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Biologically Active Saponins From *Bougainvillea spectabilis* Growing in Egypt

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Three triterpenoidal saponins 3,23-dihydroxy-12-oleanen-28-oic acid, 3-O-[α-L-rhamnopyrosyl-(1→2)-α-L-arabinopyranoside]-28-O-[α-L-rhamnopyrosyl(1→4)-β-d-glucopyranosyl-(1→6)-β-D-glucopyranoside; 3-O-β-D-glucopyranosyl-1(1→2)-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside quinovic acid and 3,23-dihydroxy-12-oleanen-28-oic acid-3-O-[α-D-glucopyranosyl-(1→4)-α-D-glucopyranoside]-28-O-[α-D-glucopyranoside] were isolated for the first time from the methanolic extract of the leaves of *Bougainvillea spectabilis* (Family: *Nyctaginaceae*). The structure of the isolated compounds had been established on the basis of several spectral techniques. Biological screening revealed that they have a significant strong antibacterial activity against *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* and no cytotoxic activity either on Ehrlich ascities carcinoma cell line or on different types of human cancer cell lines.

Key Words: *B. spectabilis*, Nyctaginaceae, Saponins.

INTRODUCTION

Bougainvillea, a member of *Nyctaginaceae* family, is a native of South America, growing extensively in the warmer climates of the United States[1]. *Bougainvillea* is an evergreen vine spreading horizontally or hanging downward, can be grown as a tree or groomed as a ground cover, its trunk tends to be gnarled[2]. The colourful papery blooms include pink, magenta, purple, red yellow and orange are not flowers, they are bracts appear on the ends of a new growth. The true flower is white, trumpet shaped and almost unnoticeable within the bracts[3]. *Bougainvilleas* are available in a variety of species, each having its unique characters[1]. *B. spectabilis* is a traditional antidiabetic plant, for its content of the active principle D. pinitol which claimed to exert insulin like effect[4]. The stem have been used in folk medicine for hepatitis[5] while the methanolic extract of white flowers showing antibacterial activities[3]. Phytochemical screening of the plant showed the presence of saponins, flavonoids, carbohydrates/glycosides and sterols/triterpenes. The flavonoidal content of the aerial part was previously investigated[6-8] but no report mentioned the saponoidal content of any part of the plant or in other bougainvillea species. So the present work aim to isolate and determine the structure of the saponins present in the leaves of *B. spectabilis* as well as studying some of its biological activities.

EXPERIMENTAL

The fresh leaves of *B. spectabilis* were collected (March-2004), from El-Orman garden, Giza, Egypt. The plant was kindly authenticated by Eng. Teresa (agriculture engineer at El-Orman botanical garden). The leaves were shade dried and powdered by electrical mill.

Chromatographic materials: Silica gel 60 (Merck, Germany) for column chromatography, silica gel 60H-type without gypsum (Merck, Germany) for vaccum liquid chromatography (VLC), silica gel G/UV254 precoated aluminiuum sheets (Machery-Nagel, Germany) for thin layer chromatography (TLC), Whatmann paper no. 1 for paper chromatography. Glucose, arabinose and rhamnose sugars (Sigma, USA).

Spray reagent[9]: Ethanolic sulphuric acid (10 %) and aniline phthalate reagent.

^{1}H NMR and ^{13}C NMR were recorded on a Varian NMR spectrometer 500 MHz, The NMR spectra were recorded in DMSO. TMS was used as an internal standard and chemical shift values were recorded in δ ppm.

Mass spectra were recorded on a Varian Mat 311A spectrometer with direct techniques, at 70 eV.

Extraction and isolation: The dried powdered leaves (1 kg) were exhaustively extracted with 70 % methanol at room temperature after defatting with petroleum ether. The methanolic extract was concentrated under reduced pressure. The residue (50 g) was fractionated on 250 g silica gel column and elution started with chloroform and polarity was increased by adding gradient mixtures of chloroform-methanol and finally pure methanol (115 fractions, 100 mL each were obtained). Fractions with the same TLC profile (solvent system; 1-butanol:acetic acid 98 %: water; 66:17:17) were combined together. Fractions showed positive reaction to saponins were further purified by different chromatographic techniques and afforded three compounds, which were identified by ^{1}H NMR, ^{13}C NMR and mass spectral data. The results are presented in Tables 1-3.

