Journal of Tropical Forest Science 37(Special Issue): 121–131 (2025)
https://doi.org/10.26525/jtfs2025.37S.SI.121
ISSN: 0128-1283, eISSN: 2521-984

ANTI-DIABETIC, ANTI-INFLAMMATORY, AND ANTIOXIDANT ACTIVITIES OF *PALAQUIUM GUTTA* (HOOK.) BAILL.

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Submitted June 2024; accepted December 2024

Palaquium gutta (Hook.) Baill. is notable for its milky white latex, i.e. gutta percha. There is no known medical use of *P. gutta* except for the latex in dentistry. In this study, we explore the potentials of *P. gutta* bark, twig, and leaf methanolic extracts as anti-diabetic, anti-inflammatory, antioxidant, anti-cancer, anti-trypanosomal, and anti-microbial agents using *in vitro* models. *In vitro* toxicity and chemical analyses were also conducted. Among the three extracts, the bark exhibited the highest anti-diabetic activity by inhibiting α-glucosidase (97.6 ± 0.5%) and α-amylase (85.8 ± 2.1%). It also showed high anti-inflammatory effect based on lipoxygenase inhibition (93.1 ± 3.5%). All the three extracts displayed high 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (97.6–98.4%). However, none of the extracts showed anti-cancer, anti-trypanosomal, or antimicrobial activities. The bark did not negatively affect the viability of Vero (kidney) cells, but it reduced the viability of WRL-68 (liver) cells to about 50%. Phytochemical screening revealed the presence of flavonoids, steroids, and tannins in the bark. High performance liquid chromatography (HPLC) analysis shows the presence of phenolic compounds and flavonoids. Based on these results, the bark of *P. gutta* showed the most promising anti-diabetic, anti-inflammatory, and antioxidant activities. The observed effects could be attributed to the presence of flavonoids and phenolic compounds.

Keywords: α-glucosidase, α-amylase, lipoxygenase, DPPH radical scavenging, *in vitro* toxicity

INTRODUCTION

Palaquium gutta (Hook.) Baill. is a timber tree from the family Sapotaceae. It is geographically distributed in the South East Asia and commonly found throughout Malaysia, including Sabah and Sarawak. It is locally known as taban merah or nyatoh taban merah (Sarah et al. 2015). *Palaquium gutta* is notable for its milky white latex, i.e. gutta percha. Gutta percha was an important commodity in the 19th century. Due to its inertness to biodegradation and its ability to be moulded, it was used to insulate copper wire for undersea cables. Thus the humble gutta percha was a significant substance that helps accelerate modern telecommunication (Wong 2016).

Long before the appreciation of gutta percha as a valuable commodity, the natives used the plant for its timber (wood). The timber is durable with moderate weight. It was also the timber name (nyatoh) that was more widely used to identify the tree/plant than taban, which is the local name of the plant (Burkill 1966). The seeds are rich in fats and said to produce a very good wax for the manufacture of soaps and candles. The fat is often used in the preparation of food (van Royen 1953). The natives also used gutta percha to make knife handles and riding whips (Burkill 1966). When gutta percha had been introduced to the Global North, other than as insulator of undersea cables, it was used for making surgical and chemical instruments, corks, golf balls, and as dental filling, especially for those who are sensitive to synthetic fillers (Burkill 1966, Sarah et al. 2015, Wong 2016).

Gutta percha is probably one of the most studied substances. A keyword search of gutta percha in PubMed database returned 4,726 results but there is no known medical use of *P. gutta*, except for its latex in dentistry. This could be due to the scarcity of the plant. The early collection method for its latex was environmentally destructive. *Palaquium gutta* has vanished from Singapore and parts of the Malay Peninsula by the 1880s. An estimated 3

million trees were felled in just one region of Sarawak over a span of 20 years and a ban on harvesting the latex was issued in 1883 (Burkill 1966, Wong 2016). A recent study by Kadir et al. (2022) reported that the heartwood extract of *P.* gutta has termiticidal and repellency effects against Asian subterranean termites, *Coptotermes gestroi* and could be used as wood preservatives.

In this study, we explore the potentials of *P. gutta* bark, twig, and leaf methanolic extracts as anti-diabetic, anti-inflammatory, antioxidant, anti-cancer, anti-trypanosomal, and anti-microbial agents based on *in vitro* experimentations. We also conducted *in vitro* toxicity screening and chemical analysis.

MATERIALS AND METHODS

Plant materials and extraction

The barks, twigs and leaves of *P. gutta* were collected from the Keruing Trail at Forest Research Institute Malaysia (FRIM) and authenticated by a botanist. A voucher specimen (TD014/15) was prepared and deposited at FRIM.

