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Original Article

Mangifera indica Linn. Waste Peel Ethanol Extract on Inducing *Citrus amblycarpa* Hassk. Ochese Antioxidant Activity

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ABSTRACT

Background: Excessive production of reactive oxygen species plays a significant role in the proliferation and evolution of numerous high-risk diseases in humans, the antioxidant capacity of bioactive substances is of immense importance. *Mangifera indica L.*, a medicinal plant has high bioactive metabolite contents and contributes to several positive biological processes in the plant. *Citrus amblycarpa* H.O. contains an excellent source of chemical compounds and the presence of these compounds can be a potential pharmacological activity as antimicrobial, anti-inflammatory, antioxidant, and others. The aim of the study is to assess the effect of *Mangifera indica* and *Citrus amblycarpa* peel ethanol extract as an antioxidant agents. **Method:** Phytochemical screening of *M. indica* and *C. amblycarpa* peeled ethanol extract was done to identify alkaloids, polyphenols, flavonoids, anthraquinone, tannins, and terpenoid contents. The antioxidant activity of the extract was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. **Result:** *Mangifera indica* and *Citrus amblycarpa* peel ethanol extract have an antioxidant activity of IC_{50} 29,28 µg/mL and IC_{50} 669,52 µg/mL separately, while combination antioxidant activity was 48,05 µg/mL. **Conclusion**: *Mangifera indica* Linn. peel ethanol extract could induce *Citrus amblycarpa* antioxidant activity producing a potential antioxidant agent.

Keywords: Antioxidant, *Citrus amblycarpa*, *Mangifera indica*, Peel Extract.

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INTRODUCTION

Reactive oxygen species (ROS) are an integral part of metabolic and cell signaling pathways in living organisms, but excessive production of ROS as a result of impaired cellular metabolism is one of the main causes behind oxidative stress in living cells, which affects a cell's own defense and repair mechanism. Various seasonal fruits can be explored as dietary supplements because many of them are potential sources of such kinds of natural antioxidants. Consumption of diets rich in such kinds of natural antioxidants should be encouraged to fight against these ROS-related fatal diseases and it would be best if these seasonal fruits are locally available and low cost.[1] Among the various food plants, fruits and vegetables are reported to have health-improving benefits, some fruits such as the citrus fruits (orange, grapefruit, lime, lemon), grapes, pomegranates, apples, dates, strawberries, carrots, have been known worldwide to contain antioxidants.[2]

Mango (*Mangifera indica L.*, Family Anacardiaceae) is one of the most popular tropical fruits of the 21st century, it has a unique pleasant taste, aroma, and excellent nutritional value. Fresh mango fruits are distributed to the worldwide market throughout the year. Indeed, mango fruit has been reported to exert nutritional, antioxidant, anti-inflammatory, metabolic, and immunomodulatory functions relevant to human health and well-being. The role of mango pulp phytocomplex in counteracting the production of reactive oxygen species (ROS) and pro-inflammatory mediators associated to cancer, cardiovascular, and neurodegenerative pathologies has been highlighted by several in vitro and in vivo studies.[3] In this sense, mango peel represents approximately 15–20% of the total fruit. They were considering that approximately 20% of the world's mango production is used to produce different products and that mango peel accounts for 15–20% of the total fruit. However, it is worth noting that mango peel may be an interesting source of phenolic compounds, even in higher proportion than the pulp. The main bioactive compounds found in mango peel are polyphenols, carotenoids, and organic acids. These compounds exhibit potent antioxidant, anti-inflammatory, and anti-aging activity.[4]

Orange plants are a significant horticultural commodity in Indonesia, with a wide distribution. In Indonesia, there are numerous types of oranges, and the agribusiness orange industry generates a lot of trash, such as fruit skins, seed residue, and pulp residue.[5] A research of the biological activity of *C. amblycarpa* revealed various interesting actions, including larvicidal Aedes aegypti[6], anti-diabetes[7], analgesic[8], and antioxidant[9]. Chemical study of its skin revealed the isolation of a number of chemicals, including flavonoids, alkaloids, phenolics[10], tannins, triterpenoids, quinones, steroids, vitamins C and A, coumarin, rosemary acid derivatives, oils, and essential[11]. The flavonoid is a source of promising antioxidant compounds.[12]

MATERIAL AND METHODS

Materials

Ethanol extract of *Mangifera indica and Cytrus amblycarpa* was grown in Bandung, West Java, Indonesia. All reagents, including gallic acid reference standard (Merck, Darmstadt, German); quercetin standard (MarkHerb, Bandung, Indonesia); ethanol, methanol, and Milli Q-water (HPLC grade, Merck); and Folin Ciocalteau 10%, sodium chloride, sodium

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acetate, $AlCl_3$ 2%, 1,1-diphenyl-2-picrylhydrazyl (DPPH), sodium carbonate, n-hexane, ethylacetate, acetonitrile, and silica gel 60 H (Merck) were of analytical grade.

