Phytochemical and biological evaluation of *Buddleja polystachya* growing in Saudi Arabia

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Abstract: Several *Buddleja* species were the target of phytochemical and biological studies; however, nothing was reported concerning the chemistry of *Buddleja polystachya* Fresen. growing in Saudi Arabia. Sixteen constituents were isolated from the aerial parts of *B. polystachya* using various chromatographic techniques and were identified by the help of different spectral techniques including 1D, 2D NMR and mass spectrometry. Moreover, the different fractions were evaluated for their anti-inflammatory and hypoglycemic activities. The isobenzofuranone derivative (4-hydroxy-7-methylisobenzofuranone) (**4**), has been isolated for the first time from this natural source, *B. polystachya*, along with fifteen known compounds namely; phenolic fatty acid ester, 1'(4-hydroxyphenyl) ethanol ester of docosanoic (**1**), uvaol (**2**), sakuranetin (**3**), kumatakenin (**5**), cirsimaritin (**6**), 5- hydroxy-3,7,4'-trimethoxyflavone (**7**), oleanolic acid (**8**), herbacetin 3,7,8-trimethyl ether (**9**), ursolic acid (**10**), verbascoside (**11**), linarin (**12**), luteolin 7-*O*-β-D-glucoside (**13**), luteolin 7-(6"-caffeoyl)-*O*-β-D-glucopyranoside (**14**), luteolin (**15**), and 6-*O*-α-L-(4"-*O*-*trans*-cinnamoyl) rhamnopyranosylcatalpol (**16**). Regarding the biological activities investigated, the ethyl acetate fraction showed the most significant anti-inflammatory activity, followed by the *n*-butanol and the aqueous fractions. As for the petroleum ether and dichloromethane fractions, their anti-inflammatory effects were moderate. The highest hypoglycemic activity was possessed by the ethyl acetate fraction, followed by the dichloromethane fraction and the *n*-butanol fraction showed the weakest activity.

Keywords: Anti-inflammatory, *Buddleja polystachya*, phytoconstituents, isobenzofuranone.

INTRODUCTION

Family Buddlejaceae consists of eight genera, growing mostly in tropical and few grow in warm temperate regions. Of the eight genera, only two, *Nuxia* and *Buddleja* are found in Saudi Arabia (Chaudhary, 2001). *Buddleja* includes100 species, which are mainly found in Southern Asia, South and East Africa and also in America (Migahid, 1989; John, 1999). *B. polystachya* is the only species found in Saudi Arabia (Chaudhary, 2001).

B. polystachya grows in the Southern region (Asir) and is known as "Afar" in Arabic (Migahid, 1989; Chaudhary, 2001). People in Ethiopia and other East African countries use *B. polystachya* traditionally for treatment of many skin disorders (Houghton, 1984). There are previous reports showing antimicrobial and cytotoxic activities of the different fractions of the plant (Fawzy *et al*., 2013; Alemu and Andualem, 2014). However, there is no report on the phytochemistry of *B. polystachya*' so it was of interest to explore its constituents and to investigate some other pharmacological activities of the plant.

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MATERIALS AND METHODS

Instrumentation and chemicals

Solvents were evaporated at 40°C using a Buchi® rotary evaporator, Model 011; UV absorption spectra were obtained using a Shimadzu UV-160/PC UV-Vis spectrophotometer; the ultraviolet lamp employed in visualizing the TLC plates was a Mineralight® device, multiband UV-254/366 nm, from UVP Inc., USA; the melting points were determined using a Mettler FP 80 Central Processor with a Mettler FP 81 MBC Cell Apparatus; infra-red spectra were recorded in potassium bromide pellets unless otherwise specified using Shimadzu, FTIR spectrophotometer; ^{1}H and ^{13}C NMR spectra including DEPT experiments were recorded in CD_3OD, $CDCl_3$, pyridine-d_5, DMSO-d_6 using a Bruker AM500 instrument (Bruker Biospin GmbH, Rheinstetten, Germany) operating at 500 MHz in case of protons and 125 MHz for carbons. Chemical shift values were given in δ (ppm) units using (TMS) as an internal standard while the coupling constants (J) were given in Hertz (Hz). Standard pulse sequences were applied for generating the COSY, HSQC and HMBC spectra. The electron