The collected plant materials were cleaned, dried, and powdered before extracting with methanol at the ratio of 1:10 (w/v). Each sample was soaked in methanol at room temperature for 3 days, filtered, and the residue extracted again for a total of three times. The methanol extract (filtrate) was concentrated under reduce pressure using a rotary evaporator at temperature not exceeding 50°C. The dried methanol extracts were kept at -20°C.

Biological Activities Analyses

Anti-diabetic activity

In vitro anti-diabetic activity was evaluated using α-glucosidase and α-amylase inhibitory assays (Norodin et al. 2018).

α-Glucosidase inhibition assay.

α-Glucosidase enzyme from *Saccharomyces cerevisiae* was used as the enzyme and p-nitrophenyl-α-D-glucopyranoside as the substrate. Test sample (10 µL), enzyme (20 µL), distilled water (20 µL), and buffer (40 µL) were mixed in a microtiter plate and incubated at 37°C in a shaker for 10 min. The first absorbance ($A^{0\ min}$) was measured at 405 nm. Substrate (10 µL) was then added into the well and incubated at 37°C for another 30 min, and the second absorbance measured ($A^{30\ min}$). The final reaction mixture contained 10 µg/mL sample, 0.1 U/mL enzyme, and 1.25 mM substrate. 1-Deoxynojirinycin (DNJ) and distilled water were used as the positive and negative controls, respectively. Percentage of enzyme inhibition was calculated from the ratio of changed of sample absorbance within 30 min over changed in the negative control.

α-Amylase inhibition assay.

Porcine pancreatic amylase was used as the enzyme and starch (0.5%, w/v) as the substrate. Test sample (10 µL), enzyme solution (50 µL), and distilled water (40 µL) were mixed in a microtiter plate and incubated at room temperature for 5 min. The substrate (100 µL) was then added to the mixture and incubated at room temperature for another 7 min. The final reaction mixture contained 10 µg/ml sample, 1 U/ml enzyme, and 0.25% (w/v) substrate. After incubation, 100 µL of DNS colour reagent (96 mM 3,5-dinitrosalicylic acid and 5.31 M sodium potassium tartrate in 2M NaOH) was added and incubated at 85°C in a shaker for 30 min. The plate was cooled to room temperature before reading the absorbance at 540 nm. Acarbose was used as the positive control and distilled water as the negative control. Percentage of enzyme inhibition was calculated from the ratio of sample absorbance over negative control absorbance, after subtraction with blank.

Anti-inflammatory activity

Anti-inflammatory activities were evaluated using lipoxygenase, xanthine oxidase, and hyaluronidase inhibition assays (Ul-Haq et al. 2004, Noro et al. 1983, Ling et al. 2003).

Lipoxygenase inhibition assay.

Soybean lipoxygenase type I-B was used as the enzyme and linoleic acid as the substrate. Test

sample (10 µL), enzyme (20 µL), and 100 mM sodium phosphate monobasic buffer, pH 8.0 (160 µL) were mixed and incubated at 25°C for 10 min. The reaction was initiated by the addition of substrate (10 µL). After another 10 min of incubation at 25°C, formation of (9Z,11E)-(13S)-13-hydroperoxyoctadeca-9, 11-dienoate was measured at 234 nm with a spectrophotometer. The final concentrations of the sample, enzyme, and substrate in the reaction mixture were 100 µg/mL, 600 U, and 0.3 mM, respectively. Nordihydroguaiaretic acid (NDGA) was used as the positive control while enzyme was not added for the negative control. Percentage inhibition was calculated by dividing the absorbance difference between negative control and test sample with absorbance from the negative control.

Xanthine oxidase inhibition assay.

Xanthine oxidase was used as the enzyme and xanthine as the substrate. Test sample (10 µL), enzyme (10 µL), and 50 mM potassium phosphate buffer, pH 7.5 (130 µL) were mixed and incubated at 25°C for 5 min. The reaction was initiated by the addition of substrate (100 µL) and incubated at 25°C for another 10 min. The enzymatic conversion of xanthine to uric acid and hydrogen peroxide was measured at 295 nm with a spectrophotometer. The final concentrations of the sample, enzyme, and substrate in the reaction mixture were 100 µg/mL, 0.016 U, and 0.06 mM, respectively. Allopurinol was used as the positive control while enzyme was not added for the negative control. Percentage inhibition was calculated by dividing the absorbance difference between negative control and test sample with absorbance from the negative control.

Hyaluronidase inhibition assay.

