

PHYTOCHEMICAL STUDIES ON PERSICARIA SALICIFOLIA PLANT AND SEEDS FROM EGYPT

ZEINAB A. EL-SWAIFY* , DOAA A. MOATY* MOHAMED M. YOUSSEF*
AND ATEF EL-HELA**

*Botany and Microbiology Department, Faculty of Science (Girls Branch), AL -Azhar University, Cairo, Egypt.

** Pharmacognosy Department, Faculty of Pharmacy (Boys Branch), Al-Azhar University, Cairo, Egypt.

ABSTRACT

phytochemical investigation of the primary (carbohydrates and amino acids) and secondary metabolites of *Persicaria salicifolia* seeds revealed the presence of free sugars and amino acids, they were analysed qualitatively and quantitatively. Also fifty-two active compounds were identified from n-hexane extract of *Persicaria salicifolia* seeds. Total phenolics and flavonoids were determined colourimetrically. The phenolics were identified using LC/MS. From this study *Persicaria salicifolia* seeds can be considered as a promising pharmaceutical agent.

INTRODUCTION

The Polygonaceae is a family of flowering plants known informally as the knotweed family or smartweed buckwheat family in the United States. The name is based on the genus *Polygonum*. The name refers to the many swollen nodes the stems of some species have. It is derived from Greek; poly means many and goni means knee or joint. Polygonaceae comprise about 1200 species distributed into about 48 genera. 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 Thirteen species of *Polygonum* L. were listed in Egypt by **Täckholm, 1974**³⁷, while **Boulos, 2009**⁷ separated the genus according to the morphological characters into two different genera viz *Polygonum* L. and *Persicaria* (**Baraka, 1985**)⁵. Some species of the Polygonaceae family were reported to have tonic, astringent, anti-septic, anti-malarial, anti-spasmodic, anti-tumour, anti-pneumonia properties (**Hussain et al., 2008**)¹⁸, in addition to being used as a fish poison and for insect and snake bites (**Hussein and Mohamed, 2013**)¹⁹, Plants belonging to this family are known to produce a large number of biologically important secondary metabolites, such as flavonoids, anthraquinones, steroids (**Fukuyama et al., 1985**)¹⁵ and alkaloids (**Korulkin and Muzychkina, 2015**)²⁵. The most characteristic compounds of the Polygonaceae family are polysaccharides, phenylpropane glycosides and stilbenes (**Li-shuang, et al 2006**)²⁷

; **Yang et al., 2016**³⁹ and **Kim et al., 1994**)²³. Numerous *Polygonum* species are frequently used in traditional medicine (**Midiwo et al., 2002**³¹ ; **Datta et al., 2007**¹¹ and **Ahmad et al., 2014**)⁴. Some species are used in the treatment of cough and diarrhea (**Baytop, 1984**⁶; **Hussein and Mohamed, 2013**)¹⁹. and several are used as diuretic agents and to treat urinary inflammation (**Liu et al., 2007**)²⁸ and **Baytop, 1984**)⁶. *Polygonum* species are characterized by the presence of drimane- sesquiterpenoids, or sesquiterpenoids and sulphated flavonoids (**Yagi, 1996**)³⁸; **Abd El-Kader et al., 2013**¹ and **Cheng et al., 2012**)⁹. Pharmacological investigations of *Polygonum* species revealed that different extracts of the plants possessed antibacterial, (**Abd El-Kader et al., 2012**² and **KUBINOVA et al., 2014**)²⁵ analgesic, anti-inflammatory **Granica et al., 2013**)¹⁷, hypothermia, diuretic and anti-oxidative properties (**Liu et al., 2008**)²⁹; **Kumar et al., 2012**)²⁶; **Yang et al., 2012**)⁴⁰ and **El-Haci et al., 2013**)¹². It worth noting that nothing was reported about phytochemical studies on *Persicaria salicifolia* Seeds was available in the literature. Therefore, this study was performed to determine the phytochemical investigation of *Persicaria salicifolia* Seeds extracts,

MATERIALS AND METHODS

Persicaria salicifolia was collected from El-Mansoura during summer season, (2013), and the species was identified according to **Boulos, 1999**)⁷. Reference herbarium specimens of studied species was prepared and kept in the

herbarium of Botany and Microbiology Department, Faculty of Science (Girls branch) Al-Azhar University.

Isolation and identification of lipodal matters

About two hundred grams of *P. salicifolia* seeds were extracted three times with 700 ml of methanol–water (70: 30, v/v). The pooled supernatant phases were filtered and concentrated under vacuum to dryness to give (21 g). It was suspended in water and defatted with hexane. The hexane extract concentrated and subjected to gas chromatographic mass spectrum (GC/MS) to analyze it (**Said et al ., 2015**)³³.

