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FULL PAPER

Preliminary study on mangrove plants used as food in Sungai Acheh, Nibong Tebal, Pulau Pinang

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Abstract

Mangroves can be sources of food, especially in scarce times. A community in Sungai Acheh Nibong Tebal Pulau Pinang had been using several mangrove plant species as food and beverages. *Sonneratia caseolaris (L.) Engl.* (Lythraceae), *Acanthus ilicifolius L.* (Acanthaceae), *Avicennia marina (Forssk.) Vierh.* (Avicenniaceae), *A. officinalis* L. (Avicenniaceae) and *Acrostichum aureum* (Parkeriaceae) are examples of mangrove species that have been used as food. A preliminary study has been carried out to identify the major phytochemical groups, total flavonoid content, radical scavenging activity, and cytotoxicity in these species. From this study, the community can develop commercial food or product with therapeutic effects by using fruits of *S. caeolaris* and leaves of *A. aureum*. This is due to their flavonoid content, antioxidant activities, and low cytotoxicity effect.

1. Introduction

In the Permanent Forest Reserve of Peninsular Malaysia, mangrove forest covers 105,824 hectares. Mangrove forest has a significant role in protecting coastal areas from disasters such as tsunamis and coastal erosion (Muhammad *et al*., 2019). The unique biodiversity in mangrove areas can be tourist attractions and recreational areas. Furthermore, mangroves also can supply firewood, building materials, medicinal herbs, and food, especially fish, prawns, crabs, shellfish, birds and more (Numbere, 2018). Moreover, the local mangrove community has been using mangrove species in their delicacies and drinks (Mukrimah and Mohd Parid, 2018).

A community in Sungai Acheh, Nibong Tebal, Pulau Pinang had consumed several mangrove plants as food and beverages. The plants also had been utilised traditionally as medicine by the elderly. The most common parts of mangrove plants that the community had been used are seeds, fruits, and leaves. Examples of mangrove species are, *Sonneratia caseolaris* (berembang), *Acanthus ilicifolius* (jeruju), *Avicennia* species (api-api), *Acrostichum aureum* (piai lasa), and *Bruguiera cylindrical* (berus). The community had turned these mangrove plants into traditional cakes and desserts, fruit jams, and drinks (Nor Azah *et al*., 2019). To ensure the safety of these food products, a preliminary study was initiated. The objectives of this study were to determine phytochemical contents, identify biological activities, and assess the cytotoxic level of mangrove species.

2. Materials and methods

2.1 Information acquisition

Information on food and beverage utilising mangrove species was gathered from the fishermen's

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community in Nibong Tebal, Pulau Pinang. This community has been approached through Penang Inshore Fishermen Welfare Association (PIFWA). Researchers conducted interview sessions, visit the mangrove site and witness how the community cooked the dishes.

2.2 Plant material

A total of six mangroves species that has been commonly used by the community are locally known as *berembang, jeruju putih, jeruju hitam, api-api putih, api-api jambu* and *piai lasa.* These species were collected from a mangrove area in Kampung Sungai Acheh, Nibong Tebal, Pulau Pinang. The plant materials were taxonomically identified by a botanist at the Forest Research Institute Malaysia (FRIM). The voucher specimens were deposited in the department herbarium for future reference.

2.3 Preparation of extracts

Fruits and leaves of mangrove species were cleaned, dried, and ground. The dried pulverized materials were soaked in ethanol (95%, AR grade) with a ratio of 1: 10 for 72 hrs. Then, samples were filtered and the solvent was removed using a rotary evaporator. Ethanolic extracts were kept at 4°C until used.

2.4 Phytochemical screening

The dried pulverized materials were subjected to qualitative phytochemical screening. The method of screening alkaloids, saponin, tannin (hydrosable and condensed), flavonoid, triterpene, and steroids was described by Saidatul Husni *et al*. (2015) and Adiana *et al.* (2019) in Table 1.