Compound 1: R_f 0.55 (*n*-butanol/acetone/water;7/11/2) and 0.46 (*n*-butanol/acetic acid/water; 66/17/17), ^{1}H NMR: δ 0.74-1.21 ppm (6-methyl groups), 5.30 (br, m, H-12), 4.60 (d, H-1′), 5.20 (d, H-1″), 5.37 (d, H-1‴), 4.45 (d, H-1*), 4.9 (H-1**). CI-MS, m/z 1221.6 $[M+H]^+$, 1097.5 $[M\text{-Rha} + H_2O]^+$, 965.5 $[M\text{-Rha-Ara} + 2H_2O]^+$, 773.4 $[M\text{-Rha-2Glu} + 3H_2O]$. ^{13}C NMR data are shown in Tables 1-3.

Compound 2: R_f 0.66 (*n*-butanol/acetic acid/H_2O; 6/17/17), ^{1}H NMR; δ 0.76-1.09 (6-Me gps), 4.72 (1H, d, H-1 of glu), 4.98 (1H, d, H-1 of Rha), 5.14 (1H, s, H-12), CI-MS; m/z 958 $[M+H]^+$,795 $[(M+H)^+\text{-Glu}]$,751 $[(M+H)^+\text{-Glu-COO-}]$, 633 $[(M+H)^+ \text{-2Glu}]$ and 487 $[(M+H)^+\text{-2Glu -Rha}]$. ^{13}C NMR data are shown in Tables 1-3.

Compound 3: R_f 0.69 (*n*-butanol/:acetic acid/H_2O ; 66/17/17), ^{1}H NMR: δ 0.84-1.14 (6-Me gps), 4.75 (1H, d, H-1 of glu), 5.38 (1H, H-12). CI-MS; m/z 974 $[M+H]^+$, 811 $[(M+H)^+ \text{-glu})]$, 649 $[(M+H)^+ \text{-2 glu})]$. ^{13}C NMR data are shown in Tables 1-3.

Acid hydrolysis of compounds: Few milligrams of each of the pure isolated compound were refluxed with 2 N HCl (2 h, 100 °C), the hydrolysate mixture after

TABLE-1
^{13}C NMR SPECTRAL DATA OF THE AGLYCONE PARTS OF SAPONINS 1, 2 AND 3

Carbon No.	Saponin 1	Saponin 2	Saponin 3
1	39.70	39.48	39.00
2	26.40	26.21	26.20
3	82.30	88.83	83.20
4	44.00	39.66	42.80
5	48.60	56.04	48.60
6	18.80	18.60	18.10
7	33.30	37.05	33.10
8	40.70	40.06	40.00
9	49.40	47.89	47.90
10	37.70	36.96	37.10
11	24.60	23.02	23.80
12	123.80	129.26	122.90
13	144.90	132.16	144.90
14	43.00	56.90	42.10
15	28.90	26.21	28.40
16	24.10	24.20	23.90
17	47.20	48.20	46.50
18	42.50	55.82	42.40
19	47.20	39.52	44.10
20	31.60	37.80	31.000
21	34.90	30.53	34.30
22	33.30	36.90	33.40
23	64.80	28.05	63.90
24	13.80	17.13	15.00
25	16.60	15.81	17.30
26	18.00	18.14	17.60
27	26.50	178.20	26.30
28	178.20	180.30	180.10
29	33.50	17.75	33.30
30	24.10	19.98	23.80

neutralization with diluted solution of $NaHCO_3$ was extracted with chloroform in a separating funnel to separate the aglycone in the organic phase, while the sugar being in the aqueous phase[10]. The aqueous layer, then filtered, concentrated and compared with standard sugars on paper chromatography using solvent system; *n*-butanol/acetic acid/water; 4:1:5. Spots were detected by spraying with a solution of aniline phthalate.

