Original Research Article

Determination of Total Phenolic Content, Antioxidant and Monophenolase Inhibition Activities of *Carica papaya* Peel Extracts in Solvents with Different Polarity

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Abstract

Dysregulation of melanin production that is catalyzed by an enzyme known as tyrosinase may lead to skin hyperpigmentation. Even though fruit peels are often regarded as waste product, they can be a good source of phytochemicals with potent biological activities that can be utilized to inhibit tyrosinase and reduce hyperpigmentation. This study was carried out to determine the phenolic content, antioxidant and monophenolase inhibition activities of *Carica papaya* (*C. papaya*) peel extracts prepared using three solvents with different polarity; ethanol, ethyl acetate and petroleum ether. The total phenolic content was determined according to Folin-Ciocalteu method while the antioxidant and monophenolase inhibition activities of the plant extracts were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and tyrosinase inhibitory assays respectively. There was no significant difference in total phenolic content and antioxidant activities of the extracts ($p > 0.05$). However, the ethanolic extract of *C. papaya* peel showed the highest phenolic content (0.58 ± 0.19 mg/g) and maximum antioxidant activity (IC_{50} = 164.27 µg/mL) compared to ethyl acetate and petroleum ether. There was a significance difference in monophenolase inhibition activities of the extracts ($p < 0.05$). Ethyl acetate extract exhibited excellent monophenolase inhibition activity (82.69 ± 2.57 %) compared to other solvents. Finding of this study can be used for future researches to study the biological activities and elucidate the chemical structure of active compounds involved.

Keywords: tyrosinase, tyrosine, peel, papaya

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1.0 Introduction

Melanogenesis is a process of melanin pigments production which determine the colour of skin, hair and also eyes in human and animals. Melanin is produced by melanocytes in melanosomes and are transported to the adjacent keratinocytes. Melanin is important as it is responsible in giving protection against ultraviolet (UV) radiation (1). Tyrosinase is an enzyme involved in the production of melanin. It catalyzes the rate-limiting activity of the first two steps of the pathway. L-tyrosine and l-3,4-dihydroxyphenylalanine (L-DOPA) act as the substrates for the tyrosinase enzyme. Both steps are known as monophenolase and diphenolase activities which involve the conversion of substrate tyrosine to DOPA and oxidation of substrate DOPA to dopaquinone, respectively (2). The pathway for biosynthesis of melanin is shown in Figure 1.

The types and amounts of melanin are determined genetically and can be greatly influenced by various factors including age, disease conditions, hormonal changes, inflammation and UV exposure (3). The imbalance production and inappropriate distribution of melanin can contribute to variation in skin diseases, such as hypopigmentation and hyperpigmentation conditions. Ones with a very concentrated amount of melanin, it causes a person to have darker skin tone compared to hypopigmented person (4).

Darker skin is viewed as unattractive by certain population, particularly in African and Asian countries (5). This belief later resulted in the desire to obtain fairer skin that can be accomplished by inhibiting the melanin production (6).

Many skin lightening agents such as hydroquinone, resorcinol and mercury disrupt the production of melanin by inhibiting the tyrosinase enzyme activities (6). Such active ingredients are potentially carcinogenic constituents and products incorporating them are banned in most countries including Malaysia, Japan and Australia (7). The World Health Organization (WHO) has warned the usage of mercury-containing compounds as it can damage the kidneys and brain if it is absorbed by the skin and accumulates in the body (8).

Due to the hazardous side effects, interest in finding inhibitors of tyrosinase enzyme from the natural sources has been increasing, mainly because plants are rich in bioactive chemical sources that has been reported for various biological activities (9).

Carica papaya is a plant from Caricaceae family. It was first cultivated in Malacca (Melaka) in the early 1600s (10). A number of pharmacological properties on this plant have been reported previously. For instance, the methanol extract of unripe *C. papaya* fruit possesses an anti-sickling and reversal of sickling properties with its minimum inhibitory concentration (MIC) of 1.0 gram (g) in 1.0 milliliter (mL) of physiological saline (11). With effective concentration at 50% (EC_{50}) of 34.6 mg/kg, the ethanol extract *C. papaya* fruit appeared to be effective in depressing the blood pressure and heart rate DOCA-salt hypertensive rats (12). The water extract of *C. papaya* seeds exhibited a strong anti-amoebic activity with its MIC of 7.81 µg/mL (13).

