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# ANTIOXIDANT ACTIVITY OF WAX JAMBU

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## ABSTRACT

Free radical contributes to more than hundred disorders in human being including atherosclerosis, arthritis, respiratory injury of many tissues, cancer, AIDS. Free radicals due to the environment pollutant, chemicals and deep fried spicy food as well as physical stress cause depletion of immune system antioxidants and change in gene expression and induce abnormal proteins. Oxidation is one of most important routes for producing free radical in food, drugs and living system. Catalase and hydrogen peroxidase enzyme convert hydrogen peroxide and hydrogen peroxide in to non- radical form and function as natural antioxidant in human body. Current available synthetic antioxidants like butylated hyroxy anisole, butylated hydroxy toluene and gallic acid ester have suspended to cause or promote negative health effect. A lot of the antioxidant activity have been reported for leaves, root, bark, fruits of the plant Wax Jambu. Edible fruits of Wax Jambu represent potential benefits for human health because they are rich source of polyphenolic antioxidants. The fruits of red and pink cultivators contain the highest amounts of pigments, flavanoids compounds and antioxidant activities. Wax apple extracts thus have the potential to be used as bioactive constituents, antioxidant natural sources for the healthcare.

**KEYWORDS:** Free radical, Oxidation, Antioxidant, Wax Jambu, Flavanoids.

## INTRODUCTION

Antioxidants are any substance that inhibits oxidative damage to a target molecule. At a time one antioxidant molecule can react with single free radicals and are capable to neutralize free radicals by donating one of their own electrons, ending the carbon stealing reaction. Thus antioxidants prevent cell and tissue damage and act as scavenger. Cells produce defence against excessive free radicals by their preventative mechanisms, repair mechanisms, physical defences and antioxidant defences.[1] When an antioxidant destroys a free radical, this antioxidant itself becomes oxidized. Therefore, the antioxidant resources must be constantly restored in the body thus while in one particular system an antioxidant is effective against free radical, in other systems the same antioxidant could become ineffective. Also in certain circumstance an antioxidant may even act as pro-oxidant. eg. It can generate toxic reactive oxygen species (ROS).

The antioxidant process can function in one of two ways; Chain breaking or prevention. For the chain breaking when a radical releases or steals an electron a second radical is formed. The last one exerts the same action on another molecule and continues until either the free radical formed is stabilized by a chain breaking antioxidant (Vitamin C, E, carotenoids, etc.) or it simply disintegrates in to an in offensive product.[2]

Polyphenolic antioxidant's recent studies have shown that many flavanoids and related polyphenols are actually better antioxidants than vitamins .Fruits and vegetables are high in flavanoid content; flavanoids impart colour and taste to flowers and fruits, and it is estimated that humans consume between a few hundred milligrams and one gram of flavanoids every day. Flavanoids appear in blood plasma at pharmacologically active levels after eating flavanoid-rich foods but do not accumulate in the body. Consuming flavanoids regularly increases longevity by reducing inflammation and contributing to the amelioration of atherosclerosis from CHD. The range of flavanoid biological activity is large; in addition to scavenging free radicals and ROS, flavonoid actions include anti-inflammatory, antiallergenic, antiviral, antibacterial, antifungal, antitumor and antihemorrhagic. The anthocyanins are the most important flower and fruit pigments; they attract pollinators and seed dispersers and protect plant tissues from ultraviolet (UV) radiation damage. Some flavonoids act as antifeedants to herbivorous pests. The isoflavones are responsible for the chemical signaling involved in legumous root node formation. It is well established that the efficacy of flavonoids as antioxidants stems from the number and position of the hydroxyl substitutions on the basic structure; an increase in number of hydroxyl groups is directly correlated with increasing activity, and the 3',4' -dihydroxy substitution is significant. Anthocyanins are the glycosides of anthocyanidins, and contribute greatly to the antioxidant properties of certain fruits. As pigments, they produce the orange, red and blue colors in fruits and flowers. The antioxidant anthocyanidin that colors blueberries and grapes bluish-red is delphinidin. Other known antioxidant anthocyanins include cyanidin (orange-red), pelargonidin (orange), malvidin (bluish-red) and peonidin (red). Fruit color is therefore an important indicator of possible polyphenolic compounds.[3]

