Photo-Protective and Anti-Oxidative Potential in the Leaves of Three Different Melastomataceae Family Species

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Abstract

Melastoma is the family of Melastomataceae species which consists of total more than 4000 species and the most common are Melastoma malalabthricum, Clidemia hirta and Melastoma decemfidum. Continues exploration from the leaves of melastoma plant has been extensively probe for its therapeutic value. Therefore, this work aimed to investigate the photo-protective ability and antioxidant potential using DPPH-free radical scavenging assay, total phenolic content and FRAP-reducing power assay. The results show all Melastoma family species have wide range of absorbance such as UVA, UVB and UVB radiation and exhibit good SPF number where Clidemia hirta leaves extract ethyl acetate recorded to have highest SPF value among others. Meanwhile, the three of antioxidant assay shows that Clidemia hirta ethyl acetate displays higher antioxidant activity against DPPH radical and contain higher phenolic and FRAP value as compared to other Melastoma species. Therefore, it can conclude that Melastoma especially from Clidemia hirta species could be one of the potential source of antioxidants as sunscreen products and also for utilization for cosmeceutical, neutraceuticals and medicinal use in the future to overcome various diseases.

Keywords : Melastomataceae, Photoprotective, Antioxidant, Sun Protection Factor, Leaves

I. Introduction

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). The high electromagnetic waves from UVA radiation reaches deep dermal layer of the epidermis and dermis and involves in the generation of reactive oxygen species (ROS) (Souza et al., 2017). UVB radiation has 30-40 times more energy than UVA radiation and can directly damage DNA and cause sunburn and skin cancer after long-term exposure to the sun (Martins et al., 2016). Meanwhile, UVC radiation is completely filtered by the atmosphere before reaching earth. 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, inflammatory response, immunosuppression, hyperplasia, photocarcinogenesis, 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. It has been recognized for their potential in promoting health by delaying or inhibit the initiation and propagation of free radicals or oxidizing chain reaction (ROS) (Maizura et al., 2011; Chanda and Nagani, 2010). Antioxidants act as scavenger for reactive free radicals by donating one of their electron to the free radical. When a free radical accepts the electron from an antioxidant compound, the radical no longer have to attack the cell as the chain reaction of oxidation is broken (Merinal and Viji, 2012).

Generally, antioxidants compounds can be found naturally in plants, fruits and whole grains. They are rich in vitamin c, vitamin e, carotenoids, flavonoids and polyphenols. Antioxidant such as plant has special characteristics and synthesizes numerous of active compounds that can defend and fight all sorts of pathogens and other substances. They can be sorted by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites.

Melastoma is the family of *Melastomaceae*, it is one of the substantial sources of natural antioxidants that provide varieties of therapeutic elements to cure various diseases. *Melastomaceae* originate in the tropic and subtropic regions with a total more than 4000 species in the world. According to Joffry et al. (2011) in the Southest Asia region alone, the Melastoma consists at least 12 species, which are used by natives in folk medicines. In Malaysia, particularly the most common *Melastomacea* can be found in three categories for example *Melastoma malabathricum, Clidemia hirta* and *Melastoma decemfidum*. In spite of being barely explored, therefore this study to investigate their photo-protective and their antioxidant activities which can fulfill the needs for

alternatives medicines remedy and can be used in pharmaceutical, cosmeceuticals and neutraceuticals for replacing synthetic medicines.

II. Methods

Plant Material

The leaves of two species of Melastoma family, *Melastoma malabathricum* (purple petals) and *Clidemia hirta* were collected at their natural habitat at Ayer Hitam, Hutan Puchong Selangor, Malaysia in August 2017. Meanwhile, *Melastoma decemfidum* (white petals) was collected in Muar, Johor – southern province of Malaysia in August 2017. The plants were further identified by Dr Paiman bin Bawon a senior lecturer from Faculty of Forestry, Universiti Putra Malaysia (UPM), Selangor, Malaysia.

Preparation of Samples

The plant materials were thoroughly washed and dried in shade (30 ^{o}C) for two weeks. According to literature, this process did not cause any destruction of the bioactive compounds of the leaves of the plants as proven by the availability of antioxidant, anti-inflammatory, anti-nociceptive and anti-bacterial activities (Zakaria, et al., 2011). Next, the air-dried leaves were ground using mechanical grinder machine into powder form. The whole leaves powder samples were packed in a sealed plastic bottle until extraction.

