Biological Activity and Isolation of Compounds from Stem Bark of Plumeria acutifolia

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ABSTRACT

Background: Plumeria acutifolia (Apocynaceae) is an ornamental plant, used in the traditional medicine and known to have a variety of constituents as alkaloids, flavonoids, and iridoids. Extracts of this plant were proved to have antimicrobial and anticancer activities. Objectives: This research was conducted for the evaluation of the biological activities of P. acutifolia stem bark and isolation and structural elucidation of various chemical compounds from the biologically active fractions. Materials and Methods: Methanol extract of stem bark of P. acutifolia was successively fractionated with petroleum ether, dichloromethane, ethyl acetate, and n-butanol. The fractions were evaluated for their antimicrobial, cytotoxic, and antioxidant activities. Fractions with promising biological activities were subjected to chromatographic techniques for the isolation of compounds, followed by structural elucidation using several spectroscopic techniques. Results: P. acutifolia stem bark showed a significant antimicrobial activity, where the ethyl acetate fraction was active against Syncrphaestrum racemosum (781 µg/ml) and Escherichia coli (3.9 µg/ml). The cytotoxic activity against HEPG-2, HCT-116, and MCF-7 cell lines was highest in the petroleum ether fraction, using concentrations of 1, 2.5, 5, and 10 µl/ml. The antioxidant activity was concentration dependent; ethyl acetate fraction showed the most predominant effect, with an IC₅₀ of 197.1 µg/ml. Five compounds were identified as narcissin (1), quercitin (2), sweroside (3), gaertneroside (4), and plumieride (5). Conclusion: P. acutifolia was proved to have significant antimicrobial, cytotoxic, and antioxidant activities; the isolated compounds were flavonoids, iridoids, and secoiridoid, some of which were reported for the first time in genus Plumeria and/or family Apocynaceae. Key words: Antimicrobial, antioxidant, Apocynaceae; flavonoids, iridoids

SUMMARY

• P. acutifolia stem bark showed a significant antimicrobial activity, where the ethyl acetate fraction was active against Syncrphaestrum racemosum and Escherichia coli. The cytotoxic activity against HEPG-2, HCT-116, and MCF-7 cell lines was highest in the petroleum ether fraction. The antioxidant activity was concentration dependent; ethyl acetate fraction showed the most predominant effect. Five compounds were identified as narcissin (1), quercitin (2), sweroside (3), gaertneroside (4), and plumieride (5).

INTRODUCTION

Plants of the genus Plumeria (Apocynaceae) are usually cultivated in gardens, for their showy and fragrant flowers. Various Plumeria species are used to cure rheumatism, diarrhea, blennorrhrea, and venereal disease. They were reported to exhibit significant antibacterial, antifungal, and anticaner activities. The chemical constituents of Plumeria include alkaloids, flavonoids, iridoids, and triterpenes. Several extracts of Plumeria acutifolia Poir. were proved to have significant antipyretic, antinociceptive, anti-inflammatory, and antimicrobial activities, in addition to potential antitumor and antioxidant activities. Previous phytochemical investigations on P. acutifolia proved the presence of alkaloids as phoebegrandine B, laurelliptine, plumerianine, and plumericidine. The flavonoids kaempferol, ayanin, and pillion were also isolated. In addition, the presence of iridoids, including includes plumieride, plumieridin A and B, 1α-plumieride, and 8-isoplumieride, was isolated. Triterpenes such as ursolic acid, stigmast-7-enol, lupeol carboxylic acid, and lupeol acetate have been isolated from P. acutifolia. The main objective of the present research was the evaluation of the biological activities of P. acutifolia stem bark and isolation and structural elucidation of various chemical compounds from the biologically active fractions.

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MATERIALS AND METHODS

General experimental methods

Materials

Precoated silica gel 60 F-254 glass plates, 0.25 mm thick, silica gel F-254, particle size 0.08 mm, 200 mesh, dimethyl sulfoxide (DMSO), malt extract broth and nutrient broth (E. Merck, Germany); gentamycin (Way Well Limited, China). Muller-Hinton agar medium (Becton Dickinson, Heidelberg); sterile nutrient agar (Immupréparate, Berlin); 2,2-diphenyl-1-picrylhydrazyl (Fluka, Germany); and amphotericin B (World Industry Co. Ltd, China) were the materials used for the study. Ampicillin, ascorbic acid, doxorubicin, in addition to all of the solvents and other materials, were purchased from Sigma-Aldrich Company.

