Antioxidant and Antimicrobial Activities of *Musaparadisiaca L.*, *Citrus Microcarpa L.*, and *Mangifera Indica L.* Peel Extracts

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Abstract

The growing awareness and concern of the public for the safety and efficacy of synthetic preservatives in food and pharmaceutical preparations demand a search for effective, safer and cheaper alternatives. This study aims to utilize the peels of Musa paradisiaca, Citrus microcarpa and Mangifera indica aqueous, ethanolic and methanolic extracts as potential food and pharmaceutical preservatives through determination of their antioxidant and antimicrobial activities. All extracts, except methanolic extract of C. microcarpa, exhibited comparable activity with standard antioxidant preservative, butylated hydroxytoluene, by half maximal inhibitory concentration (IC50) measurement using the ferric reducing antioxidant power (FRAP) assay (p>0.05). Aqueous extract of M. paradisiaca (p=0.509, n/a, 0.347), C. microcarpa (p=0.916, 0.504, 0.773) and M. indica (p=n/a,0.999, 0.942) revealed similar activity with the antimicrobial preservative, methylparaben, against Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae. These properties were attributed to the secondary metabolites present in the extracts such as glycosides, reducing substances, tannins, plant acids and flavones/flavonols. This study implies that these peel waste extracts may be used as an alternative and cheap source of preservatives in food and pharmaceutical industry.

Keywords: natural preservatives, banana, calamansi, mango, antimicrobial, antioxidant

Introduction

Food-borne diseases, food contamination and microbial spoilage of pharmaceuticals continue to become a global concern to both developing and industrialized countries due to changes in production practices, climate change, rise in global trade of food and the continuing microbial resistance of microorganisms (Havelon, 2010). Preservatives are added to prolong shelf life and limit the microbial growth on food and pharmaceutical products. Commonly, synthetic preservatives are used for such purpose. However, due to concerns about the safety and efficacy of these compounds (Wilson *et al.*, 2015), industries and research agencies are pressured to search for alternative sources of preservatives.

Preservatives act by inhibiting the growth of microorganisms and preventing lipid oxidation of different products, thus extending shelf life. Plant-occurring bioactive compounds and medicinal plants have long

been reviewed and considered as potential antimicrobial agents to replace synthetic preservatives in industries (6). Phytochemicals present in these plants such as phenolic compounds, terpenoids and essential oils, alkaloids and polypeptides (Podlubny, 1999) inhibit microbial activity by altering cellular permeability (Rucinsky and Voght, 1990), binding and inactivating proteins (Tenne, 2014) and complexing with bacterial cell walls (Whittaker and Wilson, 1927).

The Philippines has a plethora of plants that can be used as an alternative source of preservatives. In this study, peels of *Musa paradisiaca* (saba), *Citrus microcarpa* (calamansi) and *Mangifera indica* (mango), which are commonly thrown away, were evaluated for their antioxidant and antimicrobial activities.

The utilization of fruit peels as an alternative source of preservative is beneficial for as these wastes will be upcycled, they help reduce economic problems and environmental pollution.

Methodology

Preparation of Crude Extracts

Fruit peel wastes of ripe *Musa paradisiaca* (MP), *Citrus microcarpa* (CM) and *Mangifera indica* (MI) were used in the study. The peels were collected from street vendors around Paco, Manila. Voucher specimens were submitted to the National Museum Plant Division-Philippines and authenticated by curator Wilfredo F. Vendivil, PhD (control number: 170- 2014). The peels were washed thoroughly with distilled water, cut into small pieces, air-dried, and then crushed into powder. The powder was macerated into different solvents (95% ethanol, methanol and water) for 72 hours with constant shaking. Methanol and ethanol extracts were concentrated using rotary evaporator while the aqueous extract was evaporated to dryness using water bath. The dried extracts were stored in an amber container at 4°C until use. The resulting extracts were subjected to phytochemical screening, antioxidant and antimicrobial assays.

Phytochemical Screening

Extracts were screened for the presence of secondary metabolites such as alkaloids, glycosides, tannins and other polyphenols, reducing substances, plant acids, saponins and sapogenins, flavones and flavonols, and flavonoids (Aguinaldo *et al.*, 2005).

Test for Alkaloids

The presence of alkaloids was determined by acidifying 2 mL of the extracts with 1% hydrochloric acid. Two drops of Mayer's reagent (mercuric potassium iodide TS) were added to the acidified solution. The resulting precipitation indicated the presence of alkaloids.

