Arabian Journal of Chemistry (2017) **10**, 76–81

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Phenolics of selected species of *Persicaria* and *Polygonum* (Polygonaceae) in Egypt

Sameh Hussein [a], Usama EL-Magly [b], Mohamed Tantawy [b], Salwa Kawashty [a,*], Nabiel Saleh [a]

[a] *Department of Phytochemistry and Plant Systematics, National Research Centre, Dokki-12311, Cairo, Egypt*
[b] *Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt*

Received 18 January 2012; accepted 12 June 2012
Available online 21 June 2012

KEYWORDS

Persicaria;
Polygonum;
Macromorphology;
Micromorphology;
Flavonoids;
Chemosystematics

Abstract Four selected species of family Polygonaceae Juss. *viz*. *Persicaria salicifolia* (Brouss. ex Willd.) Assenov, *Persicaria senegalensis* (Meisn.) Soják, *Polygonum bellardii* All. and *Polygonum equisetiforme* Sm. were subjected to botanical, chemical and numerical studies. The botanical part deals with macro- and micromorphological characters of the whole plant. The chemical part deals with extraction and identification of 17 compounds including flavones, flavonols, flavone C-glycosides and phenolic acids. The botanical and chemical results of the four selected species were subjected to a numerical analysis.

1. Introduction

Polygonaceae Juss. is a family comprising about 50 genera and 1120 species of monoecious and dioecious herbs, shrubs and small trees (Li et al., 2003). In Egypt, Polygonaceae includes about 22 wild species under six genera; *Atraphaxis*, *Calligonum*, *Emex*, *Oxygonum*, *Polygonum* and *Rumex* (Montasir and Hassib, 1956). Täckholm (1974) had recognized 28 species and added one more genus *viz*. *Bilderdykia* to the genera cited by Montasir and Hassib (1956) while Boulos (1999) treated *Polygonum* and *Persicaria* as two different genera.

Persicaria (L.) Mill. is distributed mainly in the Northern hemisphere and seven species are recorded in Egypt (Boulos, 1999). On the other hand *Polygonum* L. is the largest genus of Polygonaceae, which comprises about 30 species of much-branched annual herbs, and distributed in the temperate regions of the northern hemisphere (Hong et al., 2005). According to Boulos (1999) only six species are found in Egypt.

Several studies dealt with the macro- and micromorphological characters of *Persicaria* and *Polygonum* (Simmonds, 1945; Haraldson, 1978; Ronse Decraene and Akeroyd, 1988; Hamed and Tantawy, 1990, 1991; Leresten and Curtis, 1992; Brandbyge, 1993; Boulos, 1999; Partridge, 2001; Li et al., 2003; Tantawy et al., 2005). The taxonomy of *Polygonum* has been unsettled since Linnaeus established it and has been subdivided into various sections and subsections, split into segregate genera, and some elements have even been treated as different tribes (Leresten and Curtis, 1992).

Quercetin glycosides were commonly reported in the Polygonaceae while myricetin glycosides and acylated flavonoids

* Corresponding author.
E-mail address: salwasharkawy@windowslive.com (S. Kawashty).
Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2012.06.002
1878-5352

were rare and methylated flavonols were detected in some species of sections *Echinocaulon* and *Persicaria* (Hegnauer, 1969; Kuroyanagi and Fukushima, 1982; Kawasaki et al., 1986; Park, 1987).

The survey involving *Persicaria salicifolia* indicated that flavonol glycosides are predominant (Calis et al., 1999). Chalcone, dihydrochalcone and quercetin glycosides were isolated and identified from *Persicaria senegalensis* (Maradufu and Ouma, 1978; Dossaji and Kubo, 1980; Midiwo and Owuor, 1992; Midiwo et al., 1994; Midiwo et al.,2002, 2007). Quercetin and three quercetin glycosides in addition to isorhamnetin were isolated and identified from *Polygonum equisetiforme* by Ghazal et al. (1992).

The specific objectives of this study are to: investigate the macro- and micromorphological characters of both vegetative and reproductive organs of the investigated taxa in comparison with those of Hamed & Tantawy (1990 and 1991) and Tantawy et al. (2005); study the flavonoid chemistry of the four investigated taxa in comparison with the other investigated taxa in the literature and clarify the relationship between *Persicaria* and *Polygonum* and should they be treated as two separate entities or as one taxonomic unit.

2. Material and methods

2.1. General

UV spectra were recorded on Shimadzu, model 2401. EIMS spectra were carried out on Finnigan-Mat SSQ 7000 spectrometer. ESIMS spectra on Micromass Quattro-LC triple quadruple mass spectrometer equipped with a Z-Spray electro-spray ion source while NMR measurements were carried out using Jeol EX-500 spectroscopy; 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) and Joel JNM-EX 270 spectroscopy; 270 MHz (^{1}H NMR) and 67.5 MHz (^{13}C NMR). Sugars of *O*-glycosides were identified by enzymatic hydrolysis (β-glucosidase and β-galactosidase) or acid hydrolysis followed by co-chromatography with reference standards. *C*-glycoside flavonoids were determined using ferric chloride degradation (Mabry et al., 1970). Authentic samples were obtained from the department of phytochemistry and plant systematics, NRC.