Analysis of lipodal matters

The prepared *Persicaria salicifolia* seeds hexane extract was subjected to GC / MS analysis using Shimadzu GC/ MS – QP 5050 A. Software Class 5000. Searched library: Wiley 229. LIB. Column: DBI, 30 m, 0.53 mm ID, 1.5 µm film. Carrier gas: Helium (flow rate 1ml / min.). Ionization mode: EL (70 Ev.). Temperature program: 40 °C (static for 2 min) then gradually increasing (160 °C at a rate of 2 °C/ min) up to 250 °C (static for 7.5 min). Detector temperature 250 °C. Injector temperature 250°C. , **Perez-Magariño et al., 1999**³¹ and **Ferran Sañchez-Rabeneda et al. , 2003**¹⁴).

Identification of lipodal matters

Qualitative identification of the hexane extract was achieved by library searched data base Willey 229LIB. and by comparing their retention indices and mass fragmentation patterns with those of the available references and with published data **Adams, 1989**³. The percentage composition of components of the volatile was determined by computerized peak area measurements.

Determination of soluble carbohydrates

Extraction

Half gram of defatted plant powder was extracted with ethanol (80%) by reflux for 2 hr. The alcohol was evaporated and the aqueous extract was clarified using Carrez reagent, then its volume was completed to 100 ml with distilled water (**Chaplin and Kennedy, 1994**⁸ and **Saszczêsn ,2007**³⁵).

Investigation of nitrogenous compounds

Free amino acids

Ten grams of defatted air-dried plant powder were extracted with 70 % ethyl alcohol. The resulting extract was concentrated and passed through a column of purified cation exchange resin. Elution was carried out with 70 % ethyl alcohol to take all carbohydrate present, then with 2 % HCl for elution of amino acids. The same steps were repeated again using 2 % NH₄OH instead of HCl to complete elution of amino acids. Each of the acidic and alkaline eluents was concentrated separately to a small volume by evaporation at 45 °C. The collected solutions were adjusted to pH 5-7, concentrated and kept for investigation with amino acid analyzer (**Chaplin and Kennedy, 1994**)⁸.

Protein amino acids

The plant powder remained after alcoholic extraction of the previous experiment was hydrolyzed with 20 ml of 6N HCl for 24 hrs. at 110 °C in a sealed tube. The resulting extract was filtered then centrifuged to remove any particles then concentrated under reduced pressure using rotary evaporator apparatus at 45 °C till a volume of 2 ml which was used for identification using the amino acid analyzer. (**Saszczêsn ,2006**)³⁶.

Total phenol and flavonoids

-Determination of total phenol:

The total phenolic contents of *Persicaria salicifolia* extract were determined according to the method described by **Malik and Singh ,1980**³⁰.

-Determination of total flavonoids:

The aluminum chloride method was used for the determination of the total flavonoid content of *Persicaria salicifolia* extract according to the method described by **Danny et al. ,2003**¹⁰)

Extraction and identification of free phenolic acids

The free phenolic acids were isolated from roots of *P. salicifolia* according to **Danny et al. ,2003**¹⁰) and separated by High Performance Liquid Chromatography (HPLC) instrument (Knauer, Germany) equipped with a Model 7125

injection valve (Rheodine, Cotati, CA, USA) with a 50 μ l sample loop, under computer control (Knauer, HPLC version 211a). **Justesen , 2001**²². The flow rate was 1.0 ml / min and detection was carried out by UV at 280 nm (The Regional Center for Mycology and Biotechnology, Al-Azhar University).

Instrument

Liquid chromatography (LC) analyses were performed using an Agilent (Wald bronn, Germany) Model 1100 quaternary pump equipped with an auto sampler and a diode-array detector (DAD). Achemstation HP Rev. A.08.03 was used for data analysis. A Luna C18 column (150 d 2.1 mm i.d. 5 m) (Phenomenex, Torrance, CA, USA) was used. The structure of the compounds was identified by spectroscopic methods including: ultraviolet and visible absorption spectrometer (UV–VIS, Labomed Inc.) for measuring UV spectral data of the isolated compounds, in the range of 200–500 nm in methanol and with different diagnostic shift reagents.

ESI-MS Positive ion acquisition mode was carried out on a XEVO TQD triple quadruple instrument.

Waters Corporation, Milford, MA01757 U.S.A, Mass Spectrometer

Column: ACQUITY UPLC - BEH C18 1.7 μ m - 2.1 \times 50 mm . **Flow rate:** 0.2 mL/min, **Solvent system:** consisted of A (water containing 0.1 % TFA) and B (acetonitrile containing 0.1 % TFA)

RESULTS AND DISCUSSION

Free amino acids

Free amino acids data of amino acid analyzer in **Table1** revealed the presence of five amino acids; tyrosine, phenyl-alanine, arginine, threonine and lysine with differences in their values, 0.22 mg/kg, 0.15 mg/kg, 0.39 mg/kg, 0.054 mg/kg and 0.134 mg/kg respectively. Results showed that total amino acids recorded 0.94 mg/kg, free amino acids recorded 0.74 mg/kg and the combined amino acids recorded 0.20 mg/kg. **Szczêsna, 2006**³⁶ found that *Polygonum bistorta* contained sixteen free amino acids the most abundant were; lysine, isoleucine,

