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ANTIMICROBIAL CONSTITUENTS FROM *PLUCHEA WALLICHIANA* DC.

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ABSTRACT

Eight compounds viz., β -amyrin **(1)**, β -amyrin acetate **(2)**, (+) – syringaresinol **(3)**, *ent*-pimara- 8 (14), 15-dien-19-oic acid **(4)**, pluviatilol **(5)**, β-sitosterol **(6)**, β -sitosterol 3-*O*-β-D – glucopyranoside **(7)**, and apigenin 7-*O*- β -D-glucoside **(8)**, were isolated for the first time from *Pluchea wallichiana* DC. and their structures were elucidated by extensive spectroscopic studies. All the isolated compounds were tested for their antimicrobial activity.

Keywords: *Pluchea wallichiana*, antimicrobial activity, phytochemistry

INTRODUCTION

The genus *Pluchea* belongs to the Family Compositae which is widely distributed in Pakistan, India, Afghanistan and Iran (Mhaskar *et al*., 2002; Kupchan *et al*., 1970). The family Compositae contains Sesquiterpene, Chlorogenic acid, lactones, orange yellow pigment, sitosterol glycoside, which are known to possess cytotoxic, antitumor and mutagenic properties (Therdore, 1908; Perry and Metzar, 1980; Jayawera, 1980). Several species have shown antifungal, antibacterial and anti inflammatory activities against number of microorganisms. The plants are traditionally used in the treatment of peptic, ulcer burns, abdominal pain and bacterial diseases. (Jafri, 1966; Ahmed and Fiza, 1988). The literature survey reveals that there are no phytochemical work has so far been carried out on *Pluchea wallichiana* DC. This led us to carry out phytochemical investigations on this plant.

MATERIAL AND METHODS

Plant Material:

The whole plant of *Pluchea wallichiana* DC. was collected by botanical authority from Karachi region in the month of July to August and identified by Dr. Surraya Khatoon, Plant Taxonomist, Department of Botany University of Karachi, Pakistan, where a voucher specimen (1037/KUH) has been deposited.

Isolation:

The shade dried whole plants of *P.wallichiana* (20Kg) was extracted with MeOH (3x50L) at room temperature. The combined methanolic extract was evaporated under reduced pressure to obtain a thick gummy mass (700gm). It was suspended in water and successively extracted with n-hexane, ethylacetate and n-butanol. The chloroform soluble fraction was subjected to column chromatography over silica gel eluting with *n*-hexane-chloroform, chloroform and chloroform- methanol in increasing order of polarity to obtain six major fractions C_A-C_F. The fraction C_A obtained from *n*-hexane-chloroform (6.0: 3.0) gave two spots on TLC which on preparative TLC in the same solvent system gave β -amyrine (**1**) (30 mg) and β -amyrine acetate **(2)** (20 mg) respectively. The fraction C_B obtained from the *n*-hexane-chloroform (5.0 : 5.0) was rechromatographed over silica gel with the same solvent and final purification with PTLC using *n*-hexane-chloroform (4.5 : 5.5) as solvent system provided (+)–syringaresinol (**3)** (20 mg). The fraction C_c obtained from *n*-hexane-chloroform (4.0: 6.0) were combined and rechromatographed over silica gel eluting with same solvent system. The final purification was achieved through PTLC using *n*-hexane-chloroform (3.0 : 7.0) as eluent to afford ent-pimara-8-(14),-15-dien-19-oic acid (**4**) (20 mg). The fraction C_D obtained from *n*-hexane-chloroform (2.0: 8.0) gave two spots on TLC which on PTLC with the same solvent system gave pluviatilol (**5**) (25 mg) and β -sitosterol (**6**) (30 mg). The fraction C_E obtained from chloroform- methanol (9.8: 0.2) gave one major spot on TLC which on PTLC in the solvent system chloroform- methanol (9.5:0.5) gave β -sitosterol 3-*O*- β-D-glucopyranoside (**7**) (25 mg). The fraction C_F obtained from chloroform- methanol (9.5 : 0.5) were combined and rechromatographed over silica gel eluting with the solvent system (9.4 : 0.6) and final purification from PTLC using chloroform- methanol (9.2 : 0.8) as developing solvent yielded apigenin 7-*O*- β -D-glucopranoside (**8**) (25 mg). All the compounds were identified through comparison of physical and spectral data with those reported in literature.