Biological activities

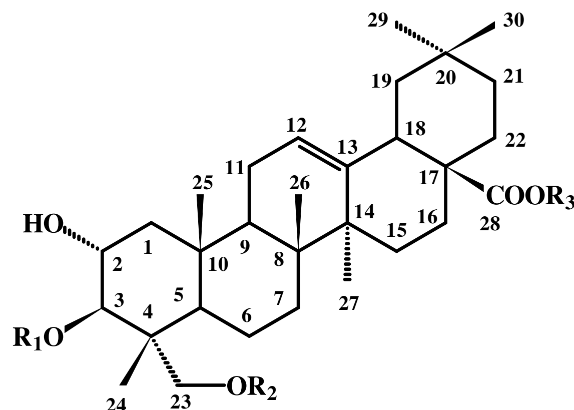
Antimicrobial activity: The filter paper disc diffusion method[11-13], was used for the *in vitro* evaluations of the antimicrobial activity. The standards used for antibacterial activity were sodium penicillin-G 2.5 unit/mL and streptomycin sulphate 10 μg/mL in sterile distilled water; for antifungal activity were salicylic acid and resorcinol 1 % each in sterile distilled water. (Test samples were 0.01 mg/mL).

TABLE-2
^{13}C NMR SPECTRAL DATA OF THE SUGAR MOIETIES AT C-3 OF SAPONINS 1, 2 AND 3

Carbon No.	Saponin 1	Saponin 2	Saponin 3
1'	104.30	105.32	104.80
2'	76.60	73.82	74.80
3'	73.70	71.20	76.80
4'	70.20	85.90	81.30
5'	64.60	68.20	69.40
6'		17.13	62.10
1"	101.90	103.91	105.20
2"	72.20	82.49	75.00
3"	72.40	77.28	78.20
4"	73.80	70.81	71.60
5"	69.10	76.17	69.70
6"	17.90	61.93	62.50
1"'		104.58	
2"'		74.46	
3"'		77.80	
4"'		71.16	
5"'		77.20	
6"'		61.67	

TABLE-3
^{13}C NMR SPECTRAL DATA OF THE SUGAR MOIETIES AT C-28 OF COMPOUNDS 1 AND 3

Carbon No.	Saponin 1	Saponin 3
1*	95.8	95.7
2*	73.9	74.1
3*	78.2	79.1
4*	71.0	71.2
5*	68.1	78.9
6*	69.4	62.0
1**	104.3	
2**	75.3	
3**	76.7	
4**	79.6	
5**	76.8	
6**	61.9	
1***	102.9	
2***	72.4	
3***	72.1	
4***	73.8	
5***	70.7	
6***	17.9	



Compd. **1**: R_1 = Rha-Arab	Compd. **2**: R_1 = Rha-Glu-Glu	Compd. **3**: R_1 = Glu
R_2 =CH_2OH	R_2 = CH_3	R_2 = CH_2OH
R_3 = Rha-Glu-Glu	R_3 = H	R_3 = Glu-Glu

***In vitro* testing for cytotoxic effect:** (Done on Cancer Biology Department, Pharmacology unit, National Cancer Institute, Cairo University).

Ehrlich ascities carcinoma[14]: A set of sterile test tubes were used, where 2.5 $\times 10^5$ tumour cells per mL were suspended in phosphate buffer saline. Then 25, 50, 100 μg/mL from the isolated compounds were added to the suspension, kept at 37 °C for 2 h. Trypan blue dye exclusion test was then carried out to calculate the percentage of non-viable cells.

Human tumour cell line: The isolated compounds were tested for any cytotoxic activity against U251 (brain tumour cell line), MCF7 (breast carcinoma cell line), HELA (Cervix carcinoma cell line), Hepg2 (Liver carcinoma cell line), H460 (Lung carcinoma cell line). Potential toxicity of the compounds was tested using the method of Skehan and Storeng[15], cells were plated in 96 multi well plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate. Different concentraions of the compounds under test (0, 1, 2.5 and 10 μg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C in atmosphere of 5 % CO_2. After 48 h cells were fixed, washed and stained with sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with *Tris* EDTA buffer. Colour intensity was measured in an ELIZA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumour cell line after the specified compound.