Hyaluronidase (1200–1500 U) in 20 mM sodium phosphate buffer was used as the enzyme and hyaluronic acid as the substrate. Test sample (25 µL) was pre-incubated with the enzyme (100 µL) at 37°C for 10 min. The reaction was initiated by the addition of substrate (100 µL) and incubated at 37°C for a further 45 min. Undigested hyaluronic acid was precipitated with acid albumin (1 mL). After standing at room temperature for 10 min, the absorbance of the reaction mixture was measured at 600 nm. The final concentrations of the sample, enzyme, and substrate in the reaction mixture were 100 µg/mL, 1200–1500 U, and 0.002% w/v, respectively. Apigenin was used as the positive control and the absence of enzyme as the negative control. Percentage inhibition was calculated by dividing the absorbance difference between negative control and test sample with absorbance from the negative control.

Antioxidant activity

Antioxidant activity was evaluated by 2,2-diphenyl-2-picrylhydrazy (DPPH) radical scavenging (Blois 1958, Peng et al. 2000). Briefly, test sample (50 µL) was mixed with DPPH solution diluted in ethanol (200 µL). The final concentrations of the sample and DPPH were 200 µg/ml and 200 µM, respectively. The mixture was shaken at 300 rpm for 15 s and left to stand for 30 min in the dark at room temperature. The absorbance was then read at 520 nm. Green tea was used as the positive control while ethanol was used as the negative control. Percentage of DPPH scavenging activity was calculated by dividing the absorbance difference between the negative control and the test sample with the absorbance of negative control.

Anti-cancer activity

Ovarian cancer (SKOV-3), breast cancer (MCF-7), and colorectal cancer (HT-29) cell lines purchased from the American Type Culture Collections (ATCC), U.S.A. were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum, 1% penicillin-streptomycin, 1% amphotericin B, and 1% gentamicin in a humidified incubator at 37°C and 5% CO2. Confluent cells were seeded in a 96-well microtiter plate and incubated for 24 h. The cells were then treated with the test samples at concentrations ranging from 0–100 µg/mL in triplicates. Cisplatin was used as the positive control. Sulphorodhomine B (SRB) assay based on the method by Nurhanan et al. (2017) and Skehan et al. (1990) was used to assess cell viability after 72 h incubation. The percentage of viable cells was calculated

by dividing the absorbance of treated cells over untreated cells. Dose-response curves (percentage of viable cells vs. concentrations) were plotted for each test sample to determine the half maximal inhibitory concentration (IC_{50}). Test sample with IC_{50} < 20 µg/mL was considered to have potential anti-cancer activity (Boyd & Paull 1995).

Antit-trypanosomal activity

Anti-trypanosomal activity was determined against the strain *Trypanosoma brucei brucei* BS221 according to the method described by Norhayati et al. (2013). The parasites were cultured in Balz Minimal Essential Medium (BMEM) in a 24-well microtiter plate and incubated at 37°C in a humidified atmosphere of 5% CO_2. Trypanosomes were inoculated into a flat-bottom 96-well microtiter plate containing different concentrations of test samples ranging from 0.01–12.5 µg/mL during experimentation, and incubated at 37°C for 72 h. Alamar Blue assay was then performed to assess toxicity when treated with the test samples (Räz et al. 1997). Pentamidine was used as the positive control, while 5% DMSO was used as the negative controls. The IC_{50} values were then calculated from the dose-response curves. Anti-trypanosomal activity was scored into three categories: score 1 (weak activity, IC_{50} > 12.5 µg/mL), score 2 (moderate activity, 1.56 < IC_{50} ≤ 12.5 µg/mL) and score 3 (strong activity, IC_{50} ≤ 1.56 µg/mL).

Antimicrobial activity

Antimicrobial activity against a panel of *Staphylococcus aureus* strains (ATCC 25923, ATCC 33591, ATCC 700699, and US 300) and *Escherichia coli* (ATCC 8739) were evaluated via broth micro-dilution method to determine the minimum inhibitory concentration (MIC) and minimum microbiocidal concentration (MMC). Media solution was incorporated with test samples in a 96-well microtiter plate to obtain the final test sample concentrations ranging from 1–10 mg/mL. A standardised population (10^6 cfu/mL) of an 18–24 h old target microbes was then added to each well and incubated at 37°C for 24 h. Turbidity was taken as an indication of growth, thus the lowest concentrations which remained clear after macroscopic evaluation was taken as the MIC. Immediately after the macroscopic MIC evaluation, the inhibitory status of each test sample was evaluated (MMC), i.e. permanent inhibition (cidal) or temporary inhibition (static), by adding 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) reagent (20 µL/well, final MTT concentration: ~0.1 mg/mL) to the microbe-test sample-media mixture of selected MIC wells and further incubated at 37°C for an additional 20 min. Clear coloured microbe-test sample-media mixture indicates cidal activity as opposed to dark purple colour mixture (static). Standardised microbial solution plus solvent used to dissolve the test samples served as the negative control. The MIC and MMC were determined in triplicates. MIC/MMC value of ≤ 1000 µg/mL was considered as active (BSAC 2005, Mastura et al. 2009).

