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Antimicrobial Activity of some Tropical Fruit Wastes (Guava, Starfruit, Banana, Papaya, Passionfruit, Langsat, Duku, Rambutan and Rambai)

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ABSTRAK

*Ekstrak buah jambu batu muda, ranum dan daun jambu batu (*Psidium guajava*); buah belimbing bintang (*Averrhoa carambola*) muda, ranum dan daun belimbing bintang; buah pisang (*Musa sapientum *variety Montel) muda dan ranum; buah betik (*Carica papaya*) muda dan ranum; kulit buah markisa (*Passiflora edulis *F. Flavicarpa); kulit buah duku dan langsat (*Lansium domesticum*); kulit rambutan (*Nephelium lappaceum*) dan kulit rambai (*Baccaurea motleyana*) diuji keaktifannya menentang bakteria gram positif, gram negatif, yis dan kulat* (Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Lactobacillus bulgaricus; E. coli, Proteus vulgaricus, Pseudomonas aeruginosa, Salmonelli typhi; Saccharomyces cerevisiae, Candida lypolytica; Rhizopus *spp.*, Aspergillus niger, *dan* Chlamydomucor *spp.*). *Keaktifan anti mikrob telah diuji menggunakan kedua-dua kaedah, iaitu resapan cakera kertas turas dan pencairan dalam tabung uji. Ekstrak dari belimbing bintang ranum, daun jambu batu dan kulit rambai menunjukkan kebolehan menentang semua bakteria yang diuji, dengan kekuatan yang lebih daripada 50ug streptomycin. Kulit buah markisa, buah jambu batu muda dan ranum, mempunyai kebolehan menentang kesemua bakteria melainkan* E. coli. *Kulit rambutan juga menunjukkan kebolehan menentang semua bakteria yang diuji melainkan* Pseudomonas aeruginosa. *Kebanyakan hasil buangan buah-buahan di atas menunjukkan kebolehan melawan bakteria tetapi tidak yis atau kulat. Ekstrak kulit pisang, betik, duku, langsat dan rambutan menunjukkan aktiviti ke atas* Candida lypolytica *dan ekstrak jambu batu menunjukkan aktiviti yang kuat melawan* Saccharomyces cerevisiae. *Selain dari jambu batu, buah belimbing ranum, kulit rambai dan rambutan menunjukkan potensi digunakan untuk menentang bakteria.*

ABSTRACT

*Extracts of ripe, unripe and leaves of guava (*Psidium guajava*); ripe, unripe and leaves of starfruit (*Averrhoa carambola*); ripe and unripe banana (*Musa sapientum *variety Montel); ripe and unripe papaya (*Carica papaya*); passionfruit (*Passiflora edulis *F. Flavicarpa) peel; two varieties of* Lansium domesticum *peel (langsat and duku); rambutan (*Nephelium lappaceum*) peel and rambai (*Baccaurea motleyana*) peel were evaluated for antimicrobial activity against gram positive bacteria, gram negative bacteria, yeast and fungi* (Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Lactobacillus bulgaricus; E. coli, Proteus vulgaricus, Pseudomonas aeruginosa, Salmonelli typhi; Saccharomyces cerevisiae, Candida lypolytica; Rhizopus *spp.*, Aspergillus niger, *and* Chlamydomucor *spp*). *The antimicrobial activities were tested using both the filter paper disc diffusion and tube dilution assays. Extracts from ripe starfruit, guava leaves and rambai peel showed strong activity against all the bacteria tested, in most cases with activity stronger than 50ug streptomycin. Passionfruit peel, ripe and unripe guava showed activity against all the bacteria tested except* E. coli. *Rambutan peel too showed activity against all the bacteria tested except towards* Pseudomonas aeruginosa. *Most of the fruit wastes showed some activity towards bacteria but poor activity against yeast or fungi. Extracts from bananas, papayas, passionfruit peel,* Lansium domesticum *peels and rambutan peels showed activity against* Candida lypolytica *while extracts from guava showed strong activity against* Saccharomyces cerevisiae. *Other than guava, ripe starfruit, rambai peel and rambutan peel showed potential for use against bacteria.*

INTRODUCTION

Various fruits (peel, flesh or seed) have been used in traditional medicine for stomach ache, sore eyes, fever, etc. Papaya has been shown to contain sulphydroxyl protease which can inhibit virus or microbial infection (Rajashekhara *et al.* 1990). Jensen *et al.* (1990) found passionfruit effective against *Bacillus subtilis* and some yeasts. The astringent compound in *Lansium domesticum* skin has been used in traditional medicine against hernia. Rambutan roots and leaves have been used by the Malays for fever, and the bark for tongue infection. Other fruits that have been shown to have some antimicrobial activity include mangosteen *Garcinia mangostana* (Sundram *et al.* 1983); cranberry (Senchyuk and Demkevich 1974); *Glaucium flavum* (Cabo *et al.* 1988); bedara *Ziziphus spinachristi* (Shah *et al.* 1986) and *Annona montana* (Wu *et al.* 1987).