Test for Glycosides

A few drops of lead acetate TS were added to the extracts (2 mL) of each sample and then

filtered. Lead sub-acetate TS was added to the filtrates until neutral or alkaline. The precipitation

or turbidity indicated the presence of glycosides.

Test for Tannins and Other Polyphenols

Few drops of 5% ferric chloride in methanol were added to the 2 mL extracts of each sample. The formation of blue-black precipitate was recorded as a positive result.

Reducing Substances

An equal amount (1 mL) of Fehling's A and Fehling's B were transferred to 2 mL extracts of each sample. The solutions were heated to boiling. A few drops of concentrated hydrophilic acid were added to the heated solution. The formation of brick-red precipitate indicated the presence of reducing substances.

Test for Plant Acids

Few milliliters of sodium carbonate TS were added to the extract of each plant sample. The occurrence of a stable and dense froth was recorded as a positive result indicating the presence of plant acids.

Test for Saponins

Extracts (2 mL) of each sample were shaken vigorously for 30 seconds then allowed to stand in a vertical position for about 30 minutes. The resulting stable froth indicated the presence of saponins.

Test for Saponins and Sapogenins

Five milliliter extracts of each sample were added with a few drops of saturated alcoholic solution of cholesterol. Formation of crystalline precipitate was recorded as a positive result.

Test for Flavones and Flavonols: Shinoda Test

Magnesium powder and few drops of concentrated hydrochloric acid were added to 2 mL extracts of each sample. Orange, pink, red to purple colors indicated the presence of flavones, flavonols, the corresponding 2,3-dihydro derivatives and/or xanthones.

Test for Flavonoids

Few grams of extracts were digested with 2N hydrochloric acid in 1-propanol for 15-30 minutes.

A slow development of a strong red or violet color was indicative of a positive reaction for the presence of flavonoids.

Ferric Reducing Antioxidant Power (FRAP) Assay

Antioxidant power by reduction of ferric iron to ferrous was measured using FRAP assay (12). Tubes containing 750 µL of phosphate buffer (0.2M, pH = 6.6) and 750 µL potassium ferricyanide (1%) were prepared to which 300µL solutions of 0.125, 0.250, 0.500, 1.000, 5.000 and 10.000 mg/mL extracts were added. The mixtures were incubated at 50°C for 30 minutes, added with 750 µL trichloroacetic acid (10%) and centrifuged for 10 minutes at 3000 g. A 500-µL upper layer of the mixtures were transferred to another tube and diluted with 500 µL distilled water. The solutions were mixed with 100 µL fresh ferric chloride (0.1%) and then measured at 700 nm using UV-Vis spectrophotometer (Hitachi UH5300).

Distilled water was used as blank while butylated hydroxytoluene (BHT) was used as positive control.

The antioxidant activity was expressed as IC50 (mg/mL), which signifies the concentration that provides 50% antioxidant power.

Antimicrobial Susceptibility Assay

Isolated cultures of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Klebsiella pneumoniae* ATCC 13381 were bought from the Department of Microbiology, College of Public Health, University of the Philippines Manila. Microorganisms were inoculated in sterile Mueller-Hinton broth and turbidity was compared to 0.5 McFarland standard equivalent to $1x10^8$ cfu/mL bacterial suspension (Lahitha 2005). Sterile cotton swabs were dipped in the standardized test inocula and immediately streaked in the Mueller-Hinton agar. Streaking was repeated up to four times to distribute the inoculum evenly in the agar surface. Paper discs (Whatman grade AA discs, 6 mm) were dipped in the crude extracts and dried at 30°C in hot air oven. The dried paper discs were incubated at 37°C for 24 h. The diameter of the inhibition was measured in mm using a transparent ruler. Solvents without the extract were used as negative control while 0.05% methylparaben was used as positive control.

Statistical Analysis

The data gathered were recorded as mean ± SEM, with all measurements done in triplicate. The difference in means between extracts and positive controls was determined by one-way one-way ANOVA, followed by Tukey's HSD test using SPSS 17.0 software.

Results

The phytochemical screening revealed the presence of glycosides in the aqueous, ethanolic and

methanolic extracts of MP, CM and MI fruit peel wastes, with pH ranging from 4 – 5 (See Tables 1-3).