In vitro toxicity

Vero and WRL-68 cell lines obtained from the American Type Culture Collection (ATCC), U.S.A. were used as kidney and liver model cells. They were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum, 1% penicillin/streptomycin, and maintained at 37°C and humidified atmosphere of 5% CO_2/95% air. Exponentially growing cells were seeded in a 96-well plate at the density of 6×10^3 cells/well in complete culture medium (100 µL/well). After an overnight incubation, the culture medium was removed and the cells washed with phosphate buffered saline (PBS) (150 µL/well). The cells were then treated with the 100 and 200 µg/ml of test samples (200 µL/well) for 72 h. After the treatment incubation period, cell viability was assessed by the MTT assay based on Mosmann (1983) with slight modifications. Taxol (0.1 and 1.0 µg/mL) was used as the positive control while the negative control contained 0.5% DMSO. Percentage of viability was calculated by dividing the blank-corrected absorbance of the sample with the blank-corrected absorbance of the negative control. Test samples were considered non-cytotoxic when the viability was ≥ 70%.

Phytochemical screening

Phytochemical screening was carried out using in-house procedures on powdered plant materials to identify the presence of alkaloids, flavonoids, triterpenoids, steroids, saponins, and tannins.

Test for alkaloids

Ammoniacal chloroform (20 mL) was added to each powdered plant material (5 g). The mixture was shaken for 1 h, filtered, and a few drops of 10% sulphuric acid were added to the filtrate (1 mL). The mixture was then shaken and left to separate. The aqueous layer was transferred to a test tube and added with a few drops of Mayer's reagent. Formation of white precipitate indicates the presence of alkaloids.

Test for flavonoids.

Chloroform (60 mL) was added to each powdered plant material (5 g) and the mixture was shaken for 1 h, filtered, and the filtrate evaporated to dryness using a rotary evaporator. The dried filtrate was then reconstituted with ether (2 mL) and 10% ammonia (2 mL) was added to the solution and shaken slowly. The change of colour solution into red, yellow, or orange indicates the presence of flavonoids.

Test for triterpenoids/steroids.

Chloroform (60 mL) was added to each powdered plant material (5 g). The mixture was shaken for 1 h and filtered. The filtrate (2 mL) was transferred to a test tube and added with a few drops of Liebermann-Buchard reagent. The change of colour solution into reddish brown, purple, or pink indicates the presence of triterpenoids while the change of colour solution into pale blue, greenish blue, or shades of yellow indicates the presence of steroids.

Test for tannins.

Methanol (50 mL) was added to each powdered plant material (5 g), shaken for 1 h, and filtered. The filtrate (2 mL) was transferred into a test tube and added with a few drops of 1% ferric chloride. The change of colour solution into blue black or formation of precipitate indicates the presence of hydrolysable tannins. The change of colour solution into shades of brown or formation of precipitate indicates the presence of condensed tannins.

Test for saponins.

Methanol (50 mL) was added to each powdered plant material (5 g), shaken for 1 h, and filtered. The filtrate (1 mL) was transferred into a test tube, added with distilled water (5 mL), and then shaken vigorously for 30 s. Formation of stable bubbles indicates the presence of saponins.

High Performance Liquid Chromatography (HPLC) analysis

HPLC analysis was carried out using a Waters HPLC system equipped with a photodiode array (PDA) and a quaternary pump. Samples for HPLC analysis were prepared by reconstituting the methanol extracts (75 mg for the bark and twig extracts, 25 mg for the leaf extract) with methanol, sonicated for 15 min, and each sample filtered (0.45 µm). A *Phenomenex* Luna C18 100A (5 µm, 4.6 mm × 250 mm) column was used with mobile phase consisting of (A) water with 0.1% formic acid, (B) acetonitrile, and (C) methanol. The gradient elution began at 90% A, 10% B, and 0% C; within 50 min, the gradient changed to 50% A, 35% B, and 15% C; the gradient was maintained for 5 min before returning to the starting gradient (90% A, 10% B, and 0% C) in 5 min; and maintained at that gradient for another 5 min. The total run time was 65 min for each sample. The flow rate was 1 mL/min and the sample injection volume was 20 µL. The column temperature was set at ambient temperature and PDA detection was carried out at 280 nm.

