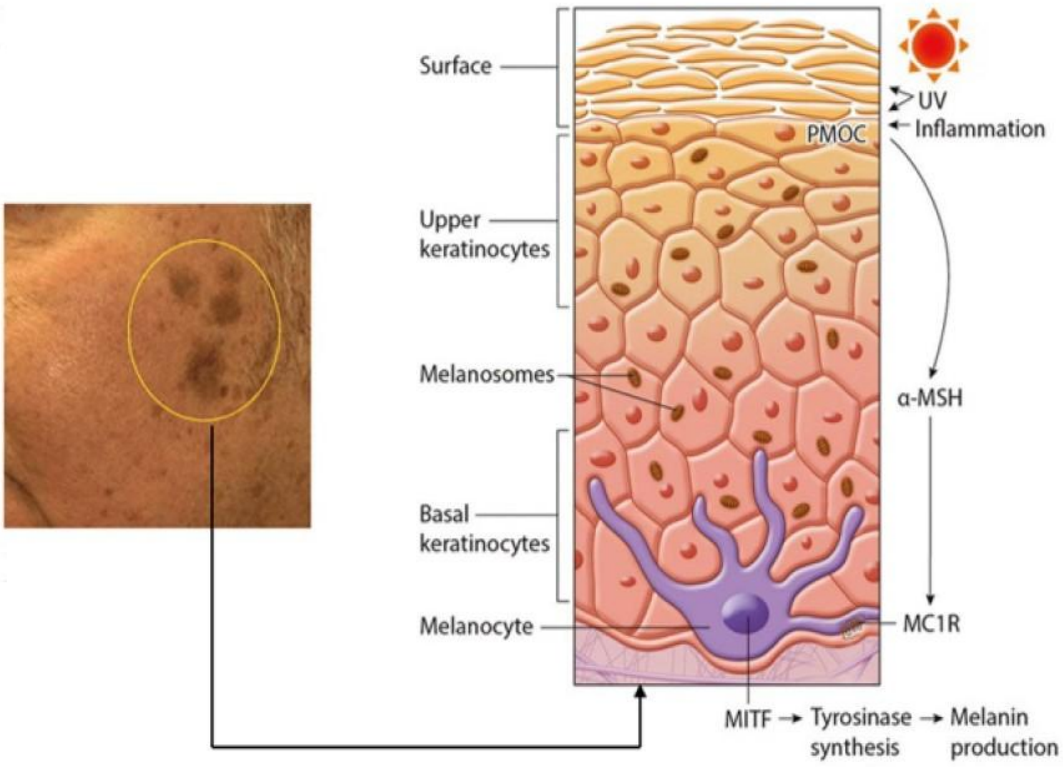


**Figure 2.1**: Overview of skin pigmentation. (Modified from https://thebab.co.uk/blog/f/the-science-of-hyperpigmentation?blogcategory=AlumierMD+Skin+Care+Products, 2018).

Melasma is frequently associated with hormonal changes, particularly during pregnancy or due to oral contraceptive use, while post-inflammatory hyperpigmentation arises following skin injury or inflammation, such as acne or eczema (Gupta et al., 2006). Treatment strategies for hyperpigmentation include topical agents like hydroquinone, retinoids, and vitamin C, as well as procedural interventions such as chemical peels and laser therapy (Callender et al., 2011). Preventative measures, including the use of broad-spectrum sunscreens and protective clothing, play a crucial role in managing and mitigating hyperpigmentation.