Equipment and techniques

Buchi® Rotavapor and water bath (Switzerland), Vacuubrand® pump (Germany), and Julabo® chiller (Germany); nuclear magnetic resonance (NMR) Bruker instrument at 500 or 700 MHz for protons and Bruker instrument at 125 or 176 MHz for carbons; and Finnigan-MAT Model TSQ 700 (San Jose, CA, USA) triple quadrupole mass spectrophotometer with an atmospheric pressure chemical ionization interface were the equipment used. Capillary temperature, 200°C; vaporized temperature, 450°C; corona needle current, 5 µA; sheath gas, nitrogen; collision gas, helium; collision energy, 50%.

Plant material

Stem bark of P. acutifolia was collected in June 2011 from Riyadh city, Kingdom of Saudi Arabia. The plant was kindly identified by Dr. Amal Hosni, Professor of Plant Taxonomy, Plant Department, College of Science, Cairo University, Cairo, Egypt. The stem bark of the plant was dried, powdered, and reserved at room temperature for biological and chemical study. A voucher specimen of the plant was kept in Pharmacognosy Department, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Extraction

The powdered air-dried stem bark of P. acutifolia (950 g) was exhaustively extracted via percolation with 70% methanol and evaporated under reduced pressure. 150 g of the methanol extract was suspended in water and successively fractionated with petroleum ether, dichloromethane, ethyl acetate, and n-butanol saturated with water. Each fraction was concentrated in vacuum to yield petroleum ether (1 g), dichloromethane (2 g), ethyl acetate, and n-butanol fractions were prepared as follows: 1 mg/ml, 0.50 mg/ml, 0.45 mg/ml, 0.40 mg/ml, 0.35 mg/ml, 0.30 mg/ml, 0.25 mg/ml, 0.20 mg/ml, 0.15 mg/ml, 0.10 mg/ml, and 0.05 mg/ml.

Biological Activity

Antimicrobial activity

The antimicrobial activity was evaluated according to the National Committee of Clinical Laboratory Standards, against the Gram-positive bacteria, Bacillus subtilis and Streptococcus pneumoniae; the Gram-negative bacteria, Escherichia coli and Pseudomonas aeruginosa; and the fungi Aspergillus fumigatus, Candida albicans, Geotrichum candidum, and Syncephalastrum racemosum. Amphotericin B, ampicillin, and gentamicin were used as positive controls and DMSO was used as a negative control. The minimal inhibitory concentration (MIC) against the sensitive microorganisms was determined by the agar diffusion technique, several concentrations of the dichloromethane, ethyl acetate, and n-butanol fractions were prepared as follows: 1 mg/ml, 0.50 mg/ml, 0.45 mg/ml, 0.40 mg/ml, 0.35 mg/ml, 0.30 mg/ml, 0.25 mg/ml, 0.20 mg/ml, 0.15 mg/ml, 0.10 mg/ml, and 0.05 mg/ml.

Cytotoxic activity

The potential cytotoxicity was tested in vitro against three human cell lines: HEPG-2, MCF-7, and HCT-116. These cell lines were obtained from American Type Culture Collection, USA. DMSO was used as a negative control, and doxorubicin was used as a positive control. Different concentrations of each sample in DMSO (1, 2.5, 5, and 10 µl/ml) were prepared.

Antioxidant activity

In addition, P. acutifolia samples were evaluated for their antioxidant activities on the basis of the scavenging activity of the stable α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical. The prepared concentrations of each fraction were 250, 500, 1000, 1500, 2000, and 2500 µg/ml.

Isolation

Dichloromethane fraction (1.8 g) was chromatographed on a silica gel column (72 g, 80 cm × 3 cm) using petroleum ether and increasing polarity with dichloromethane followed by methanol. The collected fractions (20 ml each) were pooled depending on their thin layer chromatography (TLC) behavior to give 16 fractions. Fraction number 5 (143.5 mg), eluted with 2% methanol in dichloromethane, was rechromatographed on a silica gel subcolumn (7.2 g, 60 cm × 1 cm) eluted gradually starting from 50% dichloromethane in petroleum ether (5 ml each). The collected fractions were merged into 27 subfractions according to their TLC. Subfraction number 15, eluted with 100% dichloromethane, was crystallized by ethanol and methanol, successively, to afford compounds 1 and 2. However, fraction number 6 (78.1 mg), eluted with 2% methanol in dichloromethane, was subjected to crystallization by methanol, resulting in isolation of compound 3. On the other hand, a portion of the ethyl acetate fraction (1.5 g) was dissolved in methanol and subjected to preparative TLC (silica gel plates, 20 cm × 20 cm), using 22% methanol in dichloromethane as a solvent system, resulting in the isolation of compounds 4 and 5. The isolated compounds were subjected to 1H-NMR, 13C-NMR, COSY, and HMBC spectroscopic analysis.