RESULTS AND DISCUSSION

Compound 1: The ^1H NMR spectrum exhibited signals of 6th tertiary CH_3 singlets at 0.74, 0.85, 0.95, 0.99, 1.02, 1.21 ppm, respectively. The olefenic hydrogen signal showed a broad multiplet at 5.30 ppm which suggest a pentacyclic triterpene

with oleanolic acid skeleton[16]. Five anomeric proton signals were observed in the ^{1}N NMR spectrum at 4.45, 4.60, 4.90, 5.20 and 5.37 ppm. In ^{13}C NMR, these signals were correlated with signals at 104.3, 104.1, 102.9, 101.9, 95.5 ppm, respectively, indicating the presence of five sugar moieties. The key two bond correlations between δ 82.3 (C-3), δ 4.60 (1′-H) and δ 178.2 (C-28), δ 5.37 (1‴-H), confirmed the both glycosylation sites of bisdesmoside[17]. Proton coupling patterns, as well as, the proton and carbon chemical shifts proposed the structure of compound **1** as; 3,23-dihydroxy-12-oleanen-28-oic acid, 3-O-[α-L-rhamnopyrosyl-(1→2)-α-L-arabinopyranoside]-28-O-[α-L-rhamnopyrosyl(1→4)-β-d-glucopyranosyl-(1→6)-β-D-glucopyranoside.

Compound 2: Comparison of ^{1}H and ^{13}C NMR spectrum of this compound with those in literature, it was appeared that its aglycone is quinovic acid[18]. In ^{13}C NMR spectrum (Tables 1-3), c-12 and c-13 resonatly at δ 129.28 and 132.16, respectively and these values usually observed in urs-12-ene-27,28-dioic acid and with unsubstituted carboxylic acid at c-14[18,19]. Furthermore, the downfield signals at 178.20, 180.30 in ^{13}C NMR were assignated to the c-27 and c-28 free carboxyl group[18,20]. The downfield shift of the signals for c-3 at δ 88.83 showed that a sugar chain was located at this position[18,21]. Three anomeruc protons appeared in ^{1}H NMR spectrum at 4.72, 4.80 and 4.98 which are compatible with the ^{13}C NMR signals for anomeric ether linked carbons at 105.32, 103.90 and 104.58[19,21]. Signals at 5.14 is for olefenic proton[20,21]. Downfield shift of c-4 (the rhamnosyl unit appeared at 85.90) and of c-2 of the inner glucose unit at 82.50 indicate that those carbons are the position of the sugar unit linkage[18,21]. CI-MS spectrum of saponin (2) showed peak at m/z 958 $[M+H]^{+}$, peak observed at m/z 795 $[(M+H)^{+}$-glu] and 633 $[(M+H)^{+}$-2glu], peak at m/z 487 suggested the removal of another one rhamnosyl unit at c-3 $[(M+H)^{+}$-2glu-rha] and this proved the presence of ether glycosidic linkage at c-3 of the aglycone[21]. On the basis of these data, the proposed structure of saponin (2) is; 3-O-β-D-glucopyranosyl-1(1→2)-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside quinovic acid.

Compound 3: Three anomeric proton signals were observed in the ^{1}H NMR spectrum at 4.6, 5.20, 5.37 ppm, in ^{13}C NMR these signals were correlated in ^{13}C NMR with signals at 105.2, 104.8 and 95.7 ppm, respectively, indicating the presence of 3-sugar moieties. The signals of the aglycone moiety in ^{13}C NMR spectrum were in good agreement with the occurrence of bidesmosidic saponin[17]. The downfield shift of c-3 of the aglycone part at 83.2 showed that this carbon is the position of attachment of the sugar chain[22,23]. CI-MS spectrum showed a molecular ion peak at m/z 974 $[M+H]^{+}$, fragmented to further characteristic peaks for sugar elimination at m/z 811 $[(M+H)^{+}$-glu] and 649 $[(M+H)^{+}$-2glu]. On the basis of the above data, the structure if saponin (3) was elucidated as 3,23-dihydroxy-12-oleanen-28-oic acid-3-O-[α-D-glucopyranosyl-(1→4)-α-D-glucopyranoside]-28-O-[α-D-glucopyranoside].