ionization-mass spectra (EI-MS) were obtained on a solid probe using Shimadzu QP-2010 plus (Cairo University, Micro Analytical Center). Also, electron spray ionization-mass spectrometry (ESI-MS) was measured on Agilent Triple Quadrupole 6410 QQQ LC-MS mass spectrometer. Silica gel 60 (Fluka, 70-230 mesh, Germany) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden) and Diaion HP-20 (Nippon, Rensui Co., Japan) were used for column chromatography purposes. TLC was carried out on pre-coated silica gel 60 F254 plates, (E. Merck, Darmstadt, Germany), silica gel RP-18 F254 (E. Merck, Darmstadt, Germany). The pure compounds were visualized by spraying with freshly prepared ceric sulphate. Purification of compounds was done using preparative C-18 HPLC (10µm PS, 30cm Lx2.15cm i.d.) (ODS-80 TM, TSK, Japan) fitted with a guard column (10 µm PS, 7.5cm Lx2.15cm i.d.) (ODS-80 TM, TSK, Japan).

Animals

Male Wistar rats around 200 g weight each, were used for the anti-inflammatory study, while male Swiss Albino mice, of 25 g body weight, were employed for screening the hypoglycemic activity. Animals were obtained from the Experimental Care Center, College of Pharmacy, King Saud University, Kingdom of Saudi Arabia. Animal utilization protocols were followed according to the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of King Saud University, College of Pharmacy.

Plant material

The aerial parts of *B. polystachya* have been collected from Asafra village in Saudi Arabia in March 2009. The plant was identified by Dr. Mohammad Youssef, Department of Pharmacognosy College of Pharmacy, King Saud University. A voucher specimen (#15270) has been deposited at the Department of Pharmacognosy, College of Pharmacy, King Saud University.

Extraction and compounds isolation

Two kilograms of powdered aerial parts of *B. polystachya* were extracted using 80% ethanol by cold maceration till exhaustion. The alcoholic extract was then concentrated to dryness under reduced pressure at 40°C using a rotary evaporator to give 349.5g of residue. The dried alcoholic extract was suspended in water then successively partitioned using petroleum ether, dichloromethane, ethyl acetate and finally *n*-butanol. Each of the four fractions, was dried over anhydrous sodium sulphate and evaporated to dryness to give fraction A (petroleum ether, 43.4g), fraction B (dichloromethane, 95.4g), fraction C (ethyl acetate, 23.2g), fraction D (*n*-butanol, 43.2g) and fraction E (aqueous mother liquor, 144.3g).

Fraction B (80 g) was subjected to silica gel CC packed by wet method. Elution started with petroleum ether and polarity was gradually increased with dichloromethane followed by methanol. The collected fractions (135 fractions) were concentrated to dryness under reduced pressure and monitored by TLC. Similar fractions were added together to end up with three fractions; B'-1-B'-3. Repeated silica gel column chromatography of these fractions using different solvent mixtures (petroleum ether, dichloromethane and methanol) resulted in the isolation of compounds **1** (9.4mg), **2** (35.1mg), **3** (21.5mg), **4** (23.2mg), **5** (4.3mg), **6** (6.9mg), **7** (7.8mg), 8 (30.6mg), **9** (59.5mg) and **10** (70mg).

Fraction C (21.5g) was separated using Sephadex LH-20 column and eluted with water: methanol gradient to yield 96 fractions. Similar fractions were pooled together to get 6 collected fractions. Fractions I-II (3.23g), were combined together according to behavior on TLC and were separated over silica gel column. The mobile phase mixture used was ethyl acetate-methanol-water (95: 5: 3) to afford compound **11** (0.18g). Fractions III-VI were each evaporated to dryness, re-dissolved in methanol and upon crystallization of each fraction with acetone, they yielded compounds **12** (13.5mg), **13** (13.3mg), **14** (42.8 mg) and **15** (54.4 mg).