RESULTS

Biological activities

The results of the antimicrobial activity and MIC are represented in Table 1, while the results of the cytotoxic activity are represented in Table 2 and Figure 1. Table 3 and Figure 2 show the percentage of DPPH scavenging activity of the tested samples.

Isolated compounds

Compound 1 (4 mg) was isolated as yellow needles. (melting point) m.p. 179 ± 1°C. 1H-NMR (DMSO-d6) δ ppm: 6.1 (1H, s, H-6), 6.31 (1H, d, H-8), 3.9 (3H, s, -OCH3), 7.8 (1H, d, J = 1.9 Hz, H-2’), 6.9 (1H, d, J = 8.4 Hz, H-5’), 7.36 (1H, dd, J = 8.4 Hz, H-6’), 5.35 (1H, d, J = 7 Hz, H-1’), 3.2–3.5 (m, H-2’’,3’’,4’’,5’’) and 1.1 (3H, d, H-6’’), 13C-NMR (DMSO-d6) δ ppm: 157.0 (C-2), 133.0 (C-3), 177.0 (C-4), 161.5 (C-4a), 96.0 (C-5), 163.0 (C-6), 98.0 (C-7), 157.0 (C-8), 106.0 (C-8a), 57.0 (−OCH3), 122.0 (C-1’’), 113.5 (C-2’’), 148.0 (C-3’’), 146.0 (C-4’’), 113.0 (C-5’’), 115.5 (C-6’’), 104.0 (C-1’’’), 76.5 (C-2’’’), 79.0 (C-3’’’), 71.5 (C-4’’’), 79.5 (C-5’’’), 69.5 (C-6’’’), 103.0 (C-1’’’’), 70.2 (C-2’’’’), 71.0 (C-3’’’’), 73.0 (C-4’’’’), 68.5 (C-5’’’’), and 18.2 (C-6’’’’).

Compound 2 (7 mg) white amorphous powder, m.p. of 183 ± 1°C. CI-MS m/z: 448, 433, 285, 251, 235, 146. 1H-NMR (DMSO-d6) δ ppm: 6.20 (1H, d, J = 2.5 Hz, H-6), 6.40 (1H, d, J = 2.5 Hz, H-8), 1.25 (3H, d, J = 6 Hz), 7.65 (2H, m, H-2’ and H-6’), 6.85 (1H, d, J = 7.5 Hz, H-5’), and 5.35 (1H, d, J = 2.5 Hz, H-1’).
Table 1: Antimicrobial screening and minimum inhibitory concentration values of stem bark fractions of *Plumeria acutifolia*

| Microorganism | Aspergillus fumigatus | Syncaphalastrum racemosum | Geotrichum candidum | Candida albicans | Streptococcus pneumoniae | Bacillus subtilis | Pseudomonas aeruginosa | Escherichia coli |
|---------------|-----------------------|--------------------------|--------------------|-----------------|------------------------|-----------------|------------------------|-----------------|
| Zone of inhibition (mm) | | | | | | | | |
| Sample | Petroleum ether fraction | Dichloromethane fraction | Ethyl acetate fraction | n-butanol fraction | Standard | Ampicillin | Gentamicin | | |
| | 18.6±0.34 | 17.2±0.25 | 20.1±0.39 | 18.3±0.33 | Amphotericin B | - | - | | |
| | 19.3±0.38 | 19.2±0.39 | 23.4±0.44 | 19.8±0.18 | - | - | - | | |
| | 15.1±0.38 | 17.9±0.33 | 15.2±0.34 | 25.4±0.1 | 23.8±0.2 | 32.4±0.3 | - | - | |
| | 19.1±0.44 | 22.6±0.15 | 20.8±0.26 | 23.1±0.63 | 17.3±0.1 | 19.9±0.3 | | | |
| MIC (µg/mL) | | | | | | | | | |
| Sample | Petroleum ether fraction | Dichloromethane fraction | Ethyl acetate fraction | n-butanol fraction | Standard | Ampicillin | Gentamicin | | |
| | 31.25 | 62.5 | 7.81 | 125 | 3.0 | 0.97 | 0.97 | 31.25 | 7.81 | 17.3±0.1 | 19.9±0.3 |
| | 125 | 125 | 3.9 | 125 | 7.81 | 3.9 | 125 |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |

NA: No activity; MIC: Minimum inhibitory concentration

Table 2: Cytotoxic activity of stem bark fractions of *Plumeria acutifolia* against HEPG-2, HCT-116 and MCF-7 cell lines

| Concentration (µg/mL) | Sample (percentage of cell viability) | Petroleum fraction | DCM fraction | Ethyl acetate fraction | Butanol fraction |
|-----------------------|--------------------------------------|--------------------|--------------|-----------------------|-----------------|
| HEPG-2 | 0 | 100 | 100 | 100 | 100 |
| | 1.56 | 79.14 | 87.91 | 100 | 90.64 |
| | 3.125 | 68.7 | 79.34 | 96.38 | 82.58 |
| | 6.25 | 51.38 | 66.27 | 88.76 | 70.42 |
| | 12.5 | 41.52 | 53.86 | 79.24 | 63.96 |
| | 25 | 30.91 | 37.78 | 61.98 | 51.83 |
| | 50 | 23.36 | 21.16 | 42.75 | 33.91 |
| HCT-116 | 0 | 100 | 100 | 100 | 100 |
| | 1.56 | 63.74 | 98.14 | 100 | 96.34 |
| | 3.125 | 53.71 | 92.42 | 95.88 | 90.78 |
| | 6.25 | 41.38 | 81.57 | 87.11 | 81.82 |
| | 12.5 | 37.25 | 60.88 | 80.48 | 79.46 |
| | 25 | 24.22 | 41.9 | 63.97 | 55.54 |
| | 50 | 16.46 | 15.44 | 41.72 | 18.77 |
| MCF-7 | 0 | 100 | 100 | 100 | 100 |
| | 1.56 | 82.18 | 92.56 | 100 | 97.32 |
| | 3.125 | 68.54 | 84.67 | 97.98 | 90.88 |
| | 6.25 | 54.63 | 69.38 | 94.25 | 79.63 |
| | 12.5 | 43.86 | 57.42 | 82.96 | 63.38 |
| | 25 | 35.72 | 39.39 | 65.24 | 49.62 |
| | 50 | 21.98 | 23.74 | 48.18 | 38.46 |

HEPG-2: Liver cancer cell line; MCF-7: Breast cancer cell line; HCT-116: Colon cancer cell line; DCM: Dichloromethane

Compounds 3 (4 mg) amorphous powder, m.p. 168 ± 1°C.

'H-NMR (CD_3OD) δ ppm: 5.54 (1H, d, J = 1.2 Hz, H-1), 7.58 (1H, d, J = 2.4 Hz, H-3), 3.27 (1H, m, H-5), 1.75 (2H, m, H-6), 4.35 (2H, m, H-7), 5.53 (1H, m, H-8), 2.69 (1H, m, H-9), 5.31 (1H, dd, J = 2.0, 16.8 Hz, H-10a), 5.26 (1H, dd, J = 2.0, 9.0 Hz, H-10b), 4.67 (1H, d, J = 8.0 Hz, H-1`), 3.27 (1H, dd, J = 8.0, 8.8 Hz, H-2`), 3.76 (1H, t, J = 8.8 Hz, H-3`), 3.31 (1H, m, H-4`), 3.44 (1H, m, H-5`), 3.65 (1H, dd, J = 12.0, 6.0 Hz, H-6`a) and 4.05 (1H, dd, J = 12.0, 6.4 Hz, H-6`b).

'C-NMR (CD_3OD) δ ppm: 98.3 (C-1), 152.4 (C-3), 104.6 (C-4), 27.1 (C-5), 25.4 (C-6), 70.2 (C-7), 131.9 (C-8), 47.3 (C-9), 119.5 (C-10), 167.2 (C-11), 98.4 (C-1`), 73.7 (C-2`), 76.5 (C-3`), 70.2 (C-4`), 73.3 (C-5`), and 61.3 (C-6`).

Compounds 4 (13 mg) beige, amorphous powder, m.p. 150 ± 1°C.

'H-NMR (CD_3OD) δ ppm: 5.28 (H-1), 7.52 (H-3), 3.86 (m, H-5), 6.48 (H-6), 5.53 (H-7), 2.96 (H-9), 7.39 (H-10), 4.58 (H-13), 3.77 (H-15), 7.45 (H-2`, 6`), 6.80 (H-3`, 5`), 4.71 (H-1`), 3.22 (H-2`), 3.42 (H-3`), 3.33 (H-4`), 3.55 (H-5`), 3.62 (H-6`a) and 3.86 (H-6`b, m).

