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Comparitive Study On Antioxidant Properties Of Dendrobium Officinale (tiepishihu)

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COMPARITIVE STUDY ON ANTIOXIDANT PROPERTIES OF *DENDROBIUM OFFICINALE* (TIEPISHIHU)

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DEDICATION

This thesis is dedicated to my parents, Bin Zhu and Caihong Zhang for their love and support, also to my wife, Kunxing Guo for endless encouragement throughout the years. It would not be possible without the great experiences with my Japanese family, the Watanabe's, who inspired me to start the journey of studying nutritional science. I also appreciate all the friends and colleagues for their company and experiences for improving my work in research.

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TABLE OF CONTENTS

DEDICATION ii

ACKNOWLEDGMENTS iii

LIST OF TABLES v

LIST OF FIGURES vi

LIST OF ABBREVIATIONS vii

INTRODUCTION 1

Radicals and Antioxidants in Nutrition 1

Dendrobium Officinale 3

OBJECTIVES 6

MATERIALS AND METHODS 7

Extraction 7

Total Phenolic Content (TPC) 7

DPPH Radical Scavenging Assay 8

Oxygen Radical Absorbance Capacity Assay (ORAC) 9

ABTS Radical Cation Decolorization Assay 11

Alpha-glucosidase Inhibition Assay 13

RESULTS AND DISCUSSIONS 16

CONCLUSION 23

REFERENCES 50

ABSTRACT 55

LIST OF TABLES

Table 1: Absorbance readings of Gallic acid standards for concentrations 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5mg/mL at 765nm for TPC assay 25

Table 2: GAE values in mg/mg for Dendrobium acetone extract based on absorbance recorded and mean values calculated at concentrations of 1, 2, 5, 10, 25, 50mg/mL for TPC assay 27

Table 3: Trolox Standards mean net AUC values based on FL readings for ORAC assay 34

Table 4: Calculated mean TE values from ORAC assay for DO acetone and ethanol fraction with respective concentrations 36

Table 5: Trolox standards absorbance values and calculations for ABTS assay 40

Table 6: Calculated mean TE values from ABTS assay for DO acetone and ethanol fraction with respective concentrations 42

Table 7: Analyzed results of TE values for DO acetone and ethanolic extracts from ORAC and ABTS assay for correlation evaluation 44

Table 8: Glucose standards absorbance measurements at concentrations of 0, 50, 100, 200, 400 and 500 mg/dL for plotting standard curve 46

Table 9: Percentage α-glucosidase inhibition for DO Acetone and Ethanol (EtOH) fractions based on glucose concentration mean values and absorbance mean values 48

LIST OF FIGURES

Figure 1: Beer's Law plot for Gallic acid standard curve absorbance readings at 765nm vs. concentration of 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5mg/mL for TPC assay 26

Figure 2: mg of GAE per mg of Dendrobium crude Acetone extract in different concentrations of 2mg/mL, 5mg/mL, 10mg/mL, 25mg/mL and 50mg/mL for TPC assay 28

Figure 3: Adjusted DPPH• concentration level based on Dendrobium extract reaction over 2-hour period at 10, 25, and 50mg/mL concentrations 29

Figure 4: DPPH• percentage scavenging capacity of Dendrobium antioxidant presented in extract at 10, 25, and 50mg/mL concentrations 30

Figure 5: ORAC FL readings for Trolox standards over 90 minutes period 31

Figure 6: ORAC plot expressed as FL readings for DO Acetone fraction in reaction with AAPH radicals for sample solutions at 0.05, 0.1, 0.25, 0.5 and 1.0mg/mL 32

Figure 7: ORAC plot expressed as FL for DO Ethanol fraction in reaction with AAPH radicals for sample solutions at 1.0, 0.5, 0.25, 0.1 and 0.05mg/mL 33

Figure 8: Trolox stand curve with concentrations of 0, 20, 40, 80, 100 and 200µM for calculating TE values in sample for ORAC assay 35

Figure 9: ORAC TE values comparison between DO acetone and ethanolic extracts for each individual concentration, range from 0.05mg/mL to 1mg/mL 37

Figure 10: $ABTS^{\bullet+}$ percentage scavenging capacity for DO Acetone fraction at concentrations of 0.05, 0.1, 0.5 mg/mL. 38

Figure 11: ABTS•+ percentage scavenging capacity for DO Ethanol fraction at concentrations of 0.05, 0.1, 0.5 mg/mL. 39

Figure 12: Standard curve with slope equation for Trolox scavenging power at different concentrations 0, 20, 40, 80, 100, 200 and 500µM for ABTS assay 41

Figure 13: ABTS Assay TE values comparison between DO acetone and ethanolic extracts for each individual concentration: 0.05mg/mL 0.1mg/mL and 0.5mg/mL 43

Figure 14: Comparison between TE analysis by ORAC and $ABTS^{\bullet+}$ assay for DO acetone fraction and ethanol fraction. 45

Figure 15: Standard curve plot of Glucose at different concentrations for subsequent calculations of sample glucose production and α-glucosidase inhibition 47

Figure 16: Bar graph for illustrating percentage α-glucosidase inhibition differences between DO acetone and ethanol fractions in various concentrations. 49

LIST OF ABBREVIATIONS

DO: Dendrobium Officinale Kimura et Migo

AOXs: Antioxidants

ROS/RNS: Reactive Oxygen/Nitrogen Species

AOC: Antioxidant capacity

TPC: Total Phenolic Content

GAE: Gallic Acid Equivalent

DPPH: 2, 2-diphenyl-1-picrylhydrazyl

ORAC: Oxygen Radical Absorbance Capacity Assay

TEAC: Trolox Equivalent (**TE**) Antioxidant Capacity

ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)

SD: Standard Deviation

HPLC: High Performance Liquid Chromatography

GC-MS: Gas chromatography – Mass Spectrometry

INTRODUCTION

Radicals and Antioxidants in Nutrition

Countless chronic diseases, metabolic syndromes, are caused by oxidative damages to the cells, pathways and nutrients necessary for maintaining good health. The main source of such damages is from reactive oxygen/nitrogen species (ROS/RNS) that are being generated through various external and internal effects such as radiation, smoking, regular exercises and metabolism. These free radicals have reactive and unstable properties that constantly seek available electrons through anti-oxidative reactions utilizing antioxidants (AOX) or enzymes for the protection of cells. Otherwise, accumulation of such damages will eventually lead to the development of many pathological conditions. [1] Therefore, the identification and investigation of AOX have become increasingly popular as an alternative solution for disease prevention and treatment.

Dietary antioxidants can be found in two main categories: synthetic and natural, which both are essential and serving respective functions. Their purpose is to couple with available nutrients for minimizing oxidative stress and damages, essentially by limiting and destroying free radicals, enhancing activities of antioxidant enzyme activities and regeneration.[2] Synthetic AOX including butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) etc., are frequently used by food industry for preservative purpose such as preventing spoilage of foods that containing oxidation-prone fatty acids. [3] On the other hand, natural antioxidants cover variety of subcategories including phenols, carotenoids, vitamins and nitrogenous compounds. They occur in nature such as our body and plants, where we utilize and replenish them by consumption of such antioxidant-rich foods to counteract oxidative damage. [1] This is why majority of the antioxidant researches began with identification and quantification of possible

bioactive compounds in foods. Then the ones with better efficacies would become targets of interest for subsequent in-depth analysis such as isolation of possible active compounds and elucidation of reaction mechanisms.

The anti-oxidative reactions vary based on several factors including type of radicals, chemical property of active compound, kinetics and quantity etc. For instance, radicals, such as peroxyl (O_2•), peroxynitrite (ONOO-) and hydroxyl (HO•) etc., mainly require reduction reaction via hydrogen atom transfer. Compounds that are capable of performing such reaction sometimes are limited to each individual radical type, for example, phenols are good quenchers for peroxyl radicals but carotenoids are relatively ineffective. [4] Plants that are rich in phytochemicals often have antioxidant capacities (AOC) for reducing oxidative stress caused by multiple types of radicals. [5, 6] Therefore, using analytical methods targeting scavenging capacities for different types of radicals may provide comprehensive information for determine antioxidant profile of an unknown sample. In this way, we can obtain more AOX from various natural sources to increase the efficiency of lowering oxidative stress and damages.

In the aspect of nutrition, it is crucial to understand of how ROS/RNS affect nutrients and respective metabolisms in order to evaluate physiological effect in terms of, for example, cellular senescence, signaling and other biological processes. With time, the radicals are prone to cause more severe consequences such as cardiovascular disease and carcinogenesis. [7]. For maintaining low oxidative stress, dietary intakes of macro- and micro-nutrients play important roles as modifiers in both direct and indirect cellular ROS production and removal. [8] For example, protein intake and its amino acid metabolism are closely related to glutathione oxidation and reduction. Excessive amount may increase the production of oxidative nitrogen radicals. On the other hand, deficiency would negatively affect the production of anti-oxidative

enzymes such as superoxide dismutase and glutathione reductase, which are essential for endogenous neutralization of free radicals. [9] As of lipid peroxidation, intake of PUFAs from plant or fish oil may increase the susceptibility of oxidative stress, but also can benefit cardiovascular functions in terms of reducing endogenous lipogenesis. The factor that influences which process would be predominant relies on how and what other nutrients are available in the system simultaneously. [10] Therefore, diet including essential minerals, vitamins and various beneficial phytochemicals will aid in decreasing oxidative stress and serving cellular protective functions. Amongst those, vast researchers are focusing on the investigation of phytochemical antioxidants that may serve as possible replacement therapies for people with complex pathologies or rejection rewards potent drug treatments. Those plant sourced compounds would have minimal adverse effects when treatments involve compounds like polyphenols, vitamins and carotenoids as alternative or complementary medicine. [11]

Dendrobium Officinale

Dendrobium Officinale Kimura et Migo, aka Tiepishihu (DO), is one of the species under the large genus of orchids. The medicinal properties various Dendrobium species were recorded in *Chinese Pharmacopoeia* and *Shennong Herbal Record*, which indicated various health benefits including improving immune system, anti-fatigue, anti-oxidative, ameliorating hyperglycemia, anti-carcinogenic and so on. [12] Recently, DO and similar species have become endangered due to the frequent usage and overharvesting. In addition, Dendrobium plant in nature also requires a substantial amount of years to reach harvestable maturity with significant medicinal property, which promoted an increasing number of researches for various purposes such as agriculture, pharmacology and also nutrition. [13] Although Chinese herbal medicine has

been utilized for treatments in Asia for ages, many specific mechanisms and beneficial components remain yet unclear for most of Dendrobium species. Thus the utilization of Chinese medicinal herb such as DO for illness treatment is still very rare in western culture. Nonetheless, numerous research techniques have been developed and frequently used for analyzing natural compounds for their AOC in order to understand potential health benefit as a part of diet or alternative treatments. [14]

Currently, there are many other Dendrobium species including D. nobile Lindl, D.chrysotorum, D. denonianum, D. hercoglossum and dozens more, however, they were not equal as in terms of medicinal values. They have been researched mostly in China for their AOCs, results were used for comparison among species. Essentially, researchers are trying to understand the correlation between the value and individual chemical property. [15] Multiple studies focused on identification of new species that may have similar medicinal properties as the endangered ones. Some preliminary profiles were identified throughout different studies, which gave basis for ongoing projects targeting individual component such as AOX. There are numerous known compounds that serve as antioxidants to provide health benefit, such as alkaloids, polyphenols, bibenzyls, amino acids, polysaccharides, multiple essential minerals and so on. [16, 17] Among those, there are some studies that targeted the DO polysaccharide properties, which was hypothesized to have superior AOC and other benefits.