2.2. Plant material

Four selected plant species were collected from different localities representing Egyptian taxa of the family Polygonaceae *viz Persicaria salicifolia* (Brouss. ex Willd.) Assenov, *P. senegalensis* (Meisn.) Soják, *Polygonum bellardii* All. and *P. equisetiforme* Sm. The investigated taxa were identified according to Boulos (1999), and voucher specimens were deposited in the herbarium of the National Research Centre (CAIRC). Details of the collected samples are given below, with an asterisk (∗) indicating herbarium samples: *P. salicifolia* (Brouss. ex Willd.) Assenov, Ismailia Canal and Nile-Delta region 15 Nov. 2008, leg. S.R. Hussein and U.I.A. EL-Magly, s.n.623. *P. senegalensis* (Meisn.) Soják El-Kanater, Nile-Delta region 2 Oct. 2007, leg. S.R. Hussein and U.I.A. EL-Magly, s.n.532. *P. bellardii*[*] All. Shakshouk near lake Qarun along a canal 24 Mar. 1981, leg. L. Boulos s.n.1725 and *P. equisetiforme* Sm. El-Arish & Rafah Desert Road. 25 Apr. 2007 leg. S.R. Hussein and U.I.A. EL-Magly, s.n.513.

2.3. Extraction and isolation

The aerial parts of *P. senegalensis* and *P. equisetiforme* were dried in the shade and ground. Each powder was extracted twice with 70% methanol. The extract was filtered and concentrated then partitioned with solvents of increasing polarity (petroleum ether, diethyl ether, chloroform, acetone, ethyl acetate, methanol and water). Isolation of flavonoids was carried out using column chromatography (MN-polyamide 6S) and paper chromatography (Whatman No. 1 and 3MM) using butanol–acetic acid–water 4:1:5 (BAW upper phase), water and 15% AcOH (water–acetic acid 17–3), (Harborne, 1967; Mabry et al., 1970). The compounds were further purified on a Sephadex LH-20 column with standard solvent systems (Markham, 1982). Trace compounds were identified by co-chromatography with authentic samples. Details of the isolated compound are outlined below.

2.3.1. Apigenin (5,7,3′-trihodroxy-flavone) (**1**)

R_f: 0.85 (BAW), 0.03 (H_2O), 0.15 (15% AcOH). UV/Vis λ_{max} (MeOH): 272, 332; (+NaOMe): 283, 333, 400; (+$AlCl_3$): 280, 305, 350, 386sh; (+$AlCl_3$ + HCl): 279, 305, 347, 385sh; (+NaOAc): 281, 306sh, 387; (+NaOAc + H_3BO_3): 276, 321, 347sh. EI-MS: *m*/*z* 270 equivalent to molecular formula of $C_{15}H_{10}O_5$.

2.3.2. 3,6-Dimethoxy-kaempferol (**2**)

R_f: 0.81 (BAW), 0.04 (H_2O), 0.16 (15% AcOH). UV/Vis λ_{max} (MeOH): 271, 342; (+NaOMe): 276, 327, 399; (+$AlCl_3$): 274, 306sh, 349; (+$AlCl_3$ + HCl): 280, 303sh, 352, 406sh; (+NaOAc): 276, 300sh, 351; (+NaOAc + H_3BO_3): 276, 346. ^{1}H NMR in DMSO-d_6: δ 7.92 (2H, d, J = 8.7 Hz, H-2′, H-6′), δ 6.93 (2H, d, J = 8.7 Hz, H-3′, H-5′), δ 6.52 (1H, s, H-8), δ 3.77 (3H, s, OCH_3), δ 3.74 (3H, s, OCH_3). EI-MS: *m*/*z* 330 equivalent to molecular formula of $C_{17}H_{14}O_7$.

2.3.3. 3,7,4′-Trimethoxy-kaempferol (**3**)

R_f: 0.93 (BAW), 0.01 (H_2O), 0.18 (15% AcOH). UV/Vis λ_{max} (MeOH): 276, 333; (+NaOMe): 278, 291sh, 390; (+$AlCl_3$): 263sh, 279, 303, 349; (+$AlCl_3$ + HCl): 260, 283, 300, 350; (+NaOAc): 276, 298sh, 350; (+NaOAc + H_3BO_3): 276, 336. ^{1}H NMR in DMSO-d_6: δ 8.04 (2H, d, J = 8.7 Hz, H-2′, H-6′), δ 7.12 (2H, d, J = 8.7 Hz, H-3′, H-5′), δ 6.87 (1H, d, J = 2 Hz, H-8), δ 6.61 (1H, d, J = 2 Hz, H-6), δ 3.86 (6H, s, OCH_3), δ 3.75 (3H, s, OCH_3).

2.3.4. Calycopterin (5,4′-dihydroxy-3,6,7,8-tetramethoxy-flavone) (**4**)

R_f: 0.88 (BAW), 0.05 (H_2O), 0.27 (15% AcOH). UV/Vis λ_{max} (MeOH): 273, 341; (+NaOMe): 278, 302sh, 398; (+$AlCl_3$): 274, 306sh, 350; (+$AlCl_3$ + HCl): 280, 303sh, 352; (+NaOAc): 273, 300sh, 351; (+NaOAc + H_3BO_3): 276, 346. ^{1}H NMR in DMSO-d_6: δ 7.91 (2H, d, J = 8.5 Hz, H-2′, H-6′), δ 6.92 (2H, d, J = 8.5 Hz, H-3′, H-5′), δ 3.9 (3H, s, OCH_3), δ 3.88 (3H, s, OCH_3), δ 3.78 (3H, s, OCH_3), δ 3.69 (3H, s, OCH_3). ESI-MS: *m*/*z* $[M-H]^-$ 373 corresponding to a molecular weight of 374 and a molecular formula of $C_{19}H_{18}O_8$.