The 136.4 g of the aqueous extract (fraction E) was dissolved using water and little amount of methanol and then applied onto the top of Diaion HP-20 column (350 g) and eluted by water: methanol gradient. Similar fractions were pooled together to afford 33 sub fractions. Fraction 25-33 (9.54g) was subjected to separation on silica gel (200g) column with dichloromethane: methanol: H_2O (70: 30: 3) as mobile phase, followed by further chromatographic purification using preparative HPLC to yield compound **16** (7.4mg, R_t=26min) using MeOH: H_2O (70:30), flow rate of 3ml/min, in isocratic mode of development.

Study for Biological activities

Anti-inflammatory activity

Male Wistar rats weighing around 200 g each were divided into seven groups (three rats in each group). The first group received saline to serve as negative control. The following groups received total alcohol, petroleum ether, dichloromethane, ethyl acetate, n-butanol and aqueous fractions. Rat paw-edema as a model of acute inflammation was induced using carrageenan. A dose of 0.2 ml carrageenan was injected into the paw of each rat of the seven groups. The animals were administrated 500 mg/kg of suspension of the different extracts, one hour before carrageenan injection via intraperitoneal route.

The volume of the rat paw is measured before carrageen injection and then two hours after the injection using a Hydo-Plethysmograph (Model 7150, Basile, USA). The rats were then held firmly and the right paw was immersed into a pool of mercury up to the tibiotarsic articulation. The pressure increase due to the slight rise in

the mercury level is transmitted to the pressure transducer. After amplification, the transduced signal is increased as a deflection in the pen on the chart. The results are shown as % inhibition of edema (protection against inflammation) two hours after carrageen injection compared to the control group (Winter *et al*., 1962).

Screening of hypoglycemic activity

Male Swiss Albino mice of around 25g body weight, were used in these experiments. The animals were kept under controlled environmental conditions (22±2°C and 55±5% relative humidity, a 12h light/dark cycle). The animals were fasted for 18 h before treatment, while water was provided *ad libitum*. The tested fractions were suspended in 0.25% sodium carboxy methyl-cellulose. Orbital blood was obtained using fine capillary tubes to determine the basal plasma glucose level. Then the level was determined using Reflotron Instrument and Reflotron glucose Kit (Roche Diagnostics) that uses the glucose oxidase method (Barham and Trinder, 1972). After removal of blood for the determination of the basal glucose level, the animals in the different groups (four mice in each group) were injected different fractions at a doses of 500 mg/kg (i.p.). One hour later, animals were anaesthetized using ether and the blood was collected to determine the plasma glucose level.

STATISTICAL ANALYSIS

Data were expressed as means ± SEM. Significant differences between the various treatments and control values were determined using ANOVA. Unpaired student-t-test was conducted, using the Graph PadIn Stat (ISI Software) computer program. Differences were considered significant at P values less than 0.05.

RESULTS

Characterization of compounds 1-16 (fig. 1)

The structures of the compounds were elucidated by physicochemical and spectral analysis and were in agreement with those reported in the literature.

1'(4-hydroxyphenyl) ethanol ester of docosanoic (**1**): white amorphous powder {R_f=0.51 in Pet. ether: EtOAc (90: 10)}; UV (MeOH) showed absorption bands at λ_{max} 220 and 292 nm and IR (KBr) υ_{max}3450cm^{-1} (OH), 1740cm^{-1} (ester C=O), 1510cm^{-1} (aromatic), 1455cm^{-1} (-CH_2) and 1365cm^{-1} (-CH_3); the molecular ion peak was determined using negative ESI-MS at m/z 459 $(M-H)^-$; ^1H and ^{13}C NMR data were in agreement with previous literature (Houghton, 1989).

Uvaol (2): white amorphous powder {R_f=0.59 in Pet. ether: Acetone (80: 20)}; m.p. 230-232°C; IR showed intense absorption band at 1630cm^{-1} along with broad absorptions at 3460cm^{-1}; from EI-MS, molecular ion peak m/z 442 $(M)^+$corresponding to the molecular formula $C_{30}H_{50}O_2$; ^1H and ^{13}C NMR data were in agreement with other literature (Shahani *et al*, 2012).

