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Antioxidant potential of Mondo grass (Ophiopogon japonicas) by using different extraction Solvents

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Abstract
Natural antioxidants are preferred over the synthetic antioxidants as the latter cause side effects to human. Seeking for alternative natural antioxidants to sustain the sources of antioxidants is indispensable. This study aimed to (i) compare the antioxidant content of Mondo grass leave via Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) assays, (ii) compare antioxidant activity of Mondo grass leave using Ferric Reducing Antioxidant Potential (FRAP) and ABTS radical scavenging activity, and (iii) examine whether antioxidant content is correlated with its activity. Methanol, ethyl acetate and petroleum ether were used as extraction solvents. Results revealed that ethyl acetate is effective in extracting the plant's antioxidant compounds since it displayed the highest TPC and TFC values. It also had the highest scavenging activity in ABTS assay. For FRAP assay, extract of petroleum ether showed the best performance. There were statistically significant correlation between the antioxidant activity and TFC assays. The correlation between TFC and FRAP assays was statistically insignificantly different.

Keywords: antioxidant capacity, antioxidant activity, Mondo grass, extraction solvent

1. Introduction

High content of free radicals in human body can cause severed disease such as atherosclerosis, arthritis, Alzheimer disease and cancer (Yashin *et al*., 2011) [1]. Antioxidant is a compound that can prevent the free radical, especially high reactive oxygen and nitrogen species that can cause body cell damage (Yashin *et al*., 2011) [1]. Research in the determination of antioxidant activity and its content, particularly on plant is gaining increasing attention among researchers over the years (Yashin *et al*., 2011; Dudonne *et al*., 2009) [1, 2]. Numerous efforts have been done to discover new natural resources of active antioxidant compounds (Dudonne *et al*., 2009) [2]. There are two categories of antioxidant, namely the natural and synthetic antioxidants. Synthetic antioxidants consist of compound with phenolic structures of various degrees of alkyl group substitution while the natural antioxidants are phenolic, nitrogen, carotenoids and ascorbic acid compounds (Velioglu *et al*., 1998) [3]. On contrary, Thaipog *et al*. (2006) [4] reported that the natural antioxidants in plants have defensive response, devoted by three major groups, which are vitamins, phenolics, and carotenoids. Phenolics are classified as hydrophilic antioxidants while carotenoids belong to lipophilic antioxidants (Halliwell, 1996) [5]. The natural antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene and dl-alpha-tocopherol possess higher effect of antioxidant activity compared to the synthetic antioxidant. Moreover, the natural antioxidants are always far less toxic than the synthetic antioxidant. It is imperative to point out here that the synthetic antioxidants can cause adverse health effect to human body if over consumed.

Mondo grass or its scientific name, *Ophiopogon Japonicus* is a dark green herbaceous plant with grass-like structure. This perennial plant belongs to the family of Liliacea under class liliopsida. Plants from Liliacea family have various biological active elements (Liang *et al*., 2012) [6] and around 50 species are classified under the *Ophiopogo*n species. Mondo grasses, monkey grasses, snake's beard and Aztec grass are the common names of *Ophiopogon* species (Fantz, 2008; Huxley *et al*., 1992) [7] [8]. Fantz (2009) [9] claimed that the *Ophiopogon Japonicus* species has had certain characteristics. The leaves size of the Mondo grass is approximately 10 to 50 cm in length and 2 to 4 mm in thickness. Its leaves pattern is radial with linear shapes and acuminate tips, with lengths of 6 to 12 inches. It forms tufted colonies by spreading rhizomes and a tuberous root (Edward, 1999) [10]. In summer, Mondo grass usually produces white flowers and small berries that turn from light green to blue-black. Previous studies have shown that Mondo grass is drought tolerant, anti- disease and insect resistant. As highlighted by Zhang (2003) [11], Mondo grass is a perennial evergreen herb supported by strong root system and it is a heat tolerant plant. Fig 1 shows the Mondo grass grown in Pasir Putih, Kelantan.

Fig 1: The Mondo Grass in Pasir Putih, Kelantan.