Extraction of Crude Extracts

The dried powder of Melastoma leaves were extracted two times in the ratio of (1:10) into three different solvent extraction (hexane, ethyl acetate and methanol) by maceration for 3 days. Next, the mixtures were filtered using filter paper (Whatman No.1) and evaporated until dryness using rotary evaporator (Yamato, Rotary Evaporator, model- RE 801, Japan). Prior to use, the crude extract will be dissolved in extraction solvent to a final concentration of 1000 mg/mL.

Photo-Protective Activity

The in-vitro determination of sun protection factor (SPF) was performed according to the method described by Napagoda et al. (2016). 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);

$$SPF_{spectrophotometric} = CF \times \sum_{290}^{320} \mathbf{EE}\ (\boldsymbol{\lambda}) \mathbf{x}\ \mathbf{I}\ (\boldsymbol{\lambda}) \mathbf{x}\ \mathbf{Abs}\ (\boldsymbol{\lambda}) \quad (1)$$

where EE (λ) –erythermal effect spectrum; I (λ) – solar intensity spectrum; Abs (λ)- absorbance of sunscreen extract; CF-correction factor (=10). The value of EE x I are constants. Table 1 indicates the normalized extract function used in the calculation of SPF. Commercial available sunscreen (SC) product containing TiO_2 as active ingredients was used as a standard reference. In addition, betel leaves (*piper betle*) extract was used as a control to compare with the UV filtering potential of the leaves extracts.

Table 1. Normalized function used in the calculation of SPF

Wavelength (nm)	EE x I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

Antioxidants Activity

Determination of 1,1-diphenyl-2-picryhydrazyl (DPPH)- Radical Scavenging Activity

The DPPH free radical scavenging activity method was formed according to the method described by Blois (1958) with minor modifications to fit the study test. About, 10 mg of the dried extract was accurately weighed and dissolved in 10 mL methanol in a volumetric flask (1000 mg/mL). Next, 10 mg of 1- diphenyl-2-picryhydrazyl (DPPH) radical was prepared and dissolved in methanol and made up to 100 mL in a volumetric flask (0.1 mM). Briefly, 50 uL of the extract samples were mixed with 150 uL methanolic solution of DPPH in 96-well plate microliter plate. The mixtures were incubated in the dark for 30 minutes and the absorbance values of the samples were measured at 515 nm using UV-Vis microplate reader (Infinite M200, Tecan). A sample control was prepared by omitting sample extract from DPPH working solutions. All the analyses were performed in triplicate. The DPPH scavenging activities of the extracts were calculated using the following equation:-

$$\text{DPPH scavenging activity (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Determination of Total Phenolic Content

The total phenolic compounds were performed using the Folin-Ciocalteau method (Singleton and Rossi, 1965) with minor modifications. 100 uL (1 mg/mL) of the sample extract were mixed with 50 uL Folin solution previously diluted with 7.0 mL distilled water. After 4 min, 1.5 mL of 7.5 w/v% sodium carbonate solution was added to the mixture and incubated in the

dark room at room temperature for 2 hours. The absorbance values of the samples were measured at 765 nm using a UV-VIS microplate reader (Infinite M200, Tecan). The samples were prepared in triplicate for each analysis and the mean value of absorbance were obtained. Standard of gallic acid with different concentrations (25-1000 μg/L) was prepared in this study to generate a standard calibration curve. The samples were calculated based on the standard calibration curve and expressed as mg gallic acid equivalent (mg/g GAE) of extract sample.

Determination of Ferric Ion Reducing Antioxidant Power (FRAP)

The FRAP were assayed according to the method developed by Benzie and Strain, (1996). The FRAP solution includes acetate buffer (300 mM, pH 3.6), a 10 mM TPTZ solution in 40 mM of HCl and 20 mM FeCl3.6H2O solution. A working FRAP solution was freshly prepared by mixing 100 mL acetate buffer, 10 mL TPTZ solution and 10 mL FeCl3.6H2O solution in a ratio of 10:1:1 at the time of use. A sample blank was prepared by excluding sample extract from the FRAP solution and measured by reading the absorbance at 593 nm (0 min) using a spectrophotometer UV-VIS microplate reader. Subsequently, each extract of 60 uL was mixed with 1.8 mL FRAP solution and second reading at 593 nm will be performed after 4 min. The FRAP value of the samples were determined by subtracting the final reading of the FRAP solution plus sample with the initial blank. The standard curve was prepared using ferrous sulphate solution (1 mM) at different concentrations ranging from 0.1 mM to 1.0 mM. The activity was then express as mM Fe_{2+}/g DW. Analysis was done in triplicate for standard and each extract.