2.3.5. Quercetin (3,5,7,3′,4′-pentahydroxy-flavone) (**5**)

R_f: 0.65 (BAW), 0.09 (H_2O), 0.05 (15% AcOH). UV/Vis λ_{max} (MeOH): 258, 267sh, 298sh, 360; (+NaOMe): 274, 324, 409; (+$AlCl_3$): 275, 304sh, 333sh, 429; (+$AlCl_3$ + HCl): 269,

Table 1 Distribution of the isolated flavonoids and phenolic acids in the investigated taxa.

Compound	*Persicaria salicifolia*	*Persicaria senegalensis*	*Polygonum bellardii*	*Polygonum equisetiforme*
Apigenin (5,7,3'-trihodroxy-flavone) (**1**)	–	+	–	–
Apigenin-6-*C*-arabino-pyranosyl- 8-*C*-glucopyranoside (**11**)	+	–	–	+
Apigenin-6,8 di C-gluco-pyranoside (**12**)	–	–	–	+
3,6-Dimethoxy – Kaempferol (**2**)	–	+	–	–
3,7,4′-Trimethoxy – Kaempferol (**3**)	+	+	–	–
Calycopterin (5,4′-dihydroxy-3,6,7,8-tetramethoxy-flavone) (**4**)	–	+	–	–
Quercetin (3,5,7,3',4'-pentahydroxy-flavone) (**5**)	+	+	+	–
Quercetin-3-*O*-gluco-pyranoside (**6**)	+	+	–	+
Quercetin-3,7-di-*O*-gluco-pyranoside (**13**)	+	–	–	+
Quercetin-3-methoxy-3′-*O*-glucopyranoside (**7**)	–	+	–	–
Rhamnetin-3-*O*-rhamno-pyranoside (**14**)	–	–	–	+
Myricetin (3,5,7,3',4',5'-heahyroxy-flavone) (**15**)	–	–	–	+
Myricetin-3-*O*-gluco-pyranoside (**16**)	–	–	–	+
5-Hydroxy-7-methoxy-isoflavone (**8**)	+	+	–	–
Gallic acid (**17**)	–	–	–	+
Gentisic acid 5-*O*-(6′-*O*-galloyl)-glucopyranoside (**9**)	–	+	–	–
Gentisic acid 5-*O*-(2′-*O*-glucopyranosyl)-rhamnoside (**10**)	+	+	–	–

+ = Present, – = absent.

300sh, 358sh, 403; (+NaOAc): 269, 323,373; (+NaOAc + H_3BO_3): 261, 301sh, 379. ESI-MS: m/z $[M-H]^-$ 301 corresponding to a molecular weight of 302 and a molecular formula of $C_{15}H_{10}O_7$.

*2.3.6. Quercetin-3-O-glucopyranoside (**6**)*

R_f: 0.57 (BAW), 0.08 (H_2O), 0.38 (15% AcOH). UV/Vis λ_{max} (MeOH): 256, 267sh, 298sh, 359; (+NaOMe): 272, 326, 408; ($AlCl_3$): 275, 304sh, 333sh, 429; (+$AlCl_3$ + HCl): 269, 300sh, 358sh, 403; (+NaOAc): 271, 323, 373; (+NaOAc + H_3BO_3): 262, 301sh, 379. ^{1}H NMR in DMSO-d_6: δ 7.66 (1H, d, J = 8.5 Hz, H-6′), δ 7.55 (1H, d, J = 2 Hz, H-2′), δ 6.80 (1H, d, J = 8.5 Hz, H-5′), δ 6.37 (1H, d, J = 2 Hz, H-8), δ 6.17 (1H, d, J = 2 Hz, H-6), δ 5.37 (1H, d, J = 7.5 Hz, H-1″), δ 3–4 (5H, m).

*2.3.7. Quercetin-3-methoxy-3′-O-glucopyranoside (**7**)*

R_f: 0.52 (BAW), 0.13 (H_2O), 0.39 (15% AcOH). UV/Vis λ_{max} (MeOH): 258, 264sh, 359; (+NaOMe): 272, 327, 402; ($AlCl_3$): 263, 300sh, 366, 405sh; (+$AlCl_3$ + HCl): 264, 300sh, 360, 402sh; (+NaOAc): 269, 321, 369; (+NaOAc + H_3BO_3): 262, 370. ^{1}H NMR in DMSO-d_6: δ 8.37 (1H, d, J = 2 Hz, H-2′), δ 7.26 (1H, d, J = 7.7 Hz, H-6′), δ 6.77 (1H, d, J = 8.4 Hz, H-5′), δ 6.33 (1H, s, H-8), δ 6.13 (1H, s, H-6), δ 5.11 (1H, d, J = 7.0 Hz, H-1″), δ 3.60 (3H, s, OCH_3), δ 3–4 (5H, m). ESI-MS: m/z $[M-H]^-$ 477 corresponding to a molecular weight of 478 and a molecular formula of $C_{22}H_{22}O_{12}$.