Sakuranetin (**3**): yellow residue; m.p. of 180-181°C; UV spectrum demonstrated main absorption band at λ_{max}288 nm, showing the flavanone nature; EI-MS at m/z 286 $(M)^+$, with molecular formula of $C_{16}H_{14}O_5$; ^1H and ^{13}C NMR data were in agreement with previous literature (Greccoet *al*, 2012).

4-Hydroxy-7-methylisobenzofuranone (**4**): pale yellow needles {R_f=0.55 in Pet. ether: Acetone (80: 20)} with m.p. of 223-224°C; UV (MeOH) showed absorption bands at λ_{max} 273 and 335 nm and IR (KBr) υ_{max}3215cm^{-1}, 2931cm^{-1} and 1685cm^{-1}; negative ESI-MS at m/z 163 $(M-H)^-$, corresponding to molecular formula $C_9H_8O_3$ with six degree of unsaturation; the ^1H and ^{13}C NMR (DMSO-*d6*, 500, 125 MHZ) (table 1).

Kumatakenin (**5**)**:** yellow needles; UV spectrum showed two absorption bands at λ_{max} 257 and 347 nm; molecular formula was determined from negative ESI-MS, as $C_{17}H_{14}O_6$: spectrum exhibited a molecular ion peak at m/z 313 $(M-H)^-$; the ^1H and ^{13}C NMR data were consistent with other literature (Dungêrdorzh and Petrenko, 1972).

Cirsimaritin (6): pale yellow needles {R_f=0.38 in Pet. ether: Acetone (80:20)} with m.p. of 264-265°C; UV spectrum indicated two main absorption bands at λ_{max} 267 nm and 348 nm, indicating its flavone nature; molecular ion peak was established from negative ESI-MS, at m/z 313 $(M-H)^-$ calculated for $C_{17}H_{14}O_6$; ^1H and ^{13}C NMR data were in agreement with other literature (Zhang *et al*., 1998).

5-Hydroxy-3,7,4'-trimethoxyflavone(**7**): Yellow powder; m.p. of 144-146°C; UV spectrum showed two absorption bands at λ_{max} 277 and 332 nm, which is characteristic of flavonol skeleton; molecular formula was obtained from positive ESI-MS as $C_{18}H_{16}O_6$ with a molecular ion peak at m/z 329 $(M+H)^+$(14 mass unit more than compound 5); ^1H and ^{13}C NMR data were in agreement with other literature (Miri *et al*., 2011).

Oleanolic acid (8): white amorphous powder {R_f=0.55 in Pet. ether: Acetone (80: 20)} with m.p. of 308-309°C; it showed absorption at λ_{max} 205nm in the UV spectrum; the IR spectrum showed absorption bands at 3400cm^{-1}, 1680cm^{-1} and 1630cm^{-1}; mass spectrum showed a molecular ion peak atm/z 456, calculated for $C_{30}H_{48}O_3$. The mass spectrum showed other prominent fragment ion peaks at 248 and 203m/z (248-COOH), which is characteristic for pentacyclictriterpenes of β-amyrin series with double bond at C-12; ^1H and ^{13}C NMR data were in agreement with other literature (Seebacher *et al*., 2003).