Data analysis

All experiments were conducted in at least 3 independent experiments unless otherwise stated. Data are expressed as mean ± S.E.M (standard error of the mean). For anti-diabetic, anti-inflammatory, and antioxidant evaluation, the following cut-off points were used to

determine the activity (Nurhanan et al. 2023):

High activity: ≥70% inhibition or scavenging.
Moderate activity: 40–69% inhibition or scavenging.
Weak activity: < 40% inhibition or scavenging.

RESULTS

Table 1 shows the results of anti-diabetic, anti-inflammatory, and antioxidant activities. The methanolic bark extract of *P. gutta* bark extract at the concentration of only 10 µg/mL exhibited high inhibitory activity on both α-glucosidase (97.6 ± 0.5%) and α-amylase (85.8 ± 2.1%) digestive enzymes. The leaf extract also exhibited high α-glucosidase inhibitory activity (73.2 ± 0.3%) but only showed moderate inhibitory activity on α-amylase (57.0 ± 2.3%). The twig extract was less effective as it exhibited moderate inhibitory activity on α-glucosidase (69.6 ± 2.3%) and low inhibitory activity on α-amylase (34.8 ± 2.5%).

Similar to the trend seen in α-glucosidase inhibitory activity, the bark and leaf extracts exhibited high lipoxygenase inhibitory activity (93.1 ± 3.5% and 71.5 ± 1.3%, respectively) while the twig showed moderate inhibitory activity (68.0 ± 1.9%). However, unlike in the α-glucosidase inhibition assay, the concentration tested was at 100 µg/mL. All the three extracts were less effective in inhibiting xanthine oxidase and hyaluronidase. They only inhibited xanthine oxidase moderately and hyaluronidase weakly (Table 1). Nevertheless, the three extracts at the concentration of 200 µg/mL exhibited high antioxidant activity based on DPPH radical scavenging activity (97.6–98.4%) (Table 1).

Anti-cancer activities were evaluated on ovarian (SKOV-3), breast (MCF-7), and colorectal (HT-29) cells. The results are tabulated in Table 2. None of the three extracts exhibited any anti-cancer activities as the IC_{50} values obtained were all > 100 µg/mL. Only those with IC_{50} < 20 µg/mL are considered to have potential anti-cancer activity. The three extracts were also evaluated for anti-trypanosomal activity but all scored 1, which is weak with IC_{50} > 12.5 µg/mL (Table 2). Anti-microbial activities were evaluated on a range of *S. aureus* and *E. coli* microbes but all three extracts did not show any promising results. The MIC/MBC values were all > 5000 µg/mL (Table 2).

Vero and WRL-68 cells were used as kidney and liver model cells, respectively for *in vitro* toxicity evaluation and the results are tabulated in Table 3. Extract of the bark did not negatively affect the viability of Vero cells but it did reduce about 50% of WRL-68 cell viability. The twig extract can be considered non-cytotoxic to both Vero and WRL-68 cells as the viability was still more than 70% after exposure, while the leaf can be considered cytotoxic to both Vero and WRL-68 cells.

Phytochemical screening revealed the presence of flavonoids, steroids, and condensed tannins in the bark. The twig and leaf also contain similar groups of compounds, including saponins (Table 4). The phytochemical constitutes were similar between the twig and leaf except that the twig contained hydrolysed tannins while the leaf contained condensed tannins. The bark contained more flavonoids and tannins but less steroids than the twig and leaf.

Figure 1 shows the HPLC profiles of the bark, twig, and leaf extracts. The presence of several peaks was numbered from 1 to 10, based on their ultra violet (UV) spectra and elution time. The HPLC profile of the bark shows one main peak, which is peak 6, with UV absorbance at 280.0 nm. Several peaks were detected in the twig, with peak 7 being the most intense. The UV absorbance of peak 7 was also at 280.0 nm. Two main peaks were detected in the leaf, i.e. peak 8 and peak 9. Both have UV absorbance around 290.0 nm.