Other dermatological conditions also affect human skin. Eczema, or atopic dermatitis, is a chronic inflammatory skin disease characterized by barrier dysfunction and immune dysregulation (Leung et al., 2004). Psoriasis, an autoimmune disorder, leads to excessive keratinocyte proliferation and inflammation (Lowes et al., 2007). Skin cancer, including basal cell carcinoma, squamous cell carcinoma, and melanoma, is primarily linked to UV exposure and genetic predisposition (Rastrelli et al., 2014). Additionally, the skin exhibits remarkable regenerative capabilities, progressing through inflammation, proliferation, and remodelling phases following injury (Gurtner et al., 2008). Chronic wounds, such as diabetic ulcers, pose significant medical challenges due to impaired healing processes (Sen et al., 2009). Human skin is a multifunctional organ vital for protection, sensation, and homeostasis. Its complex structure and physiological roles make it a subject of extensive research, particularly in dermatology and regenerative medicine. Advances in skin biology contribute to the development of novel therapeutic strategies for skin diseases and wound healing.

At the current stage, venturing into polysaccharides for skin health as these components have an extremely high commercial value in the cosmetic industry. In Malaysia alone, there are 7.5 million cosmetic users and the estimated revenue will be approximately USD 402 million (Statista, 2023). The hyperpigmentation is one of the major issues of skin as this scenario is giving undesirable dark patches/spots to the skin.

## 2.2 Melanogenesis

Melanogenesis is the intricate biological process responsible for the production of melanin, the pigment determining the coloration of skin, hair, and eyes in humans and other vertebrates. Beyond its cosmetic significance, melanin plays a crucial role in photoprotection by absorbing ultraviolet radiation (UVR) and scavenging reactive oxygen species (ROS). The process of melanogenesis is tightly regulated at multiple levels, including genetic, enzymatic, and environmental influences. Central to this regulation are transcription factors such as microphthalmia-associated transcription factor (*Mitf*) and key melanogenic enzymes including tyrosinase (*Tyr*), tyrosinase-related protein 1 (*Trp-1*), and tyrosinase-related protein 2 (*Trp-2*). In this study, tyrosinase is the targeted enzyme that the polysaccharides should inhibit so that the melanogenesis process or the hyperpigmentation could be avoided. Tyrosinase (E.C. 1.14.18.1) is a copper-dependent enzyme ubiquitous in living organisms. It is the featured enzyme involved in melanogenesis comprising of a central, N-terminal and C-terminal domain (Kanteev, Goldfeder, & Fishman, 2015).

Melanogenesis occurs within melanocytes, specialized pigment-producing cells located primarily in the basal layer of the epidermis and in hair follicles. Melanocytes originate from neural crest cells during embryonic development and migrate to their final

locations in the epidermis, hair bulbs, and other tissues such as the eyes and inner ear (Mort et al., 2015). The process of melanogenesis is essential for determining pigmentation and protecting the skin against UV-induced damage. Defects in melanogenesis result in a variety of pigmentation disorders, including albinism, vitiligo, and melasma (Hearing, 2011).

Regulation of melanogenesis involves a complex interplay between intrinsic genetic factors and extrinsic environmental factors, such as UV radiation and hormonal signaling. Transcription factors play a crucial role in this process, with *Mitf* acting as the master regulator of melanocyte function and melanin biosynthesis (Levy et al., 2006). *Mitf* directly controls the expression of melanogenic enzymes such as *Tyr*, *Trp-1*, and *Trp-2*, which are essential for the synthesis and processing of melanin (Vachtenheim & Borovanský, 2010). Melanocytes arise from neural crest-derived melanoblasts during embryonic development. Their differentiation and migration are governed by key signalling molecules, including Wnt proteins, endothelin-3 (EDN3), and stem cell factor (SCF), which binds to the c-Kit receptor (Hubbard et al., 2000). The activation of *Mitf* through the Wnt/β-catenin pathway ensures the proper differentiation and proliferation of melanoblasts into mature melanocytes. Mutations in c-Kit or SCF can lead to pigmentation abnormalities such as piebaldism (Commo et al., 2004).