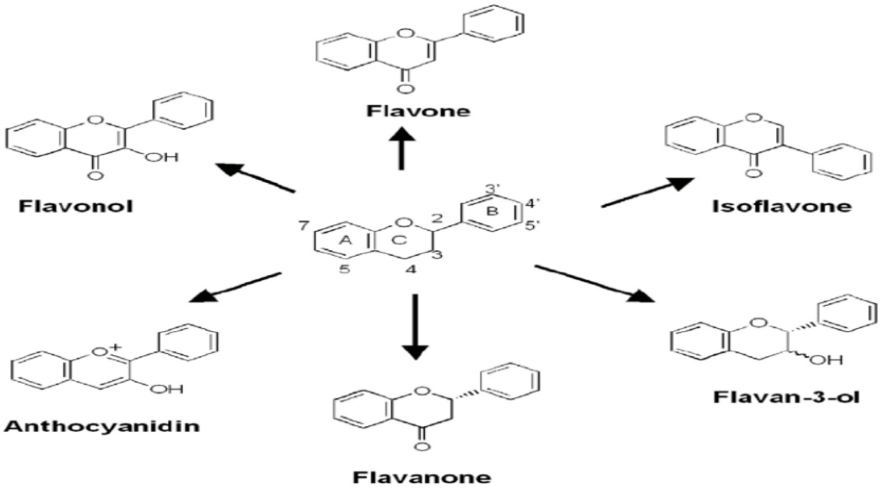


**Fig. 1: Structure of flavonoids.**

**Wax Jambu**

The wax apple is a nonclimacteric tropical fruit in the Myrtaceae family and is botanically identified as *Syzygium samarangense*. The pear-shaped fruits are usually pink, light red, or red but may be greenish-white or cream-colored, and are generally crisp, often juicy, refreshing, with a subtly sweet taste and aromatic flavour.Wax apple fruits are eaten raw with salt or cooked as a sauce.[4] The fruit is also used to add taste to salads with a faint compliment to the flavour. Wax Jambu is native to Philippines, India, Indonesia and Malaysia. Common names of the fruit include wax apple, love apple, java apple, chomphu, wax jambu, rose apple, bell fruit, makopa, tambis Philippines. The wax apple tree also grows in the Caribbean. Dominican Republic a small sub-species of the wax apple is known as cajuilito, or small cashew.[5]

**Fig. 2: Wax Jambu.**

**History**

The species presumably originated in Malaysia and other south-eastAsian countries. It is widely cultivated and grown throughout Malaysia and in neighbouring countries such Thailand, Indonesia and Taiwan. Currently in Malaysia it is cultivated mainly as small holding areas ranging from 1 to 5 hectare. In Malaysia, there is a great scope to developed wax jambu fruit industry and possible to earn huge amount of foreign capital by exporting to the other countries. According to the available records, wax apple was introduced in 17$^{th}$ century and planted in Taiwan over 300 year ago.[6]

**Cultivation, collection**

Wax Jambus need sufficient rainfall, some humidity and abundant soil for best growth. Wax jambu is a semi-deciduous tree, with height of 5-15m and width of 3-10m with yellowish-green to dark bluish-green leaves. Group of lemon-green buds appearing at the branch of the tips or in the axis of fallen leaves or point on its trunk branches will flower in 1-1.5 month's time. It contain slightly fragrant white to pale yellow color flowers with four petals.

These showy flowers bear in drooping panicles of 3 to 20 will fall within 2-3 days of blooming, leaving behind the tiny fruits to mature at the same climatic condition which ripens in about 2 months. A healthy tree will produce abundant fruits and it has two fruiting season per annum, May-September and November to March.But after the development of a technique for adjusting the production period, it advanced to December – April.[7]

**Fig. 3: Roots and flowers of the wax jambu.**

**Varieties of wax jambu**

White wax jambu: Fast growing tree in the Philippines country is white jambu, pear shaped fruit that are crunchy, tasty and stimulating on a hot summers day. Produces and crops are all well protected from frosts in subtropics when they are young.

