Journal of Applied Pharmaceutical Science Vol. 5 (09), pp. 037-040, September, 2015
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2015.50907
ISSN 2231-3354

Antioxidant and tyrosinase inhibition activities of α- mangostin and *Garcinia mangostana* Linn. pericarp extracts

Wan Nur Atiqah Wan Hassan[1], Razauden Mohamed Zulkifli[1*], Norazah Basar[2], Farediah Ahmad[2], Mohd Azizi Che Yunus[3]

[1]Department of Bioscience and Health Sciences, Faculty of Bioscience and Medical Engineering, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia. [2]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia. [3]Department of Chemical Engineering, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia.

ARTICLE INFO

Article history:
Received on: 12/06/2015
Revised on: 10/07/2015
Accepted on: 17/08/2015
Available online: 27/09/2015

Key words:
Garcinia mangostana Linn.; α-mangostin; xanthone; antioxidant; tyrosinase inhibition.

ABSTRACT

The methanol, ethyl acetate and petroleum ether crude extracts of mangosteen pericarp and α- mangostin were evaluated for the antioxidant capacity and tyrosinase inhibition properties. The ferric reducing antioxidant power (FRAP) assay was used to investigate their antioxidant capacity. Tyrosinase inhibition effect was evaluated using mushroom tyrosinase inhibition assay. Methanol extract has higher antioxidant reducing capacity (m= 1.621), compared to the rest of the extracts. Meanwhile, ethyl acetate extract and α- mangostin showed potent tyrosinase inhibition activities as compared to Kojic acid, a well- known tyrosinase inhibitor. 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*.

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).

* *Corresponding Author*
Razauden Mohamed Zulkifli,
Department of Bioscience and Health Sciences, Faculty of Bioscience and Medical Engineering, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia. Email: Mail id: razauden@fbb.utm.my

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; Park *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).

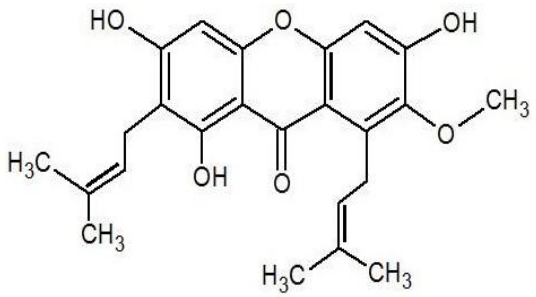

Fig. 1: 1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methyl-2-butenyl)- 9H-xanthen-9-one.

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 $FeCl_3.6H_2O$ (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 ofα-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 inihibition was calculated as follows:

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

Where $A_{control}$ is the absorbance of control treatment values corresponding to a well with reagents mixtures except the test sample and A_{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+}/ tripyridyltriazine complex, present in stoichiometric excess, to the blue colored ferrous form (Prabhune *et al.,* 2013).

Ferric reducing antioxidant power (FRAP) assay

Ferric reducing assay measured the ability of α-mangostin and *Garcinia mangostana* L. Pericarp extracts to 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	-
Petroleum ether 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, 75: 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 QJ13000.2645.09J16 and the Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia for facilities supports.

REFERENCES

Arunrattiyakarn P, Suksamrarn S, Suwannasa IN and Kanzaki H. Microbial metabolism of alpha mangostin isolated from *Garcinia mangostana Linn*. Phytochem, 2011; 72:730- 734.

Benzie FF and Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. AnalBiochem, 1996;239:70-76.

Benzie FF and Strain JJ. Ferric Reducing/ Antioxidant Power Assay: Direct Measure of Total antioxidant Activity of Biological Fluids and Modified Version for Simultaneous Measurement of Total Antioxidant Power and Ascorbic Acid Concentration. Method enzymol, 1999; 299:15-23.

Chang TS. An Updated Review of Tyrosinase Inhibitors. Int J Mol Sci, 2009; 10: 2440- 2475.

Chen LG, Yang LL and Wang CC. Anti-inflammatory activity of mangostins from *Garcinia mangostana*. Food Chem Toxicol, 2008; 46: 688–693.