leucine, methionine, phenylalanine, valine and threonine but they present with low concentrations. These results agree with present results. **Yunuskhodzhaeva et al., 2014**⁴¹ stated that the total free amino acids in *P. hydro piper* and *P. aviculare* recorded 0.7 % and 1.43% where the total bound amino acids recorded 9.6% and 9.8% respectively. The two species contain about sixteen free and bound amino acids but there were varied in their concentrations they found that asparagine, glutamine, glycine, tyrosine, and arginine, free amino acids from *P. hydro piper*; and asparagine, glycine, lysine, and arginine, free amino acids of *P. aviculare*. Asparagine, glutamine, leucine, and arginine dominated the bound amino acids of all three plants. These same amino acids dominated the bound amino acids of the alcohol-soluble fractions. These results agree with present results in the total free amino acids and the presence of all free amino acids; but disagree with present results in the total bound amino acids as it recorded 0.20 mg/kg. **Gadallah and Sayed (2014)**¹⁶ studied the *P. salicifolia* protein content, they showed that the total amino acids ranged from 10 (mg g⁻¹ DW) to 20 (mg g⁻¹ DW) during their study on three aquatic macrophytes in Assiut Province, Egypt.

Table1. Free amino acids content of *Persicaria salicifolia*.

Free amino acids	mg/kg
Tyrosine	0.22
Phenyl-alanine	0.15
Arginine	0.39
Threonine	0.054
Lysine	0.134
Total amino acids	0.94
Total free amino acids	0.74
Combined amino acids	0.20

- Free sugars

The results obtained for Free sugars using paper chromatography was tabulated and represented in **Table 2**, it is clear that *Persicaria salicifolia* contains glucose, galactose, ribose, fructose and arabinose, while it doesn't contain lactose, maltose, mannose, raffinose, sucrose and rhamnose. **Liu et al.,2008**²⁹ stated that the

concentrations of D-glucose, D-fructose, and sucrose were changed during their study the mail-lard reaction involved in the steaming process of the root of *Polygonum multiflorum*. The present results agree with **Ibrahim and El-Hela ,2012²⁰** in their using HPLC for determination sugar content of *Polygonume equisetiforme*. They found that it contained five sugars; glucose 42.3%, galactose 5.6%, arabinose 0.8%, mannose 8.6%, and rhamnose 33%.

Table 2. Free sugars of *Persicaria salicifolia* using paper chromatography.

Sugars name	Presence
Glucose	+
Galactose	+
Ribose	+
Mannose	-
Fructose	+
Arabinose	+
Maltose	+
Rafinose	-
Sucrose	-
Rhamnose	-
Lactose	+

Table 3.Identification of the separated active compounds from *Persicaria salicifolia*seeds.

Peak No	RT.	%	Name
1	3.160	1.08	alpha.-d-Galactopyranose, 6-O-[5-O-acetyl-2,3-O-(ethylborylene)-.beta.-d-lyxofuranosyl]-1,2:3,4-bis-O-(1-ethylethylidene)-
2	3.519	0.58	Heptacosane, 1-chloro-
3	3.602	1.14	Bis-[1,3]-oxazino[5,6-c:5',6'-H]quinoline,3,9-dibenzyl-3,4,9,10 (2H,8H)-tetrahydro-5-trifluoromethyl-
4	4.035	1.62	Nonadecanoic acid, 2,2,3,3,4,4,4-heptafluorobutyl ester
5	6.541	4.50	Benzene, 1,3-dimethyl-
6	7.050	1.21	3,8,12-Tri-O-acetoxy-7-desoxyingol-7-one
7	7.544	0.85	Nonadecanoic acid, 2,2,3,3,4,4,4-heptafluorobutyl ester
8	8.535	0.27	5.alpha.-Pregnane-3.alpha.,17.alpha.-diol-20-one-TMS
9	8.567	1.56	18-(1-Phenylethylcarbamoyl)-2,13-dioxabicyclo[12.2.2]octadeca-1 (17),14(18),15-triene-15-carboxylic acid, methyl ester
10	8.615	0.62	Benzoic acid N'-(4,4,5,5,6,6,6-heptafluoro-3-oxo-1-phenyl-hex-1-enyl)-hydrazide
11	8.765	0.44	N-(4,5-Diphenyloxazol-2-ylmethyl)-N-(1-phenylethyl)benzensulfonamide
12	9.545	2.05	Benzene, 1,2,3-trimethyl-
13	9.670	0.38	Piperazine, 1-(4-acetylphenyl)-4-(1-benzyl-4-piperidyl)-
14	9.706	0.54	Hexadecanoic acid, tetradecyl ester
15	10.896	0.50	24-Nor-5.beta.,14.beta.-chol-20(22)-ene-19,23-dioic acid, 1.beta.,3.beta.,5,11.alpha.,14,21-hexahydroxy-, di- gamma -lactone

-lipoidal matter from *Persicaria salicifolia* seeds n-hexane extract using GC/MS:

The results of gas chromatography analysis of pet.ether of *P. salicifolium* seeds (**Table 3**) revealed the presence of fifty two compounds of different chemical classes. The major components of pet. ether were gama -sitosterol, 14.70%, Bis (2-ethylhexyl) phthalate, 12.85 %,12 - octadecadienoic acid ethyl ester 9.69 9%, and hexadecanoic acid ethyl ester 5.84%. Where another compounds recorded lowest values as 5- alpha-Pregnane-3alpha, 17alpha-diol-20-one-TMS, Piperazine, 1-(4-acetylphenyl)-4-(1-benzyl-4-piperidyl), 2(1H)-Pyrimidinone, 4-(trimethylsiloxy)-1-[5-O-(trimethylsilyl)- beta -D-ribofuranosyl]-, cyclic benzenboronate All these compounds were isolated for the first time from *Persicaria salicifolia* seeds.