The ripe *C. papaya* fruit is known for its sweet taste, while the unripe fruit is used for salad. In contrary, the peels are frequently discarded. Previous studies suggested that the peel of the fruits may have phytochemicals with potential biological activities (14). A study on papaya peel revealed that petroleum ether extract showed antibacterial properties towards *Corynebacterium diphtheria* and *Streptococcus pneumoniae* at MIC of 5.63 mg/mL and 1.40 mg/mL respectively (15). Another study showed that aqueous and

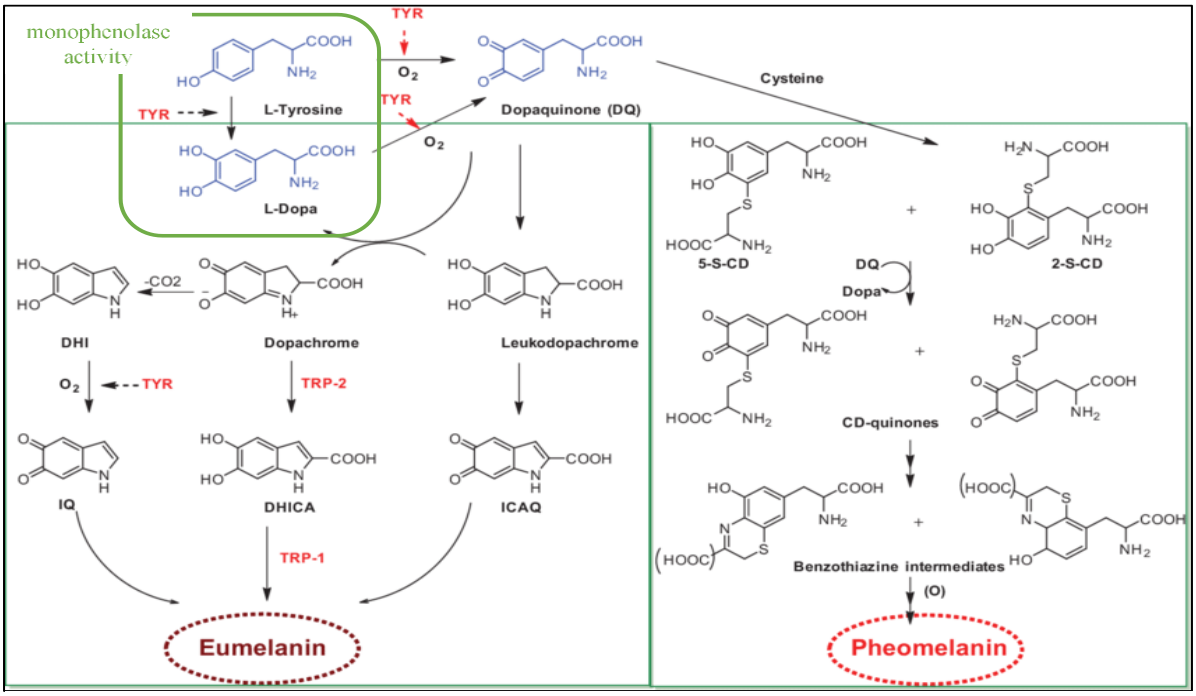

Figure 1: Melanin biosynthesis pathway

methanol extract of unripe *Carica papaya* fruit's peel demonstrated a concentration-dependent inhibition of antipseudomonal activities against multi-drug resistant clinical isolates of *Pseudomonas aeruginosa* with the mean zones of inhibition produced by methanol peel extract at 160 mg/mL was 17.9 ± 2.3 mm and larger than 14.5 ± 2.3 mm produced by aqueous peel extract at 160 mg/mL (16). Besides, the EC50 of 90% acetone extract of papaya peel assessed using DPPH scavenging activity and ABTS radical cation inhibition assays were determined at 810.43 ug/mL and 5.47 mg/mL respectively (17). In addition, antioxidant analysis of aqueous extracts of unripe *Carica papaya* fruit's peel and seed using ferric reducing antioxidant property showed that the peel contained higher antioxidant activity at 112.35 ± 0.20 (mg AAE/100 g) compared to the seed which at 102.78 ± 0.21 (mg AAE/100 g) (18).