Biological screening showed that the isolated compounds had no cytotoxic effect either on Ehrlich ascities carcinoma cell line or on human carcinoma cell line and no antifungal activity but exhibited a strong antibacterial activity. Table-4 shows result of antimicrobial activity of the three isolated compounds.

TABLE-4
ANTIMICROBIAL ACTIVITY OF THE ISOLATED COMPOUNDS

Microorganism	Zone of inhibition (mm)						
	SA	R	P	S	1	2	3
Escherichia coli	–	–	–	22	16	10	8
Salmonella typhi	–	–	–	17	10	8	8
Pseudomonas aeruginosa	–	–	24	–	20	10	12
Bacillus subtilis	–	–	–	22	15	9	10
Staphylococcus aureus	–	–	26	–	11	10	12
Sarcina lutea	20	20	–	–	–	–	–
Candida albicans	22	20	–	–	–	–	–

SA = Salicylic acid; R = Resorcinol; P = Penicillin; S = Streptomycin
1 = Saponin 1; 2 = Saponin 2; 3 = Saponin 3

REFERENCES

1. Y.O. Schleing, R.L. Jacobson and G.C. Muller, *Am. J. Trop. Md. Hyg. Oct.*, **65**, 300 (2001).
2. S.W. Balasadasivam and J.M. Walker, *Phytochemistry*, **479**, 1561 (1998).
3. M.S. Ali, S.A. Ibrahim, F.A. Ahmed and M.K. Pervez, *Nat. Prod. Res.*, **19**, 1 (2005):
4. S.H. Bates, R.B. Jones and C.J. Bailey, *Br. J. Pharmacol.*, **130**, 1944 (2000).
5. W.S. Chang, Y.J. Lee, F.J. Lu and H.C. Chiang, *Anticancer Res.*, **13**, 2165 (1993).
6. T.M. Masamichi and J.S. John, *Am. J. Bot.*, **78**, 1063 (1991).
7. K.A. Raoul and D.S. George, *Xenobiotica*, **25**, 575 (1995).
8. R.S. Heuer, J.W. Metzger, V.N. Wary and D.M. Strack, *Phytochemistry*, **37**, 761 (1994).
9. E.P. Clause, Pharmacognosy, London, edn. 4. p. 111 (1961).
10. T.J. Mabry, K.R. Markham and M.B. Thomas, The Systematic Identification of Flavonoids (1970).
11. M.A. Chakravarty, P.M. Amin, H.D. Singh, J.N. Baruah and M.S. Iyengar, *Biotech. Bioeng.*, **14**, 61 (1972).
12. Antibiotics, The Pharmaceutical Society of Great Britain, London, p. 84 (1952).
13. J.C. Maruzzella and P.A. Henry, *J. Am. Pharma. Assoc.*, *Sci. Ed.*, **47**, 471 (1958).
14. W.F. Mclimans, E.V. Davis, F.L. Glover and G.W. Rake, *J. Immunol.*, **79**, 428 (1957).
15. P.P. Skehan and R.H. Storeng, *J. Natl Cancer Inst.*, **82**, 1107 (1991).
16. A.A. Babadjamian, R. Elias, R. Faure, E. Vidal-Ollivier and G. Balansard, *Spec. Lett.*, **21**, 565 (1988).
17. M.L. Clavaud, *Phytochemistry*, **57**, 469 (2001).
18. H.A. Hassanean, M.A. El-Hamouly, S.A. El-Moghazy and D.W. Bishay, *Phytochemistry*, **33**, 667 (1993).
19. G.J. Fan and Z.S. He, *Phytochemistry*, **44**, 1139 (1997).
20. M.A. Lamidi, E.G. Ollivier and R.K. Faure, *Phytochemistry*, **38**, 209 (1995).
21. K.H. Pollmann, S.H. Gagel and H.A. El-Gamal, *Phytochemistry*, **44**, 485 (1997).
22. H.H. Hassanean, E.K. Desouky and M.A. El-Hamouly, *Phytochemistry*, **33**, 663 (1993).
23. A.U. Rahman, D.G. Shahwar, M.I. Choud and B.H. Sener, *J. Nat. Prod.*, **63**, 251 (2000).
24. X.C. Li and C.R. Yang, *Phytochemistry*, 39, 1175 (1995).

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