16	11.139	1.53	Ketone, methyl 5.alpha.-spirostan-20-yl, (20R,25R)-
17	12.350	1.26	Pregn-16-ene-11,14,18,20-tetrol, 3,9-epoxy-3-methoxy-, 11,20-diacetate, (3.alpha.,5.beta.,11.alpha.,14.beta.,20R)-
18	16.266	4.18	Thiophene, tetrahydro-, 1,1-dioxide
19	18.965	0.98	L-Alanine, N-(m-anisoyl)-, hexadecyl ester
20	19.095	0.77	Xanthine, 1,3-dipropyl-8-[4-[(4-hydroxy)phenylamino]carbonyl]methoxyphenyl]-
21	19.751	1.20	Bowdensine, 3-methoxy-
22	19.792	1.48	Diethylmalonic acid, ditridecyl ester
23	21.360	1.26	10-Acetoxy-2-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydro-2H-picene-4a-carboxyl
24	23.525	0.97	Colchicine, N-deacetyl-N-trifluoroacetyl-3-demethyl-
25	26.288	0.74	Androst-5-en-7-one, 3,17-bis(acetyloxy)-19-(methoxymethoxy)-4,4-dimethyl-, (3.beta.,17.beta.)-
26	27.865	0.40	Benzamide, N-(2'-benzoylphenyl)-2-iodo-
27	27.919	0.41	2(1H)-Pyrimidinone, 4-(trimethylsilyloxy)-1-[5-O-(trimethylsilyloxy)-beta.-D-ribofuranosyl]-, cyclic benzeneboronate
28	28.293	2.86	1,3-Xylyl-18-crown-5, 2-(benzo-1,3,2-dioxaborol-2-yl)-, methylamine(N-B)
29	28.595	0.91	Lanost-9(11)-en-18-oic acid, 20,23-dihydroxy-3-oxo-, gamma.-lactone, (20.xi.)-
30	29.285	0.50	D:A-Friedooleanan-28-oic acid, 3.beta.-hydroxy-
31	32.819	2.32	Methyl 22-methyl-octacosanoate
32	33.155	2.19	Z-23-Dotriaconten-2-one
33	33.255	0.61	Thiophene, 2,5-bis(diphenylphosphinyl)-
34	33.499	0.31	1-(4,8,11-Trihexanoyl-1,4,8,11-tetraazacyclotetradec-1-yl)-hexan-1-one
35	34.094	1.35	Hexacosanoic acid, methyl ester
36	34.125	0.29	2,3,3,3-Tetrafluoro-2-methoxy-propionic acid 3-(2-chloro-4-trifluoromethyl-phenoxy)-4-nitro-phenyl ester
37	34.320	1.70	Z-27-Hexatriaconten-2-one
38	35.471	5.84	Hexadecanoic acid, ethyl ester
39	35.555	0.99	Tritriacontane
40	35.640	1.98	1-Nickela-2-azonia-3-azacyclopentadieno[a]naphthalene, 2-(2,6-dimethyl-4-methoxyphenyl) cyclopentadieny
41	37.850	0.16	Demeclocycline
42	38.737	9.69	9,12-Octadecadienoic acid, ethyl ester
43	38.836	1.97	Ethyl Oleate
44	40.331	2.33	3.beta.-Hydroxy-5-cholen-24-oic acid
45	45.459	12.85	Bis(2-ethylhexyl) phthalate
46	45.944	0.88	Cholestane, 2-(2-nitrophenyl)-
47	45.995	0.49	Cholestan-26-oic acid, 3,7,12,24-tetrakis(acetyloxy)-, methyl ester, (3.alpha.,5.beta.,7.alpha.,12.alpha.)-
48	46.075	0.68	3,4-Dimethyl-2-(6-oxo-7,11-diazatricyclo[7.3.1.0(2,7)]trideca-2(3),4-dien-11-yl)-5-phenyl-1,3,2-oxazaphospholidine-2-sulfide
49	48.929	0.17	Silane, [(3.beta.,5.alpha.,11.beta.,20R)-pregnane-3,11,20,21-tetraol]tetrakis(oxy)tetrakis(trimethyl-
50	48.950	1.01	1,3,5,7-Tetraethyl-1,7-dibutoxytetrasiloxane
51	49.080	0.67	13-Methyl-Z-14-nonacosene
52	57.429	14.70	Gamma-sitosterol

The total phenolic and flavonoids in plant and seeds of *Persicaria salicifolia*

The qualitative analysis of the total phenol and flavonoids in seeds of *Persicaria salicifolia* using spectrophotometer were tabulated and illustrated in **table 4**. The results showed that the plant recorded 25.7 mg/g while seeds recorded 45.3 mg/g for the total phenol, while seeds have the highest value followed by the plant for the total flavonoids as they recorded 5.04 mg/g and 3.68 mg/g respectively.