***β*-Amyrin (1)**

Crystallization from ethanol (30 mg), m.P 197-198°C; $[\alpha]_D^{25}$: +100° (*c* = 0.21, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3510 (hydroxyl), 3055, 1635 and 820. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.11 (1H, m, H-12), 3.20 (1H, dd, *J* = 10.0, 4.5 Hz, H-3), 1.02, 1.01, 1.08, 0.96, 0.93, 0.88, 0.85 and 0.80 (3H, each s, Me). HREIMS *m/z*: 426.3825 (calcd. for $C_{30}H_{50}O$, 426.3861). The physical and spectral data showed complete agreement with those reported in the literature (Budzikiewicz *et al*. 1963 ; Shamma *et al*., 1962).

***β*-Amyrin acetate (2)**

Crystallization from methanol (20 mg), m.P 244-245°C; $[\alpha]_D^{25}$**:** + 81.4° (*c* = 0.20, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3055, 1710, 1660, 1460, 1382, 1180 and 810 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.10 (1H, m, H-12), 4.05 (1H, dd, *J* = 10.0, 4.5 Hz, H-3), 2.13 (3H, s, OAc), 1.00, 1.07, 0.98, 0.96 (3H, each s, Me), 0.90 (6H, s, H-29 and H-30), 0.86 and 0.81 (3H, each s, Me). HREIMS *m/z*: 468.3931 (calcd. for $C_{32}H_{52}O_2$, 468.3916). The physical and spectral data corresponded to the reported values (Chow and Quon, 1970).

(+)-Syringaresinol (3)

Crystallization from methanol (20 mg), m.P 179-180°C; $[\alpha]_D^{20}$**:** + 6.9 (C 0.02, $CHCl_3$). IR (KBr) ν_{max} cm^{-1} 3428, 1614, 1520. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.11 (1H, m, H-1), 4.73 (1H, d, *J* = 4.5 Hz, H-2), 3.89 (1H, m, H-4), 4.30 (1H, dd, *J* = 7.0, 9.0 Hz, H-4), 3.12 (1H, m, H-5), 4.73 (1H, d, *J* = 4.5 Hz, H-6), 3.89 (1H, m, H-8), 4.30 (1H, dd, *J* = 7.0, 9.0 Hz, H-8), 6.58~6.60 (4H, H-2′,6′,2′′,6′′), 3.90, (3H, s, OCH_3) HREIMS *m/z* 418. 4269 (calcd, for $C_{22}H_{26}O_8$, 418.1627). The physical and spectral data were in agreement woth those reported in literature (Deyama *et al*., 1987; Macrae and Towers, 1985; Tanaka *et al*., 1989).

ent-Pimara-8(14), 15-dien-19-oic acid (4)

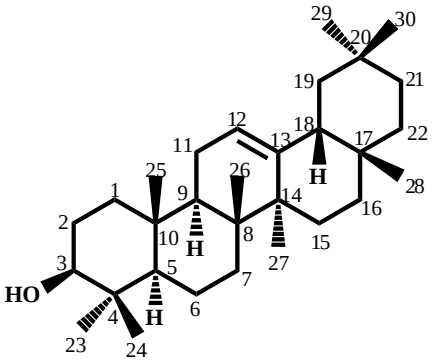
Crystallization from methanol (30 mg), m.P 165-166°C; IR ν_{max} (KBr) cm^{-1}, 3400, 1690, 1460 ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.66 (3H, s, H-20), 1.01 (3H, s, H-17), 1.23 (3H, s, H-18), 4.91 (1H, dd, *J* = 2.1, 11.7 Hz, H-16a), 4.96 (1H, dd, *J* = 1.8, 5.1 Hz, H-16b), 5.16 (1H, s, H-14), 5.71 (1H, dd, *J* = 10.5, 17.1 Hz, H-15). HREIMS *m/z* 302.4391 (calcd, for $C_{20}H_{32}O_2$, 302.4421). The physical and spectral data showed complete agreement with those reported in the litrature (Sy and Brown, 1998).