From the immunological point of view, Lin et al. used DO polysaccharide to demonstrate the enhancement in immunomodulation ability in a cell line model with chronic autoimmune disease, specifically Sjögren's syndrome that affecting salivary gland functions. The results showed positive effects on tumor necrosis factor (TNF)-α-induced apoptosis, which ROS was a considerable contributing factor in such process. [18, 19] Dendrobium also had shown anti-

fatigue effect in mouse model when subject to prolonged weight-bearing exercises. Serum lactic acid and creatine kinase activity had significantly decreases after 30 day of DO solution dietary intake, therefore exhibited beneficial effect on physiological endurance. [20] Other beneficial effects on metabolic syndromes were also studied separately on mice models with adrenaline-induced hyperglycemia and alcohol-induced hypertension. Ingestion of Dendrobium granule had shown improvement on various inflammatory markers and exerted restorative functions to those pathological conditions. [21, 22]

On the other hand, only few studies were previously done on the properties of DO in terms of alkaloids, phenols and mineral composition. Chen et al. evaluated the alkaloid characteristics between D. Officinale and D. Nobile, where both had similar chemical compounds but DO had significantly higher in quantity of some individual components. [17] Also, Zhu et al. determined that alkaloid concentration could be affected by where and how long the plant was grown. These are two of the major factors that contributed to high value of the particular specie, thus promoted the consumption and increased the rarity of DO. [23] Another study on DO mineral properties showed there was a correlation between total alkaloid and essential mineral concentration. Mineral composition in terms of beneficial ones and harmful heavy metals were determined to be related to environmental conditions and possibly modifiable based on agricultural techniques.[24] Furthermore, researches had identified a few common phenolic antioxidants via HPLC and GC-MS techniques: naringenin, moscatilin and gigantol. Again the phenolic profiles were compared among different species to evaluate the quality of certain one, which DO showed significantly low in these constituents in contrast to the highest specie, interestingly D. nobile.[13, 25] Thus the assumption was that phenolic content might not be the significant factor for the determination of medicinal values for Dendrobium.

OBJECTIVES

This study focused on the investigating of DO specific antioxidant properties based on the various chemical compositions that may have beneficial effects on combating free radicals and oxidants. Firstly, TPC assay was performed to evaluate the overall total phenol concentration in DO sample in comparison to previous studies. This would also be helpful for the determination of necessary modification on methodology for further analytical assays. Secondly, different radical species required corresponding methods such as DPPH, ORAC and ABTS, which were subsequently used to analyze AOC for DO Acetone and Ethanol fractions with respective efficacy. The comprehensive data would be evaluated for DO antioxidant properties by comparing the different assay methods and study the correlations for understanding significance and validity. In addition, α-glucosidase inhibition assay would provide an aspect of possible effect on carbohydrate metabolism, which currently no study has investigated such property in regards to chemical composition difference between acetone and ethanolic extracts. The experiment would provide crucial information on potential benefit for future postprandial hyperglycemia treatment.

MATERIALS AND METHODS

Extraction

Dendrobium plant was purchased from China, which was intended to obtain two crude extract factions, acetone and ethanol. Original whole plant contained flower buds, stem, flower and leaves, where flower and partial top stem were collected for experimentation. Fresh sample portions (900g total) were kept frozen at -80°C prior to freeze drying using Labconco Lyph Lock 6 lyophilizer (MO, USA), which were subsequently pulverized into powder form using laboratory blender. The dried DO powder was transferred into two flasks evenly with addition of 700mL of 100% acetone and 700mL of 100% ethanol respectively. The mixtures were left on stirring plate at speed of ~700rpm overnight (~8hrs) for thorough extraction. Sample solutions were then filtered using vacuum filtration with 0.45 µm filter paper to obtain the sediment-free solution possibly containing phenolic compounds and unknown antioxidants. After processed through rotary evaporation technique, where acetone and ethanol were removed, crude DO extractions were obtained. The residues from filtrations were then mixed with additional 500mL of solvent for secondary extraction. After evaporation of solvents, two sample extractions were labeled correspondingly and stored in -20°C freezer for further analysis. Acetone extract appeared to have yellow/brown color, whereas ethanolic extract had dark green pigmentation.

Total Phenolic Content (TPC)

Folin-Ciocalteau Reagent 2N (F-C), sodium carbonate (≥99%) and Gallic acid were purchased from Sigma Aldrich, MO. The TPC assay was used to determine the concentration of phenolic compound in reference to Gallic acid by detection of F-C reagent color intensity changes from yellow/green to blue color using a Spectrophotometer (Beckman DU 640, Beckman Coulter, Inc., Fullerton, CA). Sodium carbonate solution was added to maintain reaction mixture at basic pH, where F-C reaction would occur. [26] The purpose was to evaluate

whether sample contained predominant phenolic antioxidant. Also the result would provide approximate concentrations to use for further radical scavenging assay as antioxidant rich compound needed less concentrated solutions for analysis.

Procedure - The TPC method was from previous lab protocol, which was adapted from Spanos and Wrolsted. [27] F-C reagent was diluted from 2N to 0.2N with distilled deionized water (ddH_2O). 20% saturated sodium carbonate was prepared by dissolving 20g solid in 100mL ddH_2O. 5.0mL of Gallic acid stock was made at concentration of 1.0mg/mL in 50% acetone solution, which further diluted into 1mL each at concentrations of 0.5mg/mL, 0.4mg/mL, 0.3mg/mL, 0.2mg/mL, 0.1mg/mL and 0.05mg/mL. DO sample tested was acetone extracted portion, where initial stock was prepared at concentration of 50mg/mL with 50% acetone. Serial dilutions from the stock were performed to make sample solutions at 25mg/mL, 10mg/mL, 5mg/mL, 2mg/mL and 1mg/mL, which was intended to test wide range of concentrations for analysis. In each test tube, 25 µL of Gallic acid standards or sample solutions was mixed with 0.25mL of ddH_2O, 0.75mL of 0.2N F-C reagent and 0.5mL of 20% sodium carbonate solution. All reaction mixtures were prepared in duplicates and incubated in the dark at room temperature for 2 hours before absorbance readings at 765nm. Gallic acid absorbance readings were used for plotting the standard curved, and GAE of sample were calculated based on beer's law. Results were analyzed for determination of appropriate concentrations to use for further antioxidant assays.

DPPH Radical Scavenging Assay

Colorimetric analysis of antioxidant capacity was performed using 2,2-Diphenyl-1-picrylhydrazy (DPPH•) radical reagent (Sigma Aldrich, WI). Detection method included the use of clear 96-well microplate and HTS7000 bio assay reader (Perkin Elmer, CT). The simple method utilizing a stable radical with an unpaired nitrogen atom can provide basic information

on how efficient an antioxidant donor reduces the radicals with a hydrogen atom. [28] The assay would help identify suitable DO sample concentrations that could show a trend of radical scavenging capability throughout time.

Procedure - The assay methodology was adapted from Bran-Williams et al.'s method with modification suitable to experimental sample. [29] DPPH• reagent 0.8mM was prepared by diluting a 2mM stock, where DPPH• was dissolved in 50% acetone based on the solubility of the sample, DO acetone extract. 1mL each DO extract solutions in three different concentrations 50mg/mL, 25mg/mL and 10mg/mL was prepared fresh in 50% acetone prior to experiment to minimize possible degradation of antioxidant compound by solvent. The concentrations were determined according to the results obtained from TPC assays done previously. Sample blank was included with only 50% acetone solution in microplate well. Each of the other test wells contained 100µL of sample solution with 150µL of 0.8mM DPPH• solution, which all were prepared for duplicate absorbance readings. Another set of duplicate wells were prepared containing only 100µL sample solutions to minimize color interference as the DO extract solution appeared brown to light brown color at all three tested concentrations, which was labeled as sample background. Absorbance measurement was performed under room temperature at 500nm every 5 minutes for 2 hours. Sample background readings were subtracted from reaction mixture readings for further calculations of DPPH• concentration and percentage scavenged by the presence of the antioxidant in samples. Equation was also adapted from method as following:

$$\% \text{ DPPH• scavenging} = [Abs_{control} - (Abs_{sample} - Abs_{sample\ background})]/Abs_{control} \times 100$$

Oxygen Radical Absorbance Capacity Assay (ORAC)

To evaluate how sample would provide electron transferring capability with specificity and efficacy for peroxyl radicals, radical absorbance assay was designed utilizing 2,2'-Azobis (2-

amidinopropane) dihydrochloride (AAPH). Specifically, peroxyl radicals rely on the donation of hydrogen atom to reduce the oxidative form, which can be measured using spectrometry by coupling with a Fluorescein (FL) probe. Essentially, antioxidant rich compounds would provide protective ability for FL, thus retard its degradation over time caused by oxidation. Trolox as a Vitamin E analog provides a better comparable standard for mimicking the endogenous peroxyl radical reduction reaction. The methodology based on the analysis of Trolox Equivalence (TE) was adapted from Prior et al. with modification to reagent based on test sample composition. [4]

Procedure - Trolox (Acros, PA) stock solution was prepared in 15mL falcon tube at 50mM with 50% Acetone. Subsequent serial dilutions were made at the concentrations of 0.5mM, 200µM, 100µM, 80µM, 40µM and 20 µM for standard curve analysis. Phosphate buffer (PB) was prepared from sodium phosphate monobasic and sodium phosphate dibasic heptahydrate (Sigma Aldrich, MO) to a concentration of 75mM with pH adjustment using NaOH solution to 7.4. Due to the sensitivity of measurement, Fluorescein (Sigma Aldrich, MO) was required at very low concentration, which was prepared based on the optimized concentration values from previous experiments done by colleagues on similar samples. Stock FL was prepared in PB at a concentration of 0.1mM, which was diluted to 0.01mM then to final working solution at 0.008mM. AAPH (Wako, VA), was used to prepare radical solution at 0.36M by dissolving with 5mL PB in falcon tube.

DO sample test concentrations were determined according to previous DPPH• assay, therefore, stock solution of 50mg/mL was made by dissolving DO Acetone fraction extract in 50% acetone. Subsequent serial dilutions were made at the concentrations of 1.0, 0.5, 0.25, 0.1 and 0.05mg/mL based on the sensitivity of assay [30]. 100% ethanol was used for DO Ethanol fraction sample preparation, where same concentrations of dilutions were prepared. All reagent

working solutions and sample solutions were prepared fresh and enough for duplicate FL readings. A black/opaque 96-well microplate was used for assay FL measurement, which was performed on HTS 7000 bio assay reader with 485nm excitation filter and 535nm emission filter. Reaction blanks were prepare by mixing 235µL PB and 40µL 50% Acetone / 100% Ethanol. For the rest each well, 200µL 0.008mM FL solution was mixed with 40µL Trolox standards or sample solutions; then the microplate was shaken for 10 seconds and incubated for 15 minutes at 37°C. Subsequently, 35µL AAPH solution was transferred into each well for radical scavenging reactions. Fluorescence values were measured every 5 minutes for a total of 120 minutes. Data was collected and evaluated using area under the curve (AUC) calculations based on following formula:

$$AUC = 0.5 + f_1/f_0 + f_2/f_0 + f_3/f_0 + \ldots . f_{i-1}/f_0 + 0.5(f_i/f_0)$$

Where f_0/ is the initial FL reading at 0min and f_i is the final FL reading[31]

The adjustment of AUC was expressed as Net AUC by subtracting blank AUC values from Trolox/sample AUC values. Then ORAC values of the sample were calculated from linear regression equation from Trolox AUC standard curve, which was also used for analysis and verification of obtained FL measurements. [32] After computing ORAC values in TE as unit, both fractions were evaluated to compare significance of TE level. Additional data from different assay was also included for compare correlations of whether AOC would be affected significantly.