DISCUSSION

Bioprospecting of *P. gutta* bark, twig, and leaf via *in vitro* bioassays found that they were active in inhibiting α-glucosidase, α-amylase, lipoxygenase, and in scavenging DPPH free radical. α-Amylase, which is the major secretory products of the pancreas and salivary glands, catalyses the cleavage of α-D-(1-4)glycosidic linkages of carbohydrates such as starch and glycogen to produce shorter oligosaccharides. Then, α-glucosidase, which is located in the brush border surface membrane of intestinal cells, further cleaved the oligosaccharides to monosaccharide glucose, which is absorbable. Inhibitors of these enzymes can therefore delay

Table 1 Anti-diabetic, anti-inflammatory and antioxidant activities

Biological activities		Inhibition (%)			
		Bark	Twig	Leaf	Positive controls
Anti-diabetic	α-Glucosidase inhibition	97.6 ± 0.5	69.6 ± 2.3	73.2 ± 0.3	92.2 ± 0.4 (DNJ, 1 mg/mL)
	α-Amylase inhibition	85.8 ± 2.1	34.8 ± 2.5	57.0 ± 2.3	73.2 ± 0.8 (acarbose, 10 µg/mL)
Anti-inflammatory	Lipoxygenase inhibition	93.1 ± 3.5	68.0 ± 1.9	71.5 ± 1.3	99.0 ± 0.1 (NDGA, 100 µg/mL)
	Xanthine oxidase inhibition	49.4 ± 7.1	44.3 ± 3.7	35.8 ± 1.3	98.4 ± 1.2 (allopurinol, 100 µg/mL)
	Hyaluronidase inhibition	16.7 ± 0.6	6.8 ± 0.1	6.2 ± 0.3	91.46 ± 3.94 (apigenin, 200 µM)
Antioxidant	DPPH radical scavenging	98.0 ± 0.1	97.6 ± 0.2	98.4 ± 0.2	97.51 ± 0.60 (green tea, 100 µg/mL)

Data shown are mean ± S.E.M. (n ≥ 3 independent experiments). High activity: ≥ 70% inhibition or scavenging; moderate activity: 40–69% inhibition or scavenging; weak activity: < 40% inhibition or scavenging. The concentration tested for anti-diabetic was 10 µg/mL, 100 µg/mL for anti-inflammatory, and 200 µg/mL for antioxidant.

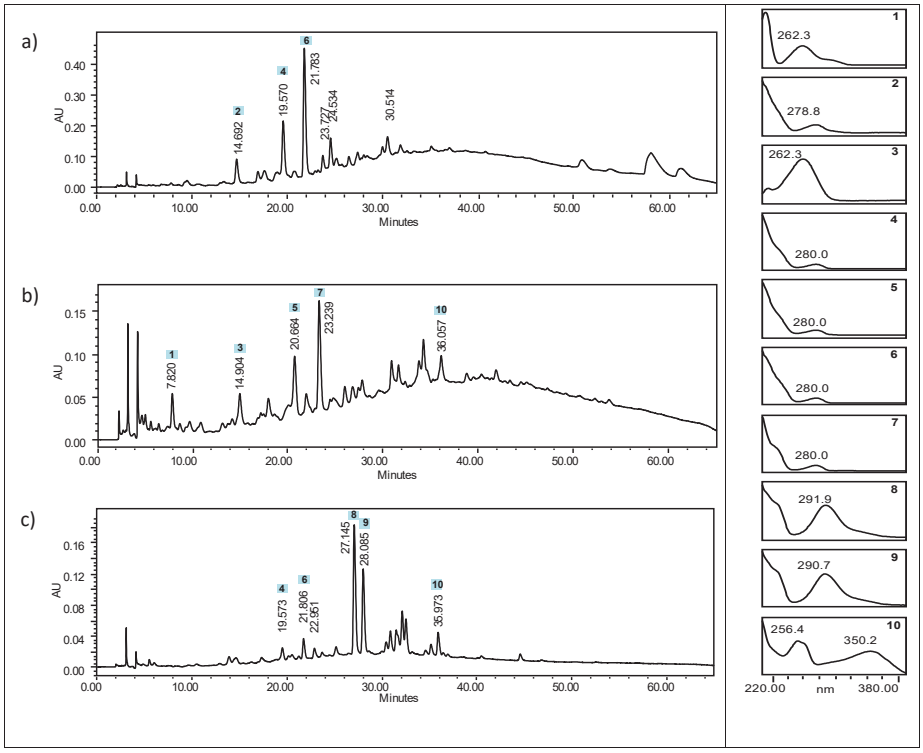

Figure 1 HPLC chromatograms and UV spectra of the (a) bark (b) twig, and (c) leaf

