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A Comparison ofAntioxidant Potential,theTotal Phenolicand Flavonoid Content of Male and Female (Ficus deltoidea)

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***Abstract*—This study aimed to evaluate a comparison of antioxidant activity, the total phenolic and flavonoid content of the female and male leaves of Ficus deltoidea (Mas Cotek.).This plant Ficus deltoidea of Moraceae family is one of the public plant very famous in Malaysia. The antioxidant activity was determined using free radical scavenging activity measured by DPPH 1, 1-diphenyl-2-picrylydrazyl and H_2O_2hydrogen peroxide scavenging assay, can be compared to Ascorbic acid as a standard. The total phenolic and flavonoid content were then compared to determine which one has a higher amount of TPC and TFC. The aqueous extract of female leaves of *F. deltoidea* is at IC_{50}= 29 µg/mL in DPPH assay compared to male ones at IC_{50} = 40.1 µg/mL and 20 µg/mL for Vitamin C. H_2O_2scavenging activity leads to female leaves of *F. deltoidea* at IC_{50} = 29 µg/mL, extracts showed ahigh antioxidant activity in DPPH and H_2O_2 scavenging assay than male leaves at IC_{50} H_2O_2 = 40 µg/mL when compared to Vit.C at IC_{50} = 23 µg/mL. Beside that showed higher inhibition after Vit. C= 90.2, 80.72 for female and 68.7 for male in DPPH. While for H_2O_2 Vit.C =83.1,female76.5and male 70.3.The result for TPC female leaves was 155.33 ± 0.15; and that for male leaves was 78.73 µg/mL Gallic acid equivalent/g. Meanwhile, TFC in female leaves was 44.11; and that in male leaves was 24.94 ± 0.01µg/mg quercetin equivalent/g. This study showed the female leaves of *F.deltoidesa* species has higher antioxidant activity, higher flavonoid and phenolic content.**

Keywords—antioxidant activity; Hydrogen peroxide scavenging activity; Total phenol; Total Flavonoid; Ascorbic acid

I. INTRODUCTION

Unstable molecules or strong reactive compounds are produced internally in the body during the metabolic functions and externally from the surroundings due to contamination, alcohol consumption and cigarette smoke. The body produces internal protection systems its own internal protection system to keep them at an acceptable level, minimizing unstable molecules and stop them before they can disrupt living cells in our body. However, the excessive oxidants or free radicals can cause cell damage and lead to chronic diseases [1]. Previous studies showed that diets with a high level of antioxidants source could prevent diseases and inflammation such as skin infection, high blood pressure, atherosclerosis and heart disease. Antioxidants enhance the functions ofimmune system and may even delay aging[2].

Ficus deltoidea, known as "Mas Cotek", is one of the famous herbal plants in Malaysia. This plant is also distributed in Africa, Indonesia, and Philippines [3]. F. *deltoidea* of *Moraceae* family is one of the public plants to have been used among the Malays. Indeed, *F. deltoidea* is used for medicinal purposes and for various diseases in the Malay Archipelago. It is also used as capsules and tea throughout Malaysia. Women who have just escaped situations like post-partum are given *F.deltoidea.* boiled and decocted, this is primarily because there is belief that this is helps the women in terms of uterus and vaginal muscle strengths and is also a great way to enhance the circulation of bloods that is going on in and around the body. There have also been its greater uses seen with respect to treating menstrual cycle disorders etc [4].

This type of plant has two groups: female and male. Each group has a certain and unique countenance which makes them distinguished from each other. For example, the male leaves are long, thin and have two or more red spots on the surface; whereas the female leaves are bigger and thicker, and have many black spots on the leaf surface. Besides that, female leaves lines can be clearly seen than those of the male ones [5]. *F.deltoidea* has a variety of effective ingredients such as flavonoids, phenolics, terpenoids and various antioxidant enzymes. The role flavonoids play in our lives, especially with respect to our health is important to note. They help in the creation of foods derived from consumption plants, preventing diseases related to degeneration and oxidative stress [6].They are usually known for their antioxidant activity and help the body maintain its levels of anti inflammation, blood circulation and protecting the estrogen and liver.

The existence of these active compounds in herbs and medicinal plants is a main source for the antioxidant. Therefore, they can be used as chemopreventive [7]. The main objective of the present study was therefore to compare the

total phenols, flavonoids and antioxidant activities in the leaves of this plant in order to determine their pharmacological properties for future studies.

II. EXPERIMENTAL

A. Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma, USA), H_2O_2 (Bendosen), Ascorbic acid, methanol (HmbG) Gallic acid, sodium nitrite, aluminium chloride, sodium hydroxide, sodium carbonate, Folin-Ciocalteu's reagent, Quercetin (Sigma–Aldrich GmbH, Sternheim, Germany).