Melanogenesis occurs within melanosomes, lysosome-related organelles that undergo a four-stage maturation process (Raposo & Marks, 2007). Premelanosomes contain Pmel17, a glycoprotein that provides a structural scaffold for melanin deposition. *Mitf* regulates melanosome maturation by controlling the expression of Pmel17 and other melanosomal proteins. The activity of melanogenic enzymes such as *Tyr*, *Trp-1*, and *Trp-*

*2* increases as melanosomes mature, leading to the synthesis of melanin in its distinct forms (Raposo & Marks, 2007). Melanin production begins with the oxidation of tyrosine by *Tyr* to generate dopaquinone. Depending on the cellular microenvironment and the presence of cofactors, dopaquinone undergoes further reactions to form either eumelanin (black/brown pigment) or pheomelanin (yellow/red pigment) (Slominski et al., 1991). *Trp-1* and *Trp-2* modulate eumelanin synthesis by influencing the conversion of intermediates, stabilizing melanin production, and affecting the final pigment outcome (Hearing, 2011). *Mitf* governs this process by regulating the transcription of *Tyr*, *Trp-1*, and *Trp-2*, ensuring coordinated melanin production. Once synthesized, melanosomes are transported along the cytoskeleton using motor proteins such as kinesin and myosin Va (Wu et al., 2002). *Mitf* also influences cytoskeletal components by regulating Rab27a, a protein essential for melanosome transport. The transfer of melanosomes to neighbouring keratinocytes occurs via exocytosis, filopodia-mediated transfer, or cytophagocytosis, where keratinocytes engulf melanosomes from melanocyte dendrites (Tarafder et al., 2014). This transfer is critical for the distribution of pigment throughout the epidermis.

Melanogenesis is regulated by a complex, integrated signalling network of autocrine and paracrine factors, typically through the modulation of melanocyte and keratinocyte proliferation or the expression of the rate-limiting enzyme tyrosinase (Bae-Harboe & Park, 2012; Costin & Hearing, 2007; Park et al., 2009). The pathway can be summarized in **Figure 2.2**.