**Red wax Jambu**

Red skin with crunchy white flesh. Highly attractive ornamental fruit, as well as sweet and tasty fruit in philippines. It's a fast growing tree with attractive red pear shaped fruits that is crunchy and refreshing on a summer day. A highly ornamental fruit due to its red colour.

**Fig. 5: Red wax Jambu.**

**Pink wax Jambu**

A popular selection and popular fruit in Asia is Wax Jambu fruit, the sw eetest selection.Pink Wax jambu fruit is very similar to that Red Wax Jambu but it has white waxy skin and is sweeter, but more cold sensitive in nature.[8]

**Fig. 6: Pink wax Jambu.**

**Nutritional value**

Wax apple fruits are crispy, juicy and tasty with apple aroma. Fruit flesh contains spongy tissue and 92.87 per cent water content and therefore, wax apple is more popular in torrid summer. The nutritional composition of wax apple fruit is given in the Table 1.[9]

**Table. 1: Nutritional Values.**

| **Nutritive value per 100 g of Wax jambu** | |
|---|---|
| **Principle** | **Nutritive value** |
| Total Fat | 0.3g |
| Cholesterol | 0 mg |
| Sodium | 0 mg |
| Carbohydrate | 5.7g |
| Magnesium, Mg | 12 mg |
| Proteins | 0.6 mg |
| Calcium | 29 mg |
| Potassium | 123 mg |
| Vitamin C | 22.0 mg |
| Riboflavin | 0.03mg |

**Chemical constituents:** Three *C*-methylated chalcones, 2′,4′-dihydroxy-3′,5′-dimethyl-6′-methoxychalcone, 2′,4′-dihydroxy-3′-methyl-6′-methoxychalcone and 2′,4′-dihydroxy-6′-methoxychalcone (cardamonin) . A number of known antioxidants are present six quercetin glycosides: reynoutrin, hyperin, myricitrin, quercitrin, quercetin and guaijaverin, one flavanone: (*S*)-pinocembrin, and two phenolic acids: gallic acid and ellagic acid.[10]

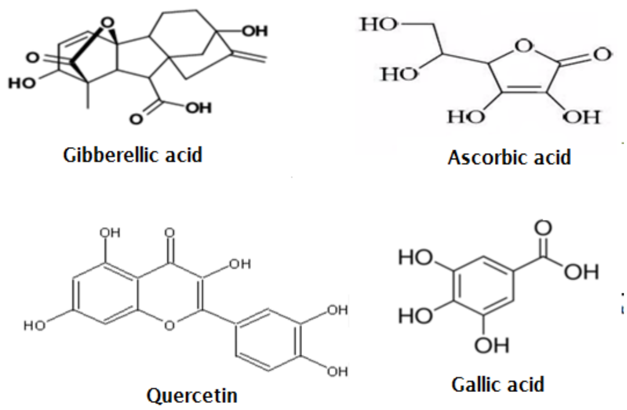


**Fig. 7: Structures of chemical constituents.**

**Uses:** Wax jambu shows antioxidant property. Wax apple contains abundant water. In Chinese medical science, wax apple fruits, leaves and seeds are antifebrile; roots are diuretic. Use of wax apple fruit,especially in summer, is beneficial in quenching thirst, releasing the sunstroke and removing harmful effects of dehydration. If used with some added salt, wax apple fruits are useful in releasing the discomfort in the intestines and stomach.[11]

**Extraction from fruit:** The fresh frozen pulp of *Syzygium samarngenese* were extracted with methyl alcohol at room temperature for 1 hr per extraction .

After the methyl alcohol was removed in vacuum, the resulting dark extract was suspended in water with hexane, ethyl acetate and n-butanol respectively. The ethyl acetate and n-butanol portions were concentrated in vacuum to give a dark brown extracts.