In China, Mondo grass is employed as a remedy although it is not scientifically proven (Tian *et al*., 2014) [12]. This plant is popular for its ornamental function. It is widely adopted for landscaping and ground covering due to its grass-like structure where it can contrast well with other mid-green shrubs. Besides being used as an ornamental plant, Mondo grass is produced as herbal tea in some of the local communities in Asia. As stated by Sari & Valioglu, (2011) [13], herbal tea is the second rank beverage consumed by people worldwide because it gives relaxation and has antioxidant property which yield health benefits. Mondo grass is a beneficial plant but researches emphasize on this plant are sheer lacking to date. Of note, study in investigating the antioxidant potential of the Mondo grass is still not in existence in the literature. This has motivated the work of this study where the main objective was to determine the antioxidant content and its activity for the Mondo grass leave. To be more detail, the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of different solvents extraction of Mondo grass were determined. Besides, the antioxidant activities of the plant using different solvent extractions were compared based on the Ferric Reducing Antioxidant Potential (FRAP) and ABTS radical scavenging activity.

2. Materials and Methods

2.1 Sample Preparation

This research was carried out for six months from September 2016 to March 2017 in the laboratory of Universiti Malaysia Kelantan Jeli Campus The Mondo grass was collected from Pasir Putih, Kelantan, Malaysia. The sample leaves were harvested by using bare hand and transported to the laboratory using proper container. The sample leaves were cleaned using tap water before they were oven dried at 45 ˚C for two days. The dried sample was pulverized using Warring blender and it was kept in - zipper bag under chilled condition upon further analysis.

2.2 Sample extraction

The -extraction of Mondo grass leave was performed using three different solvents,–namely methanol, ethyl acetate and petroleum ether. The extraction ratio considered in this study was 1:10 (m/v) between the sample and solvent. A total of 450 mL of methanol was poured into a beaker containing 45 g of samples, and the solution was then ultra-sonicated at room temperature for 30 minutes using ultrasonic cleaner. Next, the solution was filtered by - filter paper, and the solvent in the filtrate was removed by using a rotary evaporator (60°C, 2500rpm) to obtain the semi-solid extract. The solvent extraction was conducted twice and the biomass of the sample was dried in the oven prior to the next extraction of different solvents took place. The semi-solid extracts of three different solvents were stored in universal bottles at -20 ˚C -prior further study.

2.3 Total Phenolic Content (TPC) of different sample extractions

The TPC was determined - using the Folin-Ciocalteu's assay (Rabeta and Faraniza, 2013) [14]. A serial dilutions of gallic acid was prepared from 1 mg/mL of gallic acid by diluting it with DMSO for concentration ranging from 400 µg/mL to 6.25 µg/mL. About 50 µL of gallic acid for each, from the diluted solution was transferred to test tube wrapped with aluminium foil. Then, 250 µL of 10 % Folin-Ciacalteu was added into the test tubes and the mixture was incubated for 5 minutes. Subsequently, 750 µL of 7.5 % of sodium hydrogen carbonate (NaHCO3) was added to the test tubes before it can be incubated again for 2 hours. The absorbance reading was measured at wavelength of 765 nm and DMSO was served as blank for this assay and a standard graph was constructed. Sample solution of concentration 1 mg/mL for methanol, ethyl acetate and petroleum ether extract were diluted in DMSO and they were tested according to the protocols of gallic acid. The readings of absorbance were expressed in gallic acid equivalents (GAE)/mg of extract. Triple readings of absorbance were taken and the mean readings were calculated.

2.4 Total Flavonoid Content

TFC was determined based on the method of chloride colorimetric presented by Heimler *et al*. (2005) [15]. A standard serial dilution solution was prepared in DMSO with amount ranging from 1600 µg/mL to 25 µg/mL of quarcetion. A total amount of 3.4 mL aqueous methanol (30 %), 150 µL of 0.5 M sodium nitrate solution and 150 µL of 0.3 M aluminium chloride were added to a test tube containing 300 µL standard. About 1 mL of 1 M sodium hydroxide was added to the mixture after 5 minutes incubation. At the wavelength of 506 nm, the absorbance reading was taken and DMSO was used as blank. Next, a standard graph of quarcetin was constructed. TFC of methanol, ethyl acetate and petroleum ether extracts were determined by using the same procedure with 1 mg/mL concentration. The test was carried out in triplicate, the mean readings were calculated and were expressed in quarcetin equivalent (µg of QE/g extract).