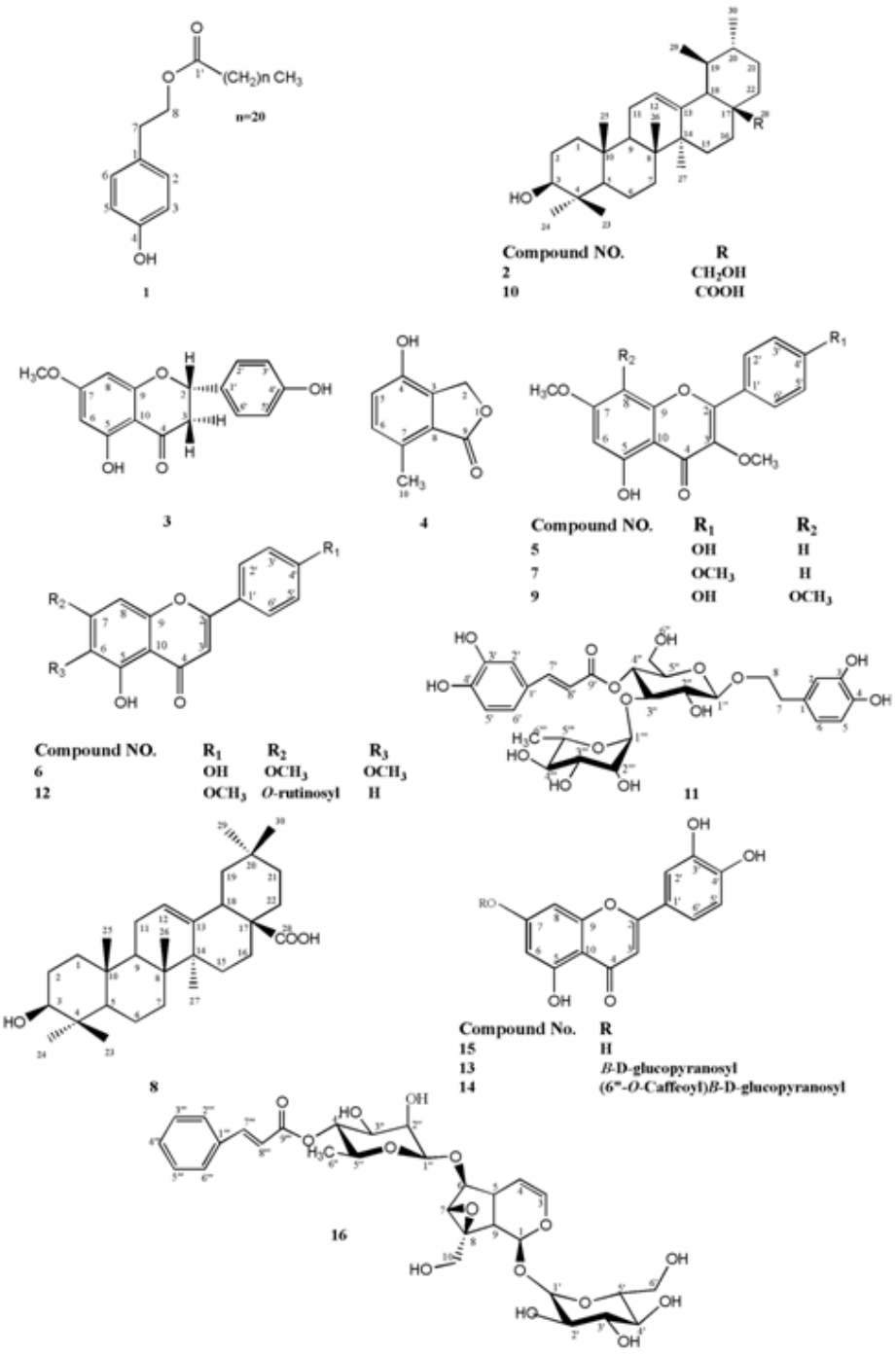

Fig. 1: Structures of compound **1-16**

Herbacetin 3,7,8-trimethyl ether (**9**): yellow amorphous powder {R_f=0.44 in Pet. ether: Acetone (75: 25)} with m.p. 266- 267°C; UV spectrum in methanol showed two major bands at λ_{max} at 271 and 343nm; molecular formula was obtained from EI-MS spectrum as $C_{18}H_{16}O_7$ as shown from the appearance a molecular ion peak at m/z 344 $(M)^+$; the ^1H and ^{13}C NMR data were consistent with other literature (Henriok and Jefferie, 1964; Ghisalberti *et al*., 1967).

Ursolic acid (**10**): white amorphous powder {R_f=0.73 in $CHCl_3$: MeOH: H2O (90: 10: 1)} and m.p. 260-262°C; IR spectrum exhibited absorption bands at 3400cm^{-1}, 1693 cm^{-1} and 1632cm^{-1}; mass spectrum disclosed a molecular ion peak at m/z 456 $(M)^+$ corresponding to the molecular formula $C_{30}H_{48}O_3$; ^1H and ^{13}C NMR data were in agreement with other literature (Mohamed and Ibrahim, 2007; Silva *et al*., 2008).