*2.3.8. 5-Hydroxy-7-methoxy-isoflavone (**8**)*

R_f: 0.91 (BAW), 0.33 (H_2O), 0.41 (15% AcOH). UV/Vis λ_{max} (MeOH): 279; 320sh (+NaOMe): 275, 361, 413sh; (+$AlCl_3$): 271; (+$AlCl_3$ + HCl): 272; (+NaOAc): 278; (+NaOAc + H_3BO_3): 279. ^{1}H NMR in DMSO-d_6: δ 8.45 (1H, s, H-2), δ 7.07 (5H, m, phenyl ring), δ 6.64 (1H, d, J = 2 Hz, H-8), δ 6.58 (1H, d, J = 2 Hz, H-6), δ 3.67 (3H, s, OCH_3). ^{13}C NMR in DMSO-d_6: δ 175.8 (C-4), 167.3 (C-7), 158.0 (C-9), 156.0 (C-5), 151.9 (C-2), 136.4 (C-4′), 132.0 (C-1′), 124.0 (C-2′), 124.0 (C-6′), 122.0 (C-3′), 122.0 (C-5′), 121.0 (C-3), 106.5 (C-10), 100.1 (C-6), 97.0 (C-8), 56.3 (OCH_3). EI-MS: m/z [M + H] 269 corresponding to a molecular weight of 268 and a molecular formula of $C_{16}H_{12}O_4$.

*2.3.9. Gentisic acid 5-O-(6′-O-galloyl)-glucopyranoside (**9**)*

R_f: 0.29 (BAW), 0.48 (H_2O), 0.43 (15% AcOH). UV/Vis λ_{max} (MeOH): 279, 361sh; (+NaOMe): 275, 361sh; (+$AlCl_3$): 277, 322sh; (+$AlCl_3$ + HCl): 276, 331sh; (+NaOAc): 278; (+NaOAc + H_3BO_3): 279. ^{1}H NMR in DMSO-d_6: δ 7.63 (1H, d, J = 2 Hz, H-6), δ 7.19 (2H, s, H-2″, H-6″), δ 6.85 (1H, d, J = 8.6 Hz, H-4), δ 6.57 (1H, d, J = 8.6 Hz, H-3), δ 4.69 (1H, d, J = 7.5 Hz, H-1′), δ 3–4 (5H, m). ^{13}C NMR in DMSO-d_6: δ 172.9 (COOH), 166.7 (CO), 157.7 (C-2), 149.5 (C-5), 146.4 (C-3″),146.4 (C-5″), 139.0 (C-4″), 122.6 (C-1″), 119.9 (C-4), 119.2 (C-3), 116.8 (C-6), 115.9 (C-1), 109.0 (C-2″), 109.0 (C-6″), 102.4 (C-1′), 76.5 (C-3′), 75.2 (C-2′), 73.8 (C-5′), 70.7 (C-4′), 65.3 (C-6′). ESI-MS: m/z $[M-H]^-$ 467 corresponding to a molecular weight of 468 and a molecular formula of $C_{20}H_{20}O_{13}$.

*2.3.10. Gentisic acid 5-O-(2′-O-glucopyranosyl)-rhamnoside (**10**)*

R_f: 0.39 (BAW), 0.65 (H_2O), 0.71 (15% AcOH). UV/Vis λ_{max} (MeOH): 266sh, 309; (+NaOMe): 272sh, 308. ^{1}H NMR in DMSO-d_6: δ 7.57 (1H, d, J = 2 Hz, H-6), δ 6.87 (1H, dd, J = 3.5, 8.6 Hz, H-4), δ 6.52 (1H, d, J = 8.6 Hz, H-3), δ

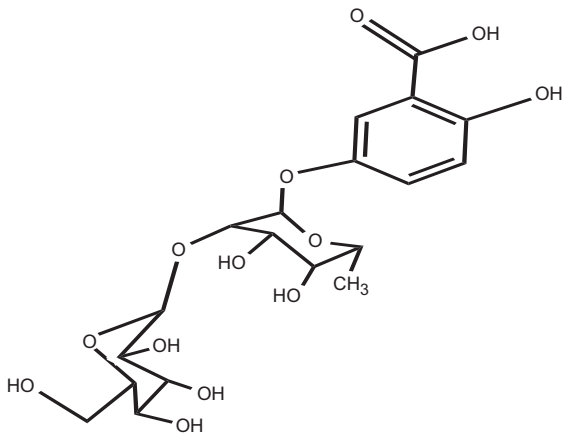

Figure 1 Structure of compound **10**.

5.41 (1H, *br. s*, $\Delta_{v1/2}$ = 2.5 Hz, H-1′), δ 4.59 (1H, d, *J* = *8.6 Hz, H-1″)*, δ 3–4 (9H, m), δ 1.12 (3H, d, *J* = 6.5 Hz, CH_3-rhamnose). ^{13}C NMR in DMSO-d_6: δ 174.2 (COOH), 157.8 (C-2), 148.5 (C-5), 122.1 (C-4), 121.3 (C-3), 118.6 (C-6), 116.8 (C-1), 104.5 (C-1″), 102.5 (C-1′), 82.1 (C-2′), 77.3 (C-3″), 77.0 (C-5″), 75.6 (C-2″), 73.6 (C-4′), 72.8 (C-3′), 70.2 (C-5′), 69.5 (C-4″), 61.1 (C-6″), 21.0 (C-6′). ESI-MS: *m*/*z* $[M+H]^+$ 463 corresponding to a molecular weight of 462 and a molecular formula of $C_{19}H_{26}O_{13}$.

