Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

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

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Submitted: 22-01-2017 Revised: 02-03-2017 Published: 11-10-2017

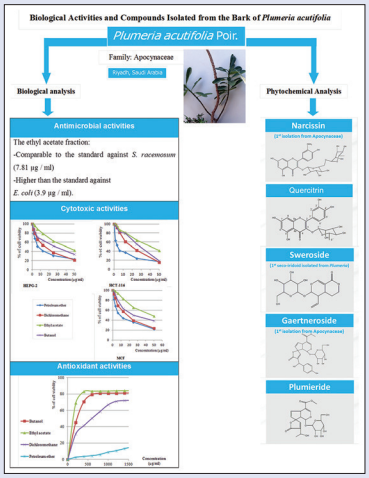
ABSTRACT

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 *Syncephalastrum racemosum* (7.81 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 IC_{50} of 197.1 µg/ml. Five compounds were identified as narcissin (1), quercitrin (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 *Syncephalastrum 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), quercitrin (2), sweroside (3), gaertneroside (4), and plumieride (5).



Abbreviations used: mp: Melting point, NMR: Nuclear magnetic resonance, s: Singlet, d: Double, t: Triplet, q: Quartet, dd: Double-double, m: Multiplet, br: Broad.

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DOI: 10.4103/pm.pm_22_17

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INTRODUCTION

Plants of the genus *Plumeria* (*Apocynaceae*)[1] are usually cultivated in gardens, for their showy and fragrant flowers.[2] Various *Plumeria* species are used to cure rheumatism, diarrhea, blennorrhea, and venereal disease.[3] They were reported to exhibit significant antibacterial, antifungal,[4] and anticancer activities.[5] The chemical constituents of *Plumeria* include alkaloids, flavonoids,[6] iridoids,[7] and triterpenes.[8]

Several extracts of *Plumeria acutifolia* Poir. were proved to have significant antipyretic, antinociceptive,[9] anti-inflammatory,[6] and antimicrobial[4] activities, in addition to potential antitumor and antioxidant activities.[10] Previous phytochemical investigations on *P. acutifolia* proved the presence of alkaloids as phoebegrandine B, laurelliptine,[11] plumerianine,[12] and plumericidine.[13] The flavonoids kaempferol, ayanin, and pillion[14] were also isolated. In addition, the presence of iridoids, including includes plumieride, plumieridin A and B,[14] 1α-plumieride, and 8-isoplumieride, was isolated.[15] Triterpenes such as ursolic acid, stigmast-7-enol, lupeol carboxylic acid, and lupeol acetate have been isolated from *P. acutifolia*.[16]

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.

Cite this article as: Alhozaimy GA, Al-Sheddi ES, Ibrahim TA. Biological activity and isolation of compounds from stem bark of *Plumeria acutifolia*. Phcog Mag 2017;13:S505-11.

MATERIALS AND METHODS

General experimental procedures

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 (Immum präparate, 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 (7 g), and *n*-butanol fractions (6.61 g).