Pluviatilol (5)

Crystallizewith benzene and petether (25 mg), m.P 161-163°C; $[\alpha]^{20}_D$ + 79.8 (C 0.16 MeOH). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.91 (1H, m, H-1), 4.42 (1H, d, *J* = 7.5 Hz, H-2), 3.32 (1H, m, H-4), 3.85 (1H, m, H-4), 3.32 (1H, m, H-5), 4.86 (1H, d, *J* = 6.0 Hz, H-6), 3.85 (1H, dd, *J* = 6.5, 9.5 Hz, H-8), 4.13 (1H, dd, *J* = 1.0, 9.5 Hz, H - 8), 6.81~6.89 (6H, H-2′,5′,6′,2′′,5′′,6′′), 5.97, (2H, s, OCH_2O), 3.91 (3H, s, OCH_3). HREIMS *m/z*: 356.3740 (calcd. for $C_{20}H_{20}O_6$, 356.1259). The physical and spectral data corresponded to the reported values .(Corrie *et al*., 1970; Banerji and Pal, 1982).

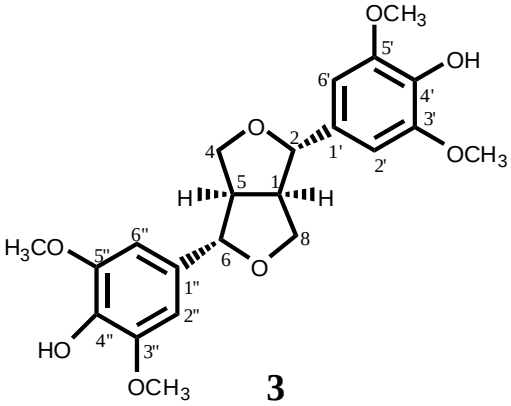
***β*-Sitosterol (6)**

Crystallization from acetone (30 mg), m.P 135°C; $[\alpha]_D^{25}$: + 35.5 (*c* = 0.22, $CHCl_3$). IR (KBr) ν_{max}: 3446, 3050, 1650 and 815 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ : 5.11 (1H,m, H-6), 3.36 (1H, m, H-3), 1.01 (3H, s, Me-19), 0.92 (3H, d, *J* = 6.2 Hz, Me-21), 0.84 (3H, t, *J* = 7.0 Hz, Me-29), 0.83 (3H, d, *J* = 6.5 Hz, Me-26), 0.81 (3H, d, *J* = 6.5 Hz, Me-27), 0.68 (3H, s, Me-18). HREIMS**:** *m/z* 414.3845 (calcd. for $C_{29}H_{50}O$, 414.3861). The physical and spectral data showed complete resemblance with those reported in the litrature (Holland *et al*., 1978; Rubintein *et al*., 1976).



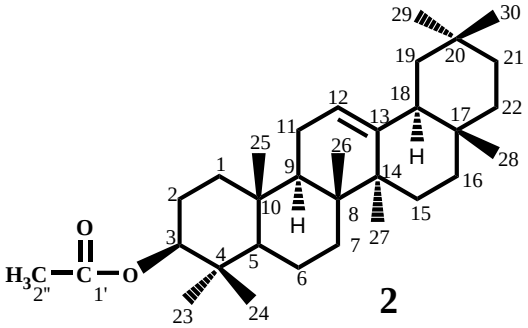
1

***β*-Amyrin (1)**



3

(+)-Syringaresinol (3)