Table 1. Phytochemical Screening of M. Paradisiaca (MP) Peel Extracts

Test MP extracts

Aqueous Ethanolic Methanolic

pH 5 5 5

Tannins (-) (-) (-)

Glycosides (+) (+) (+)

Reducing substances (+) (+) (+)

Alkaloids (-) (-) (-)

Plant acids (+) (-) (-)

Saponins (-) (-) (-)

Flavones/flavonols (-) (-) (-)

Flavonoids (-) (-) (-)

(+) indicates presence while (-) signifies absence of the secondary metabolite.

Table 2. Phytochemical Screening of C. microcarpa (CM) peel extracts

Test CM extracts

Aqueous Ethanolic Methanolic

pH 5 5 5

Tannins (-) (-) (-)

Glycosides (+) (+) (+)

Reducing substances (-) (+) (+)

Alkaloids (-) (-) (-)

Plant acids (-) (-) (-)

Saponins (-) (-) (-)

Flavones/flavonols (+) (-) (-)

Flavonoids (-) (-) (-)

(+) indicates presence while (-) signifies absence of the secondary metabolite.

Probability values less than 0.05 (p<0.05) were considered statistically significant.

Table 3. Phytochemical Screening of M. Indica (MI) Peel Extracts

Test MI extracts	Aqueous	Ethanolic	Methanolic
pH	4	4	4
Tannins	(-)	(+)	(+)
Glycosides	(+)	(+)	(+)
Reducing substances	(-)	(-)	(-)
Alkaloids	(-)	(-)	(-)
Plant acids	(-)	(-)	(-)
Saponins	(-)	(-)	(-)
Flavones/flavonols	(-)	(+)	(+)
Flavonoids	(-)	(-)	(-)

(+) indicates presence while (-) signifies absence of the secondary metabolite.

Table.4 shows the IC_{50} of each peel extracts of MP, CM and MI. Ethanolic peel extracts of CM exhibited the lowest IC_{50} value at 7.88 mg/mL (p=1.000) while the rest of the extracts showed higher IC_{50} value than standard antioxidant, BHT. Only ethanolic peel extract of MP (p<0.000) exhibited significant difference with BHT while the rest demonstrated comparable activity relative to BHT

 higher IC_{50} value than standard antioxidant, BHT. Only ethanolic peel extract of MP (p<0.000) exhibited significant difference with BHT while the rest demonstrated comparable activity relative to BHT (p>0.05).

The zones of inhibition (ZOI) of different peel extracts of MP, CM and MI against selected microorganisms are presented in Table 5. For *E. coli*, aqueous peel extracts of MP (p=0.509) and CM (p=0.916) displayed higher inhibition than the standard preservative, 0.5% methylparaben.

Table 4. IC_{50} Of M. Paradisiaca, C. Microcarpa and M. Indica Ethanolic Peel Extracts with Butylated Hydroxytoluene (BHT) As Positive Control.

	Extract IC_{50}, mg/mL		
	Aqueous	Ethanolic	Methanolic
MP	14.5 ± 4.03	58.8 ± 4.07*	25.1 ± 5.98
CM	20.4 ± 7.33	7.88 ± 0.05	-
MI	17.3 ± 3.45	20.1 ± 4.50	20.9 ± 0.87
BHT	8.72 ± 0.31	-	-

*Significant with positive control at p< 0.05. Columns with (-) indicate not measured.

Table 5. Zones of Inhibition (ZOI) of Ethanolic Peel Extracts of MP, CM And MI.

	Extract ZOI, mm		
	Aqueous	Ethanolic	Methanolic
E. coli			
MP	9.67 ± 0.88	0	4.50 ± 0.50*
CM	9.00 ± 0.58	0	0
MI	0	0	2.00 ± 0.00*
0.5% methylparaben	8.33 ± 0.33	-	-
S. aureus			
MP	0	4.33 ± 0.88*	4.33 ± 0.88*
CM	7.00 ± 0.00	5.50 ± 2.12	7.00 ± 0.00
MI	8.67 ± 0.33	0	0
0.5% methylparaben	9.07 ± 0.35	-	-
K. pneumoniae			
MP	8.50 ± 0.50	0	0
CM	7.50 ± 0.50	0	9.00 ± 2.00
MI	7.00 ± 0.00	1.00 ± 0.00*	10.0 ± 2.00
0.5% methylparaben	6.00 ± 0.58	-	-

