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Research Article

PHYTOCHEMICAL SCREENING AND BIOLOGICAL ACTIVITIES OF *BORASSUS FLABELLIFER* L.

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ABSTRACT

The extracts and fresh palm sugar of *Borrasus flabellifer* L. belonging to family Arecaceae reported to be possess the antioxidant and cytotoxic activities against human colon adenocarcinoma (Caco2 cell line) and human dermal fibroblast neonatal (HDFn) by Resazurin microplate assay and preliminary phytochemical screening. The ethanolic extracts of young fruit, ripe seed coat, cotyledon were showed inhibitory concentration 50% in anti-oxidation by 1,1-diphenyl-2-picrylhadrazyl assay at a concentrations of 1.00±0.044, 0.900±0.044, and 1.40±0.217 mg/ml, respectively. However, the palm sugar was showed antioxidant with 40% at final concentration 2.50% v/v in methanol, showed percentage of survival of HDFn C-004-5C cell lines 67.31% at concentration 10.00% v/v in distill water. The phytochemical analysis carried out revealed the presence of reducing sugar, terpenoids, tannins, flavonoids, and coumarin in all extracts, but palm sugar showed positive only for the presence of reducing sugar.

Keywords: Phytochemical, Biological activities, *Borassus flabellifer* L.

INTRODUCTION

Borassus flabellifer Linn. belongs to family Arecaceae, commonly known as palmyra palm. This plant is a tall tree (palm) growing in sandy soil and attaining height 20-30 m with a straight trunk. The fruits are large and fibrous, containing usually three nuts like portions each of which encloses a seed. Flowers and fruits were during in December to September. Sugar syrup can be purchased whole year. The plant has been used traditionally as a stimulant, antileprotic, diuretic, antiphlogistic. The fruits are used in hyperdipsia, dyspepsia, flatulence, skin diseases, hemorrhages, fever, and general debility. The roots and juice are useful in inflammatory reaction. The ash obtained by burning the inflorescence is a good antacid antiperiodic and heartburn [1]. From literature reveal that this plant had been studied antitumor activity on HeLa cell line, antioxidant and antibacterial activities of seed coat [2,3], antioxidant activity of pulp fruit [1], antibacterial of roots [4], and antioxidant activity of sugar syrup [5]. Therefore, this research has been undertaken to investigate phytochemical screening, antioxidant activity and cytotoxicity on human dermal fibroblast neonatal (HDFn) cell line and Caco2 cell lines of young fruit, ripe seed coat, cotyledon of *B. flabellifer,* and fresh palm sugar produced in Phetchaburi province, Thailand.

METHODS

Plant extraction

In July 2014, all part of *B. flabellifer* were collected from Phetchaburi Province, Thailand, while palm sugar was purchased in October 2014. The plant sample identified by Dr. Boonsanong Chourykaew, Botanist is one of the authors.

Preparation of plant extracts

The fresh young fruit, ripe seed coat and cotyledon (Fig. 1) were air dried then ground into powder which was subjected for extraction process by marceration with 95% ethanol at room temperature for 3 days 3 times. The extracts were filtered concentrated by using rotator evaporator to yield ethanolic extracts of young fruit, ripe seed coat and cotyledon and palm sugar were used immediately after purchased do not extracted. The percentage yield of extracts ranged from 12.54% to 16.22% w/w of dried plants.

Phytochemical screening

Phytochemical screenings were performed using standard procedures [6,7].

Test for reducing sugars (Fehling's test) [6]

The small quality of each extract was added to boiling Fehling solution (A and B) in the test tube. The solution was observed for a color reaction.

Test for terpenoids (Salkowski test) [6]

To small quality of each extract, 2 ml of dichloromethane was added. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for flavonoids [6]

Three methods were used to test for flavonoids. First, diluted ammonia (5 ml) was added to a portion of an aqueous filtrate of each extracts. Concentrated H_2SO_4 (1 ml) was added, a yellow coloration that disappear on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow coloration indicates the presence of flavonoids. Finally, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 minutes, the mixture was filtered, and 4 ml of filtrate

Fig. 1: Parts of *Borrasus flabellifer*: (a) Young fruit, (b) ripe seed coat, (c) cotyledon, (d) fresh palm sugar

was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for saponins [6]

To small amount of each extract, 5 ml of distilled water was added in a test tube. The solution was shaken vigorously and observed for a stable foaming. The formation for 15 minutes indicates the presence of tannins.