2.5 Flavonoid content

Flavonoid content analysis was carried out on mangrove species and was determined by the aluminium chloride colourimetric method (Chang *et al.*, 2002) with a bit of modification. 0.5 mL of ethanolic extract was mixed with 1.5 mL methanol, 0.1 mL 10% aluminium chloride, 0.1 mL 1M potassium acetate and 28 mL distilled water. After standing at room temperature for 30 min, the absorbance was measured at 415 nm. The calibration curve was prepared by using rutin at concentrations of 10 to 50 ppm in methanol. Total flavonoid content was expressed as a percentage of the weight of rutin equivalent to the dry weight of the sample (% w/w).

2.6 Antioxidant assessment

All samples were extracted with ethanol (95%, Ar grade) for 1, 2-diphenyl-2-picryl-hydroxyl (DPPH) radical scavenging activity (Shalini *et al*., 2020). A 50 µL of 1.0 mg/mL ethanolic extract was added to 50 µL of DPPH (1 mM) and 150 µL of ethanol (absolute, AR Grade) in a 96-well microtiter plate, in triplicates. The mixture was shaken using a digital shaker for 30 min at 100 rpm and left to stand in dark at room temperature. The absorbance of the resulting solution was measured spectrophotometrically at 520 nm. Data were expressed as a percentage of mean value of triplicate wells in a duplicate experiment with ± standard error of the mean (SEM) <15%.

2.7 Cytotoxicity study

Cytotoxicity of selected mangrove species was evaluated using Vero (kidney) and WRL-68 (liver) cell lines. Both cells were purchased from American Type Cell Culture (ATCC) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum and 1% penicillin/ streptomycin. For experimentation, exponentially growing cells were seeded in a 96-well plate at the

Table 1. Method of phytochemical screening groups of selected mangrove species

Phytochemical	Method	Indication
Alkaloid	The sample was extracted with ammonical chloroform and followed with 10% sulphuric acid. The aqueous layer was tested with Meyer's reagent.	Precipitate reaction occurred indicating the presence of alkaloid presence
Flavonoid	The sample was extracted with chloroform. The extract was dissolved with a combination of ether and ammonia.	The strong yellow colour in the ammonia layer indicated the presence of flavonoid
Saponin	The sample was extracted with methanol. The extract was triturated with ether and water was added the mixture was shaken vigorously.	The formation of froth indicated the presence of saponin constituent
Tannin	The sample was extracted with methanol. The extract was then dissolved in methanol and 1% of ferric chloride was added.	The blue-black colour in the lower layer indicates the presence of hydrolysable tannins while the brownish-green colour indicates the condensed tannin
Triterpene/steroid	The sample was extracted with chloroform. The extract was subjected to few drops of Liebermann-Bouchardt reagent (50% acetic acid anhydride-sulphuric acid, v/v).	The formation of bright purple indicates the presence of triterpenes. The formation of red or pink colour indicates the presence of steroids

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density of 6,000 cells/well. They were allowed to attach and spread overnight. Cells were then exposed to the ethanolic extracts at various concentrations (1.0 µg/mL up to 1000 µg/mL) and incubated for 72 hrs. After the treatment incubation period, cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). Dose-response curves were generated for each sample and the median inhibitory concentration (IC_{50} value) was determined by non-linear regression. Each sample was run in three (3) independent experiments. Data are shown as mean with ± SEM (Khoo *et al*., 2019).

3. Results and discussion

The fishermen community in Nibong Tebal, Pulau Pinang use several mangrove species in their delicacies and drinks. Based on interviews with the community, there are six mangrove species commonly used by the locals. The species were identified by the botanist as mentioned in Table 2. In this study, food and beverages from mangrove species were documented as the following, jam prepared from *S. caseolaris* fruit (Figure 1) is suitable as a spread on bread, mixture of dried leaves of *A. ilicifolius* and *A. ebracteatus* was diffused with hot water as herbal drinks (Figure 2), fruits of *A. marina* and *A. officinalis* were boiled, dried under sun and ground before being used in traditional Malay desserts such as *lepat* (Figure 3a) and *onde-onde* (Figure 3b) and shoots or young leaves of *A. aureum* were fried as fritters or *kerepek* (Figure 4).