**Extraction from root:** Root materials of *Syzygium samarangense* plant were collected. The collected root are separated and allowed to shade dry. The root sample was grounded and powdered for solvent extraction. The phyto-chemicals present in root of the collected plant were isolated using different solvent like ethyl acetate, ethanol using soxhlet extraction method.[12]

**Isolation and purification**: Ethyl acetate ethanol fraction (1.8 g, IC50 = 54.7 µg/ml in the DPPH· assay) of the pulp was subjected to column chromatography.

**Test for flavanoids**

1. Shinoda Test: The extract solution with a few fragment of magnesium ribbon and concentrated hydrochloric acid produced magenta colour after few minutes.
2. Ferric chloride test: Alcoholic solution of extract reacts with freshly prepared ferric chloride solution and given blackish green colour.
3. Lead Acetate Test: Alcoholic solution of extract reacts with 10% lead acetate solution and given yellow precipitate.[12]

**Determination of total flavanoid content**

Total flavanoid content was determined using aluminium chloride ($AlCl_3$) according to a known method using quercetin as a standard. The plant extract (1 ml) was added to 3 ml distilled water followed by 5% $NaNO_2$ (0. 3ml). After 5 min at 25°C, $AlCl_3$ (0.3 ml, 10%) was added. After further 5 min, the reaction mixture was treated with 2.0 ml of 1m NaOH. Finally, the reaction mixture was diluted to 10ml with water and the absorbance was measured at 510 nm. A calibration curve was constructed using quercetin solutions as standard and total flavanoid content of the extract was expressed in terms of milligrams of quercetin per gram of dry weigh.[8]

**Determination of antioxidant activity**

**DPPH METHOD**

Antioxidant activity of fruit or root extract was determined on the basis of their scavenging activity of the stable 1,1- diphenyl -2- picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd election in its structure and usually utilized for detection of radical scavenging activity in chemical analysis.

1ml of solution of the extract was added to 3ml of 0.004% ethanolic DPPH free radical solution. After 30 min absorbance of the mixture were taken at 517nm by a *UV* spectrophotometer which was compared with ascorbic acid concentration.

% radicle scavenging activity = (absorbance of blank - absorbance of sample/absorbance of blank) 100

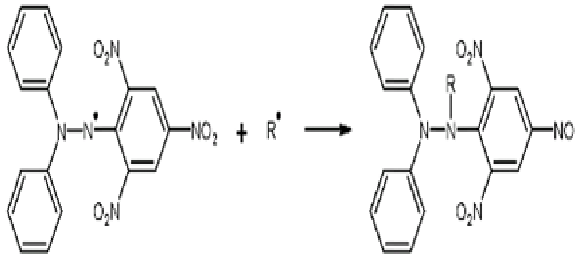


**Fig. 8: Mode of action of DPPH radical compound RH.**

**Advantage:** This technique is easy, effective, and rapid way to study plant extract profile. No sample separation is needed. Potency of sample can be known.

**Disadvantage:** Time consuming, Costly.

**FRAP METHOD**

The ferric reducing antioxidant potential (FRAP) assay was performed according to the method described by benzene and strain, direct measurement of antioxidant (reducing) ability through reduction of the complex $Fe^{3+}$ to $Fe^{2+}$ at acidic $P^H$ 3.6. It was taken at 620nm using *uv -vis* spectrophotometer during monitoring period (2hr). FRAP values were expressed in µmol trolox equivalent (TE) per g of fresh material for the extracts and µmol TE per 500µmol of pure compound.

**Advantage**

It is simple, speedy and inexpensive.

It can be performed using automated, semi -automated or manual method.

Disadvantage:

FRAP cannot detect species that act by radical quenching, particularly SH-group containing antioxidant like thiol.

**ABTS METHOD**

The ABTS cation radical which absorbs at 743nm (giving a bluish green colour) is formed by the loss of an electron by the nitrogen atom of ABTS (2,2-azino-bis(3-ethylbenthiazoline-6-sulphonic acid)).In the presence of Trolox(or of another hydrogen donating antioxidant),the nitrogen quenches the hydrogen atom yielding the solution decolourization.

