Chapter 35

The Unexplored Botanical Extracts: A New Horizon in Skin Anti-Aging Formulation

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ABSTRACT

As skin ages, it loses its natural elasticity and becomes thinner, more fragile, and laxer, taking on a wrinkle appearance. Ultraviolet light, a sequence of changes in the weather and environmental pollution are among the pivotal factors contributing to the acceleration of the natural aging process. The utilization of botanical extracts and herbs has its origins in ancient times. We tried to investigate the anti-aging potentials of senduduk which is among some of the unexplored plant sources found in Malaysia. Our main idea was to emphasize action mechanisms of these botanical extracts based products, in fighting skin aging in term of its ability to scavenge free radicals, to protect the skin matrix through the inhibition of enzymatic degradation, or to promote collagen synthesis in the skin and to provide photoprotection. The experimental data revealed the percentage of DPPH radical scavenging activity, total phenolic content (TPC), and total flavonoid content (TFC) for senduduk extracts were (DPPH: 89.66% ; TPC: 1072.92 mg/g ; TFC: 5.61 mg/g) respectively. The anti-collagenase (AC) and anti-elastase (AE) assay also exhibited good inhibition values of (AC: 94.35% ; AE: 66.67%) respectively. The sun protection factor (SPF) value obtained was 22.44 for senduduk. Next, a nanoemulsion comprising of a mixture senduduk extracts was formulated and subjected physiochemical and in-vivo analysis. Finally, the image analysis offered a quick and consistent approached for quantifying skin aging feature which showcased reduction in skin wrinkle and improvement in skin texture, thus emphasizing the formulation’s efficacy as a promising natural anti-aging source.

Key Words: Senduduk, Antioxidant, Anti-Aging, Botanical Sources, Melastoma
1. INTRODUCTION

Over the years, plant-based materials have been increasingly used due to safety concerns as they are less toxic as compared to synthetic drugs and chemicals. Moreover, according to Nizioł-Łukaszewska et al. (2018), nowadays, industry demands multifunctional cosmetic ingredients primarily in natural origins as their complex bioactive and chemical compositions are able to deliver multi-faceted activity towards problematic skin conditions such as moisturizing, soothing, and nourishing effects using just one active ingredients. Research done by Ya et al. (2015) showed that several chemicals isolated from plants can be used as whitening agents which control the overproduction of melanin synthesis. This is due to the fact that plants can synthesize major chemical compounds which can be sorted by their chemical class, functional groups and biosynthetic origin into primary and secondary metabolites (Ahirrao et al., 2011). For instance, there are a number of compounds extracted from plants such as phenols, flavones, alkaloid, and tannin that have shown antioxidant properties against unwanted radical species. A study done by Mansur et al. (2016) revealed that plant extract incorporated with antioxidant properties are of utmost interest in the phyto-cosmetic field as they provide molecules that could inactivate radical oxygen species (ROS) by restoring skin homeostasis therefore, preventing premature aging of the skin. Moreover, the biological function of the skin will be improved and in turn will improve the appearance, radiance and texture of the skin from the application of natural ingredients (Taofiq et al., 2016).

Melastomataceae are widely distributed in tropical and subtropical areas of the world and comprises 170 genera and 4600 species in total (Costa et al., 2015). The genus of M. malabathricum, Clidemia. hirta and M. malabathricum var. alba are popularly known in Malays community as “ senduduk ungu, senduduk bulu and senduduk putih” respectively. They can be found growing in mountain forests, lowland, and also on cleared land such as roadsides. This plant can be differentiated by the colour of the flower petals which are light-pink magenta, dark-purple magenta and white (Haron, Anuar and Veeramohan, 2015). Traditionally, these plants are used by old folks to treat various diseases such as inflamed wounds, diarrhea, pox scars, bleeding, dysentery, gastric ulcers, epilepsy, and remedy for skin infections (Jamalnasir et al., 2013; Lopez et al., 2016; Zakaria et al., 2011). Various pharmacological activities were also reported from M. malabathricum Linn, C. hirta and M. malabathricum var. alba such as antioxidant, antiulcer, antimicrobial and anticancer activities (Basu, Pal and Mandal, 2016; Danladi et al., 2015; Hamid et al., 2018; Ismail et al., 2017; Zabidi et al., 2012). Hence, the aim of the present study was to develop a nanoemulsion containing mixtures of extract from leaves of Melastomataceae family species (M. malabathricum Linn, Clidemia hirta and M. malabathricum var. alba) and to investigate via in vitro and in vivo evaluation of the efficacy and safety of this formulation as well as assessment of therapeutic properties of these plants for its antioxidant and functional cosmetic properties.
2. METHODOLOGY