Guava leaves are used in the Malay archipelago for diarrhoea, stomach ache, ermifuge, leucorrhoea, lotion for skin complaints and in childbirth to expel the placenta, while extracts have been on the market in Europe for diarrhoea or gastroenteritis (Burkill 1966). Starfruit flowers which are pleasantly acid are used in salads and are considered to have a vermifuge action; and applied topically for skin irritation or prickly heat. Crushed starfruit leaves and shoots are used for chicken-pox, ringworm and headache; and a decoction of the leaves and fruits is given to arrest vomiting. The fruit juice is also given as a corrective after a drinking-bout, biliousness, diarrhoea and as a cooling drink in fevers (Burkill 1966). Rambai skin is used for sore eyes, taken internally after childbirth and currently in the Malaysian and Indonesian traditional cosmetic industries, in medicated face powder preparation for treatment of acne and general skin complaints; and is also eaten as a vegetable.

The present work attempts to investigate if wastes from the fruit industry can be used for antimicrobial activity in cosmetics, food, etc. This work will also inform us whether some fruits are beneficial for consumption during sickness and help recovery as an alternative to taking antibiotics.

MATERIALS AND METHODS

Plant Material

Ripe, unripe and leaves of guava (*Psidium guajava*); ripe, unripe and leaves of starfruit (*Averrhoa carambola*); ripe and unripe banana (*Musa sapientum* variety Montel); ripe and unripe papaya (*Carica papaya*); passionfruit (*Passiflora edulis* F. Flavicarpa) peel; two varieties of *Lansium domesticum* peel (langsat and duku); rambutan (*Nephelium lappaceum*) peel and rambai (*Baccaurea motleyana*) peel were obtained from the UPM orchard or the evening market. The plant materials were chopped and dried at room temperature or 37°C oven. They were then ground to a fine powder in a cyclone mill and stored at room temperature for extraction.

Chemicals

Petroleum ether (B.P. 40-60), chloroform, and ethanol were obtained from BDH, Poole, England. Bacterial nutrient agar (NA), Malt extract agar (MEA) for yeast and fungi, peptone water and triptone soya broth (TSB) were obtained from Oxoid (Basingstoke, Hants, England, UK). Miconozole (Johnson & Johnson Malaysia Ltd.) and streptomycin sulphate (Becton Dickson & Co., Cockeysville, D 21030, USA) were used as standards.

Microorganisms

The test microorganisms include gram positive bacteria (*Staphylococcus aureus* ATCC 12598, *Bacillus subtilis* ATCC 6051, *Bacillus cereus* IFO 3457, *Lactobacillus bulgaricus*); gram negative bacteria (*E. coli* ATCC 25922, *Proteus vulgaricus* ATCC 13315, *Pseudomonas aeruginosa* IFO 3445, and *Salmonelli typhi* IFO 12529); yeasts (*Saccharomyces cerevisiae* ATCC 7754, *Candida lypolytica* ATCC 8661); and fungi (*Rhizopus* spp. ATCC 4270, *Aspergillus niger* ATCC 1015, and *Chlamydomucor* spp.) were obtained from the culture collection of the Department of Food Science, Universiti Pertanian Malaysia (ATCC = American Type Culture Collection; IFO = Institute of Fermentation, Osaka). The stock cultures were grown on TSB for 24 h at 30°C. The cells were diluted to give a final concentration of 10^5-10^6 CFU/ml (Colony Forming Unite) determined by using a haemocytometer.

Plant Extracts

The dried, powdered samples were weighed and successively extracted with petroleum ether, chloroform, and ethanol. A general extraction procedure was followed for each solvent by soaking the powdered seeds overnight and the solution filtered. The extraction was repeated

three times, each using a fresh solvent. The combined extracts of each solvent were filtered and the filtrates were evaporated under reduced pressure at temperatures below 50°C. The dark brown viscous residues were weighed, then reconstituted in 5 ml ethanol and the sterile filter paper discs (6 mm diameter) were impregnated with each plant extract solution and left to dry at room temperature.