The polarity of solvent can determine the composition of the phytochemicals extracted from the peels (19). Ethanol is categorized as polar solvent. It can dissolve polar compounds such as sugar, amino acid and glycoside compounds. On the other hand, ethyl acetate which is known as a semipolar solvent, is effective to extract alkaloid, aglycon, and glycoside compounds. Lastly, a non-polar solvent like petroleum ether, can dissolve non polar compounds such as lignin, wax, lipid, and aglycon (20). Therefore, the aim of this study was to determine the phenolic content, antioxidant and monophenolase inhibition activities possessed by *C. papaya* peel extracts in different solvents.

2.0 Materials and methods

2.1 Plant material

The peels of ripe *Carica papaya* were collected from a night market in Puncak Alam, Selangor. Samples were washed, cut into smaller pieces and dried at 40°C until constant weight was achieved before grounded into fine powder.

2.2 Preparation of plant extract

The extraction process were conducted in accordance to a research by Stankovic (21) with appropriate alterations. The dried grounded powder of samples (20 gram) were transferred into a beaker and mixed with 80 mL solvents of different polarities (petroleum ether, ethyl acetate and ethanol) respectively and stored at room temperature. The samples were then filtered through Whatman No. 1 filter paper and the residues were re-extracted with the same solvents. The process was repeated for five times for each solvent. Each extraction and re-extraction process were conducted for every 24 hours. Combined supernatants were evaporated to dryness at each boiling point of the solvent using rotary evaporator.

2.3 Total phenolic content assay

The total phenolic content of extracts were determined by using Folin-Ciocalteu's phenol reagent as described by Ramli S. et al (22) with slight modifications. Formation of blue colour complex of from the mixture of Folin Ciocalteu reagent and the extract indicates the presence of phenolic compounds after adjusted with sodium carbonate solution. 40 µl of extract in ethanol (1 mg/mL) was pipetted into 96-well microplate, followed by 50 µL of 15% Folin Ciocalteu reagent. Volume adjustment was made by adding distilled water up to 100µl. 50 µl of Na_2CO_3 aqueous solution (0.105 g/mL) was added into the microplate after the mixture was left for 5 minutes at room temperature. Next, the mixture was incubated at 30°C for 30 minutes and the absorbance of the extract was measured at 756 nm. Different concentrations of gallic acid (0.03, 0.06, 0.12, 0.25, 0.5 and 1 mg/mL) were used to prepare a standard graph. The concentration of total phenolic compounds in all extracts were expressed as mg of gallic acid equivalents per g dry weight of extract using a linear equation.

2.4 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Quantification of the activity was determined according to Ramli S. et al (22) with slight modifications. 40 µL of extract with various concentration (10, 25, 50 and 100 µg/mL) was pipetted into 96-well microplate, followed by 160 µL of DPPH solution (0.4 mM) in ethanol. The mixture was then left to stand in dark for 5 minutes at room temperature. The absorbance was measured at 517 nm and all extracts were analyzed in triplicate. Ethanol was used as a negative control whereby ascorbic acid and quercetin were used as the positive controls. The percentage inhibition of DPPH by extracts were calculated by the following equation:

DPPH radical scavenging ability (%),

= (Absorbance control – Absorbance sample) / Absorbance control x 100

2.5 Tyrosinase inhibitory assay

The tyrosinase inhibitory assay was carried out according to Salleh et al. (23) with slight modifications. Extracts in ethanol (10 mg/mL) were diluted with phosphate buffer solution at pH 6.8. 70 µL of extracts with concentration of 25 µg/mL were added into 96-well microplate followed by 30 µL of tyrosinase enzyme solution (33 units/mL). The mixture was allowed to incubate for 5 minutes at 37°C. Next, 100 µL of L-tyrosine with concentration of 4 mM was added into the microplate. The formation of DOPA chrome was measured at 475 nm by using microplate reader. Ascorbic acid and asiatic acid were used as the positive controls. The percentage of tyrosinase inhibitory activity

by the extracts were calculated by the following equation:

Tyrosinase inhibitory assay

= (Absorbance control – Absorbance sample) / Absorbance control x 100

2.6 Statistical analysis

The extract yield was calculated by dividing the final weight of the samples by initial weight of the samples. All the experimental measurements for determining total phenolics, antioxidant and monophenolase inhibition activities were carried out in triplicate. The results were expressed as means ± standard deviations, and were tested using Statistical Package for the Social Sciences (SPSS version 25.0). The differences in values between extracts and positive controls were assessed by one-way analysis of variance (ANOVA). This step was followed by pairwise comparison of the mean with positive controls by using Tukey's multiple comparison test. Values were determined to be significant when p is less than 0.05 ($p < 0.05$). The correlation coefficient between total phenolic content and antioxidant activities of the extracts was determined by using Pearson correlation coefficient.

3.0 Results and Discussion

3.1 Effect of solvents on extraction yield

Ethanol, ethyl acetate and petroleum ether extracts were prepared to examine the total phenolic content, antioxidant and tyrosinase inhibitory activities. The yield of extracts obtained from 20 g of dry grounded powder of plant material was calculated for each extract (Table 1).

Based on Table 1, the papaya peel extraction yield increased with the increase in polarity of the solvent used. The highest extraction yield was observed by using ethanol followed by ethyl acetate and petroleum ether. The result was consistent with previous studies on *Marrubium peregrinium* (21) and pine bark (24) in which their extraction yields were most obtained from the polar solvents. These results suggested that major phytochemicals in the papaya peel are mostly dissolve in polar solvent.

Table 1: The yields of extract from 20 g dried grounded powder of *C. papaya* peel in different solvents

Extract	Total yield (g)	Yield percentage (%)
Ethanol	1.76	8.80
Ethyl acetate	0.53	2.65
Petroleum ether	0.21	1.05

3.2 Total phenolic content

The total phenolic content in C. papaya peel extracts were expressed in terms of gallic acid equivalent (the standard curve equation: $y = 4.654x + 0.3198$, $r^2 = 0.9407$). Table 2 showed the total phenolic content of the extracts varied from 0.46 ± 0.01 to 0.58 ± 0.19 mg/g. The ethanolic extract contained the maximum phenolic content compared to other extracts with a value of 0.58 ± 0.19 mg/g followed by ethyl acetate extract (0.50 ± 0.01 mg/g) and petroleum ether extract (0.46 ± 0.01 mg/g).

In previous studies, orange (25) and mango (26) fruit peels showed the highest phenolic contents when the most polar solvents were used. Even though the polarity of solvents in current study seemed to influence the phenolic content presented in the extracts, the results showed the opposite situation. There is no significant difference in total phenolic content of the extracts ($p > 0.05$). Several researches revealed that the amount of phenolic compounds in plants can be greatly affected by other varying aspects including growing area of plants, climate

conditions, genetic background and extraction method (27).

3.3 DPPH free radical scavenging activity

In the present study, the determination of antioxidant activity was observed by using DPPH assay. DPPH is a stable free radical that produce deep purple colour when dissolved in solution (21). The solution turns yellowish when it is mixed with a substance that has an antagonizing effects towards the oxidation process (28). The higher the antioxidant activities, the faster the rate of colour change (29). Free radicals that are produced by oxidation process are highly reactive molecules due to their unpaired electrons. Research has discovered that excessive accumulation of radicals has a high correlation with skin diseases (30). Antioxidant is a molecule that acts as a reducing agent and has the ability to inhibit the oxidation process (31).

In this study, the DPPH free radical scavenging activity of papaya peel extracts were compared with ascorbic acid and quercetin. The antioxidant activity of the samples was expressed in terms of inhibitory concentration (IC_{50}) which is defined as the concentration to scavenge 50 % of radical presented in the reaction mixture (Table 2). The lower values of $IC_{50,}$ the better the free radical scavenging effects. There is no significant difference in the antioxidant activities of the extracts ($p > 0.05$). The ethanolic extract had the highest inhibitory effect toward the free radicals with IC_{50} of 164.27 µg/mL, followed by ethyl acetate and petroleum ether extracts with IC_{50} of 182.69 µg/mL and 246.42 µg/mL, respectively. However, the antioxidant activities of ascorbic acid and quercetin were significantly higher than the peel extracts with IC_{50} of 46.34 and 41.99 µg/mL respectively ($p < 0.05$).