2.1 Preparation of *Melastoma* Leaves Extract

The whole leaves samples were washed with tap water to remove all dust and debris and dried under shade area at room temperature (27 ± 2 °C) for one to two weeks. Next, the air-dried leaves were ground using a mechanical grinder machine into a powder form. Next, the *Melastoma* leaves were subjected into extraction by mixed with a solvent extraction for two times with stirring at room temperature. The mixtures were filtered using a filter paper (Whatman No. 1) and evaporated using rotary evaporator (Yamato, Rotary Evaporator, model-RE 801, Japan) until all crude extracts were obtained.

2.2 Antioxidant Assay

2.2.1 DPPH (1,1-diphenyl-2-picrylhydrazyl) Assay

The DPPH free radical scavenging activity method was performed according to the method described by Blois (1958) with minor modifications to fit the study test (Mathangi and Prabhakaran, 2013). 10.0 mg of the dried leaves extract was accurately weighed and dissolved in 10.0 mL methanol (1000 mg/mL). Next, 10.0 mg of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was prepared and dissolved in methanol and made up to 100.0 mL volumetric flask. Briefly, 50.0 μL of the extract samples were mixed with 150.0 μL methanolic solution of DPPH in 96-well microliter plate and incubated in the dark room for about 30 minutes for reaction takes place. The absorbance value of the samples was measured at 517 nm using UV-Vis microplate reader (Infinite M200, Tecan).

2.2.2 Total Phenolic Content (TPC)

The total phenolic compounds were determined using the Folin-Ciocalteau method of Singleton and Rossi (1965) with minor modifications. Briefly, 100.0 μL (1.0 mg/mL) of the sample extract was mixed with 50.0 μL of Folin solution previously diluted with 7.0 mL distilled water. Next, 1.5 mL of 7.5 w/v% of sodium carbonate solution was added to the mixture. The sample were incubated in the dark room at room temperature for about 2 hours for reaction takes place. The absorbance value of the samples was measured at 765 nm using a UV-VIS microplate reader (Infinite M200, Tecan). The phenolic content of each *Melastoma* leaves were calculated based on the standard calibration curve and were expressed as mg gallic acid equivalent (mg/g GAE) of extract sample.

2.2.3 Total Flavonoid Content (TFC)

A method from Stankovic (2011) was adopted to investigate the total flavonoid content in all *Melastoma* leaves extracts. Firstly, the reaction mixture was prepared by mixing 100.0
μL of each plant extract with 2% AlCl₃ solution. The mixtures then incubated for one hour at room temperature and all samples were measured spectrophotometrically at wavelength \( \lambda = 415 \) nm using microplate reader UV-VIS spectrophotometer (Infinite M200, Tecan). Based on the measured absorbance, the total flavonoids content for all *Melastoma* leaves extracts were expressed in terms of quercetin equivalent (mg of quercetin/g of extract).

### 2.2.4 Photo-Protective Activity

The in-vitro determination of sun protection factor (SPF) was performed according to the method described by Raimundo et. al (2013). The dried leaves extracts were diluted in solvent extraction at concentrations 0.5 mg/mL. Subsequently, the extracts went through spectrophotometric (Infinite M200, Tecan) scanning at wavelengths between 260-400 nm, with intervals of 5 nm. Calculation of SPF was obtained according to the equation developed by Mansur et.al (1986).