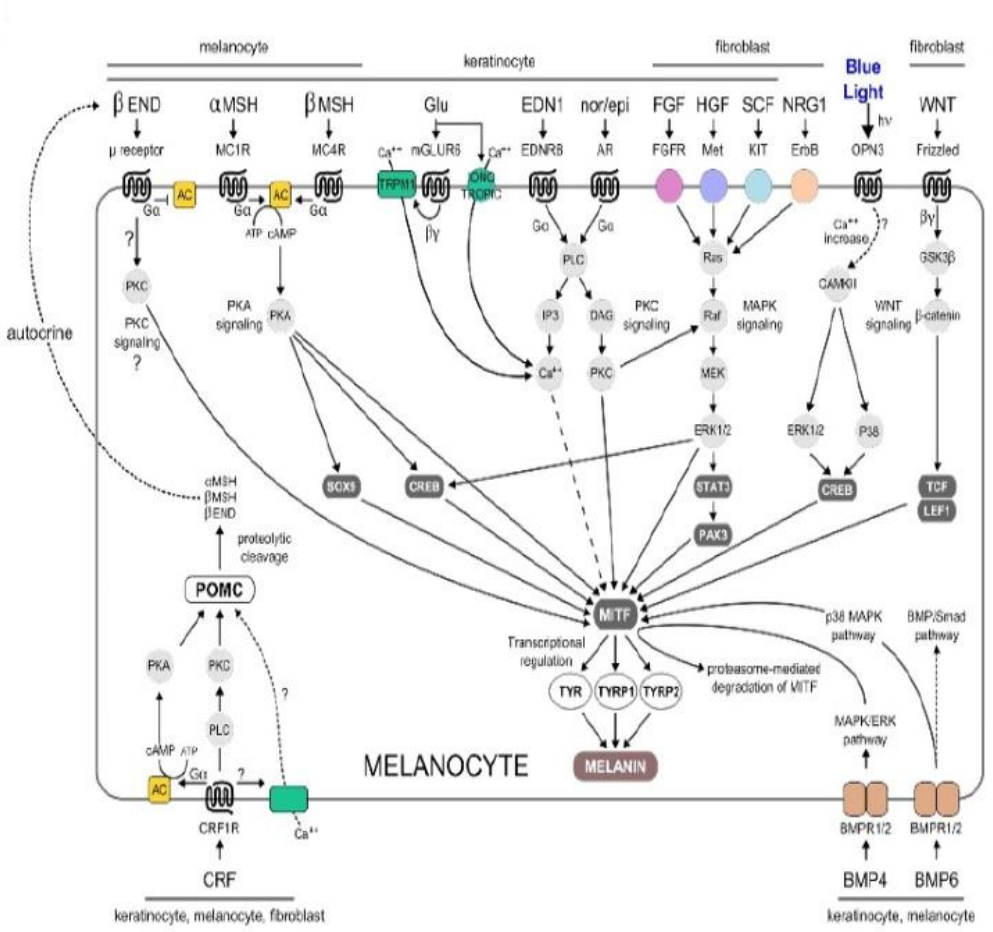


**Figure 2.2**: Summary of Melanogenesis Pathway. (Adapted from Pupesosweet, 2020).

*Mitf* is a critical transcription factor that governs melanocyte survival, differentiation, and melanogenesis. It regulates the expression of *Tyr*, *Trp-1*, and *Trp-2* by binding to their promoter regions (Levy et al., 2006). *Mitf* is activated by multiple signaling pathways, including the cAMP/PKA pathway, the Wnt/β-catenin pathway, and the MAPK/ERK pathway (Plonka et al., 2009). Additionally, *Mitf* regulates genes involved in melanosome biogenesis, transport, and survival, making it the central orchestrator of melanogenesis.

*Tyr* is the rate-limiting enzyme in melanin biosynthesis, catalyzing the hydroxylation of tyrosine to L-DOPA and its subsequent oxidation to dopaquinone (Kobayashi et al., 1994). *Trp-1* and *Trp-2* function downstream of *Tyr*, with *Trp-2* (also known as DCT) converting dopachrome into DHICA, an intermediate in eumelanin production (Del Marmol & Beermann, 1996). These enzymes are all transcriptionally regulated by *Mitf*, ensuring their synchronized expression during melanogenesis. In fact, higher tyrosinase activity gives rise to higher melanin synthesis in the cAMP-dependent pathway and this is attributed to the upregulation of the microphthalmia associated transcription factor (*Mitf*) (Chang, 2012). Therefore, if the tyrosinase could be inhibited by bioactive polysaccharides, the melanogenesis process could be stopped and hyperpigmentation will not occur.

Melanogenesis is a tightly regulated process involving multiple transcription factors, enzymatic pathways, and intracellular transport mechanisms. *Mitf* plays a central role in controlling the expression of melanogenic genes such as *Tyr*, *Trp-1*, and *Trp-2*, which are critical for melanin biosynthesis. Understanding the molecular mechanisms of melanogenesis is essential for developing targeted therapies for pigmentation disorders. Future research should focus on elucidating novel regulatory pathways and identifying potential pharmacological agents that can modulate melanogenesis for therapeutic applications.

The novelty of this research is focused on the mechanistic of cinnamon polysaccharides with anti-tyrosinase, anti-oxidant and UV protection activities to attenuate skin hyperpigmentation. This is because the commercially available tyrosinase inhibitory agents such as kojic acid, arbutin and hydroquinone are associated with harmful

side effects including contact dermatitis, irritation and even genotoxicity and carcinogenicity (Nohynek et al., 2004; Rendon & Gaviria, 2005; Westerhof & Kooyers, 2005). These drawbacks, therefore, urge the search for a cheaper, safer and more natural alternative anti-hyperpigmentation agent with high efficacy.