2.5 FRAP Assay

In this study, FRAP assay was conducted via the method proposed by Benzie and Strain (1996) [16]. The preparation of FRAP reagents were carried out by mixing 20 mM of ferric chloride solution (FeCl3.6H2O), 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) and 300 mM acetate buffer at pH of 3.6. FRAP working solution was prepared by adding 25 mL of acetate buffer, 2.5 mL TPTZ solution and 2.5 mL of ferric chloride. FRAP solution was warmed at 37 ˚C before it could be used. A serial dilution of trolox solution, ranging from 400 µg/mL to 6.25 µg/mL was prepared by dilution in DMSO. 2850 µL FRAP solution was added into Trolox of 150 µL volume. Then, the mixture was incubated in a dark room at 37 ˚C. The reading of absorbance was measured at the wavelength of 593 nm. Same procedure was applied for the methanol, ethanol and petroleum ether extract with concentration of 1 mg/mL. This test was performed in triplicate and a standard graph of trolox was constructed. The sample mean values were calculated and expressed as trolox equivalents (µg TE/mg extract). Note that the DMSO and FRAP solution mixtures were used as blank here.

2.6 ABTS Assay

ABTS radical cation decolorization assay was carried out to determine the free radical scavenging activity. This study used the method given by Demirey *et al*. (2009) [17] but with a few modifications. 14 mM of ABTS reagent was prepared by dissolving it into distilled water. Addition of 4.9 mM potassium persulfate into the same amount of ABTS reagent was done to generate the ABTS free radicals (ABTS+). Next, the mixture of ABTS and potassium persulfate was incubated for 16 hours before it could be used. ABTS+ was diluted with methanol to the absorbance of 0.700 (± 0.02) at 734 nm and it was equilibrated at room temperature. Here, the absorbance reading for the blank reagent was used as control and was denoted as. A_0 Next, the BHT stock solution of 1 mg/mL was prepared and was diluted in DMSO ranges from 400 µg/mL to 6.25 µg/mL. 50 µL of standard was added with 950 µL of ABTS+ (A 734 = 0.700 ± 0.02) and incubated in a dark room for 6 minutes. At 734 nm, the values were recorded as A_t after the sample reaction occurred. The similar procedures were repeated for the samples of methanol, ethyl acetate and petroleum ether extract. Antioxidant activity of each extract was expressed in percentage of ABTS scavenging activity (%ABTSsc) using Eq. (1).

$$\%\mathrm{ABTS}_{\mathrm{SC}} = \frac{A_0 - A_t}{A_0} \times 100 \quad (1)$$

where A_0 and A_t are the absorbance value of control and sample, respectively. Note that this test was performed in triplicate.

2.7 Statistical Analysis

Statistical Package of Social Sciences (SPSS) was used to perform the statistical analysis of data. Analysis of variance (ANOVA) was used to analyze the effects of three different solvents extraction on Mondo grass leave while Duncan Post Hoc test was done for pairwise comparisons of means at $P \leq 0.05$.

3. Results & Discussion

3.1 Mondo Grass Leaves Extraction

Table 1 shows the extract yield capacity, color intensity and state condition of Mondo grass leaves using methanol, ethyl acetate and petroleum ether. It was found that the extraction yields were affected by the solvent polarities. As the solvent polarity increased, the yield of extraction also increased. In other word, the solvent polarity has positive linear relationship with the yield of extract. Methanol recorded the highest yield (1.84%), followed by ethyl acetate (0.67%) and petroleum ether (0.52%). This result agreed with the fact that methanol is a solvent with high polarity whereas petroleum ether is always be considered as a less polar solvent. Each extract also showed different color intensity. The color intensity of semi-solid extract for methanol, ethyl and petroleum ether extract were brown, dark brown and dark green, respectively. Besides, three extracts were varied in term of their extract state conditions. Methanol extract existed in high viscous liquid state while ethyl acetate and petroleum ether extracts were both in semi-solid state.

Table 1: Extract yield capacity, color intensity and extract state condition for three different solvent extractions

Extraction Solvents	Amount of Yield (%)	Color Intensity of Semi-Solid Extract	Extract State Condition
Methanol	1.84	Brown	High Viscous
Ethyl Acetate	0.67	Dark Brown	Semi Solid
Petroleum Ether	0.52	Dark Green	Semi Solid

3.2 TPC and TFC Assays

Every plant has its own criteria of chemical composition and antioxidant compounds present in the plant have identical phenolic groups in similar species (Glash, 1983) [18]. In addition, compound with similar chemical structure gives similar reaction of chemical in specific reagents. As antioxidant compounds in plants exist abundantly, thus TPC determination test can represent the approximate amount of phenolic compounds in most plants (Jace *et al*., 2010) [19]. Table 2 displays the antioxidant content of Mondo grass leaves using three different extraction solvents.