B. Plant Material and Extraction

F. deltoidea was collected from a covered greenhouse garden of School of Bioprocess Engineering and cleaned through the running tap water to remove debris and contamination. The leaves were classified into two groups: male and female plants as shown in Figure 1 and 2 respectively. Each group was dried at room temperature for two weeks. The dried leaves were grounded. Each 100 gm was then mixed with 200 ml of methanol. Distilled water (60% v\v) was put in a beaker and covered with aluminum foil at ambient temperature for 24 h and shaken during the extraction time. The extracts were filtered through What man filter paperNo.1. Then, the solvent was removed from samples using a rotary evaporator (Switzerland). Finally, the extract was placed in airtight amber bottles and stored in a freezer to prevent the oxidation damage until further use.

Fig. 1. Male Mas Cotek leave

Fig. 2. Female Mas Cotek leave

III. ANTIOXIDANT TEST

A. Free Radical Scavenging Assay (DPPH)

Free radical scavenging assay was measured using DPPH free radical test. The primary absorbance of DPPH in methanol was measured using spectrometer at 517 nm until the absorbance remained unchanged. A total number of (20-100) µl of the extract was added to 3 ml of 0.1 mM methanolic DPPH solution. When completed, the mixture was incubated at room temperature for 30 min or less than 30 min before the change, and then absorbance was measured at 517 nm by Shimadzu uv-vis spectrometer [8].

Percentage of inhibition (%) = [(Ac of control-As of sample)/Ac of control] x100.

Ac: the absorbance of the control.

As: the absorbance of the sample.

B. Hydrogen peroxide radical scavenging assay

The solution of hydrogen peroxide (40mM) was prepared in phosphate buffer at pH 7.4. The concentration of H_2O_2wasdetermined by absorbance at 230 nm using a spectrophotometer. Extracts (20-100 µg/ml) in distilled water was added to H_2O_2 solution at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as the standard [9] . The percentage of inhibition (%) = [(Ac of control-As of sample)/Ac of control] x100.

Ac: the absorbance of the control.

As: the absorbance of the sample

C .The determination of phenolic content

The total phenolic content (TPC) was specified by uv-vis spectrophotometry, Gallic acid used as a standard, following the method of Singleton and Rossi with a simple deviation [10]. 0.5 mL of the diluted sample extract was added into the tubes containing 1.0 mL of dilution of Folin-Ciocalteu's reagent in water. Ten minutes later, 0.8 mL of a sodium

carbonate solution (7.5% w/v) was added to the sample. The tubes were then kept at room temperature for 30 min before absorbance at 743 nm was measured using TECAN Multi-mode micro plate reader Model Infinite® 200 (Mannedorf, Switzerland). The TPC was indicated as Gallic acid equivalents at (GAE) in mg/100 mL; the calibration curve of Gallic acid was illustrated in *Fig. 1*. The concentration of polyphenols in samples was taken from a standard curve of Gallic acid.

D.The Determination of Flavonoid content

This method was used for the assessment of the total flavonoids [11,12]. 0.5 ml solution of plant extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The mixture was kept at room temperature for 30 min. Then, the absorbance of the reaction mixture was measured at 415 nm using spectrophotometer (Perkin Lambda 45). Standard calibration curve was produced using quercetin as a reference standard for this study; the calibration curve of quercetin was shown in *Fig. 2*. Stock solution of quercetin was made by dissolving 10 mg in methanol and transferred to volumetric flask and completed the volume to 10 ml, After that, serial dilution was prepared to make concentration at (10-100 µg/ml) in methanol.