Table 2: Total phenolic content and antioxidant activity of the samples

Sample	Total Phenolic Content (mg/g)	Antioxidant activity (IC_{50}) (µg/mL)
Ascorbic acid	-	46.34
Quercetin	-	41.99
Petroleum ether	0.46 ± 0.01	246.42
Ethyl acetate	0.50 ± 0.01	182.69
Ethanol	0.58 ± 0.19	164.27

The antioxidant activity of the peel extracts were similar to a previous study on papaya peel which reveals that the ethanolic extract has the highest DPPH free radical scavenging activity compared to other solvents (32). Together with this present study, it can be concluded that ethanol is the best solvent to be used to extract the natural antioxidants present in the papaya peel (18). Additionally, it was shown that the scavenging activity of *Carica papaya* peel extracts were in a concentration-dependent manner from the reaction of the extracts with DPPH radical (Figure 2). However, even at the highest concentration of the extracts (100

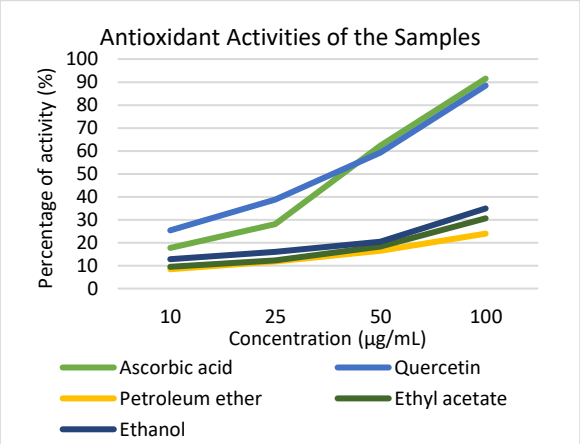

Figure 2: Percentage of DPPH radical

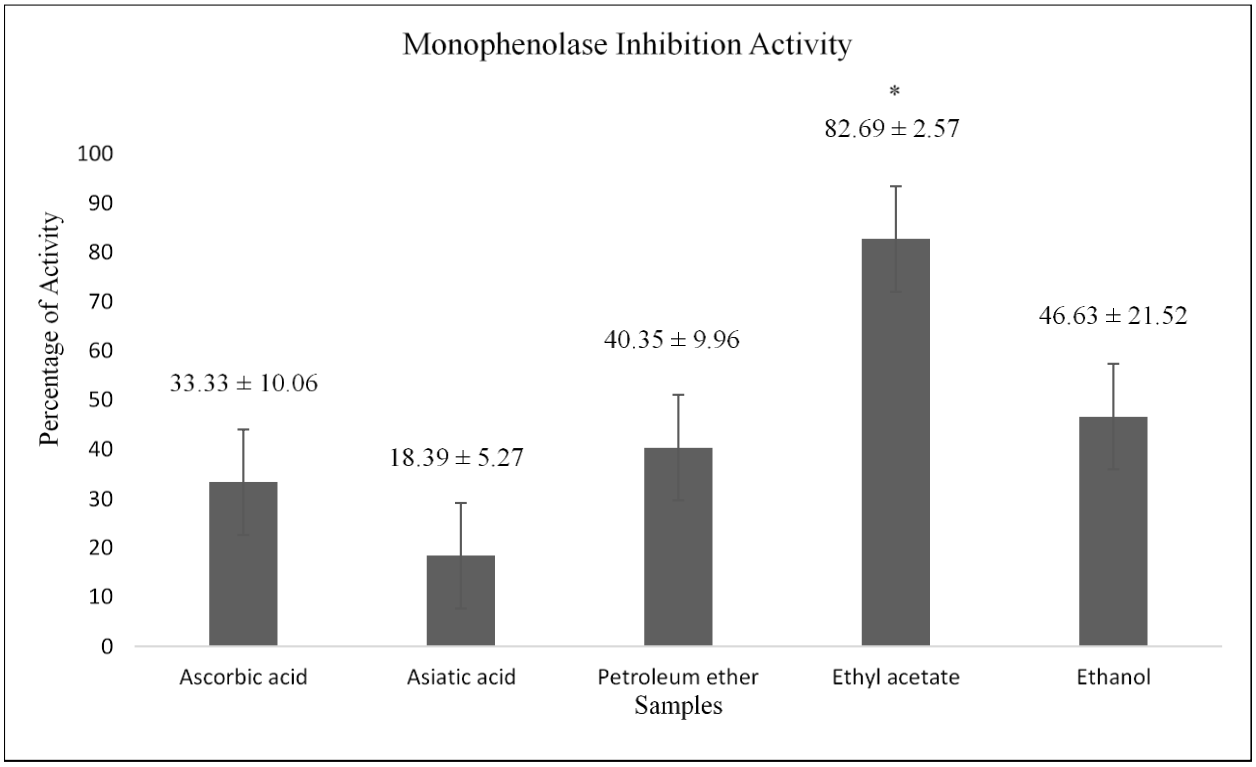

Figure 3: Monophenolase Inhibitory Activity of the samples at concentration 25 µg/mL, against 4 mM of L-tyrosine and 33 units/mL of tyrosinase enzyme. Ascorbic acid and asiatic acid as the positive controls. *$p < 0.05$ vs positive controls

µg/mL), the antioxidant activities for all extracts only ranged between 20-35 %, whereas ascorbic acid and quercetin scavenged 50% of DPPH radical at concentrations of 46.34 µg/mL and 41.99 µg/mL respectively.

The role of tyrosinase in melanocytes is to induce the transformation of tyrosine to L-DOPA which in turn converted into dopaquinone. Since dopaquinone is a high reactivity substance where it transforms into melanin after a series of oxidative polymerization reactions, therefore, any radical inhibitors and scavenging agents can lessen the tyrosinase activity (30). The antioxidant activities from *C. papaya* peel extracts shows tyrosinase suppressing function. It can thus make the skin to appear fairer through reduction of melanin and preventing skin hyperpigmentation.

3.4 Monophenolase inhibitory activity