Cruz FS. 2001. Status Report on Genetic Resources of Mangosteen (*Garcinia mangostana L.*) in Southeast Asia. New Delhi, India: National Agriculture Science Centre (NASC).

Deachathai S, Mahabusarakam W, Phongpaichit S and Taylor WC. Phenolic compounds from the fruit of *Garcinia dulcis*. Phytochem, 2005; 66: 2368–2375.

Fawole OA, Makunga PM. and Opara UL. Antibacterial, antioxidant and tyrosinase inhibition of activities of pomegranate fruit peel methanolic extract. BMC Complemen. Altern. M, 2012; 12: 200.

Halliwell B and Gutteridge JM. Free radicals and antioxidants-A historical look to the future. Ann. NY Acad Sci, 2000; 899: 136-149.

Hay AE, Hélesbeux JJ, Duval O, Labaïed M, Grellier P and Richomme P. Antimalarial xanthones from *Calophyllum caledonicum* and *Garcinia vieillardii*. Life Sci, 2006; 75: 3077–3085.

Ji X, Avula B and Khan IA. Quantitative and qualitative determination of six xanthones in *Garcinia mangostana L*. by LC–PDA and LC–ESI-MS. J Pharma Biomed Anal, 2007; 43: 1270-1276.

Jung HA, Su BN, Keller WJ, Mehta RG and Kinghorn AD. Antioxidant xanthones from the pericarp of *Garcinia mangostana* (Mangosteen). J Agric Food Chem, 2006; 54: 2077-2082.

Lim TY, Lin YY and Yule CM. Evaluation of antioxidant, antibacterial and antityrosinase activities of four *Macaranga* species. FoodChem, 2009; 114: 594-599.

Mahabusarakam W, Kuaha K, Wilairat P and Taylor WC. Prenylated Xanthone as Potential Antiplasmodial Substances. Planta Medica, 2006; 72: 912-916.

Muhammad NA. 2013. Phytochemicals and Bioactivity Studies of *Garcinia parvifolia* Miq., *Garcinia hombroniana* Pierre and *Garcinia mangostana* Linn, Master of Science (Chemistry) Thesis, Universiti Teknologi Malaysia.

Nakatani K, Nakahata N, Arakawa T, Yasuda H and Ohizumi Y. Inhibition of cyclooxygenase and prostaglandin E2 synthesis by gamma-mangostin, a xanthone derivative in mangosteen, in C6 rat glioma cells. Biochem Pharmacol, 2012; 63(1):73-79.

Noguchi N and Niki E. 1999. Chemistry of Active Oxygen Species and Antioxidants. In: Papas AM, ed. Antioxidant Status in Diet, Nutrition and Health. Boca Raton, Florida: CRC Press 2- 20.

Okonogi S, Duangrat C, Anuchpreeda S, Tachakittirungrod S and Chowwanapoonpohn S. Comparison of antioxidant capacities and cytotoxicities of certain fruit peels. Food Chem, 2007; 103: 839–846.

Park KH, Park YD, Han JM, Im KR, Lee BW and Jeong IY. Antiatherosclerotic and anti-inflammatory activities of catecholic xanthones and flavonoids isolated from *Cudrania tricuspidata*. Bioorg Med Chem Lett, 2006; 16: 5580–5583.

Prabhune *et al*. Free Radical Scavenging (DPPH) and Ferric Reducing Ability (FRAP) of some Commelinaceae members, Int J Bio Pharma App Sci, 2013; 2(5): 1128-1134.

Sakagami Y, Iinuma M, Piyasena KG and Dharmaratne HR. Antibacterial Activity of Alpha Mangostin against Vancomycin Resistant Enterococci and Synergism with Antibiotics. Phytomed, 2005; 12: 203-208.

Verheij EWM. 1991.*Garcinia mangostana L*. In:Verheij EWM,ed. Plant Resources of Southeast Asia, Edible Fruit and Nuts, Bagor a Selection. Wageningen: PUDOC177-181.

Zadernowski R, Czaplicki S and Naczk M. Phenolic acid profiles of mangosteen fruits (*Garcinia mangostana*). Food Chem, 2009; 112: 685–689.

How to cite this article:

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