Table 1: ^{1}H NMR and ^{13}C NMR data for compound 4 in DMSO-d_6 (δ in ppm, J in Hz)

Position	δ_H	δ_C
2	5.15 (2H, *brs.*)	67.4
3	-	133.4
4	-	150.2
5	7.15 (1H, *d*, *J*=7.7 Hz)	120.3
6	7.05 (1H, *d*, *J*=7.4 Hz)	131.4
7	-	127.5
8	-	123.1
9	-	171.1
10	2.33 (3H, *s*)	15.8
4-OH	10.65 (1H, *s*)	-

Table 2: Anti-inflammatory effect of different fractions of *B. polystachya* on carrageen an induced rat paw edema.

Group	Net volume of edema in ml (M±SEM)[#]		% decrease in edema	
	1 Hour	2 Hours	1 Hour	2 Hours
Control	0.4±0.05	0.56±0.03	-	-
Ehanolic extract	0.33±0.01	0.4±0.07	17.5	26.7
Pet. Ether	0.34±0.03	0.3±0.01	15	46.4
DCM	0.32±0.01	0.3±0.03	20	46.4
EtOAc	0.1±0.01	0.05±0.01[a]	75	91
n-butanol	0.14±0.01	0.13±0.03[a]	65	76.7
Aqueous	0.22±0.01	0.22±0.01[b]	45	60.7

[#]Mean of three determinations ± standard error of mean, [a]P<0.01, [b]P<0.05 compared to control

Table 3: Effect of the fractions of *B. polystachya* on mice plasma glucose level

Treatment	Blood glucose level mg% (M±SEM)[#]		% Change
	Before treatment	After 2 hour treatment	
Control	112±5.2	108±0.9	-3.50
Ethanol	114±1.9	83.3±1.7[b]	-26.95
Pet. Ether	100±4.3	117±5.6	+17
DCM	121±3.7	75.3±2.1[a]	-37.78
EtOAc	122±4.1	64.9±0.9[a]	-46.83
n-butanol	125±3.7	103.7±1.7[b]	-17.06
Aqueous	116±5.1	91.5±1.0[b]	-24.60

#Mean of four determinations ± standard error of mean, [a]P<0.001, [b]P<0.01 compared to before treatment

Verbascoside (**11**): brownish yellow mass with m.p. of 149-151°C {R_f=0.6 in EtOAc: MeOH: H_2O (20: 3: 2)}: UV (MeOH) spectrum showed absorption bands at λ_{max} 290 and 340 nm; molecular ion peak was determined using EI-MS at m/z 624 $(M)^+$, calculated for $C_{29}H_{36}O_{15}$; ^{1}H and ^{13}C NMR data were in agreement with other literature (Bajaj, 1995).

Linarin (**12**): pale yellow powder {R_f=0.35 in $CHCl_3$: MeOH: H_2O (80: 20: 2); UV spectrum exhibited two absorption bands at λ_{max} 268 and 339 nm indicating the presence of flavones; molecular formula $C_{28}H_{32}O_{14}$ was obtained from positive ESI-MS which exhibited a molecular ion peak at m/z 593 $(M+H)^+$; ^{1}H and ^{13}C NMR data were consistent with other literature (Zhang *et al*., 2007).

Luteolin 7-*O*-β-D-glucoside (**13**)**:** yellow residue {R_f=0.4 in $CHCl_3$: MeOH: H2O (70: 30: 3)} with m.p. 238-240°C; UV spectrum exhibited two absorption bands at λ_{max} 255 and 350 nm; molecular formula $C_{21}H_{20}O_{11}$, was obtained from EI-MS which showed a molecular ion peak at m/z 448 $(M)^+$; ^{1}H and ^{13}C NMR data were in agreement with other literature (Elhawary *et al*., 2011).