## 2.3 Ceylon cinnamon and the previous studies

Cinnamon, a widely used aromatic spice, is derived from the inner bark of trees belonging to the Cinnamomum genus. Traditionally, cinnamon has been valued for its distinctive flavour and medicinal properties. In this study, cinnamon is explored as an alternative source of anti-hyperpigmentation agents due to its bioactive compounds. A small group of Malaysians has long used cinnamon powder to treat skin conditions such as pimples and pigmentation, highlighting its potential dermatological benefits. However, scientific research on cinnamon's bioactive components, particularly its polysaccharides, remains limited.

One of the most prominent and well-documented uses of cinnamon extract lies in food preservation due to its natural antimicrobial properties. In a study by Black-Solis et al. (2019), tomatoes treated with biodegradable polyester nets infused with cinnamon essential oil exhibited significantly improved resistance to *Alternaria alternata*, a common spoilage fungus. The study reported a 35% reduction in fungal infection and an extended shelf life of up to 9 days longer than untreated controls, showcasing cinnamon's potential as an eco-friendly fungicide. Similarly, cinnamon oil demonstrated powerful inhibitory effects on fish spoilage bacteria, particularly *Shewanella putrefaciens* and

*Pseudomonas spp.*, by inducing membrane disruption and leakage of intracellular materials (Huang et al., 2019). Minimum inhibitory concentration (MIC) values as low as 0.125% v/v were observed, illustrating strong antibacterial activity. Complementing this, Zhang et al. (2016) found that cinnamon essential oil effectively inhibited *Escherichia coli* and *Staphylococcus aureus*, two major foodborne pathogens, through the breakdown of bacterial cell membranes and inhibition of ATP production.

In meat preservation, cinnamon bark oil was shown to maintain the quality of ground lamb during cold storage. Hussain et al. (2021) found that the addition of cinnamon oil reduced lipid oxidation by up to 45% over 9 days and maintained sensory acceptability, which is crucial for consumer satisfaction and shelf-life extension. Beyond antimicrobial action, cinnamon extract is rich in phenolic compounds such as cinnamaldehyde and eugenol, which contribute to its antioxidant properties. Shahid et al. (2018) demonstrated that incorporating cinnamon extract into palm oil significantly delayed oxidative degradation. The peroxide value of treated oil remained within safe consumption limits even after prolonged heating, highlighting cinnamon's potential as a stabilizer in lipid-rich foods. Cinnamon also displays antibiofilm activity against harmful bacteria. Lu et al. (2021) reported that cinnamon extract inhibited biofilm formation in *Vibrio parahaemolyticus* and *E. coli* by disrupting quorum sensing pathways. This property is particularly valuable in the food industry, where biofilms are notoriously resistant to cleaning and disinfection procedures.

Furthermore, Tang et al. (2019) found that yogurt enriched with cinnamon residue extract exhibited enhanced antioxidant capacity, as measured by DPPH and ABTS radical scavenging assays. The fortified yogurt also showed increased total phenolic content,

suggesting cinnamon can serve as both a functional food additive and a health-enhancing ingredient. Cinnamon extracts also demonstrate promising therapeutic effects on metabolic health, especially in diabetes management. In a randomized controlled trial, Zare et al. (2019) found that supplementation with cinnamon capsules significantly lowered fasting blood glucose (from 173.2 to 144.8 mg/dL, $p < 0.05$) and HbA1c levels in patients with type 2 diabetes. These effects are largely attributed to improved insulin sensitivity and glucose uptake modulation. Supporting these findings, Hlebowicz et al. (2007) observed that the consumption of cinnamon with a carbohydrate meal delayed gastric emptying and reduced postprandial blood glucose concentrations by up to 30% in healthy individuals. The slowed gastric emptying rate prolongs satiety and helps in better glucose regulation, making cinnamon a valuable dietary aid for people with impaired glucose metabolism.