Ethanol extraction

One hundred grams of *Mangifera indica and Citrus amblycarpa peeled* powder was extracted by stirring in 1000 mL of 96% (v/v) ethanol for 3 days at 20-22°C using a magnetic stirrer, and filtered to separate the insoluble plant material and solvent evaporated at 50 °C under reduced pressure in a rotary evaporator, resulting in 21.08 grams of *Mangifera indica* ethanol extract and 12.83425 grams of *Citrus amblycarpa.*

Phytochemical Screening

Alkaloids

Simplisia or other test material is placed in a test tube and then acidified with 2N hydrochloric acid, then filtered. The filtrate is flavored with ammonia solution 10% ammonia solution, then chloroform is added and shaken vigorously. The layer chloroform layer was pipetted while filtering, then into it added 2 N hydrochloric acid and shaken vigorously. 2 N hydrochloric acid and shaken vigorously until there were two layers, and the acid layer was pipetted and divided into three parts: 1. The Acid layer was pipetted divided into three parts: in part 1 was added Mayer reagent and the presence of a white precipitate or turbidity indicates a positive alkaloid, in section 2 is added to the Dragendorff reagent and the presence of an orange-yellow precipitate or turbidity indicates a positive alkaloid and part 3 is used as a blank. Preparation of Mayer reagent: 1.36 g of HgCl2 dissolved in 60 ml of water and 5 g of KI dissolved in 10 ml of water, then the two solutions were mixed and saturated with water until the volume was 100 ml with water until the volume is 100 ml. Preparation of Dragendorff reagent: 8 g Bi(NO3)3. H2O was dissolved in 30% b/v HNO3 and 27.2 g KI were dissolved in 50 ml of water, then the two solutions were mixed and left for 24 hours, filtered, and then saturated with water until the volume is 100 ml. The above procedure sometimes gives a false positive reaction, i.e. it does not contain alkaloids (should be negative). Chemical compounds that can give false positive alkaloid reactions are coumarins, polyphenols, purines, amino acids, proteins, and other nitrogenous compounds that the test material may also contain.

Polyphenolic Compounds

Simplisia or other test materials are placed in a test tube and then added to the test tube. The water was then heated in a water bath and filtered. To the filtrate iron (III) chloride reagent solution is added and the onset of green or blue-green, red-purple, blue-black to black color. green, red purple, blue-black to black indicate positive phenolics or the emergence of a brown precipitate indicates the presence of polyphenols. brown precipitate indicates the presence of polyphenolics.

Flavonoids

A 1 gram of simplisa was placed in a beaker, then added 100 ml of hot water and boil for 10 minutes. The mixture was filtered, and the filtrate was collected as solution C which would later be used for the examination of Flavonoids, Saponins, and Anthraquinones. 5 ml of solution C was put into a test tube, then magnesium powder and 1 ml of concentrated hydrochloric acid. The mixture was added amylalcohol, shaken vigorously then left until separation occurs. The formation of color in the layer of amylalcohol layer indicates the presence of flavonoid compounds.

Saponins

Take 5 ml of solution C, then put it into a test tube and shake it vertically for 10 seconds. vertically for 10 seconds. Left for 10 minutes. The formation of a stable 1 cm foam in the test tube indicates the presence of saponin compounds. And the foam still persists (does not disappear) after adding a few drops of hydrochloric acid chloride.

Anthraquinone

A 5 ml of solution C is put into a test tube. Added a few drops of 1 N Sodium Hydroxide solution. the formation of yellow to red color indicates the presence of quinone compounds.

Tannins

A 1 gram of simplisia is added to 100ml of hot water, then boiled for 15 minutes. The mixture was cooled, then filtered and the filtrate was divided into 3 parts in a test tube. To the first filtrate is added 1% iron(III) chloride solution. The formation of a dark blue or greenish-black color indicates the presence of the tannin compound group. Into the second filtrate was added 1% gelatin solution. The formation of a white precipitate The formation of a white precipitate indicates the presence of tannin compounds. To the third filtrate was added 15 ml of Steasny's reagent, then heated with the bath. The formation of a pink precipitate indicates the presence of concentrated tannin. The third test filtrate was filtered. Filtrate was saturated with the addition of sodium acetate, then a few drops of 1% iron(III) chloride solution were added. The formation of ink blue color indicates the presence of tannin gallate. Preparation of Steasny reagent: 2 parts of 30% formaldehyde were mixed with I part of concentrated hydrochloric acid.