ABTS Radical Cation Decolorization Assay

2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay is another frequently used analytical experiment for test antioxidant capacities in foods. Due to its simplicity and stable reagent property for storage and experimentation under various pH conditions, ABTS was chosen to analyze DO sample AOC and to compare results with previously done antioxidant assays. The technique was based on the formation of $ABTS^{\bullet+}$ from oxidative reaction between

ABTS and potassium persulfate, which would yield green/blue chromophore for decolorization test. Concentration correlated color intensity can be measure at two common frequencies around 415nm and 734nm. The reaction with the presence of antioxidant will reduce $ABTS^{\bullet+}$ to its colorless neutral form ABTS by a single electron transfer. By establishing a standard using Trolox, sample AOC can be evaluated for its capability and efficacy. [33]

Procedure – The protocol was adapted from Re et al. and Zhou et al. with modifications based on DO solvent and equipment availability. [33, 34] Both ABTS (MP Biomedicals, OH) and potassium persulfate (Acros, PA) were dissolved separately in ddH_2O. Portions of each solution were taken and combine into 1mL total volume to make stock $ABTS^{\bullet+}$ solution, which yielded finished concentration at 7mM for ABTS and 2.5mM for potassium persulfate. The mixture was stored in dark place at room temperature for 12-16 hours, where cation formation occurred and would be stable for up to 1 week. 200µL of stock solution was placed in a transparent microplate for absorbance reading using HTS7000 bio assay reader at wavelength of 405nm. $ABTS^{\bullet+}$ working solution with absorbance of 0.7(±0.02) was made by diluting stock with ddH_2O, ~ 80 fold, based on the absorbance reading obtained previously. 1.5mM Trolox stock was made by dissolving 3.75mg Trolox reagent (Sigma Aldrich, MO) in 10mL ethanol. Subsequent serial dilutions were performed to make standards with concentrations of 500, 200, 100, 80, 40 and 20µM. DO samples were selected from both acetone and ethanol fractions, which were subsequently dissolved to make 10mg/mL stock solution with 50% acetone and 100% ethanol respectively. Serial dilutions of the samples were performed to make three concentrations of each sample at 0.5, 0.1 and 0.05mg/mL. To minimize error and interferences, 210µL each of 50% acetone negative control and 100% ethanol negative control were used for absorbance readings. 10µL of Trolox standard solutions or DO sample solutions was transferred

into each microplate well in duplicates. After addition of 200µL of $ABTS^{\bullet+}$ working solution, plate was covered for absorbance measurement at 405nm every minute for 30 minutes. The $ABTS^{\bullet+}$ scavenging effect was calculated based on following equation:

$$\text{Scavenging power \%} = (Abs_{INITIAL} - Abs_{END)} / Abs_{INITIAL} \times 100$$

Trolox standard curve was plotted and used for sample % scavenging power analysis, which the slope would be used for calculating TE values for two samples based on their % Absorbance change. The end results were analyzed with values obtained from previous ORAC experiment, which gave secondary information regarding assay validity and correlation.

Alpha-glucosidase Inhibition Assay

α-glucosidase is a major digestive enzyme in carbohydrate metabolism, which hydrolyzes disaccharides such as sucrose, maltose and isomaltose to form simple glucose molecules. The purpose of this enzymatic assay is to investigate whether the test sample, DO, has any inhibitory effect on α-glucosidase activity. This would potentially indicate whether DO could decrease or delay carbohydrate metabolism, which provides beneficial effect for postprandial hyperglycemic condition, specifically in pre-diabetic and diabetic patients. Assay methodology was based on Ohta et al., which utilizes Glucose Autokit (Wako, CA) for detection of glucose production from enzymatic reaction with substrate mixture of disaccharides. As the presence of sample extract may or may not interact with α-glucosidase competitively, glucose synthesis can be measured using spectrophotometer at 505nm as autokit solution can provide pink/red color chromophore upon reaction with mutarotase. In addition, Acarbose (LKT, MN) was used as a positive inhibitory control to evaluate sample efficacy. [35]

Procedure – Protocol was based on provided glucose autokit instructions and Hogan et al. with modification. [36, 37] α-glucosidase acetone powder from rat intestine (Sigma, MO) was used for making enzyme extract with specific concentration at 25mg/mL by mixing 2.5g powder

with 100mL 0.1M phosphate buffer (pH 6.8). Mixture was shaken overnight on stirring plate and the filtered via vacuum. Filtrate was kept in -80°C overnight, and subsequently freeze dried using Labconco lyophilizor. Purified α-glucosidase powder was again weighed and dissolved in 0.1M PB to make 25mg/mL enzyme solution, which was again centrifuged at 3000 rpm 4°C for 5 minutes prior to use for clearing additional sediment. Substrate solutions of isomaltose (TCI, AL), maltose and sucrose (Fisher, IL) were made separately at 125mM each with 0.1M PB (pH 6.8), where substrate mixture was then prepared by combine equal portion (1:1:1 ratio, 200µL each) of the three solutions. Acarbose working solution at 50µg/mL was prepared by diluting 1mg/mL stock solution, which was made from dissolving 10mg Acarbose in 10mL 0.1M PB (pH 6.8). DO sample solutions were prepared with both acetone and ethanol (EtOH) fractions, which were dissolved in 25% acetone and 20% ethanol respectively to make three concentrations: 5, 2.5, 1.0mg/mL. Due to the sensitivity of assay measurement, only small aliquots of reaction mixtures were needed for each test. Thus in each 0.2mL centrifuge tube, 28µL of Acarbose (as positive control) or solvent/PB buffer (as negative control) or sample solutions from both fractions was mixed with 22µL of rat intestinal α-glucosidase solution. After addition of 11µL of disaccharide substrate solution to each tube, mixtures were vortexed well and incubated for 30 minutes at 37°C. Autokit Glucose working solution was obtained per instruction by dissolving color reagent in given buffer solution. Glucose standards were made from given 200mg/dL and 500mg/dL stock by dilution with ddH_2O to achieve additional concentration range: 50, 100, 200, 300, 400 and 500mg/dL. Post incubation 0.006mL of reaction cocktail was mixed with 0.9mL of Glucose autokit working solution in separate tubes, which were again incubated for 20 minutes at 37°C. 200µL each of the final reaction mixture was transferred into a clear 96-well microplate and

measured for absorbance at 505nm using HTS 7000 bio assay reader. Glucose concentration was then calculated based on absorbance values according following equation:

Glucose Concentration (mg/dL) = (Abs_{sample} / $Abs_{standard}$) × Standard Concentration

Percentage α-glucosidase inhibition was calculated based on corresponding negative control (PB/ 25% acetone / 20% ethanol) using following equation:

% inhibition = (1 – ($[glucose]_{sample}$ / $[glucose]_{negative\ control}$)) × 100

Where [glucose] was concentration obtained from previous calculation. Both acetone and ethanol fractions were analyzed for comparing the inhibition property.

Statistical Analysis

Assay data were collected and analyzed via Microsoft Excel and SPSS Statistics 22. Mean, SD and SEM were calculated correspondingly for determination of errors. From the multiple data sets, correlation study and value significance were calculated in SPSS, where p=0.05 was used as subset in Student's t-test.

RESULTS AND DISCUSSIONS

TPC – Gallic acid standard curve was plotted based on duplicate absorbance readings with standard deviation as error, which was shown in **Table 1**. Standard curve trend line was calculated to have a slope of 1.8864 and intercept of 0.0452 as shown in **Figure 1**. The statistical significance of the standard curve was represented by the R^2 value, which is 0.9933, indicated the accurate and valid results were obtained. Subsequent calculations of Dendrobium acetone extract concentrations were determined according to absorbance data shown in **Table 2**. Phenolic content within extract was presented as GAE per extract weight in the unit of mg/mg. The calculated concentrations of phenolic content within sample were then divided by the original sample concentrations to obtain the GAE values listed. Standard Error of Mean (SEM) was used to determine validity of analyzed data. Summary of GAE values were illustrated in **Figure 2** with SEM error bars. The results indicated that DO extract with 1mg/mL concentration showed negative value, which was subsequently removed from data presentation; extract prepared at 2mg/mL and 5mg/mL concentrations gave larger error in terms. Maximal detectable GAE phenolic content concentrations were observed among the higher concentrations of 10, 25 and 50mg/mL, which was 0.00943±0.00023 mg GAE/mg or 9.43±0.23 mg GAE /g extract in average; therefore, they were selected for further analysis of antioxidant capacity.

From the GAE values obtained for samples with different concentrations as in **Figure 2**, there was no significant difference among the 10, 25 and 50mg/mL samples. (p >0.05) The phenolic components of the DO extract did show consistent lower concentrations as other research. [25] On the other hand, results indicated that the non-phenolic constituents needed further analysis using different methods for investigating additional antioxidant property. Thus, DO was not determined to be an antioxidant poor herb yet. In addition, there might be possible

loss of water-soluble phenolic content during the processes of lyophilization and/or extraction. For further AOC analysis, higher concentrations would be more suitable for experimentation since most methods were targeting phenolic compounds.

DPPH• Assay – As previously determined, DO samples were prepared in higher concentrations for DPPH• assay. Due to the color intensity of sample solutions, sample background was also used for absorbance readings in addition to the reaction mixture, which decreased error that might be resulted from color interference. DPPH• concentration was diminished over time as DO solution reacted with radicals, thus absorbance decreased as shown in **Figure 3**. The plot illustrated already adjusted values for absorbance readings, where control was showing consistent and insignificant decrease in values (p =0.105). There was a decrease in concentration of DPPH• from absorbance of 2.866 to 2.468 for 10mg/mL sample, from 2.757 to 1.731 for 25mg/mL and from 2.513 to 1.765 for 50mg/mL sample, where all differences were significant (p <0.05). Based on the absorbance values, **Figure 4** illustrated the % DPPH• scavenged at each time point over 120 minutes. All three concentrations showed significant change, where 44.3%, 37.2% and 13.9% DPPH• was scavenged at concentrations of 50, 25 and 10mg/mL respectively. Both figures showed that there were strong DPPH• scavenging capacity for higher concentration of 25mg/mL and 50mg/mL. However, 10mg/mL concentration showed only gradual decrease in DPPH• concentration, thus scavenging capacity was diminished at lower concentration.

DPPH• assay illustrated similar trend to TPC assay that only samples with higher concentrations were able to show relatively functional AOC. However, overall scavenging capacity below 50% of initial DPPH• radical concentration indicated that there might be several

factors involved in efficacy of the anti-radical capacity. First, DO samples and DPPH• solutions were prepared with acetone as solvent, this might affect absorbance readings in comparison to method by Brian-Williams et al. [29] Secondly, unforeseen products during chemical reaction such as precipitation and turbidity occurrence might interfere with measurement accuracy. The assay method may require constant adjustment based on sample density and chemical property, which can be difficult when unknown chemical composition is presented in different samples or even fractions. The assay has been frequently used for AOC analysis due to the simplicity and ease of measurement. However, factors such as storage condition, preparation solvent may contribute to variations in reactivity with samples. Therefore, optimization of the method coupling with different assays analysis may help evaluate AOX properties for better interpretations in the future.

