**INTRODUCTION**

Natural reactive oxygen species (ROS) were produced by human body to carry out physiological functions (Noguchi and Niki, 1999). However, it also can damage some biological targets which cause oxidative stress. Oxidative stress may lead to several illnesses such as cardiovascular disease, cancer and other degenerative diseases (Noguchi and Niki, 1999). The biological antioxidant which able to delay or prevent the oxidation of other molecules (Halliwell and Gutteridge, 2000) is required as insufficient levels of antioxidants may damage or kill cells. Tyrosinase also known as monophenol monooxygenase plays an important role in synthesizing melanin (Chang, 2009). Melanin is a dark molecular pigment that gives the colour to hair, skin and other tissue. However, overexpression of tyrosinase cause hyperpigmentation that give high impact on quality of life of a patient. Tyrosinase inhibitor will organize the metabolism of pigmentation and decelerate overproduction of melanin.

Mangosteen is originated from South East Asia and belongs to the Guttiferae family, genus *Garcinia* (Verheij, 1991). Due to its petals that almost look like a crown and its taste, which is one of the best tasting tropical fruit, mangosteen has been known as the “queen of tropical fruit” (Cruz, 2001). The ripe mangosteens have dark purple pericarp with white, juicy and sweet flesh. Unlike most of tropical fruit which prone to quick ripening and rotten, ironically, it is very rare to find a rotten mangosteen. This has made this fruits so special among traditional healer. Recently, mangosteen has been popularized for its medical benefits (Sakagami et al., 2005; Mahabusarakan et al., 2006). In Southeast Asia, the pericarp has been used as a medicine for treating diarrhea, wounds and skin infection (Nakatani et al., 2007; Ji et al., 2007; Zadernowski et al., 2009). The pericarp is known as one of the best natural sources of xanthones (Deachathai et al., 2005; Jung et al., 2006). Xanthone belong to a class of polyphenolic compounds commonly found in higher plants (Zadernowski et al., 2009). Alpha mangostin syn. 1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methyl-2-butenyl)- 9H-xanthen-9-one is a biosynthetic di-prenylated tetra oxygenated xanthone derivatives which as isolated from the pericarp (Arunrattiyakan et al., 2011).
Xanthone and their derivatives have been reported to have high antioxidant (Jung et al., 2006; Okonogi et al., 2007), anti-inflammatory (Chen et al., 2008; Okonogi et al., 2008), antiantherosclerotic (Park et al., 2008) and antimalarial activities (Hay et al., 2006). Present study focuses on the assessment of the antioxidant and tyrosinase inhibition activities in α-mangostin and different solvent extracts of mangosteen pericarp.

MATERIALS AND METHODS

Chemicals and reagents

Analytical grade methanol, DMSO (dimethyl sulfoxide), glacial acetic acid and Hydrochloric acid (37%) were bought from Merck (Darmstadt, Germany). L-DOPA (L-3, 4-dihydroxyphenylalanine) was ordered from Sigma- Aldrich (China). Tyrosinase, Kojic acid, L-ascorbic acid, TPTZ (2, 4, 6-Tripyridyl- s-Triazine) were purchased from Sigma- Aldrich (USA). Potassium hydrogen phosphate, Potassium dihydrogen phosphate and Ferric Chloride hexahydrate were from Qrec and Sodium acetate trihydrate was from GCE laboratory chemicals.

Plant materials

Alpha mangostin, methanol, ethyl acetate and petroleum ether crude extracts of mangosteen pericarp were obtained from Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia. Alpha mangostin was obtained by purification of ethyl acetate fractioned after back extraction of methanolic crude extract. The structures of α-mangostin (Figure 1) was identified on the basis of their spectral data and by comparison with previously reported data including 1D NMR (1H, 13C, DEPT) and 2D NMR (COSY, HMQC, HMBC), FTIR, UV spectroscopy and MS (Muhammad, 2013).