### 2.2.5 Neutrophil Elastase and Collagenase Inhibition Assay

The determination of elastase inhibition assay of *Melastoma* nanoemulsion was carried out based on Neutrophil Elastase Colorimetric Drug Discovery Kit which was purchased from Enzo Life Sciences (BML-AK497). This kit provide a complete assay system which designed to screen inhibitors of neutrophil elastase (purified human neutrophil elastase; 2.2 μU/ μL), a potential therapeutic agent using the chromogenic substrate (MeOSuc-Ala-Ala-Pro-Val-pNA; 100.0 μM) and spectrophotometrically measured at absorbance 405 nm using microplate reader. Meanwhile, the Collagenase inhibition assay, Matrix metalloproteinase-1 (MMP-1) is interstitial collagenase or fibroblast collagenase). These enzymes play a significant role that target collagen, gelatin, entactin, pro-TNF-α, and the chemokine SDF-11-4. The MMP-1 Colorimetric Drug Discovery kit was purchased from Enzo Life Sciences (BML- AK404). This kit is a complete assay system designed to screen MMP-1 inhibitors, a potential therapeutic agent using a thiopeptide as a chromogenic substrate (Ac-PLG-[2-mercapto-4-methyl-pentanoyl]-LG-OC2H5)6,7. During this assay, the MMP cleavage site peptide bond is replaced by a thioester bond in the thiopeptide (substrate), which produced a sulfhydryl group that reacted with DTNB (5,5’ -dithiobis (2-nitrobenzoic acid). The final product was detected at absorbance 412 nm.

### 2.2.6 Wrinkles Activity

The measurement for skin condition or wrinkles activity was carried out on an initial condition for baseline and interval of 1 week for 4 weeks. Each of the volunteer was instructed to apply the cream formulation on their face (smiling line), twice daily after washing face. The measurement and evaluation of skin condition was taken in controlled in air-conditioned room (25 ± 2°C and 45 ± 2% relative humidity).
skin conditions was performed using an probe instrument Visioscan ® VC98 equipped with high resolution video camera with high-resolution optical system in a charge-coupled device. The camera consist of two individual halogen lights which were arranged to illuminate the skin uniformly and were designed to eliminate undesired reflections of sharp skin image (Karim, 2016). The parameters were studied to describe the skin condition captured by the image are texture, volume, surface evaluation of living skin and etc. These parameters were well described in the manual of the Visioscan ® VC 98.

3. RESULTS AND DISCUSSION

3.1 Antioxidant activities

Radical scavenging activity of three varieties of *Melastoma* plant extracts against stable 1,1-diphenyl-2-picryl-hydrazyl hydrate DPPH was evaluated in this study for its antioxidant activity. During the experimentation, the change in colour from deep purple to yellow light represents the antioxidant compound in *Melastoma* plant reacts with DPPH radical by donating the hydrogen radical resulting in bleaching of the DPPH solution. Higher percentage of inhibition indicates better scavenging activity of *Melastoma* plant extracts or exhibited good antioxidant potential. From the result, it shows that, *Melastoma* extracts were 89.66% (Table 1). The highest inhibition of DPPH radical activity from the *Melastoma* leaves extract can be deduce from the high antioxidant compounds in the extract such as ellagic acid, nobotannin B and anthocyanin compounds.