Biological Activity

Antimicrobial activity

The antimicrobial activity was evaluated according to the National Committee of Clinical Laboratory Standards,[17] 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; 100 μl of the petroleum ether, dichloromethane, ethyl acetate, and *n*-butanol fractions were used. The minimal inhibitory concentration (MIC) against the sensitive microorganisms was determined by the agar diffusion technique,[18] 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*[19] 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.[20] 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 ^{1}H-NMR, ^{13}C-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. ^{1}H-NMR (DMSO-d_6) δ ppm: 6.1 (1H, *s*, H-6), 6.31 (1H, *d*, H-8), 3.9 (3H, *s*,-OCH_3), 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 6``), 4.3 (1H, *s*, H-1```), 3.2-3.5 (*m*, H-2```, 3```, 4```, and 5```) and 1.1 (3H, *d*, H-6```). ^{13}C-NMR (DMSO-d_6) 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 (–OCH_3), 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, 301, 285, 251, 235, 146. ^{1}H-NMR (DMSO-d_6) 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							
	Fungi				Gram-positive bacteria		Gram-negative bacteria	
	Aspergillus fumigatus	*Syncephalastrum racemosum*	*Geotrichum candidum*	*Candida albicans*	*Streptococcus pneumoniae*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*
Zone of inhibition (mm)								
Sample								
Petroleum ether fraction	NA	NA	NA	NA	NA	NA	NA	NA
Dichloromethane fraction	18.6±0.34	17.2±0.25	19.3±0.38	15.1±0.38	19.1±0.44	20.1±0.25	10.4±0.33	13.6±0.25
Ethyl acetate fraction	20.1±0.39	19.9±0.58	23.4±0.44	17.9±0.33	22.6±0.15	26.4±0.44	16.8±0.53	22.7±0.53
n-butanol fraction	18.3±0.33	16.4±0.34	19.8±0.18	15.2±0.34	20.8±0.26	23.1±0.63	12.1±0.25	13.9±0.37
Standard								
Amphotericin B	23.7±0.1	19.7±0.2	28.7±0.2	25.4±0.1	-	-	-	-
Ampicillin	-	-	-	-	23.8±0.2	32.4±0.3	-	-
Gentamicin	-	-	-	-	-	-	17.3±0.1	19.9±0.3
MIC (µg/mL)								
Sample								
Dichloromethane fraction	31.25	62.5	7.81	125	7.81	3.9	125	125
Ethyl acetate fraction	7.81	7.81	1.95	62.5	1.95	0.24	62.5	3.9
n-butanol fraction	31.25	125	7.81	125	7.81	3.9	125	125
Standard								
Amphotericin B	0.97	7.81	0.03	0.48	-	-	-	-
Ampicillin	-	-	-	-	0.97	0.007	-	-
Gentamicin	-	-	-	-	-	-	31.25	7.81

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	80.88	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

Compound 3 (4 mg) amorphous powder, m.p. 168 ± 1°C. ^{1}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). $^{13}$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`).

Compound 4 (13 mg) beige, amorphous powder, m.p. 150 ± 1°C. ^{1}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*). ^{13}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),

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. ^{1}H-NMR (CD_3OD) 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-7), 2.96 (1H, *dd*, *J* = 3, 5 Hz, H-9), 7.38 (1H, *s*, H-10), 4.57 (1H, *m*, H-13), 1.43 (3H, *d*, *J* = 6.5 Hz, H-14), 3.77 (3H, *s*, H-16), 4.70 (1H, *d*, *J* = 8 Hz, H-1`), 3.21 (1H, *t*, *J* = 8.5 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). $^{13}$C-NMR ($CD_3OD$) 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.

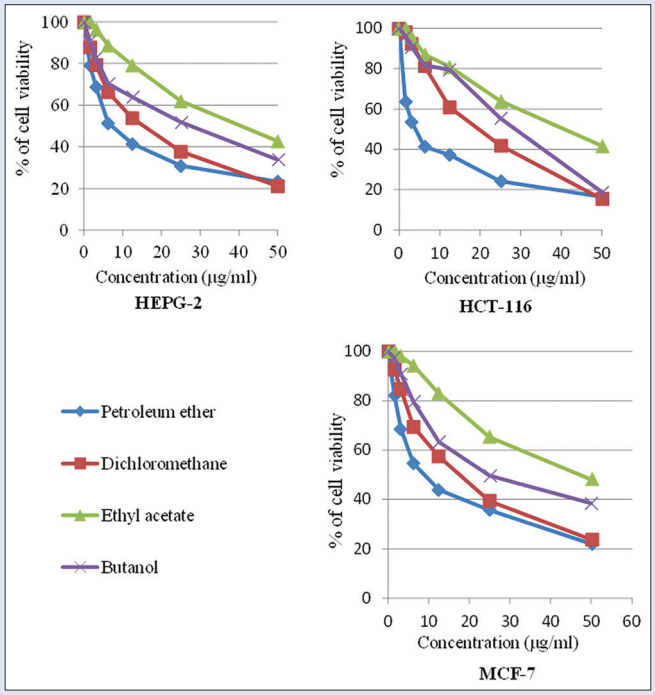

Figure 1: Cytotoxic activity of stem bark fractions of P. acutifolia against HEPG-2, HCT-116, and MCF-7 cell lines