Monoterpenes and Sesquiterpenes

Simplisia or other test material is crushed with ether and then filtered. The filtrate is placed in an evaporator cup and allowed to evaporate until dry, then a 10% solution of vanillin in concentrated sulfuric acid is added, and the appearance of colors positive sign of mono and sesquiterpene compounds.

Triterpenoids and Steroids

Simplisia or other test materials are crushed with ether and then filtered. Filtrate is placed in an evaporator cup and allowed to evaporate until dry, then Liebermann Burchard reagent solution is added and the occurrence of a red-purple color indicates positive triterpenoids, while when the green-blue color indicates positive steroids. Preparation of Liebermann Burchard reagent: 1 ml of anhydrous acetic acid was mixed with 1 ml of chloroform, then cooled at 0°C, then added 1 drop of concentrated sulfuric acid.

Antioxidant activity

The antioxidant activity of the extract was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The stock solution was prepared by diluting the extract with ethanol to obtain a concentration of 1000 ppm. *Mangifera indica L.* peel ethanol extract was prepared in various concentrations (5, 10, 15, 20 and 25 ppm), while *Citrus amblycarpa* peel ethanol extract was prepared in 100, 200, 300, 400 and 500 ppm. Each extract was combined with 1 ml of the DPPH solution stock, vortexed for one minute, and incubated at room temperature for 30 minutes. The solution absorbance was then measured using a spectrophotometer at 515.5 nm, and the content was calculated using the linear equation derived from the DPPH standard curve. The result was expressed as an inhibition concentration percentage (%IC_{50}).

Time and place

This study was carried out between May 2024, in the Pharmacy Laboratory, Faculty of Mathematics and Natural Science, Bandung Islamic University.

RESULTS

Phytochemical Screening

M. indica and *C. amblycarpa* were performed various kinds of methods to identify biological compounds and substances, the result was shown in table 1.

Results showed that both samples consists amount number of polifenolat, flavonoid and tannin, followed by anthraquinone, triterpenoid, steroid and alkaloid substances.

Antioxidant activity

In present study, DPPH were used in the damping technique to examine the antioxidant activity of *Mangifera indica and Citrus amblycarpa peeled* extract. The extract samples were used to determine the total of phenolic and total flavonoid compounds. However, in order to determine certain components present in the *Mangifera indica and Citrus amblycarpa peeled*, a preliminary test known as absorbance and inhibition was conducted as shown in Table 2.

Table 2 shows the absorbance and inhibition percentage of the applied antioxidants, the concentration of which was monitored to determine the antioxidant.

The findings indicated that the *Mangifera indica* peel ethanol extract IC_{50} values were 29,28 µg/mL (Figure 2). The lower the IC_{50} value, the stronger antioxidant activity in warding off free radicals.

The findings demonstrated that *Citrus amblycarpa* peel ethanol extract had IC_{50} values of 669,52 µg/mL (Figure 4).

Table 1. Active substances in *M.indica* and *C. amblycarpa* peel ethanol extract.

Parameter	Mangifera indica peel	Citrus amblycarpa peel
Alkaloid Mayer	+/-	+
Alkaloid Dragendorf	+/-	+/-
Polifenolat	++	++
Flavonoid	++	++
Saponin	-	-
Anthraquinone	+	++
Tannin	++	++
Monoterpene, sesquiterpene	+/-	+
Triterpenoid and steroid	+	+

Table 2. DPPH scavenging of extracts in various concentrations.

Sample	Concentration (µg/mL)	Absorbance	% Inhibition (%)
	10	0,63	20,95
	20	0,502	37,01
M. indica	30	0,398	50,06
	40	0,26	67,38
	50	0,16	79,92
	100	0,591	25,85
	200	0,492	38,27
C. amblycarpa	300	0,418	47,55
	400	0,343	56,96
	500	0,281	64,74
	10	0,648	18,70
	20	0.589	26,10
Mix fruits	40	0,446	44,04
	60	0,312	60,85
	80	0,196	75,41

Table 3. Antioxidant activity of *Mangifera indica*, *Citrus amblycarpa* and Combination samples by DPPH Method.