III. Results and Discussion

UV filtering potential

Among the nine extracts *Clidemia hirta* (EA) and *Clidemia hirta* (MeOH) have higher SPF ≥ 20 and followed with *M. malabathricum* (MeoH), *M. malabathricum* (EA), *M. decemfidum* (MeOH), *M. decemfidum* (EA), *M. malabathricum* (Hex), *Clidemia hirta* (Hex) and the least SPF value was *M. decemfidum* (Hex). SPF values of *M. malabathricum* for hexane, ethyl acetate and methanol extracts and *Clidemia hirta* and *M. decemfidum* of ethyl acetate and methanol were found to be slightly higher than the SPF of betel leaf. Meanwhile, the commercial photoprotective creams used as the standard reference substance has displayed SPF 38.2 lower than their labelled SPF value. (Table 2).

Table 2. SPF values of crude extracts

Extract	Measured SPF value
MM-Hex	13.07 ± 0.03
MM-EA	15.98 ± 0.04
MM-MeOH	16.54 ± 0.10
CH-Hex	7.89 ± 0.02
CH- EA	22.44 ± 0.03
CH-MeOH	21.01 ± 0.01
MD- Hex	5.99 ± 0.03
MD- EA	14.12 ± 0.04
MD- MeOH	15.46 ± 0.03
SC (standard reference)	38.2 ± 0.01 (labelled SPF: 50+)
Betel leaf *(piper betle)*	11.60 ± 0.05

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 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 where *Clidemia hirta* in ethyl acetate extract exhibits highest SPF values among other Melastoma species. Moreover, from the calculated SPF values, it shows that every melastoma extracts from semi polar and polar solvent displays higher SPF values as compared to non-polar solvents. Therefore, it shows that semi-polar and polar solvents play a significant role in producing good photo-protective products. Literature reported by Napagoda et al. (2016) also shows that the plant extracts using a polar solvent exhibits higher sun protection activity.

Furthermore, the study also reveals that real SPF value was found to be lower than the labelled value in the commercial sunscreen product as the protection percentage from UV radiation is different. For example, when SPF is 15, the photo-protective provides >93% protection against solar radiation (UVB) and SPF +30 gives 97% of protection from solar radiation (UVB) (Stevanato et al., 2014). Therefore, in order to protect skin against UV radiation, the formulation products should have good SPF number and wide range of absrbance between 290 to 400 nm.

The sunscreen activity in three Melastoma species is further evaluated through recording the transmission spectrum. In the present study, it shows that all melastoma species exhibit good photo-protective activity which a strong

spectrophotometric absorption peaks can be seen in the UVC, UVB and UVA region. The maximum absorption wavelength (λ max) in UVC, UVB and UVA region are 275 nm, 290 nm and 400 nm for all extracts (Figure 1, 2 and 3). Therefore, the characteristics absorption band exhibited by the melastoma extracts could be one of the ideal sunscreen potential to replace synthetic sunscreen.