7-(6"-Caffeoyl)-*O*-β-D-glucopyranoside (14): brownish yellow powder {R_f=0.5 in $CHCl_3$: MeOH: H_2O (80: 20: 2)}; molecular formula $C_{30}H_{26}O_{14}$, was obtained from negative ESI-MS which showed a molecular ion peak at m/z 609 $(M-H)^-$; ^{1}H and ^{13}C NMR data were consistent with other literature (Moharram and El-Aty, 2001).

Luteolin (**15**): yellowish white amorphous powder {R_f =0.42 in $CHCl_3$: MeOH: H_2O (90: 10: 1)}; EI-MS spectrum exhibited a molecular ion at m/z 286 $(M)^+$, calculated for $C_{15}H_{10}O_6$; ^1H and ^{13}C NMR data were in agreement with other literature (Saeidnia *et al*., 2009).

6-*O*-α-L-(4''-*O*-*trans*-cinnamoyl) rhamnopyranosylcatalpol (16): brown yellowish residue {R_f=0.57 and 0.7 in MeOH: H_2O: Acetic acid (70: 30: 1) and $CHCl_3$: MeOH: H_2O (70: 30: 3), respectively; UV (MeOH) spectrum showed absorption bands at λ_{max}220 and 278 nm and IR (KBr) υ_{max}3373, 1705, 1635, 1604, 1546 and 1363; molecular ion peak was determined using positive ESI-MS at m/z 639 $(M+H)^+$, corresponding to molecular formula $C_{30}H_{38}O_{15}$; ^1H and ^{13}C NMR data were in agreement with other literature (Tatli and Akdemir, 2003). It gave positive Trim and Hill and Molish's test indicating its preliminary iridoid and glycosidic nature (Trim and Hill, 1952).

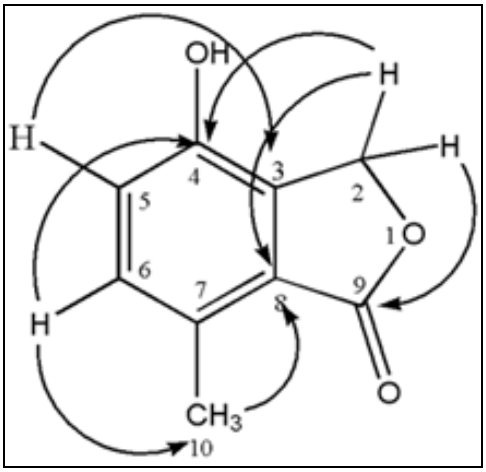

Fig. 2: Important HMBC correlations for compound 4.

DISCUSSION

The ^1H NMR spectrum of compound 4 exhibited signals attributed to aromatic methyl group at δ2.33 (3H, *s*), an oxymethylene moiety at δ5.15 (2H, *brs*.) and a hydroxyl group at δ10.65 (1H, *s*) (table1)**.** Moreover the^1H NMR spectrum exhibited two doublet signals assigned for *ortho*-coupled aromatic protons each integrated for one proton at (δ 7.05 and 7.15) with *J* values 7.4 and 7.7 Hz, respectively and assigned for H-6 and H-5. COSY spectrum confirmed that H-5 and H-6 were coupled to each other. The ^1H NMR and ^{13}C NMR data were useful to confirm the presence of isobenzofuranone skeleton. The lack of chelated hydroxyl group in^1H NMR spectrum (>12.00ppm), confirmed the position of hydroxyl at C-4 and not C-7.

The ^{13}C NMR (table 1) and DEPT spectra of compound 4 showed the presence of one aromatic methyl group (δ_C15.8), an oxymethylene moiety (δ_C67.4) and two methine carbons at (δ_C 120.3 and 131.4). This is in addition to five quaternary carbon signals at (δ_C 133.4, 150.2, 127.5, 123.1 and 171.1), the last one was attributed to a carbonyl carbon.