Agar Diffusion Technique (Nazrul *et al.* 1984)

For antibacterial activity, nutrient agar (NA), pH 7 - 7.2, was sterilized for 15 min at 110°C. Twenty ml of NA were added to each 100 mm sterile Petri dish and kept at 30°C for 24 h to confirm sterility. All tests were done by placing the dried discs impregnated with plant extract on the agar surface previously inoculated with a suspension of each microorganism (10^5 - 10^6 CFU/ml). The antifungal and anticandidal activities were similarly determined using malt extract agar.

The growth and the purity of each suspension was verified by using a Gram stain. Standard discs of Miconozole (1 µg /disc) and streptomycin sulphate (50 µg / disc) were used as positive controls. The plates were incubated at 30°C for 24 h and the antimicrobial activity was recorded as the width, in mm, of the clear inhibition zones surrounding the discs. Each test was repeated at least 3 times.

Minimum Inhibition Concentration (Tanaguchi and Satumura 1972; and Kubo *et al.* 1992)

0.5 ml of triptone soya broth containing 10^7 test organisms ml^{-1} was mixed with 100 - 2500 µg /ml extracts in two-fold dilution assay and incubated for 24 h at 30°C. Growth was measured by the optical density at 660 nm and the viability of the cultures was confirmed by incubation of the broth on agar plate (Oxoid, Basingstoke, Hants, England). Miconozole and streptomycin sulphate were used as standard antibiotics for comparison with the activities of the plant extracts against microbial species. The concentration of the tube of the highest dilution that was free from growth was recorded as the minimum inhibitory concentration (MIC, µg/ml).

RESULTS AND DISCUSSION

All the microorganisms responded differently to the various plant extracts and all the plant extracts tested showed some antimicrobial activity (Table 1). Most of the fruit plants showed good activity against bacteria but poor activity against yeasts and fungi; thus this is the reason why spoilage of fruits is often caused by fungal and yeast infection (Table 2). Ripe starfruit, guava leaves and rambai peel showed antibacterial activity against all the bacteria tested. Rambai peel and guava leaves showed activity above that of 50 µg streptomycin and appeared to be as a good antimicrobial agent. Ripe and unripe guava and passionfruit peel showed activity against all the bacteria except towards *E. coli.* Unripe banana showed activity against all the bacteria except towards *P. vulgaricus.* On the other hand rambutan peel was active against all the bacteria except *Pseudomonas aeruginosa.* Quite a number of the fruits and especially rambutan peel showed good activity against *Candida lipolytica* while extracts from guava plants showed strong activity against *Saccharomyces cerevisiae* with an inhibition zone double that of the standard.

The most active extracts appeared to be from ripe starfruits, immature guava, rambai peel, and rambutan peel.

Guava extracts of all polarity were found to be active against bacteria and yeast tested, indicating that more than one component may be responsible for the observed antimicrobial activity. Past research findings indicate the presence of polyphenolic compounds in guava, quercetin, avicularin and guaijaverin (Seshadri and Vasista 1964) being the active antimicrobial components in guava leaf.

In general, the more polar extracts showed stronger antimicrobial activity. It is interesting to note that most gram-negative bacteria: *Pseudomonas, Proteus vulgaricus, Salmonelli typhi* and *E. coli* were inhibited by rambai skin extracts, ripe starfruit, guava, immature papaya and rambutan peel.

Bioassay guided isolation of the active antimicrobial components of starfruit, rambai skin and rambutan skin extracts will be carried out to identify the compounds responsible for these activities.