Test for tannins [6]

Small amount of each extracts was boiled in 10 ml of water in the test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for alkaloids [7]

Small amount of each extracts was diluted to 10 ml with hydrochloric acid and filtered. The filtrate was used for the following test using Mayer's test and Dragendorff's test. The small amount of each extracts was treated with Mamey's reagent and Dragendorff's reagent respectively. Cream color precipitate and reddish brown precipitate by using Mayer's test and Dragendorff's test respectively, indicates the presence of alkaloids.

Test for cardiac glycoside (Keller–Killiani test) [6]

To small amount of each extracts diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides.

Test for coumarins [7]

The 3 ml of 10% NaOH was added to 2 ml of extract formation of yellow colour indicates the presences of coumarins.

Determination of biological activities

Antioxidant activity by 1,1-diphenyl-2-picrylhadrazyl (DPPH) scavenging assay

The free radical scavenging effect of all extract as well as standard ascorbic acid corresponding to the quenching ability of DPPH were determined by ultraviolet spectrophotometry at 517 nm. Radical scavenging activity was measured by a modified method previously described [8]. Each sample was assayed in triplicate and the average inhibitory concentration 50% (IC_{50}) value was calculated. The following concentrations of extracts were prepared, 0.3125-5.00 mg/ml in methanol. Concentrations of vitamin C was 3.125-12.50 µg/ml. 0.1 ml of each extracts was placed in a test tube, and 3.7 ml of methanol was add followed by 0.2 ml of 0.02 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. Absorbance value was monitored after a set at room temperature for 30 minutes in darkness. The radical scavenging activity was calculated using the following formula:

$$\%\text{inhibition} = \{[A_b - A_e]/A_b\} \times 100$$

Where A_b is the absorption of the blank sample and A_e is the absorption of the extracts.

Cytotoxicity against human colon adenocarcinoma (Caco2 cell line) and HDFn by Resazurin microplate assay [9].

These cytotoxic assays were determined by the colorimetric method as described by Brien *et al*., 2000. Briefly, the assays were performed in four replicate wells in 96-well plate. Cells at a logarithmic growth phase were harvested and diluted to 3×10^4 cells/ml for HDFn cell line (Gibco, C-OO4-5C) and 2×10^4 cells/ml for Caco2 cell line (ATCC HTB-37). First, plates were seeded with 200 µl of cell suspension or blank medium into well, and incubated at 37°C humidified incubator with 5% CO_2 for 48 hrs. Subsequently, culture medium was replace with 200 µl of fresh medium containing extract at concentration 100 µg/ml or 1% dimethyl sulfoxide (DMSO) for negative control or 10.00 µg/ml of ellipticine for positive control, and plates were further incubated for 24 hrs. After the incubation period, the plates were added with 50 µl of 125 µg/ml resazurin solution and incubated at 37°C humidified incubator with 5% CO_2 for 4 hrs. Fluorescence is measured at 530 nm excitation and 590 emission wavelengths by using the bottom reading mode of SOFT Max fluorometer (Molecula Devices, USA). The signal is subtracted with blank before calculation. Ellipticine was as a positive control.

The percentage of survival of cells is calculated by following equation:

$$\%\text{survival} = (FU_T/FU_C) \times 100$$

Where FU_T and FU_C are the mean fluorescent unit cells treated with extract and 1% DMSO, respectively. If %survival was more than 50% indicated the extract was a non-cytotoxic activity, but if %survival was <50% indicated the extract was a cytotoxic activity, and IC_{50} was determined.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screenings of *B. flabellifer* were presented some different constituents in extracts as followed in Table 1. Young fruit, ripe seed coat and cotyledon tested negative for the presence of saponins, alkaloids and cardiac glycoside, but tested positive for the presence of reducing sugar, terpenoids, flavonoids, and coumarins. Only palm sugar tested positive only for the presence of reducing sugar, while tannins were positive tested on cotyledon.