The HMBC experiment (fig. 2) revealed significant correlations. The correlation of the aromatic methyl group (δ_H2.33) with C-8 (δ123.1) and C-6 (δ131.4) by three bond correlations while with C-7 (δ127.5) by two bond, evidenced the methyl group connected to C-7. Furthermore, an oxymethylene at (δ_H5.15) displayed three bond correlations (J_3) with C-8 (δ123.1), C-4 (δ150.2) and C-9 (δ171.1) and two-bond correlation (J_2) with C-3 (δ 133.4). Moreover, H-6 (δ_H 7.05) showed three bond correlations with methyl carbon (δ15.8), C-8 (δ123.1) and C-4 (δ 150.2). In addition, H-5 (δ_H 7.15) showed three bond correlations with C-7 (δ127.5) and C-3 (δ 133.4) and two-bond correlation with C-4 (δ150.2). Finally, the hydroxyl group at C-4 exhibited three bond correlations with C-5 (δ120.3) and C-3 (δ 133.4) and two-bond correlation with C-4 (δ150.2).

The structure of compound **4** was elucidated based on DEPT, HSQC and HMBC experiments as 4-hydroxy-7-methylisobenzofuranone. This is the first report for isolation of isobenzofuranone derivative from a natural source (*B. polystachya*). In 1954, compound **4** was chemically synthesized by Charles worth, by condensation of 5-hydroxy-2-methylbenzoic acid with aq. CH_2O and HCl at room temperature (Charlesworth *et al*., 1954). Isobenzofuranone derivatives have been frequently isolated from microbial sources. Isobenzofuranone derivatives are an important class of compounds displaying a variety of biological activities, such as antioxidant, antimicrobial, antiplatelet, cytotoxic activity as well as antiarrhythmic effects (Huang *et al*., 2012).

Anti-inflammatory activity

The effect of different fractions of *B. polystachya* on rat paw edema is shown in table 2 showing that the ethyl acetate fraction revealed the most significant anti-inflammatory activity (91% decrease in edema) after two hours of injection. This activity may be attributed to the highly bioactive compounds isolated from the ethyl acetate fraction, including verbascoside (Mensah *et al*., 2001), linarin (Zhang *et al*., 2007), luteolin and its glucosidecynaroside (Kim *et al*., 1999). The ethyl acetate fraction was followed in activity by the *n*-butanol (76.7%) and the aqueous (60.7%) fractions, which could be also related to the iridoid compound (**16**), isolated from the aqueous fraction (Tundis *et al*., 2008). Both the petroleum ether and the dichloromethane fractions showed moderate anti-inflammatory effect (46.4%) after two hours of injection. The least effect was posssesed by the total ethanolic extract (26.7% decrease), which may be due to antagonistic action between the different constituents in the extract. In previous studies, the dichloromethane and

methanol extracts of the aerial parts of *Buddleja globosa* exhibited strong oral and topical anti-inflammatory activity (Backhouse *et al*., 2008).

Screening of hypoglycemic activity

The effect of the different fractions on the plasma glucose level is shown in table 3, indicating ethyl acetate fraction being the most active in decreasing the plasma glucose level (46.83%, compared with before treatment). It was followed by the DCM fraction (decrease by 37.78%), whose promising activity could be attributed to its bioactive constituents, represented by triterpene acids, fatty acid ester, flavones, flavonols, flavanone as well as the isobenzofuranone derivative, which were all reported to possess important biological activities (Huang *et al*., 2012). The total ethanolic and the aqueous fractions showed similar hypoglycemic effect. The *n*-butanol fraction showed the weakest activity (decrease by 17.06%), while petroleum ether fraction caused an increase in plasma glucose level by 17% compared with before treatment. Based on the above results, a future antidiabetic study on ethyl acetate and DCM fractions and their isolated compounds, is recommended.

CONCLUSION

Sixteen constituents were isolated from the aerial parts of *B. polystachya* for the first time, using various chromatographic techniques. Different plant fractions were evaluated for their anti-inflammatory and hypoglycemic activities. The ethyl acetate fraction showed the highest anti-inflammatory activity, followed by the *n*-butanol and the aqueous fractions. The petroleum ether and dichloromethane fractions, showed moderate degree of anti-inflammatory effect. As far as their effect on plasma glucose is concerned, the ethyl acetate fraction showed greatest efficacy and the *n*-butanol fraction showed weakest activity while the dichloromethane fraction was found moderate in action. These biological activities may be attributed to the highly bioactive constituents present in the ethyl acetate, *n*-butanol and dichloromethane fractions.

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