![Fig. 1: 1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methyl-2-butenyl)-9H-xanthene-9-one.](image)

Antioxidant determination by FRAP assay

FRAP assay was carried out according to Benzie and Strain, 1999 and was performed in 96 microwell plate. The fresh working reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM of TPTZ (2, 4, 6-Tripyridyl- s- Triazine) solution and 20 mM FeCl₃·6H₂O (ferric chloride) solution in 10:1:1 ratio and then warmed at 37°C before used.

The serial dilution was made with distilled water for a range of concentration to obtain 5 µl test sample. The total reaction mixture is 170 µl consists of 5 µl of test sample, 15 µl distilled water and 150 µl of FRAP reagent. Distilled water and FRAP reagent were used in control well as a replacement for test sample. After 4 min reaction time, the absorbance reading was measured at 575 nm using spectrophotometric micro-plate reader (Benchmark Micro-plate Reader, Bio-Rad). The analysis of FRAP assay was based on the standard regression line by plotting the FRAP value (y-axis) versus its concentrations (x-axis). From the linear regression equation, \( y = mx + c \), \((m)\) values indicates the antioxidant reducing capacity. The higher slope values showed higher antioxidant reducing capacity.

Tyrosinase inhibition activities

Tyrosinase inhibitory activity was determined spectrophotometrically as described by Lim et al., 2009 using L-DOPA as a substrate. Stock solution of α-mangostin, crude extracts, kojic acid (positive control), 100 unit/ml of tyrosinase and 2.5 mM of L-DOPA were freshly prepared. Tyrosinase stock solution was prepared by dissolving mushroom tyrosinase in 0.1 M phosphate buffer (pH 6.8). The total volume in test well was 200 µl that consist of 40 µl of test samples, 80 µl of 0.1 M phosphate buffer, 40 µl of tyrosinase solution and 40 µl of L-DOPA. Each sample was accompanied by a blank well without tyrosinase solution and sample solvent was used as a replacement for test sample in control well. After 30 minutes incubation, the dopachrome formation was measured at wavelength 515 nm with 655 nm as a reference using spectrophotometric micro-plate reader (Benchmark Micro-plate Reader, Bio-Rad). The percentage of tyrosinase inhibition was calculated as follows:

\[
\text{Percentage of inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100
\]

Where \( A_{\text{control}} \) is the absorbance of control treatment values corresponding to a well with reagents mixtures except the test sample and \( A_{\text{sample}} \) is the absorbance value of test sample treatment corresponding to well reagents mixtures with the test sample.

Statistical analysis

The experiments were performed in triplicate from three independent experiments (n=9). The statistical evaluation was performed by using SPSS (Statistical Package for the Social Science) Statistics version 15.0.

RESULTS AND DISCUSSIONS

Antioxidant determination

As described by Halliwell and Gutteridge (2000), a biological antioxidant was defined as ‘any substances that when present at low concentration compared with those of an oxidisable substrate significantly delay or prevent the oxidation of that substrate’. In this study, determination of antioxidant activities of methanol, ethyl acetate and petroleum ether extracts of mangosteen’s pericarp and α-mangostin were performed by direct method FRAP assay. FRAP assay measures the ability of the extract to donate electron to Fe (III). The higher the FRAP value, the greater the antioxidant activity. High antioxidant sample is effective to react with ferric tripyridyltriazine (Fe (III) - TPTZ). In
the FRAP assay, reductants (antioxidants) in the sample reduce Fe$^{3+}$ to Fe$^{2+}$. Table 1 showed the m slope value of all samples. Within 4 minutes, ascorbic acid demonstrates the highest antioxidant reducing capacity indicated by m slope value. In contrast, the activity of α-mangostin, ethyl acetate, petroleum ether and methanolic extract in the FRAP assay are relatively low compared to ascorbic acid. Alpha mangostin, ethyl acetate (semi-polar) and petroleum ether (non-polar) extract did not have high ability to reduce Fe$^{3+}$ to Fe$^{2+}$ compared to methanol extract which is the most polar solvent. The methanol extract might contain the compound which is effective to react with ferric tripyridyltriazine (Fe(III) - TPTZ) in FRAP assay.