Radical scavenging (antioxidant) activity

The anti-oxidation of all extracts of *B. flabellifer* were determined by using the DPPH assay. The DPPH assay use for evaluate the ability of antioxidants to scavenge free radicals which are known to be a major factor in biological damage caused by oxidative stress. The principle of this assay is based on the color change of the DPPH solution from purple to colorless as the radical is quenched by the antioxidant. Vitamin C was a positive control showed IC_{50} at 8.40 µg/ml. All extracts were exhibited IC_{50} at a concentrations of 1.00±0.044, 0.900±0.044, and 1.40±0.217 mg/ml for fruit, seed coat, cotyledon, respectively (Table 3) according to methanolic seed coat extract of this plant had showed antioxidant activity by using DPPH assay on IC_{50} 7.90 µg/ml [3] and the aqueous and methanolic pulp fruit extracts at concentration of 1000 µg/ ml had exhibited 81.34%, and 71.25% by DPPH assay, respectively [1]. Palm sugar was showed antioxidant at concentration of 2.50% V/V by DPPH assay 40.00% (Table 2), according to sugar syrup produced in Songkhla province was showed antioxidant activity by DPPH assay (µmol trolox equivalent/g sample) in range 13.27-18.49 [5].

Cytotoxic activities

All extracts of *B. flabellifer* were subjected to at concentration of 100 µg/ml were evaluated *in vitro* cytotoxicities against to HDFn cell

Table 1: Phytochemical constituents of each extracts of *B. flabellifer*

Test	Young fruit	Ripe seed coat	Cotyledon	Palm sugar
Reducing sugar	+	–	+	+
Terpenoids	+	–	+	–
Flavonoids	+	+	+	–
Saponins	–	–	–	–
Tannins	–	–	+	–
Alkaloids	–	–	–	–
Cardiac glycoside	–	–	–	–
Coumarins	+	+	+	–

B. flabellifer: Borrasus flabellifer

lines and Caco2 cell lines following the procedure of Brien *et al*., 2000. All were inactive against HDFn cell line and Caco2 cell lines, except, palm sugar at 10% V/V in distilled water showed 67.31% survival of HDFn cell lines (Table 4), but not cytotoxic against Caco2 cell lines. While ellipcine showed IC_{50} at a concentration of 2.66 and 21.53 µg/ml, respectively (% cytotoxic data not show). However, phosphate buffered saline extract of seed coat showed 50% inhibition to anti-tumor on carcinoma of the cervix (HeLa cell lines) at a concentration of 750 µg/ml [2]. The presence of antioxidant and cytotoxic activities in palm sugar was probably due to the presence of reducing sugar.

Table 2: The percentage of inhibition and IC_{50} of palm sugar on DPPH antioxidant method

Final concentration (% V/V)	% Inhibition
0.0025	0.00
0.025	0.00
0.25	5.80
2.50	40.00

IC_{50}: Inhibitory concentration 50%, DPPH: 1,1-diphenyl-2-picrylhadrazyl

Table 3: The percentage of inhibition and IC_{50} of *B. flabellifer* extracts on DPPH antioxidant method

Final concentration (mg/ml)	% Inhibition		
	Young fruit	Ripe seed coat	Cotyledon
0.3125	30.22	36.26	30.22
0.625	44.51	48.90	36.26
1.25	62.64	58.24	49.45
2.50	65.93	64.28	65.38
5.00	42.31	64.84	65.93
IC_{50} (±SD)	1.00±0.044	0.900±0.044	1.40±0.217

IC_{50}: Inhibitory concentration 50%, DPPH: 1,1-diphenyl-2-picrylhadrazyl, *B. flabellifer: Borrasus flabellifer*, SD: Standard deviation

Table 4: The percentage of survival of HDFn C-004-5C cell lines on fresh palm sugar of *B. flabellifer*

Final concentration (%V/V)	% Survival of cell lines
10.00	67.31
5.00	77.46
2.50	80.34
1.25	83.15
0.625	95.06
0.313	98.29

B. flabellifer: Borrasus flabellifer, HDFn: Human dermal fibroblast neonatal

CONCLUSIONS

In conclusion, it was observed from the present study that all extract as well as fresh palm sugar of *B. flabellifer* have natural constituent as antioxidant, although all showed inactively cytotoxicities except sugar was showed weak activity on cell lines. The further next studies are required to study another biological activities as well as need to isolate, purify, and characterize active phytochemicals responsible their biological activities.

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