Table 4. The total phenol and flavonoids in plant and seeds of *P. salicifolia*

Sample	Total phenol mg/g	Total flavonoids mg/g
Seeds	45.3	5.04

From the total phenolic and flavonoid profile of the studied taxa; *P. salicifolia* seeds extract is characterized from plant extract in presence of phenolic and flavonoid high content, this may explain why the antioxidant activity of *P. salicifolia* is slightly stronger than the plant extract. This is in accordance with **Jay et al. (2006)**²¹, who mentioned that plants rich in flavonoid compounds give high antioxidant effect. All the previous results indicated that the extracts of the studied taxa have a noticeable effect on the scavenging of free radicals and can be regarded as promising candidates for a plant derived antioxidant compounds and reveal that Egyptian species offer an interesting source of new antioxidative plant extracts, such as those of *P. salicifolia* there being a potential for their use in different fields (foods, cosmetics, pharmaceuticals) and may encourage their consumption for health protection. The results of presence of phenol and flavonoid compounds agree with different studies.

II-Identification of free phenolic acids using HPLC:

The qualitative and quantitative analysis of phenolic acids for *P.salicifolia* seeds using HPLC **Smolarz, 2000**³⁴). were tabulated and illustrated in **table5**. The results showed that *P.salicifolia* seeds contain four phenolic acids, caffeic acid recorded the highest value 28.6 mg/ml followed by fumaric acid (21.4 mg/ml), while coumaric acid and ferulic acid have the lowest values 15.6

mg/ml and 12.55 mg/ml respectively. **Sawicka et al., (2002)** used gradient thin-layer chromatography and densitometric determination has been applied to the qualitative and quantitative analysis of three phenolic acids, gallic acid, caffeic acid, and protocatechuic acid in *P.bistortae L.* from the Polygonaceae family. Where **Hussein et al., 2013**¹⁹ isolated and identified gallic acid from *Persicaria Sp.* during their study on four selected species of *Persicaria* in Egypt .

Table 5. HPLC analysis of Free phenolic acids of *P.salicifolia* seeds.

Seeds phenolic acids	R _t of stander	R _t	mg/ml
Ferulic acid	3.8	3.8	12.55
Caffeic acid	6.8	6.8	28.6
Fumaric acid	7.8	7.8	21.4
Coumaric acid	10.6	10.6	15.6

Identification of the phenolics from *P. salicifolia* seeds LC-MS:

The LC/DAD method previously used was modified to be compatible with the LC/MS system; acetic acid was replaced by the more volatile formic acid and its concentration was reduced to 0.1%. As a consequence, the ionic strength decreased and the signal-to-noise ratio increased in the negative ion mode. The gradient profile used in this study allowed the separation of all the compounds studied with a retention that, in general, followed the expected reversed-phase pattern of flavonoid aglycones Twelve different polyphenols were identified in the methanol extract of *P. salicifolium* seeds growing in Egypt using LC-ESI/MS (**Table 6** and **Fig.1**). displays the chromatogram analysis of the methanol extract at the negative mode. The reconstructed MS spectra from the chromatogram revealed the deprotonated molecular-ions [M+H]⁻ of eight flavonoids and three phenolic acids with the following m/z values; 290, 287, 376,,436,516, 580, 462, 610, 442, 288, 302, 286 and 302 belong .(-)-epicatechin, luteolin, pentamethoxy quercetin , 2'-O-methylcajanone, 2'',5'',4',5,6'',7 methoxy isovitrxin, sciadpitysin, .diosmetin 7-O- glucoside, rutin , quercetin-3-O-rhamnoside (quercitrin), dehydrokeampferol, 4' methoxy dehydrokeampferol, kaemphero and quercetin respectively. In addition to m/z values;

162, 108 and 148 belongs to 6-Hydroxycoumarin, cinnamic acid and p-cresol.

The spectra generated for cinnamic and by ions pray in the positive ion mode gave the de protonated molecule $[M+ H]$ and some fragments even at relatively low de clustering potentials. For instance, loss of CO_2 was observed for cinnamic acid, and loss of $C=O$ was observed for hydroxy coumarin .

The flavonoids aglycones gave Retro-Diels–Alder fragmentation as described. (Fabre *et al.*,2001)¹³ For instance, the m/z 151 ion is common for all the aglycones studied, the m/z 117 ion is characteristic for apigenin and its derivatives, whereas the m/z 119 ion is characteristic for quercetin and derivatives. Isorhamnetin exhibits specific fragmentation with the loss of $\dot{C}H_3$ radical from the deprotonated aglycone molecule, thus giving m/z 315 m/z 300 as described.³⁸⁻⁴²

Table 6. Tentative identification of the phenolics compounds from *P.salicifolia* seeds using LC-MS.