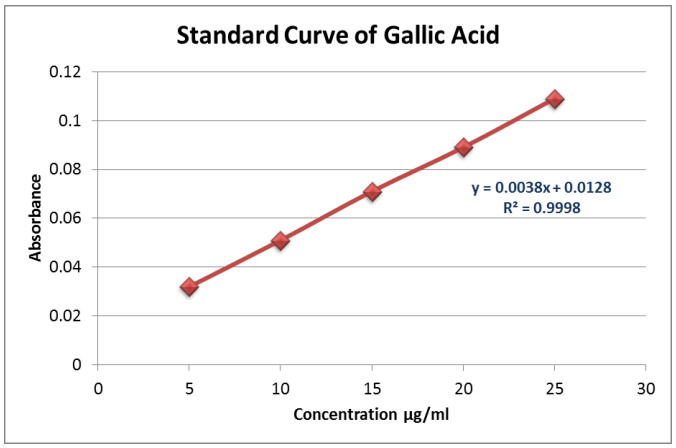

Fig. 3. Standard Curve of Gallic Acid

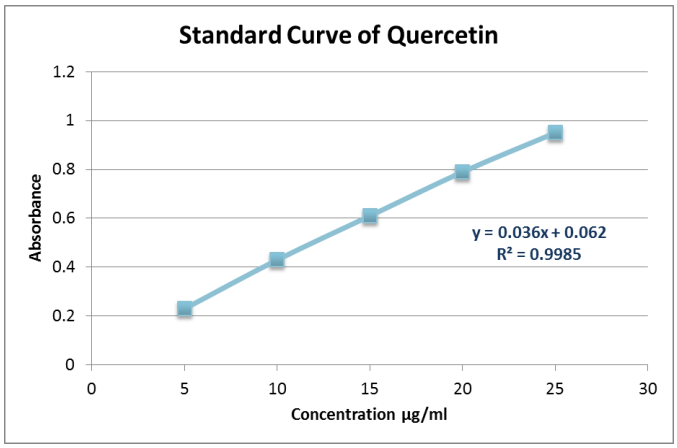

Fig. 4. Standard curve of Quercetin acid

IV. RESULTS AND DISCUSSIONS

During normal physiological which occur inside the human body process formation reactive oxygen species (ROS)and radicals, an unbalance between the defense system and oxidative leads to the accumulation of ROS, which results in the destruction of proteins, lipids , carbohydrates and DNA, and then leads to disorders and diseases. The body was protected from ROS and free radicals by antioxidants which prevent the chronic disease progression. An antioxidant is defined as any substance that significantly delays or inhibits the oxidation of a substance when it is present at low concentrations compared to that of substrate[13].

In this study, the antioxidant activities of female and male leaves of *F. deltoidea* extracts were determined by DPPH radical scavenging assay. All the results obtained from Ficus deltoidea leaves extracts for female and male were compared with Vit.C as the standard references.

Extracts showed a gradual increase in activity with an increase in the concentration for the two leaves, but the extract of female leaves showed a higher activity than that of male leaves. In this study, the highest DPPH scavenging activity was showen 80.72% at 100 µg/mL (IC_{50} 29 µg/mL), followed by male leaves extract 68% at 100µg/mL (IC_{50} 40.1 µg/mL) compared to vit. C 90.2% at 100 µg/mL showed inhibition activity and with(IC_{50} 20 µg/mL). The results of DPPH in female leaves were stronger than those in male leaves extracts at all concentrations. The DPPH method allows a direct measurement of the extract in the antioxidant to donate hydrogen or electrons to overcome the DPPH radical. Hydrogen peroxide is sometimes, causes cell toxic and damage due to the hydroxyl radical, which can accumulate in the cells. Therefore, it is very important to remove H_2O_2 by internal antioxidant or externally from the food system.

H_2O_2 scavenging assay at all concentrations results showed that the extract of female leaves had a higher inhibition activity than that of male leaves. This activity increases when the concentration of extract increases. At 100 µg/mL, the concentration of female leave inhibition was approximately 80.72%. The male leaves at 100 µg/mL showed scavenging activity with approximately 68.7% but lower than female leaves of *F. deltoidea* compared with ascorbic acid as standard at 100 µg/mL 90.2%,as shown in Figures 3and 4. The TPC in female leaves was 155.33 ± 0.15 µg /mg equivalent/g Gallic acid and that in male leaves was 78.73 µg /mg. Flavonoid in female was 44.33 ± 0.06 µg /mg quercetin equivalent/g, but TPC in the male leaves was 24.94 ± 0.01 µg /mg quercetin equivalent/g. The scavenging of H_2O_2 and DPPH by extracts due to their phenolics and flavonoid compounds [14] can donate electrons. The ability of the extracts to scavenge hydrogen peroxide compared with ascorbic acid as standard is very effective. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. IC_{50} values for scavenging of H_2O_2 for female leaves was 29 µg/ml, and 40 µg/ml for male leaves. The IC_{50} values for

vitamin C was 23 µg/ml. The studies demonstrated a correlation between phenol and flavonoid content and antioxidant capacity [15].

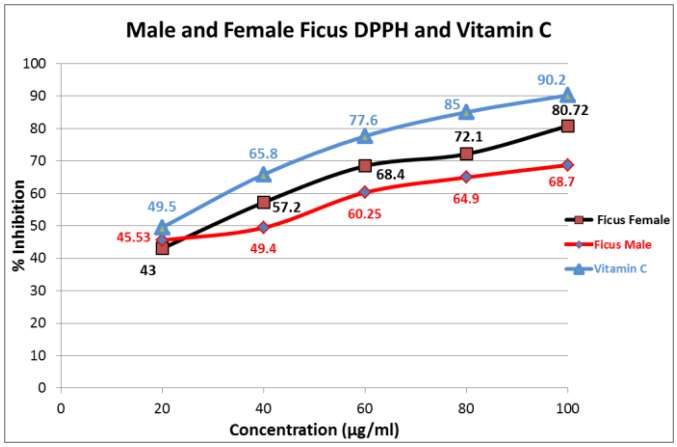

Fig. 5. Graphical representation of anti-oxidant activities (DPPH) of Ficus deltoidea (Mas Cotek.) female and male leaves.