*Significant with positive control, 0.5% methylparaben, at p<0.05. Columns with (-) indicate not measured

All extracts showed lower inhibition than the standard preservative against *S. aureus*. However, aqueous extracts of CM (p=0.504) and MI (p=0.999), and ethanolic (p=0.072) and methanolic extracts (p=0.504) of CM demonstrated comparable activity. For *K. pneumoniae*, aqueous extracts of MP (p=0.347), CM (p=0.773) and MI (p=0.942), and methanolic extracts of CM (p=0.342) and MI (p=0.177) displayed similar activity with the standard preservative.

Discussion

Utilizing fruit peel wastes is necessary in order to reduce the disposal of these wastes, and decrease economic and environmental problems in the country. In this study, the antioxidant and antimicrobial activities of the aqueous, ethanolic and methanolic extracts of *M. paradisiaca*, *C. microcarpa* and *M. indica* peels were investigated in order to find out how to utilize these wastes as potential sources of preservatives.

The FRAP assay revealed comparable activity of all extracts relative to commonly used antioxidant

in ready-to-eat foods, BHT (14), except ethanolic extract of *M. paradisiaca*, implying that these

extracts are potent antioxidants for food preservation. This may be due to the presence of different secondary metabolites, including plant acids, glycosides, reducing substances, flavones/flavonols and tannins, found in the phytochemical screening of the extracts. Phenolic compounds and those that contain aromatic rings, such as tannins, flavones and flavonols, are able to interrupt the free radical chain reaction occurring in the oxidation process by donating electrons to the free radicals formed. As a result, these antioxidants become radicals themselves but are stabilized through electron delocalization in the aromatic ring and formation of quinone structures (15). Reducing substances and glycosides, with their active hydroxyl groups, are also potent reducing agents capable of scavenging free radicals, leading to termination of oxidative chain reactions that are harmful to cells (16). *M. paradisiaca* peel extracts contain high amounts of catecholamines, such as L-3,4- dihydroxyphenylalanine and dopamine, which contribute to their significant antioxidant activity (17). Study (18) revealed that *C. microcarpa* peels are a good source of phenolic acids, with p-coumaric acid and ferulic acid as the dominant free and bound phenolic acids, respectively. The peels of *M. indica* were also found to have large amounts of polyphenols (19-20) which are known antioxidants. Most of the aqueous peel extracts of *M. paradisiaca, C. microcarpa* and *M. indica* displayed comparable activity with the standard antimicrobial preservatives for pharmaceutical preparations, methylparaben (21), against gram negative bacteria, **E. coli** and *K. pneumoniae*, and gram positive bacteria, *S. aureus*. Glycosides, the phytochemical present in the aqueous peel extracts of the three selected fruits, are known antimicrobials that work by disrupting the bacterial inner cell membrane. This results in the inactivation of bacterial enzymes and transport proteins, inhibiting cell respiration and growth, which ensues bacterial cell death. This has been demonstrated in one study (22) which showed increased concentrations of reducing sugars and proteins in the bacterial growth medium as a result of leakage caused by cell wall damage. Delphinidin-3- glucoside and cyaniding-3- glucoside are anthocyanins found in ripe *M. paradisiaca* peels (23). These anthocyanins work by chelating the metal ions important in the

activity of many enzymes; thus, increasing the lag phase and inhibit the maximum growth of bacteria (24). Phenolic compounds, such as p-coumaric acid and ferulic acid, present in the peels of C. microcarpa (18) also showed to successively inhibit the growth of the food contaminant, *S. aureus*, in chicken soup (25).

Conclusion

Based on the results presented, aqueous extracts of *M. paradisiaca, C. microcarpa* and *M. indica* peels displayed comparable antimicrobial activity with standard antioxidant preservative, methylparaben. On the other hand, all the extracts except methanolic peel extracts of *C. microcarpa* demonstrated similar antioxidant activity in terms of IC_{50} with standard antioxidant preservative, butylated hydroxytoluene.

These findings suggest the potential use of these peel waste extracts as an alternative source of preservatives. Isolation of the metabolite/s responsible for these bioactivities is recommended to develop a cheaper, safer and novel agent to replace synthetic preservatives for food and pharmaceutical use. Further analyses such as toxicological tests are recommended to prove the safety of the extracts.

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