2

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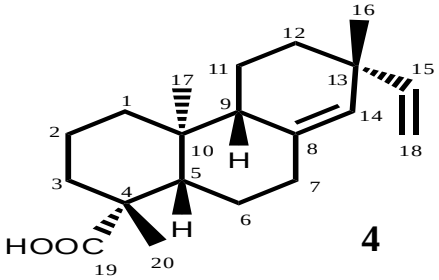
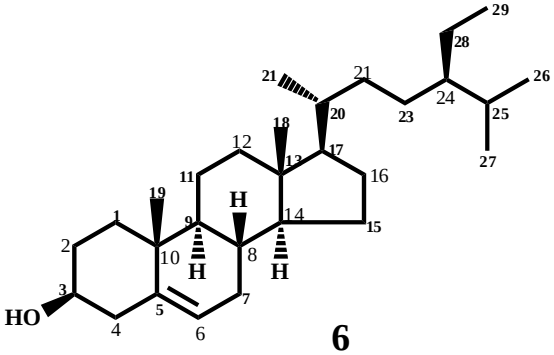


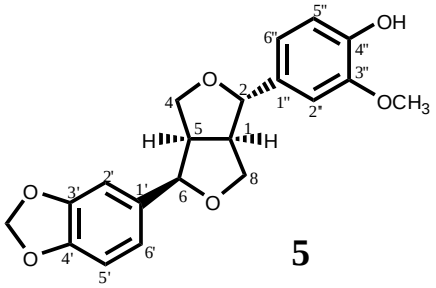
4

ent-Pimara-8(14), 15-dien-19-oic acid (4)



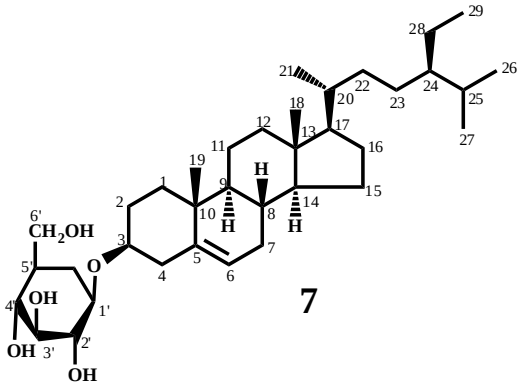
6

***β*-Sitosterol (6)**



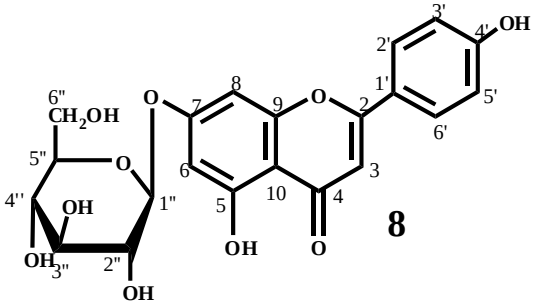
5

Pluviatilol (5)



7

***β*-Sitosterol 3-*O*-*β*-D-glucopyranoside (7)**



8

Apigenine 7-*O*-β-D-glucoside (8)

β-Sitosterol 3-*O*-β-D-glucopyranoside (7)

Crystallization from methanol (25 mg), m.P 279-280 °C; $[\alpha]_D^{25}$ -14.5 (*c* = 0.3, MeOH) IR (KBr) ν_{max}: 3452, 3044, 1646, 1618, 1595-1550 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300 MHz) *δ*: 5.33 (1H, d, *J* = 7.2 Hz, H-1′), 5.12 (1H, br d, *J* = 5.4 Hz, H-6), 3.85 (1H, m, H-3), 3.82-4.42 (m, Glc-H), 1.01 (3H, s, Me-19), 0.92 (3H, d, *J* = 6.2 Hz, Me-21), 0.84 (3H, t, *J* = 7.0 Hz, Me-29), 0.83 (3H, d, *J* = 6.5 Hz, Me-26), 0.81 (3H, d, *J* = 6.5 Hz, Me-27), 0.68 (3H, s, Me-18). HRFABMS**:** *m/z* 576.4386 (calcd for $C_{35}H_{61}O_6$ 576.43897). The physical and spectral data corresponded to the reported values.(Iribarren *et al*., 1983; Wyllie *et al*., 1976).