*2.3.11. Apigenin-6-C-arabinopyranosyl-8-C-glucopyranoside (**11**)*

R_f: 0.13 (BAW), 0.36 (H_2O), 0.48 (15% AcOH). UV/Vis λ_{max} (MeOH): 273, 333, (+NaOMe): 284, 332, 399; (+$AlCl_3$): 279, 305, 344, 387sh; (+$AlCl_3$ + HCl): 280, 304, 343, 387sh; (+NaOAc): 282, 308sh, 392; (+NaOAc + H_3BO_3): 275, 320, 347sh. ^{1}H NMR in DMSO-d_6: δ 7.9 (2H, d, *J* = 7.6 Hz, H-2′, H-6′), δ 6.85 (2H, d, *J* = 7.9 Hz, H-3′,H-5′), δ 6.5 (1H, s, H-3), δ 4.8 (1H, d, *J* = 8 Hz, H-1″), δ 4.6 (1H, d, *J* = 8 Hz, H-1‴), δ 3.2–4 (9H, m). ^{13}C NMR in DMSO-d_6: δ 182.6 (C-4), 164.5 (C-2), 161.6 (C-5), 161.6 (C-7), 158.7 (C-4′), 155.6 (C-9), 129.4 (C-2′), 129.4 (C-6′), 121.9 (C-1′), 116.4 (C-3′), 116.4 (C-5′), 108.4 (C-6), 105.3 (C-8), 103.9 (C-10), 103 (C-3), 82.2 (C-5‴), 79 (C-3″), 74.6 (C-1″), 74 (C-2″), 73.7 (C-1‴), 71.5 (C-2‴), 71 (C-3‴), 70.6 (C-4‴), 69.6 (C-4″), 69 (C-5″), 61.4 (C-6‴). ESI-MS: *m*/*z* $[M-H]^-$ 563 corresponding to a molecular weight of 564 and a molecular formula of $C_{26}H_{28}O_{14}$.

*2.3.12. Apigenin-6,8-di-C-glucopyranoside (**12**)*

R_f: 0.13 (BAW), 0.34 (H_2O), 0.48 (15% AcOH). UV/Vis λ_{max} (MeOH): 273, 333, (+NaOMe): 283, 332, 400; (+$AlCl_3$): 280, 305, 346, 388; (+$AlCl_3$ + HCl): 279, 305, 345, 387; (+NaOAc): 282, 309sh, 3902; (+NaOAc + H_3BO_3): 274, 323, 349sh. ^{1}H NMR in DMSO-d_6: δ 7.9 (2H, d, *J* = 8.5 Hz, H-2′, H-6′), δ 6.85 (2H, d, *J* = 8.5 Hz, H-3′,H-5′), δ 6.5 (1H, s, H-3), δ 4.8 (1H, d, *J* = 8.6 Hz, H-1″), δ 4.6 (1H, d, *J* = 8.6 Hz, H-1‴), δ 3–4 (10H, m). ESI-MS: *m*/*z* $[M-H]^-$ 593 corresponding to a molecular weight of 594 and a molecular formula of $C_{27}H_{30}O_{15}$.

*2.3.13. Quercetin-3, 7-di-O-glucopyranoside (**13**)*

R_f: 0.18 (BAW), 0.33 (H_2O), 0.63 (15% AcOH). UV/Vis λ_{max} (MeOH): 258, 267sh, 355; (+NaOMe): 270, 308sh, 405; ($AlCl_3$): 270, 309sh, 436; (+$AlCl_3$ + HCl): 266, 303sh, 357sh, 406; (+NaOAc): 261, 310sh, 387; (+NaOAc + H_3BO_3): 263, 306sh, 377. ^{1}H NMR in DMSO-d_6: 7.55 (2H, dd, *J* = 2.5 Hz, H-2′; *J* = 7.5 Hz, H-6′), δ 7.15 (1H, d, *J* = 8.5 Hz, H-5′), δ 6.80 (1H, d, *J* = 2 Hz, H-8), δ 6.45 (1H, d, *J* = 2 Hz, H-6), δ 5.35 (1H, d, *J* = 8 Hz, H-1″), δ 4.85 (1H, d, *J* = 8 Hz, H-1‴), δ 3–3.8 (10H, m).

*2.3.14. Rhamnetin-3-O-rhamnopyranoside (**14**)*

R_f: 0.71 (BAW), 0.25 (H_2O), 0.54 (15% AcOH). UV/Vis λ_{max} (MeOH): 257, 264 sh., 350; (+NaOMe): 267, 303sh, 398; (+$AlCl_3$): 274, 304sh, 427; (+$AlCl_3$ + HCl): 271, 301sh, 352, 399; (+NaOAc): 260, 302sh, 366; (+NaOAc + H_3BO_3): 262, 290sh, 372. ^{1}H NMR in DMSO-d_6: δ 7.33 (1H, d, *J* = 2 Hz, H-2′), δ 7.27 (1H, dd, *J* = 2, 8.2 Hz, H-6′), δ 6.85 (1H, d, *J* = 8.1 Hz, H-5′), δ 6.69 (1H, d, *J* = 2 Hz, H-8), δ 6.39 (1H, d, *J* = 2 Hz, H-6), δ 5.28 (1H, d, *J* = 2.5 Hz, H-1″), δ 3.86 (3H, s, OCH_3), δ 0.81 (3H, d, *J* = 5.5 Hz , CH_3-rhamnose).