Table 2 Anti-cancer, anti-trypanosomal, and anti-microbial activities

Biological activities		IC_{50} or MIC (µg/mL)			
		Bark	Twig	Leaf	Positive controls
Anti-cancer	SKOV-3	> 100	> 100	> 100	$1.9\times10^{-4} \pm 6.5\times10^{-6}$ (taxol)
	MCF-7	> 100	> 100	> 100	$1.7\times10^{-4} \pm 8.5\times10^{-6}$ (taxol)
	HT-29	> 100	> 100	> 100	$8.0\times10^{-5} \pm 2.4\times10^{-6}$ (taxol)
Anti-trypanosomal		> 12.5 Score 1	> 12.5 Score 1	> 12.5 Score 1	$4.5\times10^{-3} \pm 0.6\times10^{-4}$ (pentamidine)
Anti-microbial	*S. aureus* (ATCC 25923)	> 5000	> 5000	> 5000	-
	S. aureus (ATCC 33591)	> 5000	> 5000	> 5000	-
	S. aureus (ATCC 700699)	> 5000	> 5000	> 5000	-
	S. aureus (ATCC US 300)	> 5000	> 5000	> 5000	-
	E. coli (ATCC 8739)	> 5000	> 5000	> 5000	-

Data shown are mean ± S.E.M. (n ≥ 3 independent experiments). Anti-cancer and anti-trypanosomal activities are reported based on IC_{50} while anti-microbial activities are reported based on MIC/MBC. IC_{50} < 20 µg/mL is considered to be positive anti-cancer activity; anti-trypanosomal activity is categorised as score 1 (weak: IC_{50} > 12.5 µg/mL), score 2 (moderate: 1.56 < IC_{50} ≤ 12.5 µg/mL), and score 3 (strong activity: IC_{50} ≤ 1.56 µg/mL); anti-microbial with MIC/MMC ≤ 1000 µg/mL is considered as active.

Table 3 *In vitro* toxicity

Plant extracts	Viability (%)			
	Vero cells		WRL-68 cells	
	100 µg/mL	200 µg/mL	100 µg/mL	200 µg/mL
Bark	100.6 ± 1.9	109.7 ± 8.7	40.7 ± 7.2	56.6 ± 17.3
Twig	128.7 ± 13.9	128.3 ± 5.5	92.6 ± 6.3	74.2 ± 12.3
Leaf	17.2 ± 3.7	23.66 ± 5.1	65.9 ± 13.1	47.0 ± 1.1
Positive control	0.1 µg/mL	1.0 µg/mL	0.1 µg/mL	1.0 µg/mL
Taxol	66.2 ± 7.0	39.0 ± 10.2	30.6 ± 12.6	16.4 ± 10.2

Data shown are mean ± S.E.M. (n ≥ 3 independent experiments). Viability ≥ 70% is considered non-cytotoxic.

Table 4 Qualitative analysis of phytochemical constituents

Plant parts	Phytochemical constitutes					
	Alkaloids	Flavonoids	Triterpenoids	Steroids	Saponins	Tannins
Bark	ND	3+	ND	1+	ND	C: 2+
Twig	ND	2+	ND	2+	1+	H: 1+
Leaf	ND	2+	ND	2+	1+	C: 1+

1+ = Low, 2+ = Medium, 3+ = High; ND = Not detected, C = Condensed, H = Hydrolysed.

postprandial increase of blood glucose level after a carbohydrate-rich diet and is a strategy for diabetes management (Lordan et al. 2013). This study shows that at only 10 µg/mL, *P. gutta*, especially the bark extract is required to decrease postprandial hyperglycaemia.

Lipoxygenase is a rate-limiting enzyme that catalyses fatty acids to generate leukotrienes, which are pro-inflammatory mediators. Although inflammation is a natural occuring healing process but inflammation can sometimes also be harmful. Inhibition of lipoxygenase can block leukotrines production, thereby producing an anti-inflammatory effect (Hu & Ma 2018). The lipoxygense type 1-B enzyme used in this assay is also known as 15-lipoxygenase, and has been linked to disease conditions such as asthma, atherosclerosis, rheumatoid arthritis, and cancer (Wisastra & Dekker 2014). The bark, leaf, and twig extracts of *P. gutta* at 100 µg/mL exhibited anti-inflammation potential.

Free radicals are unstable molecules with at least one unpaired electron. They are reactive and in order to become stable, they tend to form electron pairing with biological macromolecules such as proteins, lipids, and nucleic acids, which will cause damage to protein, cell membrane, and DNA. Antioxidants are able to neutralise free radicals to prevent their deleterious roles. DPPH free radical scavenging is a common technique used to screen for antioxidant activity and extensively used to predict antioxidant activity due to its simplicity (Rahman et al. 2015). All the three *P. gutta* extracts displayed high antioxidant potential, though we are aware that the concentration used in this study was quite high, i.e. at 200 µg/mL.