'C-NMR (CD_3OD) δ ppm: 92.8 (C-1), 151.4 (C-3), 109.6 (C-4), 39.0 (C-5), 140.1 (C-6), 128.6 (C-7), 96.5 (C-8), 49.2 (C-9), 149.0 (C-10), 137.2 (C-11), 171.5 (C-12), 62.1 (C-13), 167.1 (C-14), 50.6 (C-15), 91.4 (C-16), and 83.7 (C-17).
131.5 (C-1’), 130.0 (C-2’,6’), 104.3 (C-3’,5’), 156.6 (C-4’), 98.6 (C-1’’), 73.3 (C-2’’), 76.4 (C-3’’), 70.1 (C-4’’), 76.8 (C-5’’), and 61.1 (C-6’’). Compound 5 (5 mg) yellow crystals, m.p. 225 ± 1°C. ^1H-NMR (CD$_3$OD) δ ppm: 5.29 (1H, d, J = 5 Hz, H-1), 7.52 (1H, d, J = 1 Hz, H-3), 3.95 (1H, dd, J = 1.5, 6.5 Hz, H-5), 6.49 (1H, dd, J = 5.5, 2.5 Hz, H-6), 5.53 (1H, dd, J = 5.5, 2.5 Hz, H-2’), 3.35 (1H, t, J = 8.5 Hz, H-3’), 3.35 (1H, t, J = 8.5 Hz, H-4’), 3.38 (1H, d, J = 8 Hz, H-5’), 3.71 (1H, d, J = 5.5 Hz, H-6’a), and 3.92 (1H, s, H-6’b). ^13C-NMR (CD$_3$OD) δ ppm: 92.8 (C-1), 151.1 (C-3), 109.6 (C-4), 39.0 (C-5), 140.0 (C-6), 128.6 (C-7), 96.5 (C-8), 49.3 (C-9), 148.9 (C-10), 137.2 (C-11), 171.4 (C-12), 62.1 (C-13), 21.0 (C-14), 167.1 (C-15), 50.5 (C-16), 98.7 (C-1’), 73.3 (C-2’), 77.1 (C-3’), 69.9 (C-4’’), 76.4 (C-5’’), and 61.1 (C-6’’). [Figure 4]. The COSY and HMBC correlations of compound 4 are shown in Figure 3.

**DISCUSSION**

**Biological activities**

Ethyl acetate fraction showed the highest antimicrobial activity against the tested microorganisms. The dichloromethane and n-butanol fractions showed weak to moderate activities against the tested microorganisms, while petroleum ether fraction exhibited no antimicrobial activity against the tested microorganisms. The activity of ethyl acetate fraction was comparable to the standard amphotericin B against *S. racemosum*(7.81 µg/ml) and showed higher activity than that of the standard gentamicin against *E. coli*(3.9 µg/ml). Dichloromethane fraction exhibited interesting antimicrobial activity against *B. subtilis*(20.1 ± 0.25 mm) and *E. coli*(13.6 ± 0.25 mm). On the other hand, Gram-negative bacterium, *P. aeruginosa*, was resistant to all of the tested fractions. The antimicrobial activity of bark extract against different bacteria such as *B. subtilis* and *E. coli* has also been reported previously.[21]

The results of the cytotoxic activity proved that the petroleum ether fraction was the most active fraction against HEPG-2, HCT-116, and MCF-7 cell lines, with IC$_{50}$ values of 23.36, 16.46, and 21.98 µg/ml, respectively. The dichloromethane fraction (21.16, 15.44, 13.24, and 8.92 µg/ml), and 61.1 (C-6’’). [Figure 4]. The COSY and HMBC correlations of compound 4 are shown in Figure 3.

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**Table 3:** Percentage of 2,2-diphenyl-1-picrylhydrazyl scavenging of *Plumeria acutifolia* stem bark fractions

| Concentration (µg/mL) | Petroleum fraction | Dichloromethane fraction | Ethyl acetate fraction | n-butanol fraction |
|-----------------------|--------------------|--------------------------|-----------------------|-------------------|
| 0                     | 0                  | 0                        | 0                    | 0                 |
| 200                   | 2.58               | 31.25                    | 68.98                | 45.31             |
| 400                   | 3.75               | 41.32                    | 82.81                | 70.88             |
| 600                   | 4.52               | 50.29                    | 83.27                | 79.37             |
| 800                   | 6.14               | 58.68                    | 83.36                | 80.91             |
| 1000                  | 8.92               | 66.89                    | 83.45                | 81.00             |
| 1200                  | 10.72              | 71.16                    | 83.76                | 81.09             |
| 1400                  | 13.24              | 71.97                    | 83.90                | 81.18             |
| 1600                  | 15.48              | 72.11                    | 83.95                | 81.36             |
| IC$_{50}$              | 5931.9             | 641.5                    | 197.1                | 308.5             |