ABTS can be oxidized by potassium persulphate or manganese dioxide, giving rise to the ABTS cation radical whose absorbance at 743nm was monitored in the presence Trolox chosen at standard.

PFRAP (Potassium ferricyanide reducing power method)

An absorbance increase can be correlated to the reducing ability of antioxidant. The compounds with antioxidant capacity react with ferricyanide to form potassium ferrocyanide. The latter react with trichloride, yielding ferric ferrocynide a blue coloured complex, with a maximum absorbance at 700nm.[13]

## DISCUSSION

### Physiochemical and phytochemical properties of wax apple affected by growth regulator application

The study represents the effects of growth regulators on the physiochemical and phytochemical properties of the wax apple fruit. Net photosynthesis, sucrose phosphate synthase (SPS) activity, peel color, fruit firmness, juice content, pH value, total soluble solids (TSSs) and the sugar acid ratio were all significantly increased in growth regulators (PGRs) treated fruits. The application of gibberellin ($GA_3$), naphthalene acetic acid (NAA), and 2,4-dichlorophenoxy acetic acid (2,4-D) significantly reduced titratable acidity and increased total sugar and carbohydrate content compared to the control. The 50 mg/L $GA_3$, 10 mg/L NAA and 5 mg/L 2,4-D treatments produced the greatest increases in phenol and flavonoid content; vitamin C content was also higher for these treatments. PGR treatment significantly affected chlorophyll, anthocyanin and carotene content and produced higher phenylalanine ammonia lyase (PAL) and antioxidant activity levels. There was a positive correlation between peel color and TSS and antioxidant activity and both phenol and flavonoid content and PAL activity and anthocyanin formation. A taste panel assessment was also performed and the highest scores were given to fruits that had been treated with $GA_3$ or auxin. The study showed that application of 50 mg/L $GA_3$, 10 mg/L NAA and 5 mg/L 2,4-D once a week from bud development to fruit maturation increased the physiochemical and phytochemical properties of wax apple fruits.[14]

### Antioxidant activity of root extract of *Syzygium samarangense*

Phytochemical screening of the plant root extracts with ethyle acetate, methanol and water showed the presence of flavanoids, terpenoids and phenolic compounds. Total flavanoid content was determined using aluminium chloride ($AlCl_3$) according to a known method using quercetin as a standard. Total flavanoid content of the extract was expressed in terms of milligrams of quercetin per gram of dry weight. 1 ml of each solution of different concentrations (1- 500g/m1) of the extracts was added to 3 ml of 0.004% ethanolic DPPH free radical solution. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. In aqueous extracts, Terpinoids with highest quantity (81.923 micrograms per gram extract) were estimated, whereas flavanoids are present only in methanoic extract and with an estimated quantity of 33.687 µg /per gram extract. Results reveals that methanolic extracts which consist of flavanoids shows the high antioxidant activity (88.021%) than the aqueous and ethyl acetate extracts at 40µg/ml extract concentration.[15]

### Cytotoxic chalcone and antioxidant activity from fruits of a *Syzygium samarangense*

This study reveals the antioxidant activity of fruit extract of wax jambu. The flavonoid and antioxidant property was determined by two methods DPPH and FRAP assay. On FRAP assay values are expressed in µmol TE per gram of fresh material for the extract and µmol of pure compound. DPPH assay was performed on purified isolates. Gallic acid was used as positive control and percent $IC_{50}$. Total flavonoid was determined by taking absorbance at 510nm.The result expressed as milligrams of quercetin equvalents per 100g of fresh material.[10]

### Morphology, phytochemistry and pharmacological aspect of fruit extracts of wax jambu.

Wax jambu consists of flavonoids, terpenoids. Antioxidant activity of fruit was determined by DPPH method. Antioxidant activity of fruit extract was determined according to the ability of scavenging 1,1-diphenyl -2 picryl hydrazylfree radical. The ethanolic fruit extract showed antioxidant activity. $IC_{50}$ of fruit extract was 200µpermL with respect to ascorbic acid.[16]