On the other hand, based on the results in this study, none of *P. gutta* extracts exhibited sufficient killing effects to measure up as anti-cancer, anti-trypanosomal, or antimicrobial potentials. In terms of toxicity, the leaf extract was more toxic towards kidney cells compared to the bark and twig. On liver cells, toxicity of the leaf was almost comparable to the bark, and both of the extracts were more toxic than the twig. It will be more meaningful if dose-response curves for α-glucosidase, α-amylase, lipoxygenase inhibitions and DPPH radical scavenging were generated to determine the lowest concentration required for the possible activities and compare it with a toxicity dose-response curve. This will enable a risk-benefit ratio determination of *P. gutta* potential benefits.

Information is scarce on the medicinal use of *P. gutta*, except for its gutta percha in dentistry. The plant is listed in the Medicinal Herbs and Plants Monograph by GlobinMed but there is no information on plant part used, preclinical data, clinical data, or even for traditional use (GlobinMed, 2022). The genus *Palaquium* is represented by more than 100 species (van Royen 1953, Mohtar, 2002). The production of gutta percha is not solely exclusive to *P. gutta*. Other species such as *Palaquium maingayi, Palaquium odoratum, Palaquium hexandrum, Palaquium herveyi,* and *Palaquium hispidum* can also yield gutta percha but *P. gutta* produces the highest quality gutta percha (Burkill 1966).

An exploratory study to find novel photosensitisers for cancer treatment from Malaysia biodiversity, *P. maingayi* leaf and stem methanolic extracts exerted negative activity (Ong et al. 2009). Another exploratory study of rain forest trees in Palawan Island in the Philippines, *Palaquium* cf. *tenuipetiolatum* Merr methanolic extracts of the twig + leaf, root, and stem exhibited no cytotoxic activity towards human lung cancer (Lu1), human epidermoid carcinoma (KB cells), vinblastine-resistant KB cells tested with and without vinblastine, murine lymphoid neoplasma (P-388), human

hormone-dependent prostate cancer (LNCaP), and human-dependent breast cancer (ZR-75-1) cells (Horgen et al. 2001). The extracts also did not exhibit antiplasmodial activity against chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum* (Horgen et al. 2001). However, the n-butanol-soluble fraction of *Palaquium formosanum* kernels ethanolic extract and some of the compounds isolated such as 16α-hydroxyprotobassic acid glycosides arganin C and butyroside B were cytotoxic towards PC-3 prostate cancer cells (Chen et al. 2018). Water extract of *Palaquium stehlinii* bark showed marginal antibiotic activity against E. *coli* and *Penicillium chrysogens* but still not as good as garlic extract (Sotheeswaran et al. 1997).

To the best of our knowledge, there is no published data on the pharmacological activity of *P. gutta*. It could be said that this study is the first reported anti-diabetic, anti-inflammatory, and antioxidant activities of *P. gutta*.

A study on the chemical composition of *P. gutta* heartwood extracted with methanol identified the presence of monoterpenes, diterpenes, sesquiterpenes, and alkaloids using gas chromatograph-mass spectrometry (GC-MS) (Kadir et al. 2022). The positive biological activities seen in this study could be attributed to the presence of high levels of flavonoids. Flavonoids are known to have a broad spectrum of pharmacological effects and potent inhibitors of enzymes such as xanthine oxidase, cyclo-oxygenase, lipoxygenase, and phosphoinositide 3-kinase (Panche et al. 2016). The activities could also be due to the presence of phenolic compounds. Based on the UV spectra from HPLC analysis, the 10 peaks observed in the 3 extracts can be divided into 2 groups of chemical compounds. The first group, peaks numbered from 1–9, with λ_{max} range from 260–290 nm, can be categorised as phenolic compounds. According to Chen et al. (2012), the presence of phenolic acids with benzoic acid skeleton was characterised with λ_{max} range between 200–290 nm. While the second group, refering to peak number 10, with λ_{max} of 256 nm and 350 nm can be characterised as flavonoids because flavonoids is characterised by the presence of two λ_{max} in the range of 250–280 nm (band II) and 310–360 nm (band I) (Chen et al. 2012). Findings from this study is still too preliminary to pinpoint which chemical constituent is responsible for the observed biological activities.

CONCLUSIONS

The methanolic extract of the *P. gutta* bark exhibited the most promising biological activities. It displayed high anti-diabetic, anti-inflammatory, and antioxidant activities in vitro. It did not affect the viability of Vero cells, although it reduced the viability of WRL-68 cells to a certain degree. The biological activities observed could be attributed to the presence of flavonoids and phenolic compounds.

ACKNOWLEDGEMENTS

This research was supported by the Developmental Project, Tenth and Twelfth Malaysia Plan. The authors would like to thank everyone who has directly or indirectly contributed to this research, especially colleagues from the Natural Products Division.

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