ORAC Assay – ORAC method illustrated FL degradation when reaction occurring with the presence of radical, AAPH. The higher concentration of antioxidant is in the sample, the more FL will remain detectable via spectrophotometry. The efficiency of AOX can also be visually seen through graphic plot. Trolox standards with 0µM (negative control), 20, 40, 80, 100, 200µM were analyzed for subsequent TE calculation. Trolox reactivity was shown in **Figure 5**, where higher concentration of 200µM showed slowest degradation over time. **Figure 6** showed FL degradation for DO acetone fraction with multiple concentration range from 0.05mg/mL to 1.0mg/mL, which all showed significant decrease in FL from the initial point. DO ethanol fraction was also tested for ORAC value shown in **Figure 7**, where all but 1mg/mL sample (p = 0.075) showed significant decrease in FL readings. Further analysis of specific concentrations in TE for each sample was done using Trolox standard net AUC values and linear regression curve

shown in **Table 3** and **Figure 8**. From the equation and respective sample concentrations, mean TE values were shown in **Table 4**: 193.39±51.2 µmol/ TE/ g acetone extract and 299.79±99.3 µmol/ TE/ g ethanolic extract.

By comparing two fractions illustrated in **Figure 6** and **Figure 7**, it was clear that acetone fraction had slightly better efficiency, where active AOX scavenged AAPH radicals in a shorter period of time. This could also mean that ethanol fraction might have higher concentration of AOX that slowed down the degradation of FL caused by radicals. Furthermore, from the average TE values, DO ethanol fraction showed significance in higher AOX concentration relative to acetone fraction, which was shown in **Figure 9**. Due to the limited research in AOX area for DO specifically using ORAC assay, it was not able to determine accuracy of measurement in the isolated ethanol or acetone extract. However, Zhang et al. had evaluated certain compounds from ethanol fractionation product, where higher activity was observed in comparison to vitamin C as a standard. [38] Whether ethanol fraction remains superior in AOC, it was necessary to evaluate using different methods. FL probe remains sensitive for spectrometry detection but loses capacity due to various factors such as storage duration, temperature and light exposure. The correlation between experimental assays performed on same sample with corresponding concentrations would be analyzed for determine the acceptance or rejection of the theory.

ABTS Assay – Following ORAC assay, ABTS radical cation scavenging assay was performed to compare AOC difference between DO acetone and ethanol fraction. **Figure 10** and **Figure 11** respectively showed the $ABTS^{\bullet+}$ scavenging trend in percentage among three different concentrations 0.05, 0.1 and 0.5mg/mL for acetone fraction and ethanol fraction. Acetone fraction had a range from only 1.70% to 4.77% scavenging capacity, as ethanolic extract showed

3.50% to 11.1% scavenging power. Based on the observation of percentage change, the Trolox standard values and linear regression curve were used to calculate TE values for the two tested fractions in respective concentrations, as shown in **Table 5** and **Figure 12**. Both fractions had same concentrations selected because they were used for better comparison under same method. TE was calculated as μmol TE / g of extract since original extraction weights were not obtained. Data and illustration in **Table 6** and **Figure 13** showed that significantly elevated TE antioxidant concentrations were expressed in DO ethanol fraction. ($p < 0.05$) Combining previous ORAC data (**Table 7**), correlation study determined that positive correlation was shown between ORAC and ABTS in radical scavenging capacity for DO. Overall, it was also theorized that neither DO acetone nor ethanol fraction had significantly strong cation scavenging properties. It is possible to have further research in regards to DO as a whole plant may have improved cation quenching capability.

The comparative analysis shown in **Figure 14** indicated that both ORAC and ABTS assay had similar trend with no significant differences in terms of calculated TE values for respective acetone and ethanol fraction. It was also observed that higher concentrations did have greater scavenging capacity for both extracts. The difference was that substantial variations between samples might indicate individual active compounds presented within each fraction. As Luo et al. showed in Dendrobium polysaccharide research, further purification of ethanolic extracts would yield bioactive compounds that might contribute to AOC and other beneficial effects. [39] $ABTS^{\bullet+}$, similar to other colorimetric assays, is highly dependent on absorbance measurement under specified wavelength. As sample having color in nature, detection of true $ABTS^{\bullet+}$ compound color may be interfered, thus cause error in results if there are inconsistency

between assays. [40] Therefore, future experiments including background readings for samples with obvious intense color would be beneficial for more accurate measurement.

Alpha-glucosidase Inhibition Assay – Autokit Glucose was optimized for glucose analysis in test solutions, which standard curve had to be established for references and concentration calculations. **Table 8** showed absorbance data, which were necessary to plot standard curve as shown in **Figure 15**. Data provided a linear relationship with a slope of 0.0014 and an intercept of -0.0328, which were suitable for accurate analysis of sample data as shown in **Table 9**. Adjusted values were calculated by subtracting background absorbance from actual readings for each control and samples. Acarbose showed working inhibition of glucose production from substrates, which gave 56.425% and 66.424% inhibition based on calculated glucose concentration and absorbance readings respectively. For samples, individual DO fraction absorbance readings were adjusted based on background readings of each corresponding solvent used for sample preparation. Subsequent calculations of enzymatic inhibition were done by using previous standard curve slope and intercept. Results showed significantly different inhibition level among tested concentrations of acetone fraction (p <0.05) but no significant change between two concentrations of the Ethanol fraction (p >0.05). Furthermore, analysis was performed to evaluate correlation between two inhibition calculations, where no significant differences were observed between using concentration and absorbance to compute % inhibition (p >0.05). However, it is more accurate and representative when using glucose production to calculate DO AOC in terms of α-glucosidase inhibition. Therefore, DO acetone fraction with 1mg/mL concentration showed highest enzymatic inhibition of 17.99%, and both EtOH fraction samples with different concentrations showed average inhibition of 5.3%. DO EtOH fraction also had 1mg/mL concentration tested, however, negative value was obtained due to unknown error

that might have affected measurement. **Figure 16** showed the comparison between DO acetone and ethanol fraction, which was determined to have significant higher inhibition for acetone extracted sample (p <0.05).

Current assay utilizing Autokit Glucose method was able to show α-glucosidase inhibition by samples in reference to working Acarbose inhibitor. At 50µg/mL concentration for acarbose, 56.4% inhibition was determined to be a low observation, which could be affected by the loss of activity after a long-term storage (reagent from 2008). The glucose kit also carries a limitation on enzyme/substrate stability, where storage condition/period might have decreased assay quality. Thus fresh reagents may serve better in determination of enzymatic activity with inhibitor as a positive control for analysis of test samples. Furthermore, the trend of an increasing inhibition with decreasing sample concentrations indicated that enzymatic mechanism might be affected by concentration specificity for compounds presented in DO acetone fraction. Lowering the concentration, possibly additional range below 1 mg/mL, may provide better inhibition by DO, which could be investigated in the future. In addition, traditional method utilizing p-nitrophenyl-α-d-glucopyranoside (pNPG) can also be performed in order to analyze correlation between assays and to evaluate possible variations. [41, 42]

CONCLUSION

Based on the analyses, phenolic content being a hallmark of anti-radical component, showed a moderate to low amount within the acetone extract of DO sample. It was similar to some of the findings from different studies on different Dendrobium species. [25, 43] Although TPC values were not as substantial as some of the known antioxidant rich plants such as cinnamon or clove extracts, other non-phenolic components that exert anti-oxidative ability may not be able to show in such experiment. [30] Therefore, it was determined that other possible chemical components may aid in beneficial effects for reduction of radicals, which was also based on the result evaluations from subsequent antioxidant assays. The current project was also focused on separate fractions of acetone and ethanol eluded solutions, which contained different chemical solubility types in terms of antioxidants. Data from DPPH, ORAC and ABTS assays indicated the limitation of DO reactivity as an antioxidant, the effects may take longer time or/and higher concentration to show more significantly beneficial properties compare to some known plants such as berries and spices. ORAC and ABTS exhibited validity and correlation while DO ethanol fraction had more AOC with consistent ability to scavenge radicals compare to acetone extracted samples as in **Table 7** and **Figure 14**. From various research articles, anti-oxidative effect of DO was also expressed in polysaccharide components, which consists a large portion of the DO active compounds. [15, 44, 45] The significant difference indicated that ethanol fraction did contain substantial amount of AOX, possibly from polysaccharide content. However, DO still remains at a lower rank when considering the phenolic antioxidant capability in radical scavenging. On the other hand, it was also suggested that plant harvesting and preparation techniques could have effect on nutrient loss if not done properly such as appropriate drying timing and condition control. [46] It is possible that sample preparation in organic solvent might

contribute to certain AOC loss. In terms of α-glucosidase, autokit glucose method produced results for comparison of the two fractions, where acetone portion had significantly higher enzymatic inhibition power than ethanol extracted fraction as in **Figure 16**. It was also concluded that different extraction methods yielded groups of compounds with separate antioxidant property, which there was no correlation between AOC and α-glucosidase inhibition. In addition, the low TPC values could explain that possible water-soluble polysaccharide may contribute to different aspect of antioxidant ability, which did not react properly in organic solvents. Therefore, future in depth studies on carbohydrate metabolism, specifically effective mechanism by phenol and polysaccharide may be helpful for creating more comprehensive profile.

Moreover, due to the lower risk of cytotoxicity from herbal derived compounds, the ultimate goal is to isolate essential active compounds in natural plant for prevention and alternative treatment of chronic diseases or even cancers. These may include extended projects in research of some different types of antioxidants. Prospective studies can be proposed including additional fractionation using different solvents and chromatography, polysaccharide isolation for specificity analysis, correlation between α-Amylase inhibition and α-glucosidase inhibition ability, cytotoxicity, lipid oxidation inhibition, thermal and pH stability, variation on the effects of additional storage conditions and bioavailability. Furthermore, the possibility of synergistic effect can also be investigated according to the traditional use of Chinese medicine, where multiple herbs or plants are combined to achieve enhanced functions. [47] Thus, HPLC and GC-MS can be utilized for identification and quantification of specific active compounds that may have predominant anti-oxidative power, and perform additional analysis on how each compound reacts with and without one another using known antioxidants.

Table 1: Absorbance readings of Gallic acid standards for concentrations 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5mg/mL at 765nm for TPC assay

[GA] mg/mL	Mean Absorbance	SD*
0	0.0057	±0.0014
0.05	0.1034	±0.0089
0.1	0.2023	±0.0018
0.2	0.4345	±0.0153
0.3	0.6390	±0.0049
0.4	0.8684	±0.0083
0.5	1.0522	±0.0253
1.0	1.8665	±0.0824

*All absorbance had duplicate readings performed and mean values were used for plotting standard curve. Standard deviation values were calculated based on duplicate readings.