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,

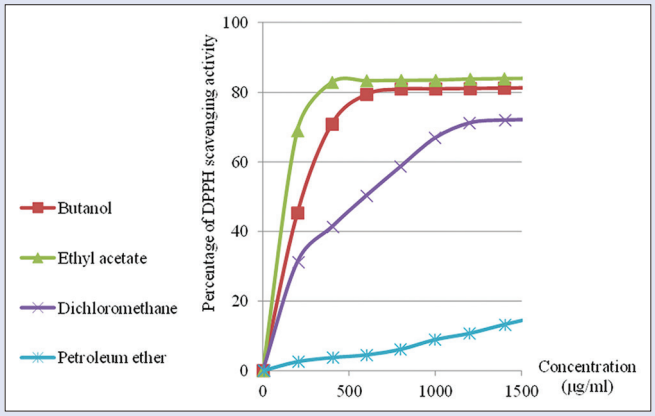

Figure 2: Percentage of α, α-diphenyl-β-picrylhydrazyl scavenging activity of stem bark fractions of *Plumeria acutifolia*

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

Concentration (µg/mL)	Sample (percentage of DPPH scavenging)			
	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)	0	5	10	15	20	25	30	35	40
Percentage of DPPH Scavenging	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

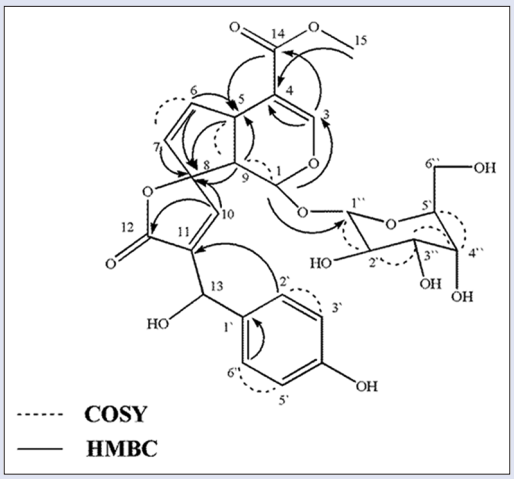

Figure 3: COSY and HMBC correlations of compound 4

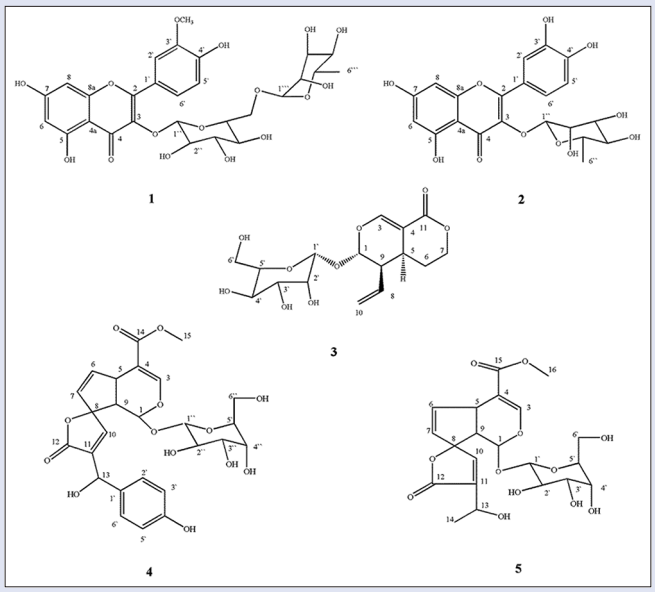

Figure 4: The structures of compounds 1-5 isolated from stem bark fractions of *Plumeria acutifolia*

and 23.74 µg/ml) and *n*-butanol fraction (33.91, 18.77, and 38.46 µg/ml) showed moderate activity against the tested cell lines, while the ethyl acetate fraction showed the lowest cytotoxic activity against HEPG-2, HCT-116, and MCF-7, with IC_{50} values of 42.75, 41.72, and 48.18 µg/ml, respectively. These kinds of cytotoxic effects have been also observed by petroleum and chloroform extracts of *Cinnamomum zeylanicum* bark on KB and L1210 cells.[22]

The free radical scavenging activity of the tested samples was concentration dependent. The ethyl acetate fraction showed the highest antioxidant activity, with an IC50 of 197.1 µg/ml, followed by the *n*-butanol fraction (308.5 µg/ml) and then the dichloromethane fraction (641.5 µg/ml), whereas the petroleum ether fraction was inactive.