The effect of ascorbic acid, asiatic acid and *C. papaya* peel extracts on tyrosinase enzyme activities by using L-tyrosine (monophenolase) as the substrate were reported in Figure 3. A significant difference was found between the extracts, ascorbic acid and asiatic acid ($p < 0.05$). Ethyl acetate extract exhibited the highest monophenolase inhibition levels among all the samples with percentage of 82.69 ± 2.57 % followed by ethanol (46.63 ± 21.52 %) and petroleum ether (40.35 ± 9.96 %) extracts. The results obtained were similar to the report from another study on monophenolase activities in which ethyl acetate of *C. papaya* fruit with peel showed the highest inhibition percentage against mushroom tyrosinase enzyme compared to other organic solvents and aqueous extract (33).

Some phenolic compounds are able to demonstrate abilities to compete for tyrosinase enzyme. Thus inhibiting the enzymatic reaction process (34). The hydroxyl groups of the phenolic compounds in the peel extracts can form a hydrogen bond with the active site of the enzyme. The bond between phenolic compounds and tyrosinase enzyme may result in steric hindrance or conformational changes, leading to a lower enzymatic activity (35).

5.0 Conclusions

In the present study, the ethanolic extracts of *C. papaya* peel showed the highest phenolic contents and antioxidant activities, whereas the ethyl acetate extract showed the highest monophenolase inhibition activities (82.69 ± 2.57 %) at concentration of 25 µg/mL, better than ascorbic acid and asiatic acid which with 33.33 ± 10.06 % and 18.39 ± 5.27 % of tyrosinase inhibition activity, respectively. Consequently, these finding indicated the potential of *Carica papaya* peel extracts to inhibit melanin production. Therefore, further researches need to be carried out to study the biological activities and elucidate the chemical structure of active compounds involved.

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Conflict of interest

Authors declare no conflict of interest in the present work.

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