Figure 1: Beer's Law plot for Gallic acid standard curve absorbance readings at 765nm vs. concentration of 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5mg/mL for TPC assay

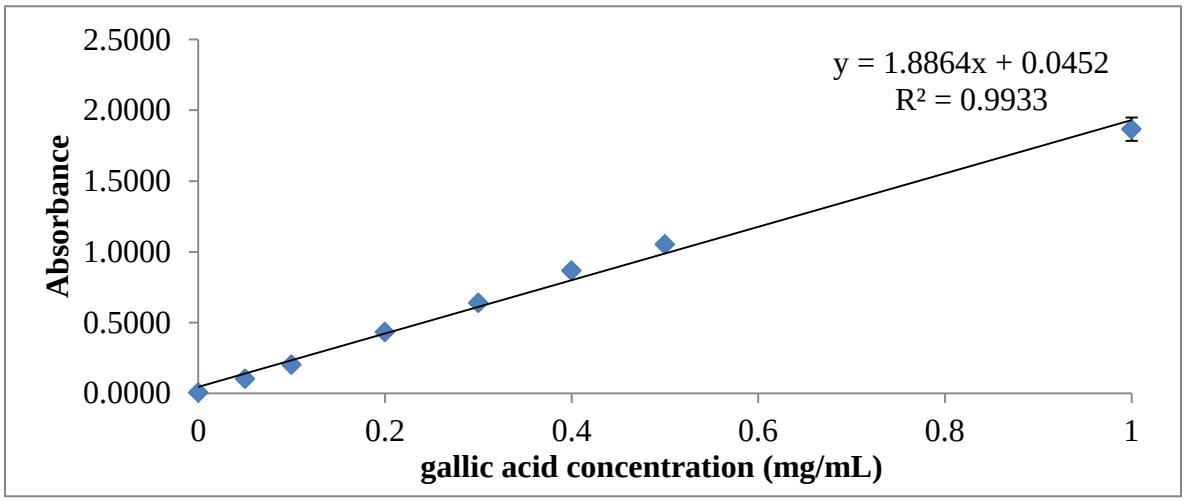
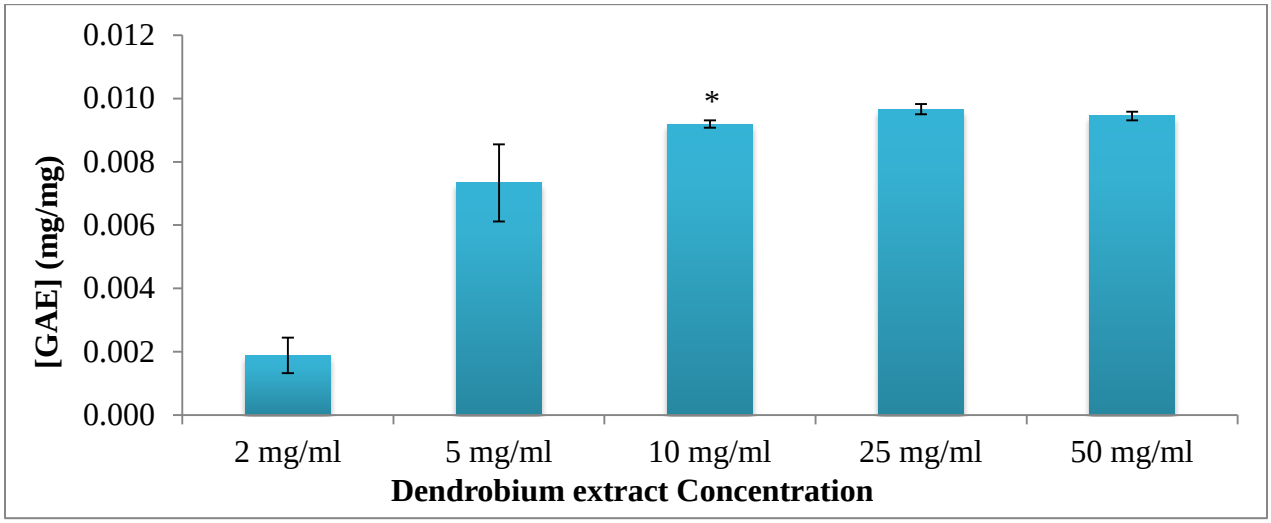


Table 2: GAE values in mg/mg for Dendrobium acetone extract based on absorbance recorded and mean values calculated at concentrations of 1, 2, 5, 10, 25, 50mg/mL for TPC assay

Sample conc. mg/mL	GAE/sample (mg/mg)	SD*
1	-0.00994	±0.00079
2	0.00188	±0.00172
5	0.00734	±0.00016
10	0.00919	±0.00023
25	0.00966	±0.00019
50	0.00945	±0.00013

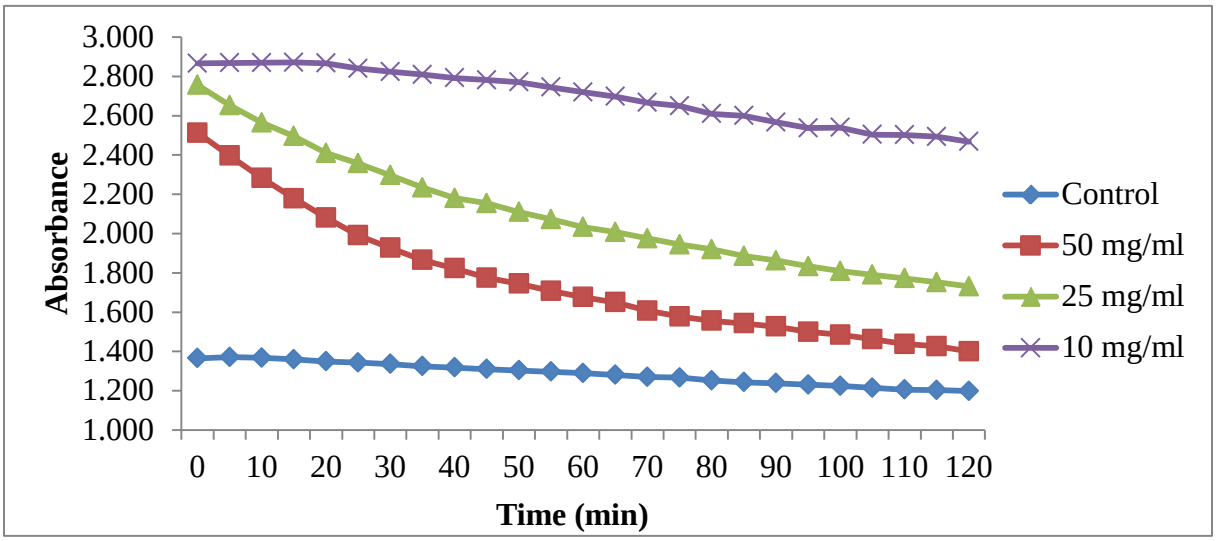
*Duplicate readings for sample were performed for mean calculations. Standard deviations were calculated based on the two sample readings for evaluation of significance. Mean GAE between 10 and 50mg/mL sample was calculated to be 0.00943±0.00023 mg GAE /mg or 9.43±0.23 mg GAE /g extract

Figure 2: mg of GAE per mg of Dendrobium crude Acetone extract in different concentrations of 2mg/mL, 5mg/mL, 10mg/mL, 25mg/mL and 50mg/mL for TPC assay



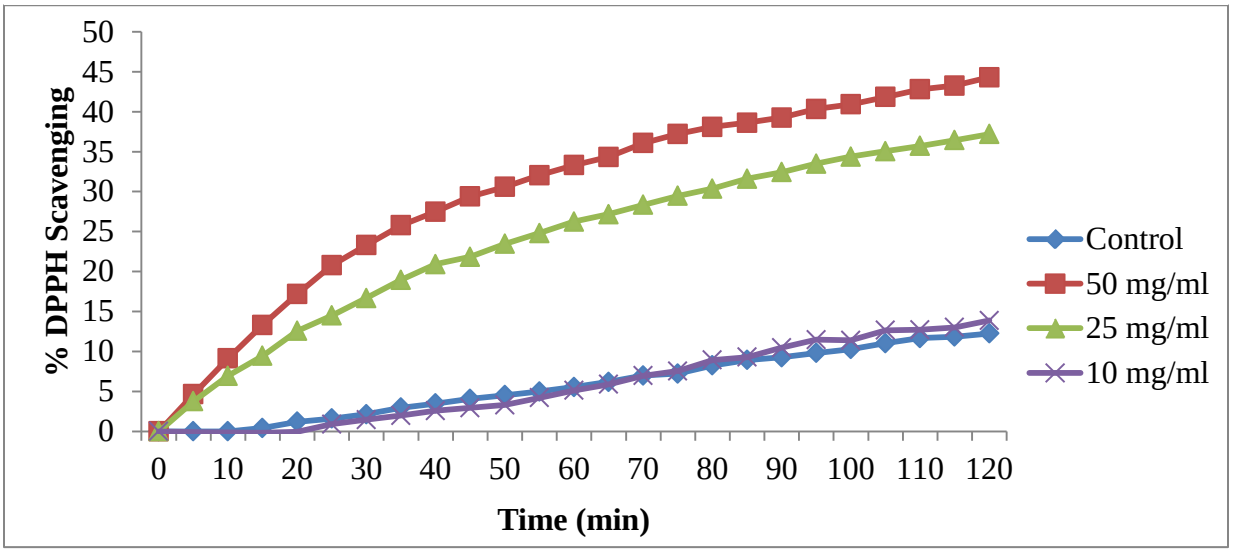
* Error bars consist of SD values from **Table 2**

Figure 3: Adjusted DPPH• concentration level based on Dendrobium extract reaction over 2-hour period at 10, 25, and 50mg/mL concentrations



Sample control values showed steady decrease with no significant difrerence (p =0.105).All three concentrations showed significant decrease from initial reaction time to 120 minute mark (p <0.05).

Figure 4: DPPH• percentage scavenging capacity of Dendrobium antioxidant presented in extract at 10, 25, and 50mg/mL concentrations



Conversion of DPPH• concentrations from **Figure 3** had same significance in differences for all three samples.