Isolated compounds

The ^{13}C-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 anomeric carbons were found at δ 104.0 (C-1``) and 103.0 (C-1```). Accordingly, the ^{1}H-NMR spectrum showed two protons resonating at δ 5.35 (*d*, *J* = 7 Hz, H-1``) and δ 4.3 (*d*, *J* = 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 the protons H-2`, H-6`, and H-5` (at δ 7.93, *d*, *J* = 1.8 Hz; 7.59, *dd*, *J* = 8.3, 1.9 Hz; and 6.82, *d*, *J* = 8.3 Hz, respectively) of the flavonoid nucleus. Based on the spectral data and comparison with references,[23,24] compound 1 could be identified as narcissin, which has been previously isolated from many genera including *Coyza* (*Compositae*),[25] *Strumpfia* (*Rubiaceae*),[26] *Ferula* (*Apiaceae*),[27] *Daniellia* (*Fabaceae*),[28] and *Atractylis* (*Asteraceae*).[29]

On the basis of mass and ^{1}H-NMR spectra, and by further comparison to the published spectral data,[30-32] compound 2 could be identified as quercitrin. The compound was previously isolated from the genus *Plumeria* including *P. acutifolia*[33] and *Pityriasis rubra*.[34]

The ^{13}C-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 ^{1}H-NMR spectrum showed a downfield doublet signal at δ 7.58 with *J* = 7.4 Hz (H-3), it indicated the presence of an oxyolefinic hydrogen of the secoiridoids.[33,34] The signal at δ 4.35 (2H, *m*, H-7) was assigned to the proton of C-7 by comparative analysis with reported data.[35,36] 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,[37,38] a secoiridoid derivative, previously isolated from many plants as *Scabiosa atropurpurea* (*Dipsacaceae*),[39] *Lonicera angustifolia* (*Caprifoliaceae*),[37] and *Gentiana loureirii* (*Gentianceae*).[40] It also presents in *Apocynaceae* family as it was isolated from *Alstonia*[41,42] and *Tabernaemontana*[43,44] species.

Compound 4 exhibited six signals in ^{13}C-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.[43,44] Moreover, ^{1}H-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 ^{1}H-NMR spectrum also exhibited one part of an AA`BB` system, corresponding to a *para* disubstituted benzene ring, with δ of 7.45 and δ 6.8, assigned for H-2`, 6` and H-3`, 5`, respectively. The ^{1}H-^{1}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 of 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.[43,44] Gaertneroside has been isolated from *Rubiaceae* family.[45,46]

NMR data of compound 5 in CD_3OD showed characteristic signals assigned to plumieride, which was supported by comparison with the reported spectral data.[15,28,47] Plumieride has been isolated previously from many species belonging to *Plumeria*,[5,7,48] including *P. acutifolia*.[15]

The antimicrobial activity of the dichloromethane fraction against *B. subtilis* and *E. coli* might be related to the presence of quercetrin[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 quercetrin and/or narcissin, both flavonoids where proved to posses antioxidant activities.[24,51] On the other hand, the ethyl acetate fraction showed positive antioxidant activity; this may refer to the presence of loganin as it was proved to possess antioxidant activity.[52]

CONCLUSION

Stem bark of *P. acutifolia* was proved to have significant antimicrobial, cytotoxic, and antioxidant 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 and plumieride. 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*.

Acknowledgement

This research project was supported by a grant from the "Research Center of the Female Scientific and Medical Colleges," Deanship of Scientific Research, King Saud University.

Financial support and sponsorship

This research project was supported by a grant from the "Research Center of the Female Scientific and Medical Colleges," Deanship of Scientific Research, King Saud University.

Conflicts of interest

There are no conflicts of interest

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