TABLE 1a
Antibacterial activity of the tested fruits and fruit leaves extracts on gram positive and gram negative bacteria after 24 h incubation at 30°C

Plant / Bacteria	Average Diameter of Inhibition (mm)						
	SA	BS	BC	LB	EC	PV	PA
Ripe starfruit							
Pet ether	-	-	-	-	-	-	-
$CHCl_3$	**15**	10	7	7	-	-	-
Ethanol	**18**	15	**25**	8	10	20	15
Immature starfruit							
Pet ether	-	10	**18**	-	-	20	-
$CHCl_3$	-	10	**18**	-	-	20	-
Ethanol	-	20	**30**	-	-	15	-
Starfruit leaves							
Pet ether	-	10	12	n.d.	7	-	-
$CHCl_3$	-	7	-	n.d.	-	-	-
Ethanol	**10**	18	**18**	n.d.	-	20	-
Ripe guava							
Pet ether	**15**	13	10	10	-	-	-
$CHCl_3$	**18**	13	10	10	-	-	-
Ethanol	**19**	20	10	12	-	20	10
Immature guava							
Pet ether	**25**	18	15	15	-	25	-
$CHCl_3$	**25**	20	**20**	10	-	20	10
Ethanol	**20**	**25**	**35**	15	-	28	10
Guava leaves							
Pet ether	**20**	20	15	15	-	20	15
$CHCl_3$	**20**	**25**	**20**	**18**	-	20	17
Ethanol	**19**	**25**	**30**	15	10	**30**	15
Rambai (*Baccaurea motleyana*) **peel**							
Pet ether	**12**	15	**18**	n.d.	10	10	-
$CHCl_3$	**10**	15	14	n.d.	9	7	-
Ethanol	**20**	**25**	**35**	n.d.	**12**	**35**	20
Standard (streptomycin)	10	25	18	18	12	30	n.d.

SA: *Staphylococcus aureus*
BS: *Bacillus subtilis*
BC: *Bacillus cereus*
LB: *Lactobacillus bulgaricus*
EC: *E. coli*
PV: *Proteus vulgaricus*
PA: *Pseudomonas aeruginosa*

TABLE 1b
Antibacterial activity of the tested fruits and fruit leaves extracts on gram positive and gram negative bacteria after 24 h incubation at 30°C

Plant / Bacteria	Average Diameter of Inhibition (mm)						
	SA	BS	BC	EC	PV	PA	ST
Ripe Cavendish banana (Montel variety)							
Pet ether	-	-	-	-	-	-	-
$CHCl_3$	-	-	-	-	-	-	-
Ethanol	**15**	**27**	-	-	-	26	-
Immature Cavendish banana (Montel variety)							
Pet ether	-	-	-	-	-	-	-
$CHCl_3$	-	-	-	-	-	-	-
Ethanol	8	7	8	7	-	26	8
Ripe papaya							
Pet ether	-	-	-	-	-	-	-
$CHCl_3$	-	-	-	-	-	-	-
Ethanol	-	**28**	13	10	**21**	-	-
Immature papaya							
Pet ether	-	-	-	-	7	7	7
$CHCl_3$	-	-	-	-	-	-	-
Ethanol	-	-	7	8	**24**	26	**26**
Passionfruit peel							
Pet ether	-	-	-	-	-	-	-
$CHCl_3$	-	-	-	-	-	-	-
Ethanol	8	12	14	-	8	8	7
Langsat (*Lansium domesticum*) peel							
Pet ether	-	-	-	-	-	-	-
$CHCl_3$	-	-	-	-	-	-	-
Ethanol	-	7	7	-	-	-	-
Duku (*Lansium domesticum*) peel							
Pet ether	-	-	-	-	-	-	-
$CHCl_3$	7	8	8	7	-	-	-
Ethanol	-	-	8	8	-	-	-
Rambutan (*Nephelium lappaceum*) peel							
Pet ether	**11**	-	-	-	-	-	-
$CHCl_3$	8	-	7	-	8	-	-
Ethanol	**27**	14	15	15	**15**	-	**26**
Standard (streptomycin)	**10**	**25**	**18**	**18**	**12**	**30**	**9**

SA: *Staphylococcus aureus*
BC: *Bacillus cereus*
PV: *Proteus vulgaricus*
ST: *Salmonelli typhi*
BS: *Bacillus subtilis*
EC: *E. coli*
PA: *Pseudomonas aeruginosa*

TABLE 2a
Antimicrobial activity of the tested fruits and fruit leaves extracts on yeast and fungi after 24 h incubation at 30°C