No	Rt	Mol. wt.	Fragmentation	Compounds
1	9,12	290	290(25%), 152(35%), 139(100%), 123 (25%)	(-)-Epicatechin
2	10.03	287	287(100%)	Luteolin
3	13.2	376	376(25%), 210(30%), 195(30%), 167(100%).	Pentamethoxyquercetin
4	14.251	436	436(100%), 421(95%), 219(35%)	2'-O-Methylcajanone
5	17.05	516	526(30%), 501(15%), 435(100%),341(50%),363(30%)	2'',5'',4',5,6'',7 methoxyisovitrxin
6	18.02	580	580(100%),290(20%), 167(20%)	Sciadpitysin
7	18.55	462	300(100%), 257(25%),	Diosmetin7-O-glucoside
8	18.91	611	611 (100%), 301 (40%)	Rutin
9	20.28	442	447 (25%), 301 (100%)	Quercetin-3-O-rhamnoside (quercitrin)
10	20.88	288	288(40%),257	dehydrokeampferol
11	21.49	302	302(70%), 150(80%), 137(95%)	4'methoxy dehydrokeampferol
12	22.37	162	162(100%),134(88%), 78(44%)	6-Hydroxycoumarin
13	22.4	148	148(100%)	Cinnamic acid
14	22.6	108	108 (100%)	P-Cresol
15	22.9	286	286(100%)	Kaempferol
16	23.1	302	302(100%),137 (25%), 153(22%)	Quercetin