### Studies on bioactive constituent, antioxidant and antimicrobial activites of wax jambu

In this study, antioxidant activities of ethanolic fruits extracts of three cultivars were examined by using DPPH reducing power assays. The IC50 values of DPPH assay of these extracts were compared with that of ascorbic acid presented in table 2. The highest antioxidant strength was observed in red cultivar followed by pink cultivar while the lowest antioxidant strength was recorded in the green cultivar. We also examined the antioxidant activity of leaf, bark and fruit extracts of three cultivars of wax apple measuring by the rabbit erythrocytes haemolysis. The negative control (without pre-treatment) obtained a mean of 60% haemolysis whereas ascorbic acid could reduce the haemolysis percentage to 8% in the positive control. All plant extracts (leaf, bark and fruit) of three cultivars of wax apple examined in this study could reduce the erythrocytes haemolysis significantly when compared to negative control. The results also showed that leaf extracts of three cultivars could reduce the erythrocytes significantly than the bark and fruit extracts. Among the three cultivars, green cultivar showed the better effects to reduce the erythrocytes compared to red and pink cultivars.[17]

**Table. 2: Evaluation of antioxidant activities of fruit extracts of three cultivars of wax apple.**

| Concentration ($\mu mL^{-1}$) | Red % inhibition | Pink % inhibition | Green %inhibition | Ascorbic acid |
|---|---|---|---|---|
| 1 | 15± 1.75 | 15±1.06 | 10± 1.95 | |
| 5 | 19 ±1.40 | 17± 1.80 | 13 ±1.69 | |
| 10 | 22 ± 2.14 | 20 ±1.09 | 17± 1.12 | ------ |
| 50 | 28 ±2.53 | 21 ± 1.68 | 19 ±1.63 | |
| 100 | 31 ±3.05 | 29 ±1.22 | 24 ±1.65 | |
| 500 | 73± 5.05 | 68 ±3.49 | 64± 2.78 | |
| **$IC_{50}$** | **283** | **320** | **358** | **15.87** |

**Antioxidant and α-glucosidase inhibitory activities of 40 tropical juices from Malaysia and identification of phenolics from the bioactive fruit juices of Barringtonia racemosa and Phyllanthus acidus**.

The study compared pH, total soluble solids, vitamin C, and total phenolic contents, antioxidant activities, and α-glucosidase inhibitory activities of 40 fresh juices. The juice of Baccaurea polyneura showed the highest yield (74.17 ± 1.44%) and total soluble solids (32.83 ± 0.27 °Brix). The highest and lowest pH values were respectively measured from the juices of Dimocarpus longan (6.87 ± 0.01) and Averrhoa bilimbi (1.67 ± 0.67). The juice of Psidium guajava gave the highest total phenolic (857.24 ± 12.65 µg GAE/g sample) and vitamin C contents (590.31 ± 7.44 µg AAE/g sample). The juice of Phyllanthus acidus with moderate contents of total phenolics and vitamin C was found to exhibit the greatest scavenging (613.71 ± 2.59 µg VCEAC/g sample), reducing (2784.89 ± 3.93 µg TEAC/g sample), and α-glucosidase inhibitory activities (95.37 ± 0.15%). The juice of Barringtonia racemosa was ranked second in the activities and total phenolic content. Gallic and ellagic acids, which were quantified as the major phenolics of the respective juices, are suggested to be the main contributors to the antioxidant activities. The α-glucosidase inhibitory activity of the juices could be derived from myricetin and quercetin (that were previously reported as potent α-glucosidase inhibitors) in the hydrolyzed juice extracts. The juice of Syzygium samarangense, which was found to be highest in metal chelating activity (82.28 ± 0.10%), also was found to have these phenolics.[18]

**Antioxidant, Polygalacturonase, Pectin Methylesterase and Polyphenol Oxidase Activities of Fresh-Cut Wax Apple (*Syzygium samarangense*) Treated with Organic Acids**