Meanwhile, the total phenolic constitutes one of the main groups of compounds acting as primary antioxidants or free radical scavengers. Hence, it was reasonable to detect and estimate their amount in the plant extracts. In this assay, higher phenolic content denotes higher antioxidant activity in a studied samples. From the results, the amount of extracted phenolic contents from *Melastoma* leaves was 1072.92 mg/g GAE (Table 1). It is claimed that phenolic compounds are one of the powerful chain breaking of radical species and mainly the scavenging activity of phenolic constituents is due to its hydroxyl group (Padmanabhan and Jangle, 2012). Thus, it can be deduce that the studied *Melastoma* plants contain significant numbers of antioxidant activity to act as scavenger for hazardous radical species. Flavonoids are plant secondary metabolites and potent class of natural products (Mierziak, Kostyn and Kulma, 2014; Panche, Diwan and Chandra, 2016). They cater major benefits which consist of large group of polyphenolic compounds (Alam et al., 2018). Higher flavonoids content or value indicates higher antioxidant activity of an extract. From the result, it shows that the total flavonoid content of *Melastoma* leaves extract was 5.61 mg/g QE of extract (Table 1). The present data also shows that the high content of the flavonoid could be associated with the high level of antioxidant activity in the extracts due to the fact the flavonoids are the main group of polyphenols which able to scavenge radical oxidizing species (Alnajar et al., 2012). Literature has been reported that, the isolation and identification of phytochemical constituents from this plant yielded quercetin, quercitrin, kaempferol-3-O-
(2",6"-di-O-p-trans-coumaroyl) glucoside which responsible for the flavonoid activity (Susanti, Sirat, Ahmad and Ali, 2008).

Table 1 Antioxidant Activity of *Melastoma* Leaves Extract

<table>
<thead>
<tr>
<th>Sample/ Assay</th>
<th>DPPH %</th>
<th>TPC (mg/g GAE)</th>
<th>TFC (mg/g QE)</th>
<th>Sun Protection Factor (SPF)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>89.66</td>
<td>1072.92 ± 0.1</td>
<td>5.61 ± 0.90</td>
<td>22.44 ± 0.03</td>
</tr>
</tbody>
</table>

3.2 Functional Cosmetics Properties of *Melastoma* Leaves Extract

3.2.1 Sun Protection Factor (SPF)

The skin accounting 15% of the whole weight and their function as a barrier against chemical, physical and biological attacks. It also acts as the main defense system for protecting body from external exposure such as ultraviolet (UV) radiation. The sun utters a wide spectrum of electromagnetic waves in which it can be divided into three regions UVA: from 315 to 400 nm, UVB: from 280 to 315 nm and UVC: from 100 to 280 nm (Dipali Gupta, 2013). UV radiation, which represents approximately 6-7% of the total amount of sun radiation that reaches the earth’s surface, accounts for most of the sun-induced damages to the skin (Souza et al., 2017). According to Martins et al. (2016) the over exposure of ultraviolet radiation such as UVA and UVB radiation to the skin may have several health effects such as erythema, pigmentation, photo-carcinogenesis, genetic material abnormalities, neoplasia development and photoaging. Therefore, in order to encounter this, antioxidant acts as an agent to prevent many unwanted diseases especially UV radiation. From the result, it was found that the calculated SPF values of *Melastoma* leaves species was 22.44 (Table1). The calculated SPF value represents as an indicator that is mentioned in sunscreens which indicates that how much photo-protection provides against UV radiation by sunscreen when it is applied thickness of 2.0 mg/cm on skin (Napagoda et al., 2016). According to Stevanato et al. (2014) any commercial sunscreen products can be classified according to their SPF values such as (SPF <12) is minimal, (SPF 12-30) is moderate and high sun protection products is (SPF ≥30). Therefore, the present results show that the *Melastomataceae* family species falls into moderate sun protection factor.