Sample	Concentration (µg/mL)	Category
Mangifera indica	29,28	Very strong
Citrus amblycarpa	669,52	Weak
M.indica + C.amblycarpa	48,05	Very strong

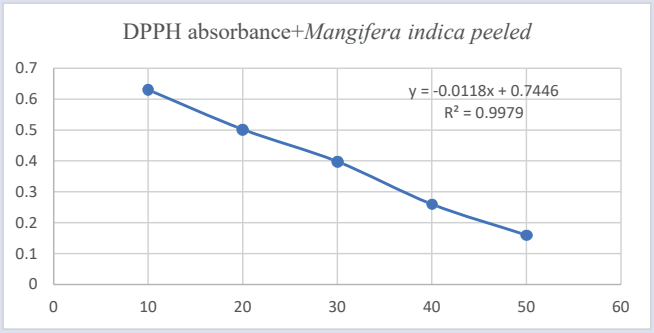

Figure 1: Concentration–absorbance graph of *Mangifera indica peeled*.

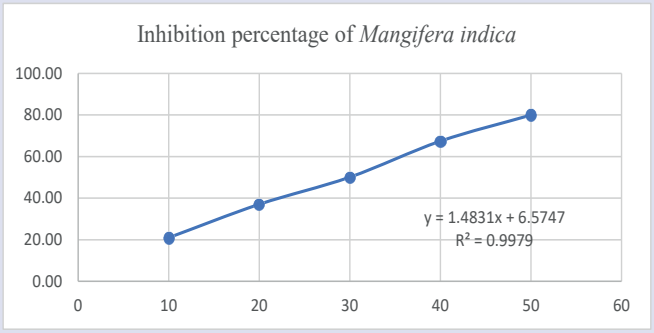

Figure 2: The inhibition percentage of *Mangifera indica in various* concentration.

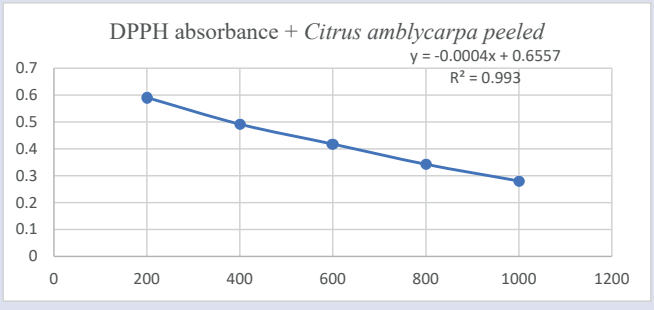

Figure 3: Concentration–absorbance graph of *Citrus amblycarpa peeled.*

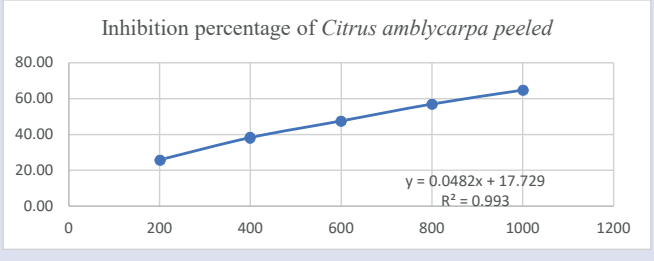

Figure 4: The inhibition percentage of *C.amblycarpa* in various concentration.

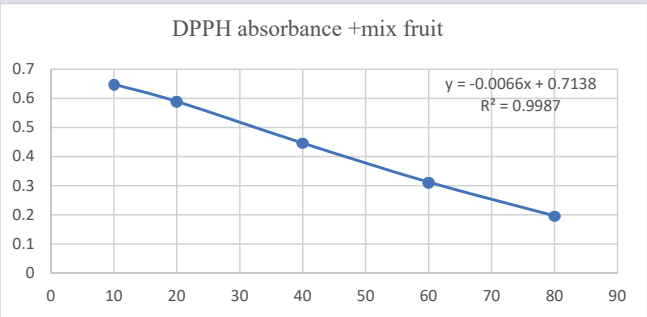

Figure 5: Concentration–absorbance graph of mix fruits.

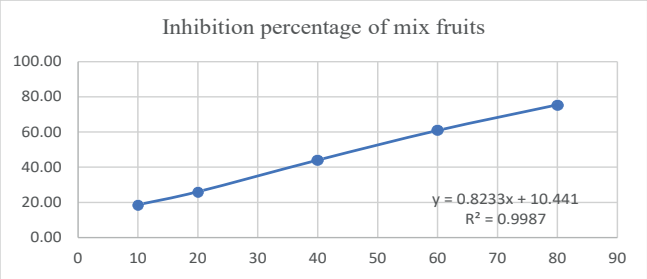

Figure 6: The inhibition percentage of mix fruits in various concentration.