Figure 5: ORAC FL readings for Trolox standards over 90 minutes period

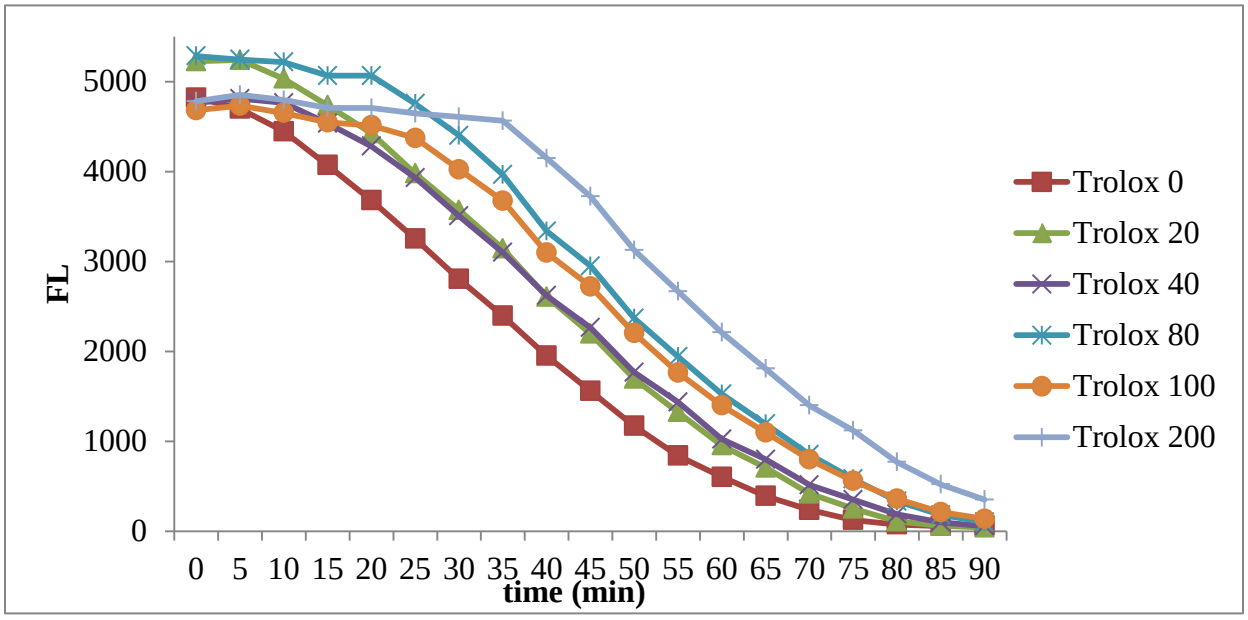
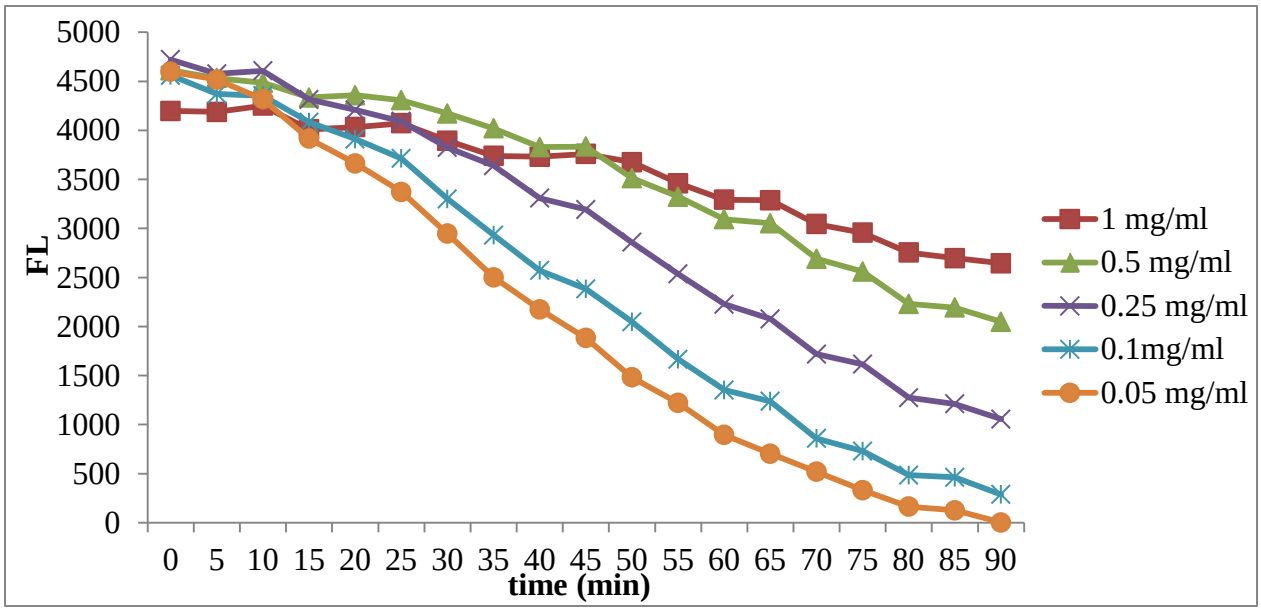
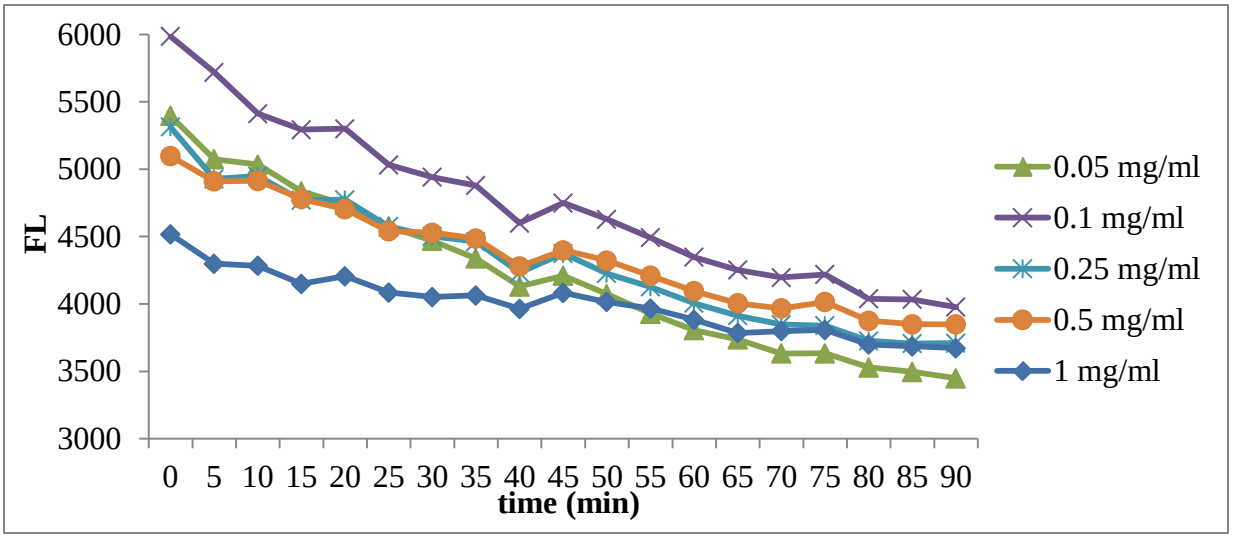


Figure 6: ORAC plot expressed as FL readings for DO Acetone fraction in reaction with AAPH radicals for sample solutions at 0.05, 0.1, 0.25, 0.5 and 1.0mg/mL



Statistical analysis indicated that all concentrations had significant decreases from the beginning to the 90 minute mark. ($p < 0.05$)

Figure 7: ORAC plot expressed as FL for DO Ethanol fraction in reaction with AAPH radicals for sample solutions at 1.0, 0.5, 0.25, 0.1 and 0.05mg/mL



Statistical analysis indicated that 1mg/mL sample did not have significant change in terms of FL readings ($p = 0.075$). All other concentrations had significant decreases from initial readings to the end points. ($p < 0.05$)

Table 3: Trolox Standards mean net AUC values based on FL readings for ORAC assay

Trolox concentrations (µM)	Mean net AUC	SD*
0	0	±0
20	1.010	±0.0571
40	1.676	±0.0057
80	2.541	±0.1096
100	2.846	±0.0348
200	4.694	±0.4726

*SD values were obtained based on the calculations from duplicate FL readings

Figure 8: Trolox stand curve with concentrations of 0, 20, 40, 80, 100 and 200µM for calculating TE values in sample for ORAC assay

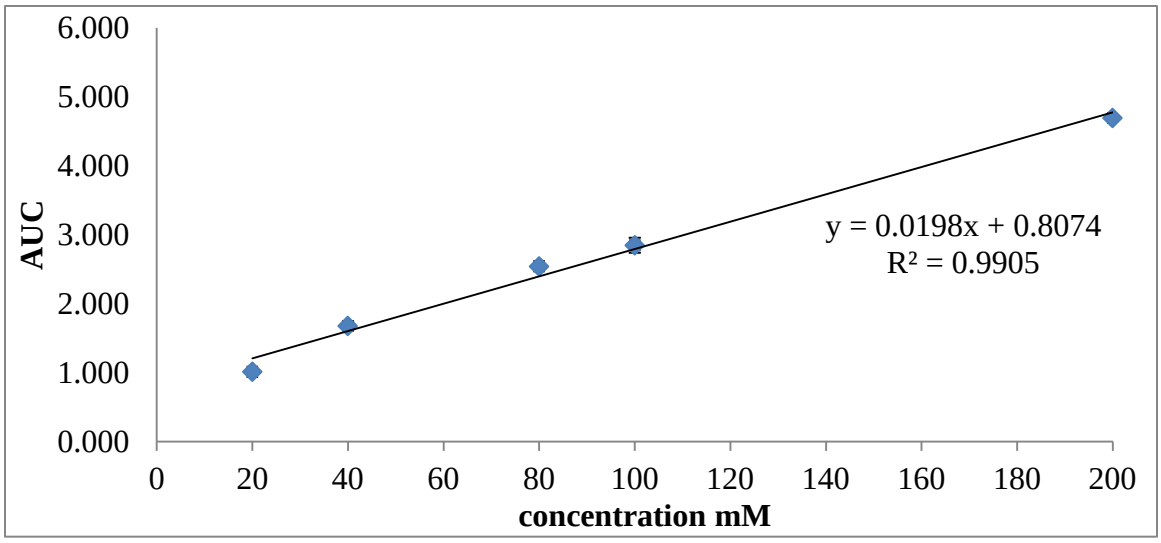
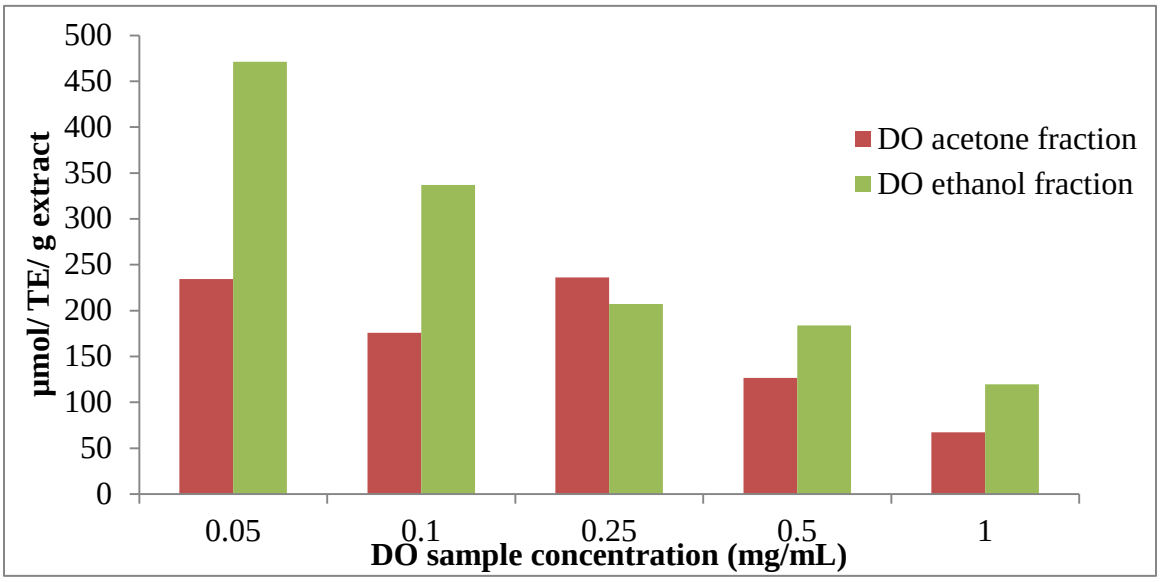


Table 4: Calculated mean TE values from ORAC assay for DO acetone and ethanol fraction with respective concentrations

Sample concentration	**Mean µmol/ TE/ g extract**	
Mg/mL	**DO acetone fraction**	**DO ethanol fraction**
0.05	234.597	471.319
0.1	175.816	336.937
0.25	236.314	207.213
0.5	126.831	183.691
1	67.301	119.577
mean*	193.39±51.2	299.79±99.3