Table 2. Identified mangrove species that have been commonly used by the community

Local name	Latin name	Family
Berembang	*Sonneratia caseolaris (L.) Engl.*	Lythraceae
Jeruju putih	*Acanthus ilicifolius L.*	Acanthaceae
Jeruju hitam	*Acanthus ebracteatus*	Acanthaceae
Api-api jambu	*Avicennia marina (Forssk.) Vierh.*	Avicenniaceae
Api-api putih	*Avicennia officinalis* L.	Avicenniaceae
Piai lasa	*Acrostichum aureum*	Parkeriaceae

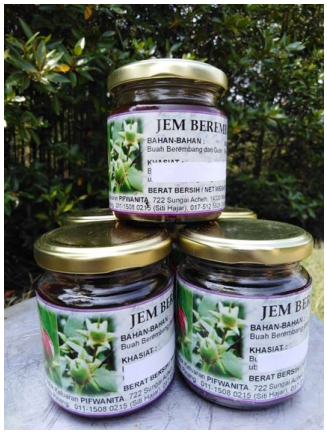

Figure 1. Jam from fruit of *S. caseolaris*

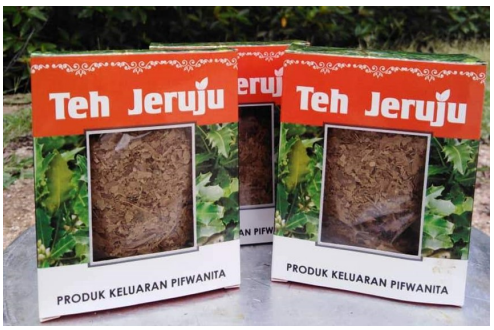

Figure 2. Herbal infusion drinks from leaves of *A. ilicifolius* and *A. ebracteatus*

Figure 3 (a). Sweet dumpling (*lepat*) (b) *Onde-onde* from fruits of *A. marina* and *A. officinalis*

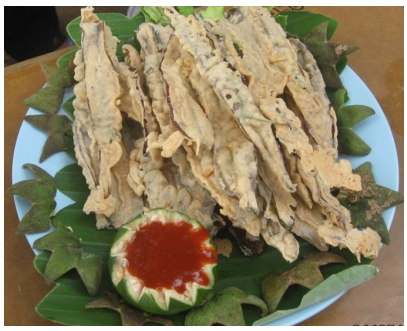

Figure 4. Fritters or *kerepek* of *A. aureum*

In phytochemical screening, all samples were tested for seven major phytochemicals groups (Table 3) except for *A. aurem* due to a shortage of samples. The steroid was detected in all samples. However, saponin, alkaloid, and triterpene were not present in all samples. Determination of flavonoid content was carried out in samples that had been detected with flavonoids in phytochemical screening. However, fruits of *A. marina* and *A. officinalis* were not tested due to the absence of samples. This situation is related to the short fruiting season. The fruiting season for *A. officinalis* lasted for two months (June-July) while for *A. marina* was recorded in June-August (Noraliza *et al*., 2020). Fruit of *S. caseolaris* showed the highest flavonoid content among other samples. Based on a previous study by Sadhu *et al*. (2006) also discovered flavonoids in *S. caseolaris* fruits.

DPPH radical scavenging is a simple approach to determining the antioxidant capacity of plants. The antioxidant property is confirmed by the discolouration of the deep violet colour of DPPH into light yellow

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Table 3. Major phytochemical groups of selected mangrove species

Sample	Parts	Phytochemical screening						
		Hydrosable tannin	Condensed tannin	Flavonoid	Saponin	Alkaloid	Triterpene	Steroid
S. caseolaris	Fruit	1+	-ve	1+	-ve	-ve	-ve	1+
A. ilicifolius	Leaf	3+	-ve	2+	-ve	-ve	-ve	3+
A. ebracteatus	Leaf	3+	-ve	-ve	-ve	-ve	-ve	3+
A. marina	Fruit	2+	-ve	2+	-ve	-ve	-ve	1+
A. officinalis	Fruit	3+	-ve	1+	-ve	-ve	-ve	2+
A. aureum	Leaf	NT	NT	2+	NT	NT	NT	1+

-ve: not present, +1: low, +2: moderate, +3: high, NT: not tested.