The interaction between the types of organic acids used in the dipping treatment and the concentration of the acids was significantly affecting antioxidant activity. Among the three acids used, fruit treated with oxalic acid possessed the highest antioxidant activity (90.64%), followed by those treated with citric acid (86.15%) and ascorbic acid (81.17%). Compared to treatment with the other two acids, increasing concentration of ascorbic acid in the dipping solution reduced the antioxidant activity. Antioxidant activity in those treated with oxalic acid tended to increase slightly from 87.69-93.66% when the oxalic acid concentration has increased from 0-2.0%. Phytochemicals such as reynoutrin, hyperin, myricitrin, quercitrin, quercetin, guaijaverin, gallic acid and ellagic acid are the well known antioxidant compounds found in wax apple. Results of the study also show that the activity of antioxidant of fresh-cut wax apple has increased as the period of storage from 0-9 days by 13.45% ($p \leq 0.001$).[19]

**Isolation and Immunomodulatory Effect of Flavonoids from *Syzygium samarangense***

Sixteen flavonoids were isolated from the acetone extract of the leaves of *Syzygium samarangense*. The isolated flavonoids were evaluated for immunopharmacological activity. Human peripheral blood mononuclear cells (PBMC) were used as target cells, and cell proliferation was determined by *H*-thymidine uptake. Among them, (-)-strobopinin (**2**), myricetin 3-*O*-(2″-*O*-galloyl)-α-rhamnopyranoside (**8**), (-)-epigallocatechin 3-*O*-gallate (**10**) and myricetin 3-*O*-α-rhamnopyranoside (**11**) showed inhibitory potency on PBMC proliferation activated by phytohemagglutinin (PHA). The $IC_{50}$ values of compounds **2**, **8**, **10** and **11** on activated PBMC proliferation were 36.3, 11.9, 28.9 and 75.6 µM, respectively. The inhibitory mechanisms may involve the blocking of interleukin-2 (IL-2) and interferon-γ (IFN-γ) production, since compounds **2**, **8**, **10** and **11**reduced IL-2 and IFN-γ production in PBMC in a dose-dependent manner.[20]

**An Extract from Wax Apple (*Syzygium samarangense* (Blume) Merrill and Perry) Effects Glycogenesis and Glycolysis Pathways in Tumor Necrosis Factor-α-Treated FL83B Mouse Hepatocytes**

FL83B mouse hepatocytes were treated with tumor necrosis factor-α (TNF-α) to induce insulin resistance to investigate the effect of a wax apple aqueous extract (WAE) in insulin-resistant mouse hepatocytes. The uptake of 2-[*N*-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl)amino]-2-deoxyglucose (2 NBDG), a fluorescent d-glucose derivative, was performed, and the metabolism of carbohydrates was evaluated by examining the expression of glycogenesis or glycolysis-related proteins in insulin-resistant hepatocytes. The results show that WAE significantly improves the uptake of glucose and enhances glycogen content in insulin-resistant FL83B mouse hepatocytes. The results from Western blot analysis also reveal that WAE increases the expression of glycogen synthase (GS), hexokinase (HXK), glucose-

6-phosphate dehydrogenase (G6PD), phosphofructokinase (PFK) and aldolase in TNF-α treated cells, indicating that WAE may ameliorate glucose metabolism by promoting glycogen synthesis and the glycolysis pathways in insulin-resistant FL83B mouse hepatocytes.[21]

## CONCLUSION

Damaging free radicals and reactive oxygen species (ROS) are produced naturally through oxidative metabolism and have also been linked to some cancers. Damage is generally reduced by endogenous antioxidants, but additional protection is necessary, and nutritive elements from food are critical in disease prevention. Repeated damage caused by ROS throughout the span of a human life increases with time, and is a major cause of age-related cancers and other oxidatively induced diseases. Polyphenolic antioxidant's recent studies have shown that many flavanoids and related polyphenols are actually better antioxidants than vitamins. Fruits and vegetables are high in flavanoid content. The edible fruits of *Syzygium samarangense* represent potential benefits for human health because they are a rich dietary source of polyphenolic antioxidants. In addition, the seeds are a rich source of the cytotoxic chalcones, it is present in high concentration (35.0 mg per kg fresh weight).

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