**Standard ascorbic acid (IC$_{50}$: 13.8 µg/mL)**

| Concentration (µg/mL) | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 |
|-----------------------|---|----|----|----|----|----|----|----|
| Percentage of DPPH    | 0 | 22.40 | 43.95 | 72.47 | 85.58 | 85.67 | 85.99 | 86.08 | 86.39 |

DPPH: 2,2-Diphenyl-1-picrylhydrazyl; IC50: Concentrations of 50% inhibition
The structures of compounds 1-5 isolated from stem bark fractions of Plumeria acutifolia

Isolated compounds

The 13C-NMR spectrum of compound 1 established 15 carbon signals for the flavonoid nucleus and 12 carbon resonances belonging to two sugar moieties, in addition to one carbon signal indicating the presence of one methoxy group. The anomic carbons were found at δ 104.0 (C-1) and 103.0 (C-1`). Accordingly, the 1H-NMR spectrum showed two protons resonating at δ 5.35 (δ 7 Hz, H-1`) and δ 4.3 (δ 8.7 Hz, H-1`) assignable to the anomeric β-glucopyranosyl proton and α-rhamnopyranosyl protons, respectively. In the aromatic area of the proton spectrum, an ABX system was evident which was attributed to two olefinic protons at δ 7.93 (δ 1.8 Hz; H-6`) and 7.59, dd (δ 8.3, 1.9 Hz; H-5`). The proton spectrum, an ABX system was evident which was attributed to two olefinic protons at δ 7.93 (δ 1.8 Hz; H-6`) and 7.59, dd (δ 8.3, 1.9 Hz; H-5`) respectively, in the aromatic area of the proton spectrum, an ABX system was evident which was attributed to two olefinic protons at δ 7.93 (δ 1.8 Hz; H-6`) and 7.59, dd (δ 8.3, 1.9 Hz; H-5`) respectively. In the aromatic area of the proton spectrum, an ABX system was evident which was attributed to two olefinic protons at δ 7.93 (δ 1.8 Hz; H-6`) and 7.59, dd (δ 8.3, 1.9 Hz; H-5`).

On the basis of mass and 1H-NMR spectra, and by further comparison to the published spectral data, compound 2 could be identified as quercitrin. The compound was previously isolated from the genus Plumeria including P. acutifolia and Pityrias rubra.

The 13C-NMR spectrum of compound 3 showed 16 signals; 10 carbon signals attributed to the aglycone part and 6 signals to the sugar moiety. While the 1H-NMR spectrum showed a downfield doublet signal at δ 7.58 with J = 7.4 Hz (H-3), it indicated the presence of an oxofinic hydrogen of the secoiridoids. The signal at δ 4.35 (2H, m, H-7) was assigned to the proton of C-7 by comparative analysis with reported data. In addition, there are two double doublets at δ 5.31 (1H, dd, J = 2.0, 16.8 Hz, H-10a, trans) and δ 5.26 (1H, dd, J = 2.0, 9.0 Hz, H-10b, cis) that were assigned to two protons of methylene group. Furthermore, resonances for sugar moiety include an anomeric proton signal at δ 4.67 (1H, d, J = 8.0 Hz, H-1`), together with five proton signals for the remaining sugar protons. The spectral data are strongly reminiscent with those reported for sweroside, a secoiridoid derivative, formerly isolated from many plants as Scabiosa atropurpurea (Dipsacaceae), Lonicera angustifolia (Caprifoliaceae), and Gentiana loureirii (Gentianaceae). It also presents in Apocynaceae family as it was isolated from Alstonia and Tabernaemontana species.