Rt.=Retention time Mol. Wt.= Molecular weight

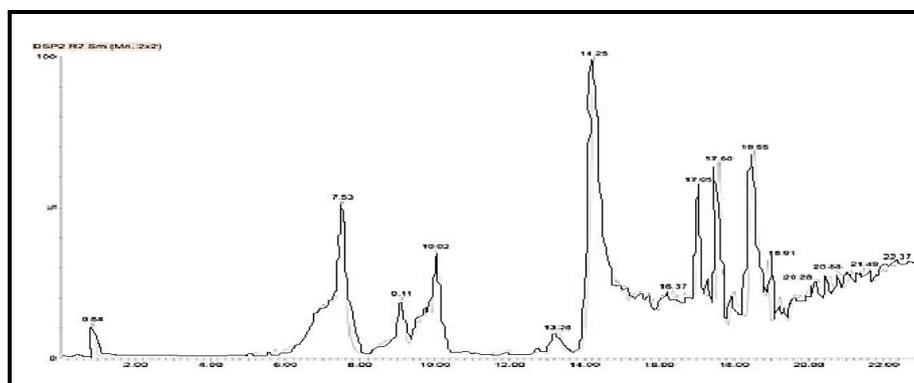


Fig.1. LC- Ms Spectrum of phenolics of *P. salicifolia* seeds

REFERENCES

1. **Abd El-Kader, A.M.; El-Readi, M.Z.; Ahmed, A.S.; Nafady, A.M.; Wink, M. and Ibraheim, Z.Z.**(2013). Polyphenols from aerial parts of *Polygonum bellardii* and their biological activities. *Pharm Biol.*, 51(8):1026-1034.
2. **Abd El-Kader, A.M.; Nafady, A.M.; Ahmed, A.S. and Ibraheim, Z.Z.**(2012). Antioxidant, hepatoprotective and antimicrobial activities of the aerial parts of *Polygonum bellardii* All. *Bull. Pharm. Sci.*, 35(1): 43-54
3. **Adams, R.P.** (1989). Identification of Essential Oils by Ion Mass Spectrometry, Academic Press, New York.
4. **Ahmad ,R.; Baharum, S.N.; Bunawan, H.; Lee, M.; Noor, N.M.; Rohani, E.R.; Ilias, N. and Zin, N.M.**(2014). Volatile Profiling of Aromatic Traditional Medicinal Plant, *Polygonum minus* in Different Tissues and Its Biological Activities. *Molecules*, 19: 19220-19242.
5. **-Baraka DM, (1985).** Ecological and Phytochemical Study on one Species of Polygonaceae. M.Sc. Thesis, Fac. Sci. Zagazig University (Benha) Egypt, p. 171,
6. **-Baytop, T.**(1984). Therapy with medicinal plants in Turkey (past and present). *Istanbul Univ. Pub. Istanbul*. P313 .
7. **Boulos , L.** (1999): Flora of Egypt. Vol 1. Al Hadara Publishing Cairo, Egypt. 153 -187.
8. **-Chaplin, M.F., Kennedy, J.F., (1994):** Carbohydrate Analysis, A practical Approach. Oxford Univ. Press, Oxford, New York, Tokyo. 2nd Ed., pp. 324.
9. **-Cheng ,H.B.; Liu, X.Q. and Chen, K.L.**(2012). Chemical constituents of ethyl acetate extract from *Polygonum perfoliatum*, *Zhong Yao Cai.*, 35(7):1088-1090
10. **-Danny, K. A.; J. H. Yun; M. B. Dian and E. M. Alyson (2013).** Comparison of total phenolic content of freeze dried and air dried Marino-berry, Strawberry and Corn, growing using conventional organic and sustainable agricultural practices. *J. of Food and Agricultural Chem.* 51: 1237 – 1241.
11. **-Datta, B. K.; Rahman, M. M.; Gray, A. I.; Nahar, L.; Hossein, S. A.; Auzi, A. A. and Sarker, S. D.** (2007). Polygodic acid, a new cadinane sesquiterpene from *Polygonum viscosum*, inhibits the growth of drug-resistant *Escherichia coli* and *Staphylococcus aureus* (MRSA) in vitro. *J Nat Med*, 61(4): 391-396.
12. **-El-Haci, I.A.; Bekkara, F.A.; Mazari, W.; Hassani, F. and Didi, M.A.** (2013). Screening of biological activities of *Polygonum maritimum* L. from Algerian coast. *Asian Pac J Trop Biomed*, 3(8): 611-616.
13. **-Fabre, N.; Rustan, I.; de Hoffmann, E. and Quetin-Leclercq, J.** (2001) :Determination of flavone, flavonol, and flavanones aglycones by negative ion liquid chromatography electrospray ion trap mass spectrometry. *J. Am. Soc. Mass Spectrom.* Vol 12: 707.
14. **-Ferran Sa´nchez-Rabateda; Olga- Ja´uregui ; Isidre- Casals and Cristina -Andre´s-Lacueva (2003):** Liquid chromatographic/electrospray ionization tandem mass spectrometric study of the phenolic composition of cocoa (*Theobroma cacao*) *J. Mass Spectrom.* Vol, 38: 35–42.
15. **-Fukuyama, Y.; Sato, T.; Miura, I.; Asakawa, Y. and Takemoto, T.**(1983). Hydropiperoside, a novel coumaryl glycoside from the root of *Polygonum hydropiper*; *Phytochemistry*, 22(2): 549–552.
16. **Gadallah, M.A.A. and Sayed, S.A.** (2014). Impacts of different water pollution sources on antioxidant defense ability in three aquatic macrophytes in Assiut province, Egypt. *Journal of stress physiology & Biochemistry*, 10(3):47-61.
17. **-Granica ,S.; Czerwińska, M.E.; Żyżyńska-Granica, B. and Kiss, A.K.** (2013). Antioxidant and anti-inflammatory flavonol glucuronides from *Polygonum aviculare* L., *Fitoterapia*, 91: 180–188.
18. **-Hussain, F.; Ahmad B.; Hameed, I.; Dastagir , G.; Sanaullah, P. and Azam ,S.**(2008). Antibacterial, antifungal and insecticidal activities of some selected medicinal plants of polygonaceae. *African Journal of Biotechnology*, 9(31): 5032-5036.
19. **-Hussein, S.R. and Mohamed, A.A.** (2013). Antioxidant activity and phenolic profiling of two Egyptian medicinal herbs *Polygonum salicifolium* Brouss ex Wild and *Polygonum senegalense* Meisn, *Analele Universităţii din Oradea, Fascicula Biologie*, 20(2):59-63..
20. **-Ibrahim, T.A. and El-Hela, A.** (2012). Polysaccharide from the root of *Polygonum equisetiforme* Sibth. & Sm.: isolation, chemical composition, antioxidant potential and cytotoxic activity. *Int J Pharma Bio Sci*, 3(4):478-492.
21. **-Jay, M.; Viricel , M.R. and Gonnet, J.F.** (2006): C Glycosyl flavonoids. p. 898. In Andersen, Q.M., Markham, K.R., *Flavonoids: Chemistry, Biochemistry and Applications.* CRC press, New York.
22. **-Justesen , U.** (2001): Collision-induced fragmentation of deprotonated methoxylated flavonoids, obtained by electrospray ionization mass spectrometry. *J. Mass Spectrom.* Vol 36: 169.
23. **-Kim, H.J.; Woo, E.R. and Park, H.** (1994). A Novel Lignan and Flavonoids from *Polygonum aviculare*. *Journal of Natural Products*, 57 (5): 581–586
24. **-Korulkina, D. Yu. and Muzychkina, R. A.** (2015). Correlation of Structure and Antiviral Activity of Alkaloids of *Polygonum* L. Plants Growing in Kazakhstan *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering* Vol:9, No:7,
25. **-KUBINOVA, R.; Porizkova, R.; Bartl, T.;**