This study also measure the antioxidant activity from combination samples from *M. indica* and *C. amblycarpa* with 50:50 percent ratio (Figure 5) , and showed IC_{50} value from combination samples were 48,05 µg/mL (Figure 6).

With an IC_{50} value of 29,28 µg/mL; 669,52 µg/mL for separate and 48,05 µg/mL for combined peeled extract, respectively, the results of the DPPH radical scavenging activity were presented in Table 3. Very strong antioxidant activity was defined as having an IC_{50} value of less than 50 µg/mL, strong antioxidant activity as 50–100 µg/mL, moderate antioxidant activity as 100–250 µg/mL, weak antioxidant activity as 250–500 µg/mL, and inactive antioxidant activity as more than 500 µg/mL.[13]

DISCUSSION

Antioxidants are compounds that inhibit the oxidation of other molecules and protect the body from the effects of free radicals, produced either by normal cell metabolism or as an effect of pollution and exposure to other external factors and are responsible for premature aging and play a role in cardiovascular disease.[14] A balanced and nutritious diet is the cornerstone of good health. The nutritional pyramid advises eating 5 servings of fruit and vegetables a day as a basic principle of a healthy diet, as they provide essential nutrients such as vitamins and minerals, as well as fiber and antioxidants. Consuming them in adequate quantities improves the immune system and helps prevent diseases such as diabetes, obesity, heart disease and even certain types of cancer. Among the most nutrient-dense nutrients in fruit and vegetables are antioxidants.[15]

A previous study by Garcia-Villegas demonstrated the mango peel extract was shown to be effective against the •NO radical with an IC_{50} of 7.5 µg/mL,[4] has the same category in this study, which is very strong antioxidant with IC_{50} 29,28 µg/mL. *Mangifera indica* L. compounds such as flavonoids and phenolic acids are capable of inhibiting enzymes through the interaction of their hydroxyl group with the functional group of the enzyme.[16] Flavonoids will induce changes in the secondary structure of enzymes and consequently lead to changes in their activity. Mango peel contains phenolic compounds including gallic acid and hydrolysable tannins, which are known to be metal chelators and therefore, can bind to the active site and prevent the substrate from being enzymatically hydrolyzed.[17] Therefore, the inhibitory activity of mango peel extract could again be explained by the presence of phenolic compounds.[18,19]

Citrus fruits are well accepted by consumers owing to their pleasant flavors and abundant phytochemicals, such as vitamins A and C, mineral elements, carotenoids, and phenolics.[10] This study showed that the antioxidant level in *C. amblycarpa* peel ethanol extract IC_{50} was 669,52 µg/mL which in very weak categories[13] almost similar with another study that demonstrated *C. amblycarpa* exhibited the antioxidant activity (IC_{50} value of 112.71 ± 4.50 µg/mL µg/mL).[20] Previous studies tested the antioxidant activity of n-hexane, ethyl acetate, and methanol extracts of lime citrus peel using the DPPH method.[14] The IC_{50} value of the three extracts was 162.16 g/mL (weak category antioxidant), 134.02 g/mL (medium category antioxidant) and 94.01 g/mL (strong category antioxidant).[21] Citrus is an excellent source of many nutrients and phytoconstituents and can supply a healthy diet. Phenolic acids and flavanones are the two main groups of phenolic compounds in citrus fruit juices.[22] Antioxidant effects might occur due to oxygenated monoterpenes, sesquiterpenes[12], flavonoids, phenolic acids, stilbenes, lignans, and other phenolic compounds. Citrus fruit phytochemicals may have antioxidant properties via raising liver protective enzyme activity, blocking lipids to prevent deoxyribonucleic acid (DNA) damage, and bolstering the immune system.[23]

This study also showed the combination of *M. indica L.* and *C. amblycarpa* peel ethanol extract demonstrated an increase in antioxidant activity on *C. amblycarpa* from IC_{50} 669,52 µg/mL to IC_{50} 48,05 µg/mL, which convert from a very weak antioxidant category into a very strong antioxidant. It might be due to the main bioactive compounds identified in mango including phenolic acids (coumaric acid, ferulic acid, and hydroxybenzoic acid), polyphenols (quercetin, mangiferin, catechins, tannins, kaempferol, anthocyanins, gallic acid, ellagic acid), carotenoids, which are the most abundant.[24]

CONCLUSION

Mangifera indica Linn. peel ethanol extract has an inducing effect on *Citrus amblycarpa* antioxidant activity.

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CONFLICTS OF INTEREST

None

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