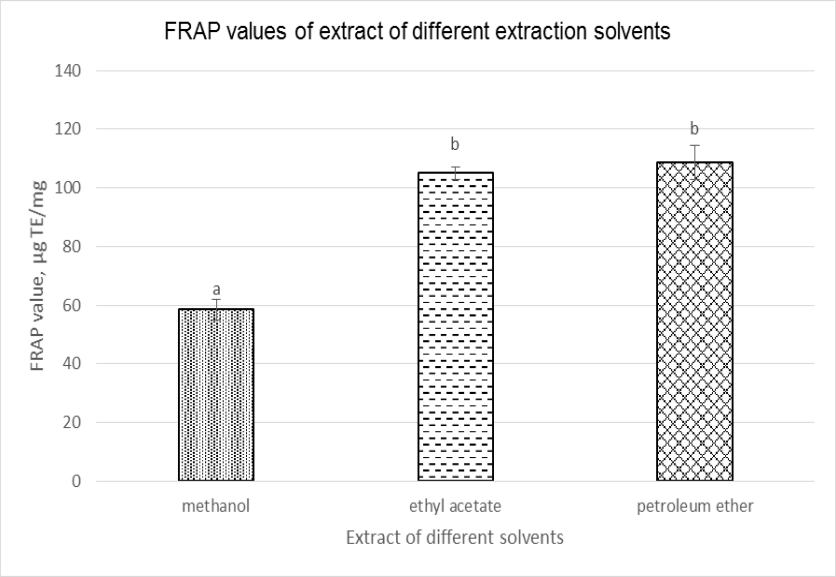
Table 2: Antioxidant content of Mondo grass leaves using TPC and TFC assays based on three different extraction solvents

Extraction Solvents	Tpc	Tfc
	µg Gae/Mg	µg Qe/Mg
Methanol	7.616 ± 0.765	25.889 ± 5.092
Ethyl Acetate	16.825 ± 2.134	619.222 ± 15.031
Petroleum Ether	9.498 ± 0.7	247 ± 13.333

In this study, the extract of Mondo grass leaves extracted by using ethyl acetate shown the highest value of phenolic content which was 16.825 ± 2.134 µg GAE/mg, followed by petroleum ether (9.498 ± 0.7 µg GAE/mg)and methanol (7.616 ± 0.765 µg GAE/mg), respectively. The previous study conducted by Horng *et al*. (2014) [20] found that the species of Mondo grass extracted by 80% of aqueous methanol using reflux extraction for three hours contains 17.29 ± 0.69 mg GAE/g of polyphenolic content, 18.60 ± 0.47 mg RE/g of flavonoids and 312.65 ± 0.64 mg/g polysaccharides. Study conducted by Fan *et al*. (2015) [21] mentioned that the variation in the TPC of Mondo grass might associated with the presence of monosaccharide composition such as rhamnose, fucose, arabinose, xylose, mannose, glucose and galactose.

3.3 FRAP and ABTS Assays

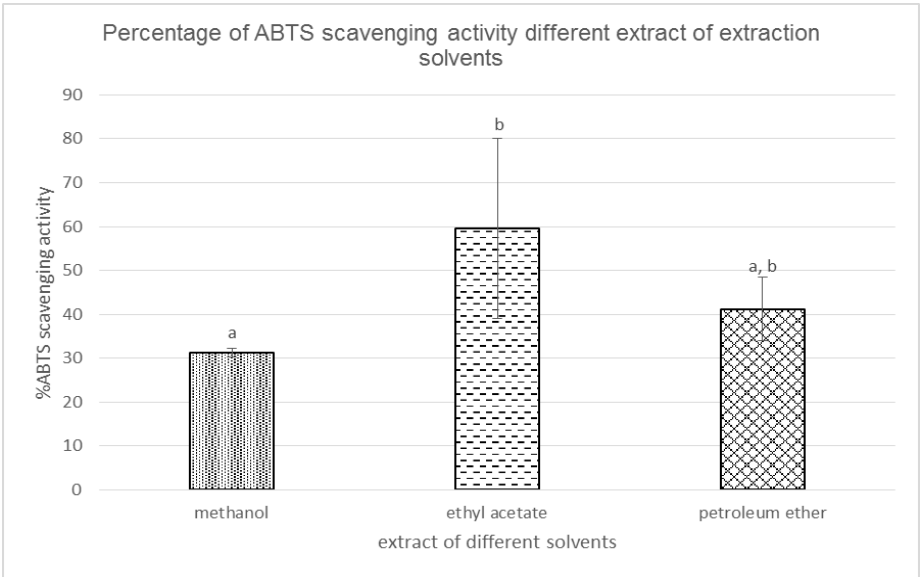
The FRAP values of Mondo extract using different extraction solvents were expressed as trolox equivalent, µg TE/mg (Fig. 1). Sample extracted by methanol exhibited the lowest FRAP value (58.528 ± 3.481 µg TE/mg) as compared to other tested samples. Meanwhile, the absorbance reading for petroleum ether extract was 108.869 ± 5.869 µg TE/mg and followed by the ethyl acetate, which recorded the highest absorbance reading (105.009 ± 2.103 µg TE/mg) among all.