*2.3.15. Myricetin (3,5,7,3′,4′,5′-heahyroxy-flavone) (**15**)*

R_f: 0.43 (BAW), 0.05 (H_2O), 0.26 (15% AcOH). UV/Vis λ_{max} (MeOH): 265, 300 sh, 361; (+NaOMe): 269, 328sh, 408; (+$AlCl_3$): 272, 310, 427; (+$AlCl_3$ + HCl): 271, 306, 362, 411; (+NaOAc): 270, 401; (+NaOAc + H_3BO_3): 266, 391. ^{1}H NMR in DMSO-d_6: δ 7.15 (2H, d, *J* = 2 Hz, H-2′, H-6′), δ 6.33 (1H, d, *J* = 2 Hz, H-8), δ 6.16 (1H, d, *J* = 2 Hz, H-6).

*2.3.16. Myricetin-3-O-glucopyranoside (**16**)*

R_f: 0.48 (BAW), 0.07 (H_2O), 0.27 (15% AcOH). UV/Vis λ_{max} (MeOH): 264, 285 sh, 357; (+NaOMe): 272, 397; (+$AlCl_3$): 272, 361sh, 411; (+$AlCl_3$ + HCl): 270, 361, 402; (+NaOAc): 269, 362; (+NaOAc + H_3BO_3): 265, 384. ^{1}H NMR in DMSO-d_6: δ 7.20 (2H, d, *J* = 2 Hz, H-2′, H-6′), δ 6.35(1H, d, *J* = 2 Hz, H-8), δ 6.20 (1H, d, *J* = 2 Hz, H-6), δ 5.42 (1H, d, *J* = 8.5 Hz, H-1″), δ 3–3.8 (5H, m).

*2.3.17. Gallic acid (**17**)*

R_f: 0.81 (BAW), 0.61 (H_2O), 0.60 (15% AcOH). UV/Vis λ_{max} (MeOH): 215, 272; ^{1}H NMR in DMSO-d_6: δ 6.9 (2H, s, H-2, H-6). EI-MS: *m*/*z* 170 equivalent to molecular formula of $C_7H_6O_5$.

3. Results and discussion

3.1. Botanical investigations

3.1.1. Macromorphological and micromorphological characters

The macro, and micromorphological characters of the stem, petiole and lamina were similar with those of Hamed and Tantawy (1990 and 1991) and the floral characters were given in Tantawy et al. (2005).

3.2. Chemical investigations

Seventeen compounds (14 flavonoids and 3 phenolic acids) were isolated from the investigated taxa and identified using chemical and physical investigations. Ten compounds were isolated from *P. senegalensis* and identified as: Apigenin (5,7,4′-trihydroxyflavone) (**1**), 3,6-dimethoxy-kaempferol (**2**), 3,7, 4′-trimethoxy-kaempferol (**3**), calycopterin (5,4′-dihydroxy-

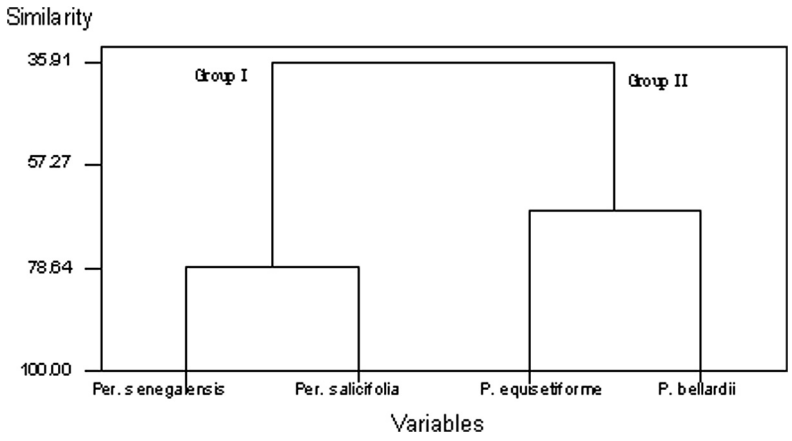

Figure 2 Dendrogram for the morphological, anatomical and chemical characters.

3,6,7,8-tetramethoxy-flavone) (**4**), quercetin (3,5,7,3',4'-pentahydroxy-flavone) (**5**), quercetin-3-*O*-glucopyranoside (**6**), quercetin-3-methoxy-3′-*O*-glucopyranoside (**7**), 5-hydroxy-7-methoxy-isoflavone (**8**), gentisic acid-5-*O*-(6′-*O*-galloyl)-glucopyranoside (**9**) and gentisic acid-5-*O*-(2′-*O*-glucopyranosyl)-rhamnoside (**10**).

Eight compounds were isolated from *P. equisetiforme* and identified as: quercetin-3-*O*-glucopyranoside (**6**), Apigenin-6-C-arabinopyranosyl-8-C-glucopyranoside (**11**), apigenin-6,8-di-C-glucopyranosyl (**12**), quercetin-3,7-di-*O*-glucopyranoside (**13**), rhamnetin-3-O-rhamnopyranoside (**14**), myricetin (3,5,7,3',4',5'-pentahydroxy-flavone) (**15**), myricetin-3-*O*-glucopyranoside (**16**) and gallic acid (**17**).

Compound **10** (Fig. 1) which was isolated from *P. senegalensis* and identified as gentisic acid-5-*O*-(2′-*O*-glucopyranosyl)-rhamnoside, is a new compound identified for the first time in nature. Six compounds **2, 3, 4, 7, 8** and **9** were isolated for the first time from the plant and the remaining three compounds were previously isolated from the same plant by Midiwo and Owuor (1992), Midiwo et al. (1992, 1994, 2002). Compounds **6, 11, 12, 13, 14, 15, 16** and **17** from *P. equisetiforme* were isolated for the first time from the plant. The distribution of these compounds was shown in Table 1.