(Ihsan *et al.*, 2018). High antioxidant activities were detected in leaves of *A. aureum* and fruits of *S. caseolaris*, 77.3±1.3 and 99.1±0.1, respectively (Table 4). The presence of flavonoids in leaves of *A. aureum* and fruits of *S. caseolaris*, may, or at least partially, contribute to the antioxidant activity. Badsheeba and Vadivel, (2018) also recorded that leaves of *A. aureum* have antioxidant activity (IC_{50} value 36.54µg/mL) was comparable to IC_{50} value of ascorbic acid, 32.84µg/mL, respectively. *A. ebracteatus* has the lowest antioxidant activities among the samples, 18.5±0.3% and flavonoid were also not detected in this sample. In contradiction, flavonoid was detected present for *A. ilicifolius* but has low antioxidant activity. This finding indicates that flavonoids in *A. ilicifolius* might be not a potent antioxidant. According to Kumar and Pandey, (2013), flavonoid glycosides were less potent than aglycone flavonoids. Similar findings were reported by Andriani *et al*. (2020) for *A. ilicifolius*, whereby, flavonoid was detected to be present in the methanolic extract of *A. ilicifolius* but has moderate antioxidant activity (41%).

In the cytotoxicity study, fruits of *S. caseolaris,* leaves of *A. aureum*, and *A. ilicifolius* were tested using the MTT test on Vero (kidney cell lines) and WRL-68 (hepatic cell lines). The result was presented in IC_{50} values as shown in Table 4. Fruits of *S. caseolaris* and leaves of *A. aureum* were recorded to have moderate cytotoxicity effects, while leaves of *A. ilicifolius* have a low cytotoxicity effect. However, IC_{50} values for all sample is much higher than IC_{50} values of paclitaxel for Vero and WRL-68, 0.05±0.0012 and 0.007±0.007, respectively. According to Khoo *et al.* (2019), understanding the dosage of a substance is important to differentiate whether the substance can be considered lethal or non-lethal. Therefore, samples with moderate or low toxicity effects still can be developed into food or product with therapeutic effects but must be used in appropriate dosage.

Conflict of interest

The authors declare no conflict of interest.

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Table 4. Flavonoid content, antioxidant activity and cytotoxicity of selected mangrove species

Sample	Parts	Flavonoid content	Antioxidant	Cytotoxicity	
		Rutin equivalent (ppm)	DPPH radical scavenging (%)	IC_{50} Vero (µg/mL)	IC_{50} WRL-68 (µg/mL)
S. caseolaris	Fruit	11.92	77.3±1.3	116.4±14.1	125.6±38.5
A. ilicifolius	Leaf	10.42	30.0±0.5	252.93±24.8	254.7±70.4
A. ebracteatus	Leaf	NT	18.5±0.3	NT	NT
A. marina	Fruit	NT	40.4±1.2	NT	NT
A. officinalis	Fruit	NT	12.5±0.6	NT	NT
A. aureum	Leaf	4.95	99.1±0.1	96.4±18.8	101.5±20.9
Paclitaxel	-	-	-	0.053±0.012	0.007±0.007

Antioxidant activity; low: 0-49%, moderate: 50-69%, high: 70-100% NT: not tested.
Cytotoxicity; high: $IC_{50} < 20$ µg/mL, moderate: $20 < IC_{50} < 250$ µg/mL, low: $250 < IC_{50} < 625$ µg/mL, non toxic: $IC_{50} > 625$ µg/mL

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