3.3 Efficacy of *Melastoma* leaves extract as active ingredient in Nanoemulsion as anti-aging

Skin aging can be defined as a chronological aging that happens over time and it is known to be caused by an intrinsic, natural and extrinsic mechanism (Taofiq et al., 2016). Therefore, in accordance to these factor, the skin slowly begins to change in hormonal secretion which often result in collagen degradation, degeneration of elastic fibers,
dryness and wrinkle skin. Therefore, to date more research on finding inhibitor of these enzymes as it is one of the significant ingredients in cosmetics and medications to protect the skin against aging. In this study, the in vitro inhibitory potential of bioactive compounds presented from the *Melastoma* nanoemulsion was carried out against the activity of elastase and collagenase enzymes. From the result in Table 2, the elastase reduction activity of *Melastoma* nanoemulsion gave a good $R^2$ (0.9099) and it presented that the studied sample exhibited about 66.67% of inhibition of elastase enzyme by *Melastoma* nanoemulsion versus positive inhibitor which exhibited 72.23%. Meanwhile, for collagenase inhibition activity, it can see that *Melastoma* nanoemulsion exhibited almost two times better activity than elastase activity. From the result highest percentage of inhibition collagenase enzyme activity which accounted to have 94.35%. In this present study, the higher percentage inhibition activity for both elastase and collagenase can be supported from the therapeutic bioactive compounds that present in *Melastoma* extract. For instance, the rutin and quercetin which is a class of polyphenols compounds exerted a high level of antioxidant compounds that can inhibit or slow down the generation of radical species in the body. Moreover, *Melastoma* nanoemulsion exhibited numerous terpenoid compounds that significantly increases the rate of inhibition enzyme activity as it was previously reported that terpenoid compounds in plant extract can act as natural inhibitor agents (Karim et al., 2014). Therefore, it is interesting to note that the *Melastoma* nanoemulsion demonstrated a good potential to act as anti-elastase and anti-collagenase activity against premature skin aging.

<table>
<thead>
<tr>
<th>Assay</th>
<th>$R^2$</th>
<th>Inhibition %</th>
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<tbody>
<tr>
<td>Elastase</td>
<td>0.9099</td>
<td>66.67</td>
</tr>
<tr>
<td>Collagenase</td>
<td>0.9654</td>
<td>94.35</td>
</tr>
</tbody>
</table>

### 3.4 Anti-Wrinkles Properties

Skin aging is associated with morphological changes that happens over time. It is part of natural chronological aging that people may experience later and cannot resists. Aging can be known caused by an extrinsic and intrinsic processes. For example, extrinsic aging known as photoaging is a result of the skin exposure to the environmental stressor such as ultraviolet radiation and pollution. Meanwhile, intrinsic involved some biochemical events for instance hormonal changes that can accelerates the rate of aging process. Prolong to exposure of this phenomenon, the skin will start to express many signs such as irregular pigmentation, dryness, roughness, fewer elasticity and increased wrinkling on the facial skin (Ali, Akhtar and Chowdhary, 2014). Therefore, an approach in skin protection against reducing skin aging is continue to be explored by using the mixture of *Melastoma* leaves extract as an active ingredient topically. In this study, the efficacy of the *Melastoma* nanoemulsion as anti-aging product was determined by using the Visioscan ® VC98 and software SELS 2000. In this study, the application of *Melastoma*
nanoemulsion may enhance a fewer fine wrinkles of the skin after 4 weeks of used. Figure 1 shows overall an improvement in skin condition where it can be seen on visual image, during first week until week four, the skin becomes more smooth and reduces its roughness. Moreover, based on the color combinations of the neighboring pixels, the image on 1st week of application seems darker as compared to the last 4th weeks of applications which the skin appears more bright. In addition, based on the images less wrinkles present on the structure of the skin which result in reduced deeper line and furrow of the skin and simultaneously increase the level of skin hydration. Thus it can be deduce that, an improvement in skin condition is significantly caused by the application of active nanocream from *Melastoma* extract.

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
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4. CONCLUSION AND RECOMMENDATION

Cosmetic products containing *Melastoma* nanoemulsion and the chemist behind them can be extraordinarily valuable to the skin as anti-aging cream. The efficacy of *Melastoma* nanoemulsion as anti-aging cream showed high antioxidant and functional cosmetic properties and present good anti-elastase and collagenase activity. Further works on the wound healing properties of *Melastoma* are under progress.

REFERENCES