**Table 1:** m slope value of samples at 4 minute reaction in FRAP assay.

| Samples                | m slope value |
|------------------------|---------------|
| Ascorbic acid          | 10.789        |
| α-mangostin             | 0.697         |
| Ethyl acetate extract  | 0.704         |
| Petroleum ether extract| 0.545         |
| Methanolic extract      | 1.621         |

Data represent mean of triplicate of three independent experiments (n=9). The data presented were statistically significant at p≤0.05.

**Tyrosinase inhibition activities**

Good tyrosinase inhibition was described by Fawole *et al.*, 2012 as tyrosinase inhibition activity with percentage above 50%. Alpha mangostin and ethyl acetate extract have moderately inhibited tyrosinase activity (percentage inhibition >50%) though the value were significantly lower than positive control, kojic acid. Meanwhile, methanol and petroleum ether extracts showed no inhibition. Table 2 clearly showed the percentage of tyrosinase inhibition of α-mangostin and mangosteen extracts.

**Table 2:** Percentage of tyrosinase inhibition.

| Sample               | Percentage of inhibition (%) |
|----------------------|-----------------------------|
| Kojic acid           | 78.66                       |
| α-mangostin          | 57.03                       |
| Ethyl acetate extract| 61.11                       |
| Methanol extract      | ...                         |

Data represent percentage of inhibition of three independent experiments. ND denotes not detected.

**Combination of α-Mangostin and Kojic Acid as Tyrosinase Inhibitor**

Kojic acid (5-hydroxyl-2-hydroxymethyl-4H-pyran-4-one) was discovered by K. Saito as the most extensively studied tyrosinase inhibitors discovered by K. Saito in 1907. Kojic acid shows a mixed inhibitory effect on the diphenolase activity and a competitive inhibitory effect on the monophenolase activity of mushroom tyrosinase. Unfortunately, instability during storage limits its use and new tyrosinase inhibitors of novel kojic acid derivatives are needed in cosmetics industry. In this study, α-mangostin showed potential inhibitory properties towards tyrosinase. Therefore, the combination study was design to investigate any agonist effect of kojic acid and α-mangostin. Kojic acid was combined with α-mangostin at different ratio (100: 0, 25: 75, 50: 50, 25: 25 and 0: 100).

**Table 3:** Tyrosinase inhibition by combination of alpha mangostin with kojic acid.

| Sample                  | Percentage of inhibition (%) |
|-------------------------|-------------------------------|
| 100% α-mangostin        | 57.034                        |
| 25% α-mangostin: 75% Kojic Acid | 72.755            |
| 50% α-mangostin: 50% Kojic Acid | 72.065            |
| 75% α-mangostin: 25% Kojic Acid | 69.889            |
| 100% Kojic Acid         | 78.657                        |

Data represent percentage of inhibition of three independent experiments.

Table 3 suggest that there are no additional tyrosinase inhibition effects and kojic acid is the main contributor in inhibiting tyrosinase activity instead of α-mangostin. The percentage of tyrosinase inhibition is proportional to the kojic acid ratio. In conclusion, it is observed that tyrosinase inhibition effect is antioxidant independent as ethyl acetate extract possessed low antioxidant capacity. This study suggests direct tyrosinase inhibition by ethyl acetate extract of *Garcinia mangostana*.

**CONCLUSION**

The antioxidant capacity of methanol extract and strong tyrosinase inhibitory properties of α-mangostin and ethyl acetate extract could contribute to its potential as antioxidant and lightening agent.

**ACKNOWLEDGEMENT**

The authors acknowledge to Research University Grant (GUP) from the Ministry of Higher Education (MOHE) for financial support under vote QJ13000.2645.09H16 and the Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia for facilities supports.

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How to cite this article:
Wan Nur Atiqah Wan Hassan, Razauden Mohamed Zulkifli, Norazah Basar, Mohd Azizi Che Yunus, Farediah Ahmad. Antioxidant and tyrosinase inhibition activities of α- mangostin and *Garcinia mangostana* Linn. pericarp extracts. J App Pharm Sci, 2015; 5 (09): 037-040