Apigenine 7-*O*-β-D-glucoside (8)

Amorphous powder (25 mg), $[\alpha]_D^{25}$**:** -64.0 (*c* = 0.14, MeOH). UV λ_{max} (log ε) (MeOH) nm: 6.9 (4.42) and 269 (3.03). IR (KBr) ν_{max} cm^{-1}: 3370 and 1660. ^{1}H-NMR (CD_3OD, 400 MHz) *δ*: 7.86 (2H, d, *J* = 9.0 Hz, H-2′ and H-6′), 6.92 (2H, d, *J* = 9.0 Hz, H-3′and H-5′), 6.75 (1H, d, *J* = 3.0 Hz, H-8), 6.68 (1H, s, H-3), 6.43 (1H, d, *J* = 3.0 Hz, H-6), 5.04 (1H, d, *J* = 7.5Hz, H-1′′), 4.52 (1H,m, H-4′′), 3.84 (1H,m, H-2′), 3.78 (2H,m, H-6′′), 3.59 (1H,m, H-3′′) and 3.38 (1H,m, H-5′).
HRFABMS (+ve) $[M+H]^+$ *m/z*:433.1135 (calcd. for $C_{21}H_{20}O_{10}$, 433.1125). The physical and spectral data showed complete resemblence with those reported in literature (Sadikum *et al*., 1980; Redaclli *et al*., 1980).

Antimicrobial Assay:

The antibacterial and antifungal were determined by agar diffusion method proposed by (Jaffer *et al*., 1988), one loop full of 24hr- old culture of selected bacteria Spread on the surface of Muiller Hinton Agar Plates wells were drug in the medium with the help of sterile borer. A stock solution of extract 2mg/ml was prepared in DMSO and dilution of the stock solution containing hundred microlitre of each dilution was added to their respective wells and after 24hr-growth inhibition of bacteria was observed.

Table 1. Antibacterial and antifungal activity of compounds 1-8 from *Pluchea wallichiana.*

Microorganism	**Zone of inhibition (mm) Compounds (1-8)**								
Bacteria	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Control**
Gram positive									
Bacillus subtiles	10	15	28	20	5	0	20	0	+
Shigelta flenneri	15	20	22	15	15	15	25	25	+
Staphylococcus aureus	5	20	16	12	15	12	18	20	+
Streptococcus pyogens	12	5	12	10	10	20	20	15	+
Streptococcus agalacteae	12	0	20	13	10	18	20	20	+
Gram negative									
Escherichia coli	15	18	20	18	7	5	22	15	+
Pseudomonus aeruginosa	12	0	20	17	12	3	18	0	+
Salmonella paratypi	10	15	25	15	12	4	16	16	+
Shigella bydi	18	16	15	10	10	6	15	14	+
Salmonella typhi	20	12	12	15	8	2	15	12	+
Fungi									
Aspergillus nigar	2	0	58	25	7	12	15	16	+
A. fumigatus	0	10	30	12	5	10	20	20	+
A. flavus	5	12	20	17	0	10	17	25	+
Alternaria solani	7	14	25	15	5	5	12	17	+

RESULTS AND DISCUSSION

The methanolic extract was fractionated into n-hexane, chloroform, ethyl acetate, n-butanol and water soluble fractions. Of these chloroform fraction has been studied in the present investigations. A series of column chromatographic techniques resulted in isolation of eight known compounds reported for the first time from this species. These could be identified as β -amyrin **(1)**, β -amyrin acetate **(2)**, (+) – syringaresinol **(3)**, *ent*-pimara- 8 (14), 15-dien-19-oic acid **(4)**, pluviatilol **(5)**, β -sitosterol **(6)**, β -sitosterol 3-*O*- β -D – glucopyranoside **(7)**, and apigenin 7-*O*- β-D-glucoside **(8).** The isolated compounds were tested for their antimicrobial activity (Table 1). The compounds 3, 4, 7 and 8 showed significant antimicrobial activity against *Bacillus subtilis*, *Shigella flenneri*, *Staphylococcus aureus*, *Streptococcus pyogens*, *Streptococcus agalacteae.* Other compounds showed weak to moderate activity.

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