- Navratilova, A.; Cizek, A. and Valentova, M. (2014). *Biological activities of polyphenols from Polygonum lapathifolium*, *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, 13 (6): 506 – 516.
- 26 -Kumar, M.D.; Deepmala, J. and Sangeeta, S. (2012). Antioxidant, antipyretic and choleric activities of crude extract and active compound of *Polygonum bistorta* (Linn.) in albino rats. *Int. J. Pharm. Bio. Sci.*, 2 (1):25-31.
- 27 -Li-shuang, L.V.; Gu, X.; Ho, C. T. and Tang, J. (2006). Stilbene glycosides from the roots of *Polygonum multiflorum* Thunb and their *in vitro* antioxidant activities. *Journal of Food Lipids*, 13 : 131–144.
- 28 -Liu M, Luo C, Zhang Y and Qiu D, Analgesic, (2007). anti-inflammatory, and diuretic effects of *Polygonum capitatum* and *Toddalia asiatica*. *Guizhou Medical Journal*. 31: 370-371,
- 29 -Liu Z, Qi J, Zhu D and Yu B, (2008). Chemical constituents from *Polygonum capitatum* and their anti-oxidation activities *in vitro*. *Zhong Yao Cai (Journal of Chinese Medicinal Materials)*. 31: 995-998,
- 30 -Malik, E.P. and Singh, M.B. (1980). *Plant Enzymology and Histochemistry* (1st Edn.) Kalyani Publishers: New Delhi; 286.
- 31 -Midiwo, J. O.; Yenesew, A.; Juma, B. F.; Derese, S.; Ayoo, J. A.; Aluoch, A.O. and Guchu, S. (2002). Bioactive compounds from some Kenyan ethnomedicinal plants: Myrsinaceae, Polygonaceae and *Psiadia punctulata*. *Phytochemistry Reviews*, 1 (3): 311-323.
- 32 -Pérez-Magariño, S.; Revilla, J.; González-Sanjosé, M.L. and Beltrán, S. (1999). Various applications of liquid chromatography–mass spectrometry to the analysis of phenolic compounds. *J. Chromatogr. A*. Vol. 847: 75.
- 33 -Said, A.A. ; Aboutabl, E. A. ; Hussein A. A. and Abdel Raouf, G. F. (2015). The composition of the lipoidal matter of the seeds of *Pleio gynium timorense* (DC.) Leenh. *Egyptian pharmaceutical J.* 14 (1) , 65-68
- 34 -Smolarz, H. D. (2000). Chromatographical analysis of phenolic acids in some species of *Polygonum* L. genus. Part 2. Quantitative determination of the major components by high performance liquid chromatography (HPLC). *Acta Soc. Bot. Pol.*, 69(1):21-23.
- 35 -Szczêsna, T. (2007 b). A Study On The Sugar Composition Of Honeybee-Collected Pollen *Journal of Apicultural Science* Vol. 51 No. 1 ,15-22.
- 36 -Szczêsna, T. (2006). Protein Content and Amino acids Composition of Bee-collected Pollen Originating from Poland, South Korea and China, *Journal of Apicultural Science* Vol. 50 No. 2 .81-90.
- 37 -Tackholm V, Students' flora of Egypt. Beirut: Cairo University, Cooperative Printing Co. (1974).
- 38 -Yagi, A. (1996). Effect of *Polygonum hydropiper* sulfated flavonoids on lens aldose reductase enzymes. *J Nat Prod*. 59: 443- 445,
- 39 -Yang, Y.X.; An, M.M.; Jin, Y.S. and Chen, H.S. (2016). Chemical constituents from the rhizome of *Polygonum paleaceum* and their antifungal activity. *Jasian Nat Prod Res*, 16: 1-6.
- 40 -Yang, Y.; Yu, T.; Jang, H.; Byeon, S.E.; Song, S.; Lee, B.; Rhee, M.H.; Kim, T.W.; Lee, J.; Hong, S. and Cho, J.Y. (2012). *In vitro* and *in vivo* anti-inflammatory activities of *Polygonum hydropiper* methanol extract. *Journal of Ethnopharmacology*. 139(2):616-625.
- 41 -Yunuskhodzhaeva, N.A.; Abdullabekova, V.N.; Ibragimova, K.S. and Mezhlumyan, L.G. (2014). Amino-Acid Composition of *Urtica dioica* Leaves and *Polygonum hydropiper* and *P. aviculare* Herbs. *Chemistry of Natural Compounds* , 50(5):970-971.

Phytochemical Studies on *Persicaria salicifolia* Seeds growing in Egypt

دراسات كيميائية على بذور نبات الزلف (*Persicaria salicifolia*) النامي بمصر

*زينب أحمد السويقي و *دعاء أحمد معاطي و *محمد محمد يوسف و *عاطف أحمد الحيله

*قسم النبات والميكروبيولوجي - كلية العلوم - جامعة الأزهر (فرع البنات) - مدينة نصر

**قسم العقاقير - كلية الصيدلة - جامعة الأزهر (فرع البنين) - مدينة نصر

تم عمل مسح كيميائي لبذور نبات الزلف *Persicaria salicifolia* وتم تعيين الأحماض الأمينية والدهنية والفينولية كمياً ونوعياً. وقد تم استخلاص وتعريف اثنين وخمسون مركب فعال من مستخلص الهكسان للبذور باستخدام كروماتوجرافيا السائل المدمج جهاز الكتلة. وقد مثل ستيروول نسبة عالية سجلت %14.7 من بين المركبات وباي -2-اثيل هكسيل فثاليت %12.85 والاوكتاديكانويل استر %9.69 و%9 والهكساديكانويك اثيل استر %5.84. وكذلك تم دراسة المحتوى الفينولي والفلافونيدى الكمى والكيفى وكذلك التعرف على المحتوى الفينولى والفلافونيدى باستخدام جهاز كروماتوجرافيا السائل المتصل بمطياف الكتلة.