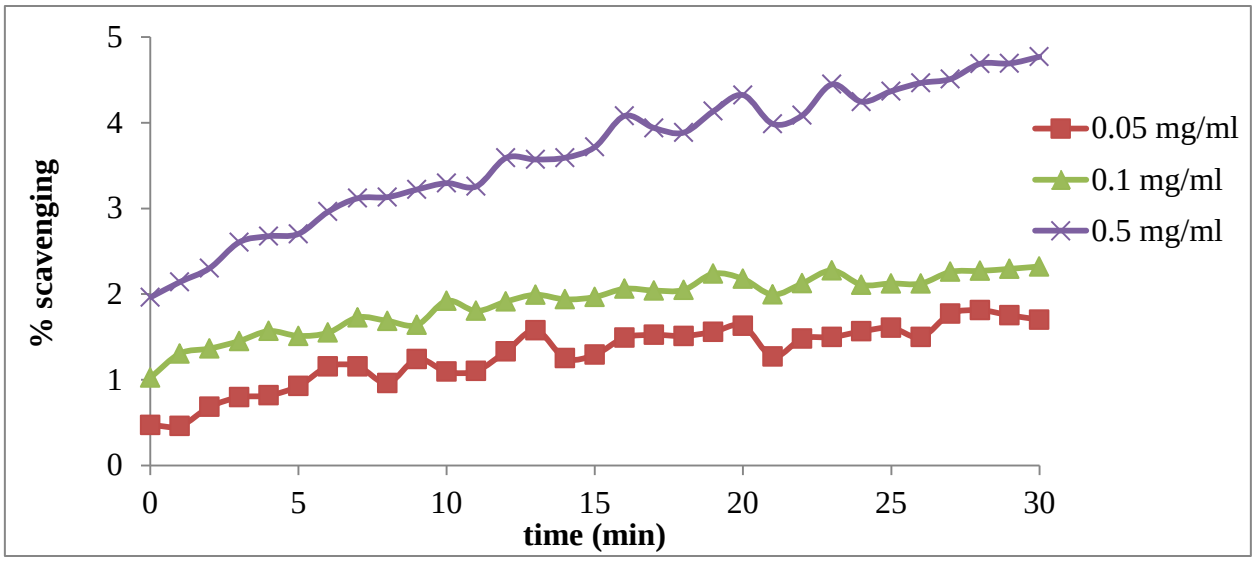
* Mean TE concentrations were calculated without values from 1mg/mL due to the non-significant changes in FL readings from **Figure 6** and **Figure 7**. Calculated values were expressed as mean ± SEM from duplicate easements.

Figure 9: ORAC TE values comparison between DO acetone and ethanolic extracts for each individual concentration, range from 0.05mg/mL to 1mg/mL



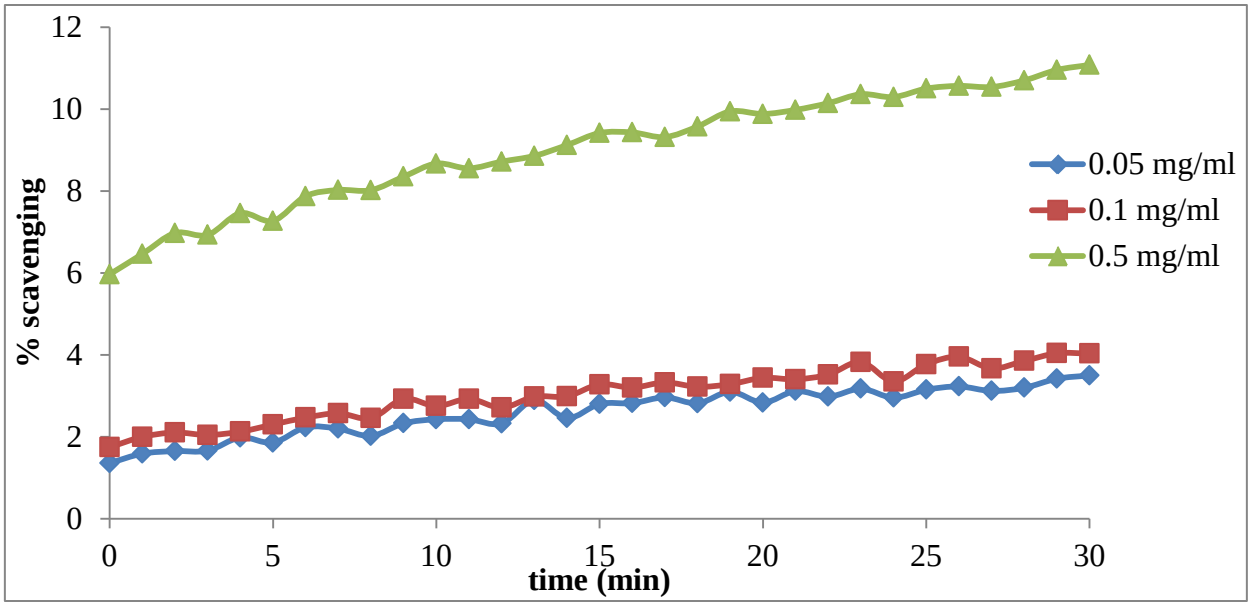
Comparing two sets of data, DO ethanol fraction had significantly higher concentrations of TE than acetone extracts. ($p < 0.05$)

Figure 10: ABTS•$^{+}$ percentage scavenging capacity for DO Acetone fraction at concentrations of 0.05, 0.1, 0.5 mg/mL.



Assay results indicated a 1.70%, 2.32% and 4.77% scavenging capacity for 0.05, 0.1 and 0.5mg/mL respectively.

Figure 11: ABTS•+ percentage scavenging capacity for DO Ethanol fraction at concentrations of 0.05, 0.1, 0.5 mg/mL.



Assay results indicated an 11.1%, 4.03% and 3.50% scavenging capacity for 0.5, 0.1 and 0.05mg/mL respectively.

Table 5: Trolox standards absorbance values and calculations for ABTS assay

Trolox Standards (µM)	Mean Absorbance	SD*	scavenging%
0	1.5819	±0.00343	0.00
20	1.5288	±0.00351	3.36
40	1.4911	±0.00261	5.74
80	1.4357	±0.00303	9.24
100	1.3964	±0.00510	11.73
200	1.2679	±0.00591	19.85
500	0.7448	±0.00444	52.92

* SD values based on 30 readings over 30 minutes of absorbance measurement

Figure 12: Standard curve with slope equation for Trolox scavenging power at different concentrations 0, 20, 40, 80, 100, 200 and 500µM for ABTS assay

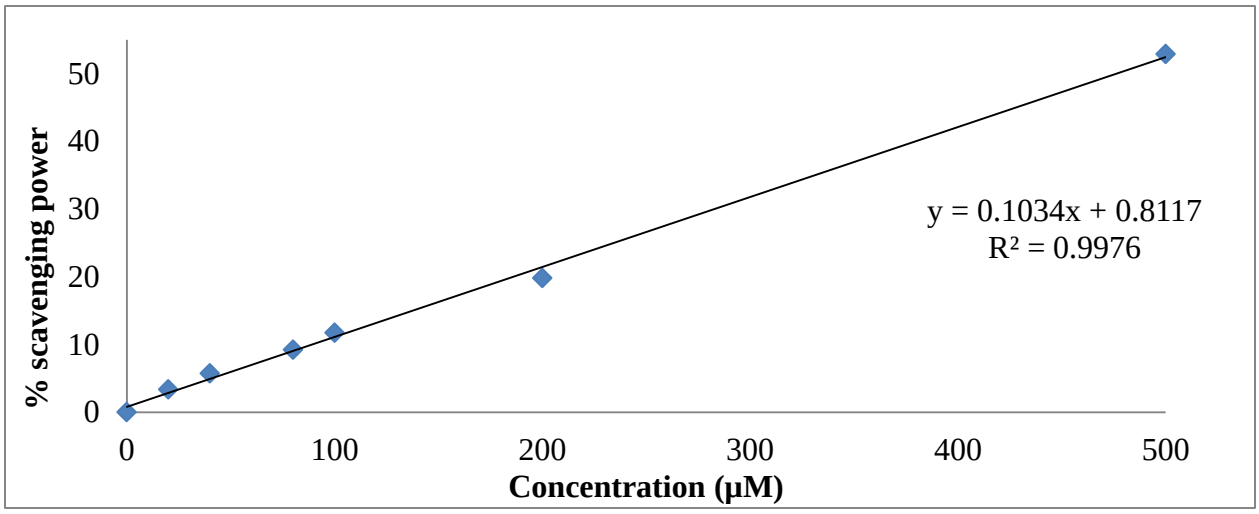
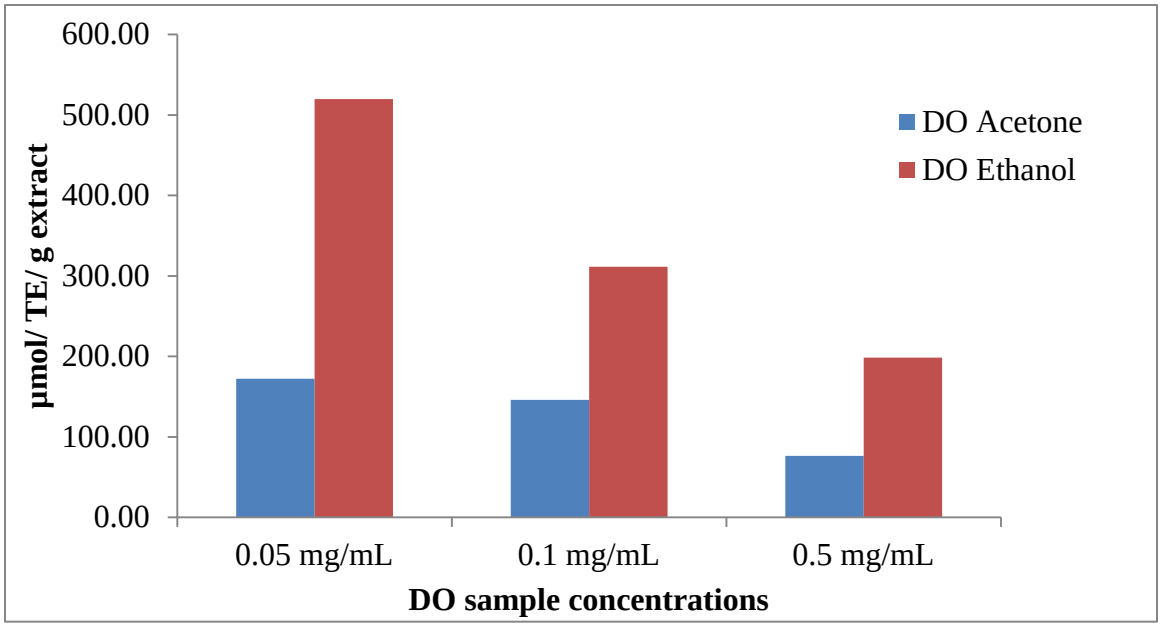


Table 6: Calculated mean TE values from ABTS assay for DO acetone and ethanol fraction with respective concentrations

Sample concentration	**Mean µmol/ TE/ g extract**	
Mg/mL	**DO acetone fraction**	**DO ethanol fraction**
0.05	172.26	519.74
0.1	146.14	311.43
0.5	76.544	198.55
mean*	131.65±28.6	343.24±94.1

* Mean TE concentrations were calculated based on Trolox standards from **Table 5** and **Figure 12**. Calculated values were expressed as mean ± SEM from duplicate easements.