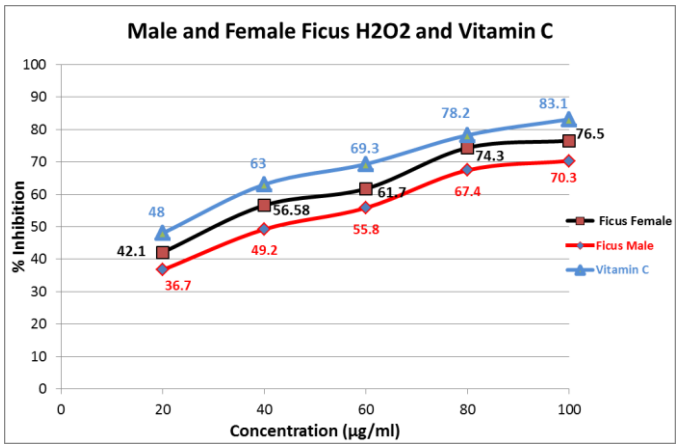

Fig. 6. Graphical representation of anti-oxidant activities (H_2O_2) of Ficus deltoidea (Mas Cotek.) female and male leaves.

A class of active ingredient compounds Phenolic act as natural antioxidant and vital activities may involve their abilities to inhibit lipoxygenase, metals chelation and scavenge free radical [16]. Phenols can combine with protein and precipitate medicinally in hemostatic, antidiarrheal, antihemorrhoidal compounds.Flavonoid is one of fundamental chemical compounds of F. deltoidea that gives it yellow pigmentation. Studies confirmed that any herbs containing flavonoid have the ability to acts as anti-inflammatory, anti-allergy, anti-cancer and anti-microbial agents, so this explains that how the plant protect itself from insects and microorganisms [17,18]. Flavonoids besidesthe antioxidant supplementation can block NF-KB (nuclear factor kappa-light-chain enhancer of activated B cells) activation as well as inhibit NF-KB activity. Since NF-KB is responsible to cancer and inflammatory, thus it indirectly plays important role to inhibit cancer and inflammatory through mechanisms distinct from redox regulation[19,20].The total phenolic was measured with the Folin-Ciocalteut Reagent. A standard compound was used in this experiment Gallic acid and the total phenolics was indicated as µg /g gallic acid equivalent using the standard curve equation as follows: y = 0.0038x + 0.0128, R^2= 0.999,where y is absorbance at 743 nm, and x is the total phenolic content in the extracts of F.deltoideafemale and male leaves indicatedby µg/gm. The higher phenolic content was found in the aqueous extract of female leaves.The total content of flavonoid was specified with the quercetin reagent. Quercetin was used as a standard compound, and the total flavonoid was indicated as mg/g Quercetin equivalent using absorbance at 415nm, and x is the total flavonoid in the extracts of female and male indicated by µg/gm. Table1 shows the content of the total Flavonoid and phenol.The flavonoid content was determined by $AlCl_3$ reagent from Quercetin acid equivalent. The total flavonoid of the extract gained from the ficus leaves was calculated from the equation of the standard Absorbance = 0.036× + 0.062 R^2 = 0.9985 varied from 44.33± 0.06 to 24.94±0.01 µg /mg quercetin equivalent/g, as shown in Table 1.The extract of female leaves, which contain the highest amount of flavonoid and phenolic compounds, exhibited greate antioxidant activity than male leaves. The activities of leaves of *F.deltoidea* plant as a result ofthe accumulation of the flavonoids and phenolic compounds which have ability to act as natural antioxidants,whichexist naturally in vegetables, fruits and seeds [21].It contained highe amount of phenolic and flavonoid; therefore, it can be used to determinenew medicines for various inflammation and diseases.

TABLE I. TOTAL FLAVONOID AND PHENOLIC CONTENT IN FEMALE AND MALE LEAVES OF *FICUS DELTOIDEA* STYLES

Different Plant Extracts (mg\ml)	Total Flavonoid (µg /mg)	Total phenol (µg /mg)
female	44.33 ± 0.06	155.33 ± 0.1 5
male	24.94 ± 0.01	78.73

V. CONCLUSION

Both antioxidant activities test were higher in female of *F.deltoidea*. There was an increase in antioxidant activity due to the flavonoid, and Phenolic content. Hence, there is a need to conduct further studies on the anti-oxidants activities which were found in the extracts of this plant. The findings of this study are also supported by previous studies. Where it showed that the plant contains high antioxidant properties. Therefore, active ingredient should be isolated from a female leave of *F.deltoidea* and needs further evaluation.

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