Compound 4 exhibited six signals in 13C-NMR spectrum, corresponding to a glucose moiety, and 18 signals corresponding to an iridoid aglycone. Two characteristic signals were observed at δ 96.5 (C-8) and δ 171.5 (C-9), together with two signals for a downfield double bond, appeared at δ 149 (C-10) and δ 137.2 (C-11), all of these signals were attributed to an α, β-unsaturated lactone ring. Furthermore, two signals due to methyl ester resonating at δ 50.6 (C-15) and δ 167.1 (C-14) were observed. A quaternary carbon appeared at δ 109.6 (C-4) and a downfield methine group at δ 151.4 (C-3) both signals are characteristic to most iridoids. Moreover, 1H-NMR spectrum clearly showed a downfield olefinic proton signal, appeared at δ 7.52, assigned for H-3, in addition to two olefinic protons at δ 6.48 and δ 5.53, indicating the presence of another double bond and assigned for H-6 and H-7, respectively. The 1H-NMR spectrum also exhibited one part of an AA’BB’ system, corresponding to a para disubstituted benzene ring, with δ 7.45 and δ 6.8, assigned for H-2’, 6’ and H-3’, 5’, respectively. The 1H-H COSY spectrum showed a coupling between H-5 (δ 3.86) and H-9 (δ 2.96), in addition to a coupling between H-6 (δ 6.48) and H-7 (δ 5.53), and thus confirming the iridoid nucleus. Furthermore, a coupling between H-2’, 6’ (δ 7.45) with H-3’, 5’ (δ 6.80) was exhibited. HMBC spectrum revealed the presence of two bond correlations between a carbon resonating at δ 39.0 (C-5) and protons appeared at δ 2.96 (H-9) and δ 6.48 (H-6), in addition to coupling between the proton of C-3 (δ 7.52) with C-4 (δ 109.6) and C-14 (δ 167.1). HMBC spectrum also indicated two bond correlations between carbon appeared at δ 96.5 (C-8) and protons with δ 2.96 (H-9), δ 5.53 (H-7), δ 7.39 (H-10), in addition to three bond correlations between the same carbon and protons...
resonating at δ 3.86 (H-5) and 6.48 (H-6). Compound 4 was identified as gaertneroside. Gaertneroside has been isolated from Rubiaceae family.\[44,46\] NMR data of compound 5 in CD$_2$OD showed characteristic signals assigned to plumieride, which was supported by comparison with the reported spectral data.\[23,24,47\] Plumieride has been isolated previously from many species belonging to Plumeria,\[5,7,44\] including P. acutifolia.\[13\] The antimicrobial activity of the dichloromethane fraction against B. subtilis and E. coli might be related to the presence of quercitrin\[49\] and/or sweroside,\[50\] which showed activity against theses microorganisms. The same fraction also showed moderate antioxidant activities, which could be related to the presence of quercetin and/or narcissin, both flavonoids where proved to possess antioxidant activities.\[24,45,46\] On the other hand, the ethyl acetate fraction showed positive antioxidative activity; this may refer to the presence of loganin as it was proved to possess antioxidant activity.\[51\]

### CONCLUSION

Stem bark of P. acutifolia was proved to have significant antimicrobial, cytotoxic, and antioxidative activities, where the ethyl acetate fraction showed the highest antimicrobial and antioxidant effect, whereas the petroleum ether fraction was the most predominant cytotoxic fraction. The isolated compounds were identified as flavonoids – narcissin and quercitrin, a secoiridoid – sweroside, and iridoids – gaertneroside. On the basis of review of published data, this is the first report of isolation of narcissin and gaertneroside from the family Apocynaceae and the first report of isolation of sweroside, as well as a secoiridoid compound from the genus Plumeria.