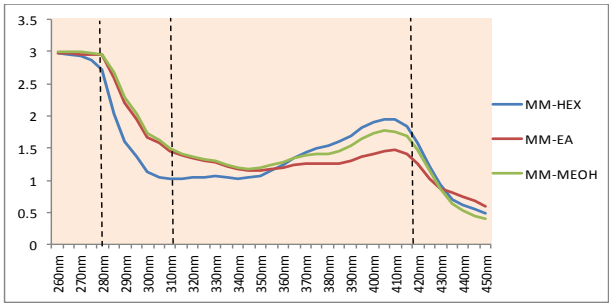

Fig. 1. UV spectra of *M. malabatricum* leaves extract

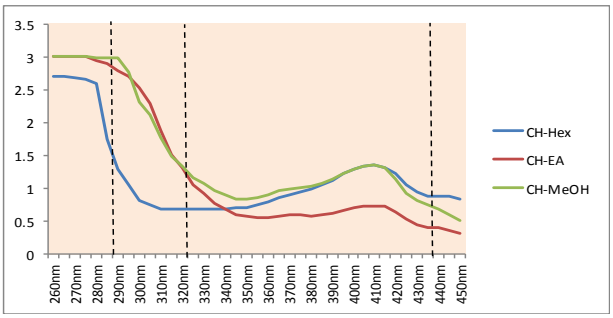

Fig. 2. UV spectra of *Clidemia hirta* leaves extract

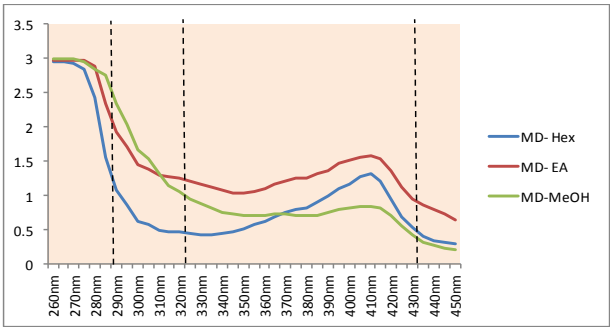

Fig. 3. UV spectra of *M. decemfidum* leaves extract

Antioxidant Activity

The antioxidant capacity of total nine crude extracts are present in Table 3. There are three types of antioxidant activities which are DPPH-radical scavenging activity, total phenolic content and FRAP-reducing antioxidant assay involved in this study. From the results, the highest radical scavenging activity is *C. hirta* (EA) which exhibits 89.66% followed by *M. decemfidum*

(MeOH) >*C. hirta* (MeOH) >*M. malabathricum* (MeOH) > *M. malabathricum* (EA) > *M. decemfidum* (EA) > *M. malabathricum* (Hex) > *C. hirta* (Hex) > *M. decemfidum* (Hex). Meanwhile, the standard reference used in this study such as ascorbic acid (AA) and butylated hyroxytoluene (BHT) have recorded to exhibit 93.53 % and 93.08% respectively (Figure 4).

The content of phenols in melastoma crude extracts was measured from the standard calibration of gallic acid and express as gallic acid equivalent (mg/g GAE). From the results, it shows that the *C. hirta* (EA) has the highest phenolic content among other melastoma crude extracts which is 1072.92 mg/g GAE. Meanwhile, *C. hirta* (MeoH) is the second highest phenolic content followed by *M. decemfidum* (MeOH) > *M. malabathricum* (MeOH) > *M. decemfidum* (EA) > *M. malabathricum* (EA) > *C. hirta* (Hex) > *M. malabathricum* (Hex) > *M. decemfidum* (Hex) (Figure 5). The present study also shows the standard reference for both ascorbic acid and BHT exhibit lower phenolic content as compared to the C. hirta (EA) crude. This shows, melastoma plant has highest antioxidant activity due to high phenolic content.

The reducing power assay in total nine melastoma crude extracts shows the antioxidative activity range from 1.89- 51.06 Fe_{2+}/g DW. From the table 3, it shows *C. hirta* (EA) has the highest FRAP value followed by *C. hirta* (MeOH) > *M. decemfidum* (MeOH) > *M. malabathricum* (MeOH) > *M. malabathricum* (EA) > *M. decemfidum* (EA) > C. hirta (Hex) > *M. malabathricum* (Hex) > *M. decemfidum* (Hex). Meanwhile, the standard reference for ascorbic acid and BHT are recorded to have 53.94 and 16.11 Fe_{2+}/g DW respectively (Figure 6).

From all antioxidant activities, it shows that semi polar and polar solvents such ethyl acetate and methanol of *clidemia hirta* extract has the highest total antioxidant activity for radical scavenging, highest phenolic and FRAP content. Therefore, they show they are effective solvents in extracting of antioxidant compounds as compared to hexane which has the lowest polarity among the solvents used. Hence, it can be considered that the solvents could play significant roles in increasing and decreasing the solubility of the extracts (Awang et al., 2016). The higher antioxidant compounds in the extracts also due to the presents of secondary metabolite of active constituents such as phenolic in the extracts that able to scavenge the radical species. In general, antioxidant means “against oxidation” where antioxidant works to protect lipids from peroxidation by free radicals which are generated during metabolism and other activities in human body (Merinal and Viji, 2012). Therefore, melastoma plants especially from *Clidemia hirta*, *M. malabathricum* and *M. decemfidum* leaves could be of the sources for natural antioxidants against many diseases.

Table 3. Antioxidant activity of crude extracts

Samples	Antioxidant activity		
	DPPH %	Total phenolic content (mg/g) GAE	FRAP mM Fe_{2+}/g DW
MM-Hex	70.08	96.43 ± 0.2	2.11 ± 0.1
MM-EA	85.04	223.00 ± 0.1	10.63 ± 0.2
MM-MeOH	85.12	415.92 ± 0.1	12.66 ± 0.3
CH-Hex	45.31	102.43 ± 0.0	2.17 ± 0.2
CH-EA	89.66	1072.92 ± 0.1	51.06 ± 0.0
CH-MeOH	85.9	763.58 ± 0.4	42.98 ± 0.0
MD-Hex	35.35	42.50 ± 0.3	1.89 ± 0.1
MD-EA	81.77	309.92 ± 0.2	5.40 ± 0.3
MD-MeOH	88.83	623.00 ± 0.1	13.88 ± 0.4
AA (standard reference)	93.53	946.08 ± 0.1	53.94 ± 0.0
BHT (standard reference)	93.08	797.58 ± 0.1	16.11 ± 0.1