Cinnamon's anti-inflammatory effects are equally noteworthy. Haidari et al. (2020) reported that rats exposed to acrylamide-induced oxidative stress exhibited significantly reduced levels of malondialdehyde (MDA) and increased antioxidant enzyme activities when supplemented with cinnamon extract. This suggests a protective role against oxidative damage from environmental toxins and processed foods. Ho et al. (2013) further demonstrated that cinnamon extract and its components, particularly cinnamaldehyde, inhibited neuroinflammation by downregulating nitric oxide and pro-inflammatory cytokines such as TNF-α and IL-6 in microglial cells. These findings point to its potential in managing neurodegenerative diseases like Alzheimer’s and Parkinson’s.

In term of cardiovascular health, cinnamon has been found to regulate lipid profiles and prevent platelet aggregation. Mehrpouri et al. (2020) reported that cinnamon

extract reduced total cholesterol and triglyceride levels in animal models, while also enhancing HDL cholesterol. In diabetic rats, cinnamon extract helped manage dyslipidemia and reduced cardiovascular risk markers (Shahrestan et al., 2020). Neurologically, cinnamon shows promise in improving cognitive outcomes after traumatic brain injury (TBI). Qubty et al. (2021) found that orally administered cinnamon extract enhanced memory and learning in TBI-induced mice models by promoting neuronal survival and reducing inflammation in the hippocampus. On the immune front, Ose et al. (2020) demonstrated cinnamon's ability to inhibit allergen-specific immune responses in both human and mouse models. This immune modulation suggests a potential role for cinnamon in allergy prevention or management. Moreover, in cancer research, Kwon et al. (2010) discovered that cinnamon extract could induce apoptosis in tumor cells by inhibiting key transcription factors such as NFκB and AP-1, indicating its potential as a complementary therapy in cancer treatment. A lesser-known but significant benefit of cinnamon is its ability to alleviate migraines and chronic headaches. Zareie et al. (2020) conducted a double-blind placebo-controlled trial showing that cinnamon intake significantly reduced migraine frequency and levels of inflammatory biomarkers such as C-reactive protein (CRP). Participants taking cinnamon reported fewer migraine attacks per month compared to the placebo group ($p < 0.01$).

There were some researchers identified a significant yet understudied class of bioactive compounds in cinnamon: pectic-polysaccharides. These natural biopolymers exhibit diverse pharmacological activities, including anti-angiogenic, hypoglycemic, anti-inflammatory, and neuroprotective effects (Ding et al., 2020; Xiang et al., 2020; Zhang et al., 2020; Zhong et al., 2020). Furthermore, polysaccharides have been associated with

anticancer properties. Lin and Lin (2020) reported that a polysaccharide from guava (*Psidium guajava L.*) seeds have strong anti-inflammatory and immunomodulatory effeects, whereas Xu et al. (2021) demonstrated that *Cordyceps cicadae* polysaccharides suppressed cervical cancer (HeLa) cell growth. Shang et al. (2020) also discovered that a polysaccharide extracted from Taishan *Pinus massoniana* pollen inhibited influenza virus infection.

Despite these promising biological properties, many potential applications of polysaccharides remain unexplored. Pectin is one of the most abundant polysaccharides found in plant and fruit cell walls. It is composed mainly of homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II, which contain varying levels of galacturonic acid. Dominiak (2014) described pectic-polysaccharides as complex mixtures consisting of homogalacturonan "smooth regions" composed of homopolymeric α-1,4-linked-D-galacturonic acid, which accounts for approximately 50-70% of primary cell wall pectic-polysaccharides. This backbone is connected to various neutral sugars, including rhamnose, galactose, arabinose, xylose, and glucose, forming "hairy regions" or branched structures. Additionally, trace amounts of phenolic compounds, such as coumaric acid, may be conjugated within the pectic-polysaccharide structure. The unique structure of pectic-polysaccharides suggests a possible mechanism by which they may inhibit melanogenesis. Specifically, their ability to form aggregates with tyrosinase may prevent the enzyme from catalyzing the formation of melanin, thereby reducing pigmentation. Furthermore, the conjugated phenolic compounds in pectic-polysaccharides provide antioxidant activity and UV-absorbing properties, which could further enhance their efficacy as skin-lightening agents.