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### Conflicts of interest

There are no conflicts of interest

### REFERENCES

1. Endress ME, Bruyns PV. A revised classification of the Apocynaceae s.l. Bot Rev 2000;66:1-56.
2. Kamariah AS, Lim LB, Basar KH, Oezek T, Demirci B. Composition of the essential oil of Plumeria obtusa L. Flavour Frag J 1999,14:237-40.
3. Perry LM, Metzger J. Medicinal Plants of East and Southeast Asia: Attributes and Uses. Cambridge: The MIT Press; 1980. p. 28-9.
4. Rasool SN, Jaheenunzisa S, Chitta SK, Jayaveera KN. Antimicrobial activities of Plumeria acutifolia. J Med Plants Res 2008;2:77-80.
5. Dohbel MP, Li G, Gryshuk A, Graham A, Bhatawadekar AK, Khaja SD, et al. Structural modifications of plumieride isolated from Plumeria bicalcar and the effect of these modifications on in vitro anticancer activity. J Org Chem 2004;69:6176-72.
6. Gupta M, Mazumder UK, Gomathi P. Evaluation of antipyretic and antinociceptive activities of Plumeria acuminata leaves. J Med Sci 2007;7:825-9.
7. Periyasamy G, Gupta M, Mazumder UK, Gebrielanos M, Sintayehu B. Antioxidant and antitumor activity of Plumeria acuminata in ethrlic ascites carcinoma bearing Swiss albino mice. British Journal of Pharmaceutical Research 2013;3:671-85.
8. Almahy HH, Elegami AA. Alkaloids and antimicrobial activity of Plumeria acutifolia (rubal) J Int J Mold Adv Sci 2007;3:12-9.
9. Hassan EM, Shabat AA, Ibrahim NA, Vletinck AJ, Apers S, Pieters L. A new monoterpene alkaloid and other constituents of Plumeria acutifolia. Planta Med 2008;74:1749-50.
10. Ye G; Li J; Xia G; Peng H; Sun Z; Huang C; et al. A new diosid alkaloid from the flowers of Plumeria rubra L. cv. Acutifolia. Helv Chim Acta 2009;92:2790-4.
11. Ye G, Yang YL, Xia GX, Fan MS, Huang CG. Complete NMR spectral assignments of two new diosid diastereoisomers from the flowers of Plumeria rubra L. cv. Acutifolia. Magn Reson Chem 2008;46:1195-7.
12. Furniko A, Rong-Fu C, Yamauchi T. Minor iridoids from the roots of Plumeria acutifolia. Chem Pharm Bull 1998;36:2784-9.
Lonicera angustifolia. Fitoterapia 2000; 71:420-4.
38. de Oliveira PR, Testa G, Medina RP, de oliveira CM, Kato L, da Silva CC,  et al. Cytotoxic activity of Guettarda pohliana Müll. Arg. (Rubiaceae). Nat Prod Res 2013; 27:1677-81.
39. Polat E, Alankuş-Caliskan Ö, Karayıldırım T, Bedir E. Iridoids from Scabiosa atropurpurea L., subsp. maritima Arc. (L.). Biochem Syst Ecol 2010; 38:253-6.
40. Wu M, Wu P, Liu M, Xie H, Jiang Y, Wei X. Iridoids from Gentiana jourei.ii. Phytochemistry 2009; 70:746-50.
41. Changwichit K, Khorana N, Suwanboonruuk K, Waranuch N, Limpeanchob N, Wisuitiprot W, et al. Bisindole alkaloids and secoiridoids from Alstonia macrophylla Wall. ex G. Don. Fitoterapia 2011; 82:798-804.
42. Koeawpradub N, Takayama H, Aimi N, Sakai SI. Indole alkaloids from Alstonia glaucescens. Phytochemistry 1994; 37:1745-9.
43. Achenbach H, Benirschke M, Torrenegra R. Alkaloids and other compounds from seeds of Tabernaemontana cymosa. Phytochemistry 1997; 45:325-35.
44. van Beek TA, Lanikhorst PP, Verpoorte R, Svendsen AB. Isolation of the secoiridoid-glucoside sweroside from Tabernaemontana psorocarpa. Planta Med 1982; 44:30-1.
45. Krohn K, Gehle D, Dey SK, Nahar N, Moshiuzzaman M, Sultana N, et al. Prismatomerin, a new iridoid from Prismatomeris tetrandra. Structure elucidation, determination of absolute configuration, and cytotoxicity. J Nat Prod 2007; 70:1339-43.
46. Cimanga K, Hermans N, Apers S, Van Miert S, Van den Heuvel H, Claey S, et al. Complement-inhibiting iridoids from Morinda morinda. J Nat Prod 2003; 66:97-102.
47. Begum S, Naeed A, Siddiqui BS, Siddiqui S. Chemical constituents of the genus Plumeria. J Chem Soc Pak 1994; 16:280-99.
48. Akhtar N. Isolation & structural studies on the constituents of Calotropis procera, Plumeria and Amberboa ramos. Pak Res Repository 1992; 46-127.
49. Morel AF, Dias GQ, Porto C, Simonatto E, Stuker CZ, Dalcol II. Antimicrobial activity of extracts of Solidago microglossa. Fitoterapia 2006; 77:463-6.
50. Kumarasamy Y, Nahar L, Cox PJ, Jaspars M, Sarker SD. Bioactivity of secoiridoid glycosides from Centaurea erythraea. Phytomedicine 2003; 10:344-7.
51. Aderogba M, Kgatle D, Mcgaw L, Eloff J. Isolation of antioxidant constituents from Combretum apiculatum Subsp. apiculatum. South Afr J Bot 2012; 79:125-31.
52. Yokozawa T, Kang KS, Park CH, Noh JS, Yamabe N, Shibahara N, et al. Bioactive constituents of Cori fructi: The therapeutic use of morroniside, loganin, and 7-O-galloyl-D-sedoheptulose as renoprotective agents in type 2 diabetes. Drug Discov Ther 2010; 4:223-34.