Compound **10** was isolated from the H_2O fraction as white crystals after separation and purification on a Sephadex LH-20 column. Complete acid hydrolysis of **10** afforded rhamnose and glucose as sugar moiety, identified by co-chromatography with authentic samples. The ESI-MS of **10** showed the molecular ion peak as the base peak $[M+H]^+$ at 463 m/z which is equal to the *Mt* 462 corresponding to the molecular formula $C_{19}H_{26}O_{13}$. ^{1}H NMR spectrum of **10** showed signals at δ 7.57, 6.87 and 6.52 assigned for H-6, H-4 and H-3, respectively, which is equivalent to gentisic acid protons (Sakushima et al., 1995). In addition, there is a broad signal at δ 5.41 for the rhamnose sugar and a doublet signal at δ 4.59 for the anomeric sugar protons with $J = 8.6$ Hz which is consistent for the glucose moiety. The ^{13}C NMR spectrum confirmed that **10** is gentisic acid (Sakushima et al., 1995) with rhamnose and glucose sugar. The C-2′ of rhamnose was shifted downfield from 71 to 82.1 ppm and the upfield shift of C-1′ from 105 to 102.5 ppm, indicated that the interglycosidic linkage between the glucose and rhamnose is glucosyl ($1'' \rightarrow 2'$) rhamnoside (Agrawal, 1989). From the above data, compound **10** was identified as gentisic acid-5-*O*-(2′-**O**-glucopyranosyl)-rhamnoside.

3.3. Chemosystematics

P. salicifolia and *P. senegalensis* are characterized by the presence of 3,7,4′-trimethoxy-kaempferol, 5-hydroxy-7-methoxy-isoflavone and gentisic acid glycosides. These compounds were not detected in the *Polygonum* species while quercetin 3,7-di-*O*-β-glucopyranoside and apigenin-6-*C*-α-arabinopyranosyl-8-*C*-β-glucopyranoside are present in *P. salicifolia* and *P. equisetiforme*. In addition, no methylated flavonoids were detected in *P. bellardii* and only one methylated flavonoid (rhamnetin-3-*O*-α-rhamnopyranoside) is detected in *P. equisetiforme*. *P. equisetiforme* is also characterized by the capability to form 3,4,5-trihydroxy nuclei in the form of myricetin and gallic acid.

The distribution of methylated flavonoids within the genus *Persicaria* in the present study agrees with the observations that methylated flavonols were detected in some species of sections *Echinocaulon* and *Persicaria* (Kuroyanagi and Fukushima, 1982; Kawasaki et al., 1986; Park, 1987). Furthermore; and according to Harborne (1977) and Kawasaki et al. (1986); methylation of flavonoids hydroxyl groups represents a more advanced step in the biosynthesis of flavonoids.

3.4. Numerical analysis

In order to determine the position of *Persicaria* compared to *Polygonum*, a dataset for numerical analysis was constructed. These data included 111 macro-, micromorphological and chemical characters. Each character used in the numerical analysis was treated as a binary character in data matrix using Minitab statistical software 13 (Minitab Inc., 1999).The dendogram formed based on the numerical analysis is illustrated in Fig. 2. At similarity level of 35.91%, the applied operational taxonomic units were separated into two main groups. Group (I) includes *P. salicifolia* and *P. senegalensis* while group (II) includes *P. bellardii* and *P. equisetiforme*. The taxa of group (I) separated at similarity level of 78.22% while the taxa of group (II) separated at similarity level of 66.76%. The grouping of *Persicaria* species and *Polygonum* species under investigation in two distinct groups is evidence for the treatment of *Persicaria* and *Polygonum* as two distinct entities.

4. Conclusion

The isolation and identification of 14 flavonoids and three phenolic acids in the four investigated taxa along with their macro- and micromorphological data formed the basis for a numerical analysis of the four investigated *Persicaria* and *Polygonum* species. The data presented above supported the treatment of *Persicaria* and *Polygonum* as two distinct entities and the same result was confirmed by using the numerical analysis.

References

Agrawal, P.K., 1989. Carbon-13 NMR of Flavonoids. Elsevier, Amsterdam, Oxford, NewYork, Tokyo.

Boulos, L., 1999. In: Flora of Egypt, vol. 1. Al Hadara Puplishing, Cairo, Egypt.

Brandbyge, J., 1993. Polygonaceae. In: Kubitzki, K., Rohwer, J.G., Bittrich, V. (Eds.), . In: The Families and Genera of Vascular Plants, vol. 2. Springer Verlag, Berlin Heidelberg, pp. 531–544.

Calis, I., kuruüzüm, A., Demirezer, O., Sticher, O., Ganci, W., Rüedi, P., 1999. Phenylvaleric acid and flavonoid glycosides from *Polygonum salicifolium*. J. Nat. Prod. 62, 1101–1105.

Dossaji, S., kubo, I., 1980. Quercetin 3-(2″-O-galloylglucoside), a molluscicidal flavonoid from *Polygonum senegalense*. Phytochemistry 19, 482.

Ghazal, S.A., Abuzarqa, M., Mahasneh, A.M., 1992. Antimicrobial activity of *Polygonum equisetiforme* extracts and flavonoids. Phytother. Res. 6, 265–269.