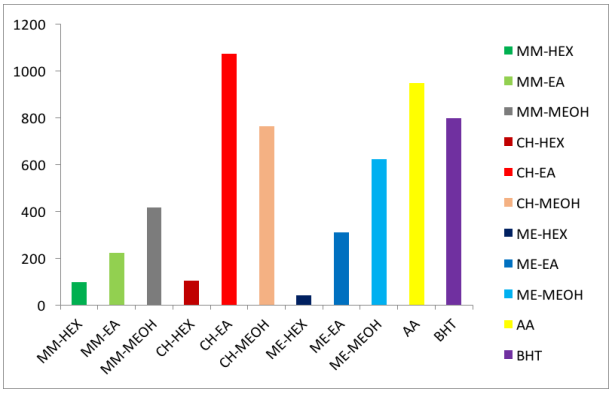

Fig. 4. Percentage of DPPH in Melastoma crude extracts

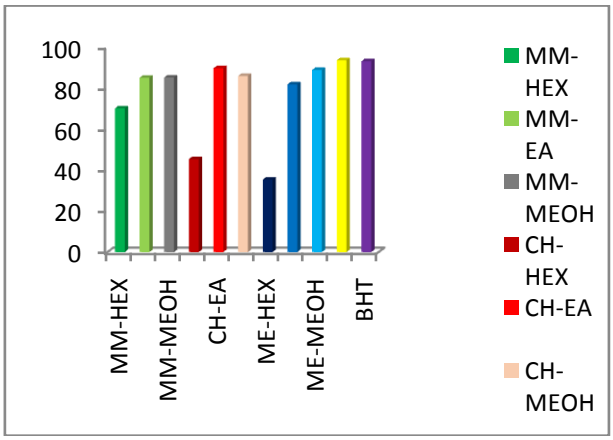

Fig. 5. Total phenolic content of Melastoma crude extracts

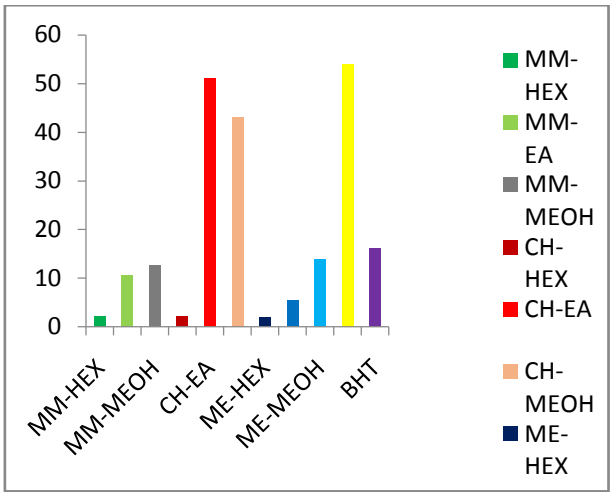

Fig. 6. FRAP assay of Melastoma crude extract

IV. Conclusion

Melastomataceae family species such as *M. malabathricum*, *Clidemia hirta* and *M. decemfidum* may act as good antioxidant potential in a variety of assay and good photo-protective ability against sun radiation. From the results, it shows that, *Clidemia hirta* extract shows a potent antioxidant activities against all antioxidant assay and present high phenolic and FRAP content. Besides, the solvent extraction also plays significant roles in order to extract more bioactive polar compounds that able to scavenge radical activity. Moreover, the natural antioxidant compound from plant not only be able to eliminate radical but also capable of absorbing energy in the UV spectral range as it shows good SPF value which are more than SPF >20 for both *Clidemia hirta* in (EA) and MeOH extract. The photo-protective ability of all Melastoma family species shows wide range of absorbance of UVA, UVB and UVC

region. Therefore, antioxidants ability found in melastoma species of *M. malabathricum*, *Clidemia hirta* and *M. decemfidum* incorporated with good photo-protective activity can enhance the quality of any sunscreen products derived from natural based. Therefore, these findings could be a clear potential for the utilization of melasoma species as a good source for many cosmeceuticals and neutraceuticals application in the future.

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