Figure 13: ABTS Assay TE values comparison between DO acetone and ethanolic extracts for each individual concentration: 0.05mg/mL 0.1mg/mL and 0.5mg/mL



Comparing two sets of data, DO ethanol fraction had significantly higher concentrations of TE than acetone extract samples. ($p < 0.05$)

Table 7: Analyzed results of TE values for DO acetone and ethanolic extracts from ORAC and ABTS assay for correlation evaluation

	µmol TE / g of extract	
	ORAC	**ABTS**
DO acetone faction	193.39±51.2	131.65±28.6
DO ethanol fraction	299.79±99.3	343.24±94.1

TE values were calculated from mean concentrations and expressed as Mean ± SEM; Ethanol fraction appeared to have significant higher TE AOC in both ORAC and ABTS assays compare to acetone fraction. ($p < 0.05$)

Correlation studies was done in Microsoft Excel where R^2 for DO acetone fraction is 0.953 and R^2 for ethanol fraction is 0.9785, which both indicated good correlation between two methods.

Figure 14: Comparison between TE analysis by ORAC and ABTS•$^{+}$ assay for DO acetone fraction and ethanol fraction.

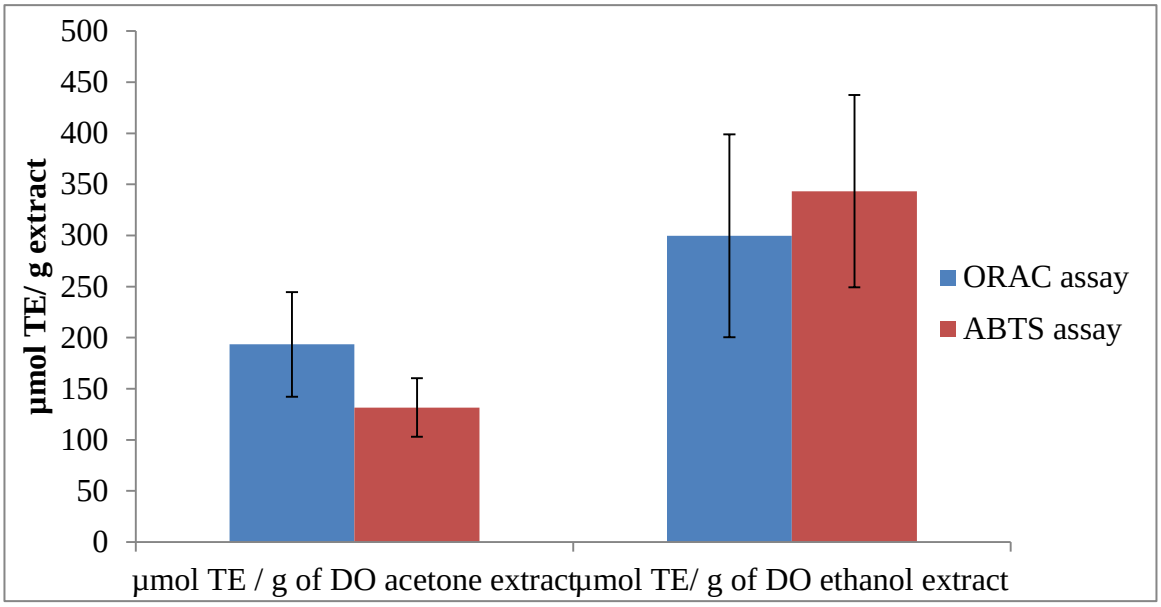


Illustration was based on **Table 7** information. Statistical analysis resulted SEM as error values and there was no significant difference between two assay results corresponding to each fraction. Acetone: (p=0.564), Ethanol: (p=0.720)

Table 8: Glucose standards absorbance measurements at concentrations of 0, 50, 100, 200, 400 and 500 mg/dL for plotting standard curve

Standard: [glucose] mg/dL	Mean Absorbance	Adjusted Mean Absorbance*	SD**
0	0.0453	0.0000	±0.0000
50	0.0758	0.0305	±0.0052
100	0.1202	0.0749	±0.0040
200	0.3025	0.2572	±0.0134
400	0.5050	0.4597	±0.0151
500	0.7506	0.7053	±0.0095

* Adjusted Mean Absorbance was calculated based on the subtraction of mean background readings of duplicated values for glucose at 0mg/mL (blank).

**SD calculated based on duplicate readings of glucose standards

Figure 15: Standard curve plot of Glucose at different concentrations for subsequent calculations of sample glucose production and α-glucosidase inhibition

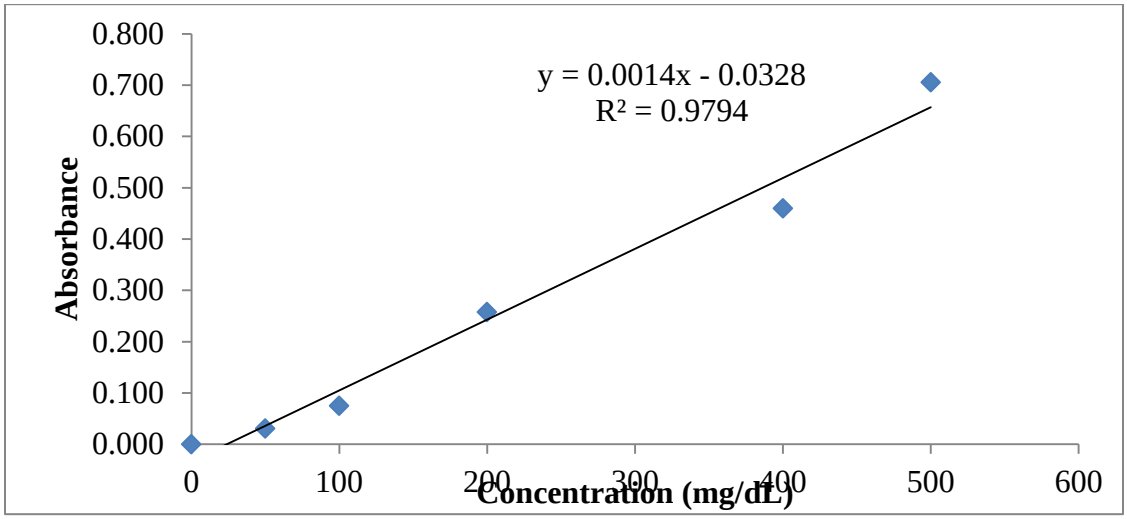


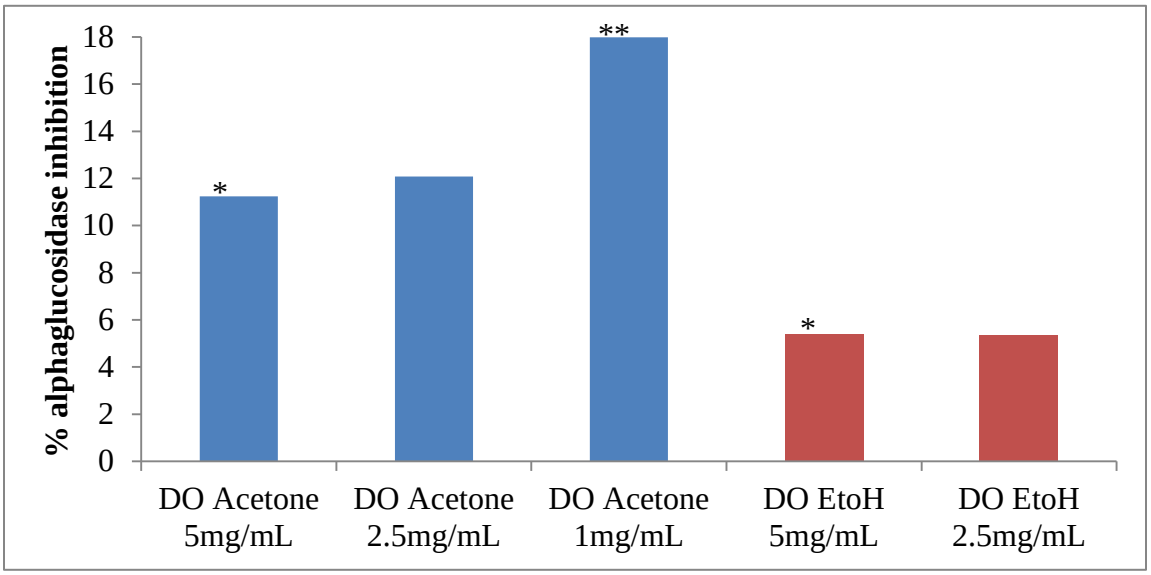
Table 9: Percentage α-glucosidase inhibition for DO Acetone and Ethanol (EtOH) fractions based on glucose concentration mean values and absorbance mean values

Sample	Adjusted Mean Absorbance*	Calculated Glucose Concentration (mg/dL)	% α-glucosidase inhibition (concentration)	% α-glucosidase inhibition (absorbance)
- control (PB)	0.1851±0.0062	155.643	N/A	N/A
+ control (Acarbose)	0.0622±0.0018	67.821	56.425	66.424
- control (25% Acet)**	0.1857±0.0101	156.036	N/A	N/A
DO Acetone 5mg/mL	0.1611±0.0013	138.500	11.238	13.224
DO Acetone 2.5mg/mL	0.1593±0.0022	137.179	12.085	14.220
DO Acetone 1mg/mL	0.1464±0.0052	127.964	17.990	21.169
- control (20% EtOH)**	0.1839±0.0071	154.786	N/A	N/A
DO EtOH 5mg/mL	0.17230±0.0067	146.464	5.376	6.335
DO EtOH 2.5mg/mL	0.1723±0.0099	146.500	5.353	6.308

* SD values were calculated based on duplicate measurements for each control/sample

** DO acetone fraction results were calculated based on 25% acetone absorbance, ethanol fraction was based on 20% ethanol absorbance values as negative control.

Figure 16: Bar graph for illustrating percentage α-glucosidase inhibition differences between DO acetone and ethanol fractions in various concentrations.



* Available sample concentrations of 5mg/mL and 2.5mg/mL for both Acetone and EtOH had significant differences in % inhibition values between the two groups. (p <0.05)

** Due to unknown error, only acetone fraction was able to obtain value for 1mg/mL, ethanol fraction had an outlier of negative value, thus eliminated.

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ABSTRACT

COMPARITIVE STUDY ON ANTIOXIDANT PROPERTIES OF *DENDROBIUM OFFICINALE* (TIEPISHIHU)

by

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For ages, Chinese herbs, such as Dendrobium Officinale (DO), have been used in Asian regions for treating various illnesses due to the high medicinal value. In this study, the DO plant Acetone and Ethanolic extracts possibly containing antioxidants were tested using different methodologies for comparison and determination of specific antioxidant capacity. The total phenolic content assay expressed as Gallic Acid Equivalent showed 9.43±0.23 mg GAE /g of extract. Further analysis using DPPH• assay indicated that antioxidants presented in the sample were able to significantly scavenge 37% and 44% radicals at the concentrations of 25mg/ml and 50mg/mL respectively, after a period of 2 hours ($p<0.05$). ORAC and ABTS assays showed consistent radical scavenging results that Ethanol extracted DO sample had significantly higher AOC than the Acetone fraction. In addition, α-glucosidase assay was included to test efficacy in postprandial hyperglycemic condition, where the result indicated a significantly higher inhibitory effect by DO Acetone fraction compare to Ethanol fraction, 11~18% vs. 5.4% ($p<0.05$). Although DO did not contain significantly high level of phenolic antioxidants, additional researches on different purified fractions such as polysaccharide compounds including

evaluations of possible synergistic effects would be part of prospective experiments to show possible improvement on antioxidant capacity.