Hamed, K.A., Tantawy, M.E., 1990. The stem anatomy of some polygonaceae and its diagnostic significance in identification. J. Fac. Educ. 15, 249–268.

Hamed, K.A., Tantawy, M.E., 1991. The microcharacters of the petiole and blade of certain polygonaceae as an identificatory tool. Ain Shames Sci. Bull. 28B, 389–406.

Haraldson, K., 1978. Anatomy and taxonomy in Polygonaceae subfam. Polygonoideae Meisn. Emend. Symb. Bot. Upsal. 22, 1–95.

Harborne, J.B., 1967. Comparative Biochemistry of Flavonoids. Academic, London.

Harborne, J.B., 1977. Flavonoids and the evolution of the angiosperms. Biochem. Syst. Ecol. 5, 7–32.

Hegnauer, R., 1969. Polygonaceae. In: Hegnauer, R. (Ed.), . In: Chemotaxonomie der pflanzen, vol. 5. Birkhäuser Verlag, Basel, pp. 361–383.

Hong, S.P., Oh, I.C., Ronse De Craene, L.P., 2005. Pollen morphology of the genera *Polygonum* s. str. and *Polygonella* (Polygoneae: Polygonaceae). Plant Syst. Evol. 254, 13–30.

Kawasaki, M., Kanomata, T., Yoshitama, K., 1986. Flavonoids in the leaves of twenty-eight polygonaceous plants. Bot. Mag. Tokyo 99, 63–74.

Kuroyanagi, M., Fukushima, S., 1982. Highly oxygenated flavonoids from *Polygonum orientale*. Chem. Pharm. Bull. 30, 1163–1168.

Leresten, N.R., Curtis, J.D., 1992. Foliar anatomy of *Polygonum* (Polygonaceae): Survey of epidermal and selected internal structures. Plant Syst. Evol. 182, 71–106.

Li, A., Bao, B., Borodina, A.E., Hong, S.P., Neill, J.Mc., Mosyakin, S.L., Ohba, H., Park, C.W., 2003. Polygonaceae. Flora China 5, 277–350.

Mabry, T.J., Markham, K.R., Thomas, M.B., 1970. The Systematic Identification of Flavonoids. Springer Verlag, New York.

Maradufu, A., Ouma, J.H., 1978. A new chalcone as a natural molluscicide from *Polygonum senegalense*. Phytochemistry 17, 823–824.

Markham, K.R., 1982. Techniques of Flavonoid Identification. Academic Press, New York.

Midiwo, J.O., Owuor, F.A.O., 1992. Epicuticular flavonoids of *Psiadia puntulata* and *Polygonum senegalense*. In: 5th NAPRECA Symposium on Natural Products, Antananarivo, Madagascar, p. 81.

Midiwo, J.O., Gikonyo, N.K., Wanjau, D.O., Matasi, J.J., Waterman, P.G., 1992. Flavonoids of *Polygonum senegalense* (Meisn) part II: more surface and internal tissue flavonoid aglycones. Bull. Chem. Soc. Ethiop. 6, 119–122.

Midiwo, J.O., Owino, N.O., Dagne, E., 1994. Flavonoids of *Polygonum senegalense* Part III: Isolation of dihydrochalcone glucoside and quercetin glycosides. Bull. Chem. Soc. Ethiop. 8, 79–84.

Midiwo, J.O., Yenesew, A., Juma, B.F., Derese, S., Ayoo, J.A., Aluoch, A.O., Guchu, S., 2002. Bioactive compounds from some Kenyan ethnomedicinal plants: Myrsinaceae, Polygonaceae and *Psiadia punctulata*. Phytochem. Rev. 1, 311–323.

Midiwo, J.O., Omoto, F.M., Yenesew, A., Akala, H.M., Wangui, J., Liyala, P., Wasunna, C., Water, N.C., 2007. The first 9-hydroxyhomoisoflavanone, and antiplasmodial chalcones, from the aerial exudates of *Polygonum senegalense*. Arkivoc (ix), 21–27.

Minitab, Inc., 1999. Minitab version 13 statistical software.

Montasir, A.H., Hassib, M., 1956. Illustrated manual flora of Egypt. Part 1, Ed. 1, Bull. Fac. Sc. Ain Shams Univ., Cairo, Egypt.

Park, C.W., 1987. Flavonoid chemistry of Polygonum sect. Echinocaulon: a systematic survey. Syst. Bot. 12, 167–179.

Partridge, J.W., 2001. *Persicaria amphibia* (L.) Gray (*Polygonum amphibium* L.). J. Ecol. 89, 487–501.

Ronse Decraene, L.P., Akeroyd, J.R., 1988. Generic limits in *Polygonum* and related genera (Polygonaceae) on the basis of floral characters. Bot. J. Linn. Soc. 98, 321–371.

Sakushima, A., Coskun, M., Maoka, T., 1995. Hydroxybenzoic acids from *Boreava orientalis*. Phytochemistry 40, 257–261.

Simmonds, N.W., 1945. *Polygonum Persicaria* L. J. Ecol. 33, 121–131.

Täckholm, V., 1974. Students' Flora of Egypt, second ed. Cairo University.

Tantawy, M.E., Hamed, K.A., EL-Magly, U.I., 2005. Floral morphology of some Taxa of Polygonaceae in Egypt. J. Biotechnol. 21, 308–329.