Note: different alphabets within bar indicate significant difference, in term of FRAP value by Duncan test at $P \leq 0.05$.

Fig 1: FRAP of methanol extract, ethyl acetate extract and petroleum ether extract of Mondo grass leaves.

This study found that the extract of ethyl acetate could scavenge the ABTS+ to 59.486 ± 20.548 % (Fig. 2) which was the highest percentage of scavenging effect compared to methanol (31.336 ± 0.971 %) and petroleum ether (41.179 ± 7.198 %), respectively. In fact, ethyl acetate is the best solvent in extracting the antioxidant compounds in leaves of *Ophiopogon japonicas* species due to its high tendency to extract more phenolic and flavonoid compounds thus effectively reduced the amount of ABTS- radicals throughout the assay. The above result might attributed by the use of polar solvents which managed to extract and liberate out more polar phenolic components (Peschel *et al*., 2006) [23]. Besides, the structural of extracted phenolic components (Rababah *et al*., 2010) [24] as well as the feasibility of electron and hydrogen atom transfer (Perez-Jimenez and Saura-Calixto, 2006) [25] using solvent with different polarities were responsible for the above result too.



Note: Different Alphabets Within Bar Indicate Significant Difference, In Term of Percentage of ABTS Scavenging Activity by Duncan Test At $P \leq 0.05$.

Fig 2: ABTS of methanol extract, ethyl acetate extract and petroleum ether extract of Mondo grass leaves.

3.4 Correlations between Antioxidant Content and Antioxidant Activity of Mondo Grass

Pearson correlation values between antioxidant content (TPC & TFC) and antioxidant activity (FRAP & ABTS) were delineated in Table 3. It was found that the correlation between TPC and FRAP assays was statistically insignificant different. Similar trend can be noticed between TPC and ABTS assays. Nevertheless, this result is in contrast with previous research that has been conducted, TPC has strong correlation with antioxidant properties for plants (Dudonne *et al*., 2009) [2]. As for TFC and FRAP assays, statistically significant

correlation (r^2 = 0.729) was found. There was also a statistically significant correlation between TFC and ABTS assays (r^2 = 0.759) It is worthy to mention here that previous researches have similar findings where the antioxidant activity was significantly relate to the flavonoid content in the plant (Wang *et al*., 2006; Lin *et al*., 2010) [26, 27]. Nonetheless, the opposite was true for phenolic content in the plant versus the antioxidant activity.

Table 3: Pearson correlation value between antioxidant content (TPC & TFC) and antioxidant activity (FRAP & ABTS)

	TPC	TFC
FRAP	0.586	0.729*
ABTS	0.852	0.759*

Note: *Correlation is significant at $P \leq 0.05$ (2-tailed).

4. Conclusions

The results obtained from this study indicated that ethyl acetate was the best solvent in extracting both the active and antioxidant compounds of Mondo grass leave. Ethyl acetate showed the highest yield of phenolic and flavonoid compounds with TPC and TFC values of 16.825 ± 2.134 µg GAE/mg and 619.222 ± 15.031 µg QE/mg, respectively. It also recorded the highest percentage of ABTS scavenging activity, 59.486 ± 20.548%. However, in FRAP, the antioxidant reducing power of ethyl acetate extract was inferior to the extract of petroleum ether. The findings of this study are beneficial to the health medicinal sector as the antioxidant property of the Mondo grass can definitely be used as an alternative to the synthetic antioxidants. Further studies will emphasize on the elucidation of the antioxidant potentials using different parts of the Mondo grass especially their flowers. Besides, it is worth to identify the purified active phenolic compounds that attributed for its bioactivities.

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