Plant / Bacteria	Average Diameter of Inhibition (mm)				
	SC	CL	RS	AN	CS
Ripe starfruit					
Pet ether	-	-	-	-	-
$CHCl_3$		-	-	-	-
Ethanol	-	-	-	-	-
Immature starfruit					
Pet ether	-	-	-	-	-
$CHCl_3$	-	-	-	-	-
Ethanol	-	-	-	-	-
Starfruit leaves					
Pet ether	-	-	-	-	-
$CHCl_3$	-	-	-	-	-
Ethanol	-	-	-	-	-
Ripe guava					
Pet ether	-	-	-	-	-
$CHCl_3$	**20**	-	-	-	-
Ethanol	**20**	-	-	-	-
Immature guava					
Pet ether	-	-	-	-	-
$CHCl_3$	-	-	-	-	-
Ethanol	**20**	-	-	-	-
Guava leaves					
Pet ether	-	-	-	-	-
$CHCl_3$	**15**	-	-	-	-
Ethanol	**15**	-	-	-	-
Rambai *(Baccaurea motleyana)* peel					
Pet ether	-	-	-	-	-
$CHCl_3$	-	-	-	-	-
Ethanol	-	-	-	-	-
Standard (1µg Miconozole)	**10**	**25**	**18**	**18**	**n.d.**

SC: *Saccharomyces cerevisiae* CL: *Candida lypolytica*
RS: *Rhizopus* spp. AN: *Aspergillus niger*
CS: *Chlamydomucor* spp.

TABLE 2b
Antimicrobial activity of the tested fruits and fruit peels extracts on yeast and fungi after 24 h incubation at 30°C

Plant / Bacteria	Average Diameter of Inhibition (mm)			
	SC	CL	RS	AN
Ripe Cavendish banana (Montel variety)				
Pet ether	-	-	-	-
$CHCl_3$	-	-	-	-
Ethanol	-	7	-	-
Immature Cavendish banana (Montel variety)				
Pet ether	-	-	-	-
$CHCl_3$	-	-	-	-
Ethanol	-	12	-	-
Ripe papaya				
Pet ether	-	-	-	-
$CHCl_3$	-	10	-	-
Ethanol	-	21	-	-
Immature papaya				
Pet ether	-	-	-	-
$CHCl_3$	-	10	-	-
Ethanol	-	17	-	-
Passionfruit peel				
Pet ether	-	9	-	-
$CHCl_3$	-	12	8	-
Ethanol	-	21	-	-
Langsat (*Lansium domesticum*) peel				
Pet ether	-	7	-	10
$CHCl_3$	-	9	-	11
Ethanol	-	10	-	10
Duku (*Lansium domesticum*) peel				
Pet ether	-	15	-	-
$CHCl_3$	-	9	-	-
Ethanol	-	18	-	-
Rambutan (*Nephelium lappaceum*) peel				
Pet ether	-	10	-	11
$CHCl_3$	-	12	-	12
Ethanol	-	**30**	-	-
Standard (1µg Miconozole)	10	25	18	18

SC: *Saccharomyces cerevisiae*
RS: *Rhizopus* spp.
CL: *Candida lypolytica*
AN: *Aspergillus niger*

TABLE 3
Minimum inhibitory concentration of the tested fruits and fruit leaves crude extracts after 24 h incubation at 30°C

Strain	Minimum Inhibitory concentration (mg/ml medium)							
	RB	UB	RP	UP	PP	LP	DP	RS
Staphylococcus aureus	2.5	2.5	-	-	2.5	-	-	0.5
E. coli	-	2.5	2.5	2.5	-	-	2.5	1.0
Bacillus subtilis	2.5	2.5	0.5	-	1.0	2.5	-	1.0
Bacillus cereus	-	2.5	1.0	2.5	1.0	2.5	2.5	1.0
Proteus vulgaricus	-	-	0.5	0.5	2.5	-	-	1.0
Pseudomonas aeruginosa	2.5	-	-	0.5	2.5	-	-	-
Salmonelli typhi	-	2.5	-	-	2.5	-	-	0.5
Rhizopus spp.	-	-	-	-	-	-	-	-
Aspergillus niger	-	-	-	-	-	2.5	-	-
Saccharomyces cerevisiae	-	-	-	-	-	-	-	-
Candida lypolytica	2.5	2.5	0.5	1.0	1.0	2.5	2.5	0.5

	(g crude* extract/100g dried sample)
RB: Ripe banana (Cavendish, Montel variety)	61.54
UB: Unripe banana	15.22
RP: Ripe papaya	36.40
UP: Unripe papaya	55.39
PP: Passionfruit peel	15.66
LP: Langsat peel	23.57
DP: Duku peel	23.30
RS: Rambutan skin/Peel	39.51

* Crude extracts obtained by extraction with ethanol and evaporating off the solvent.

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