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Research Article

Chemical profiling of *Oxalis* species growing wild in Egypt using HRLC/MS Spectrometry

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Abstract: Three medicinally promising *Oxalis* species, namely *O. pes-caprae*, *O. corymbosa & O. latifolia* were collected from Egyptian flora and their methanolic extracts were subjected to LC-QTOF-MS analysis to annotate their chemical profiles. Subsequently, 50 compounds belonging to various chemical classes were identified and characterized, of which 34 compounds were first reported from *Oxalis* L.. Moreover, five flavone compounds were separated and identified from *O. pes-caprae*; their structures were elucidated using acid hydrolysis, UV/vis, ¹H-NMR, and HR-ESI-MS. The chemotaxonomic relationship of the studied species was evaluated and the extracted data were statistically analyzed and classified *Oxalis* sp. into two distinct clusters. Each cluster was characterized by special chemical features that helped in distinguishing between them.

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1. INTRODUCTION

Oxalis L. is a genus of family Oxalidaceae R.Br. representing nearly 500 accepted species being worldwide distributed, with special richness in the area from South America to the southern region of North America and at the South-Western Cape region of South Africa (Oberlander *et al.*, 2002; The plant list, 2013). The genus is represented in Egypt by five species and one variety (Täckholm, 1974; El-Khanagry, 2005).

The pharmacological and therapeutic potential of some *Oxalis* plants was evaluated by identifying their biologically active chemical constituents, such as *O. pes-caprae* L. which has antioxidant and neuroprotective activities (Gaspar *et al.*, 2018). While some other species have been reported for their antioxidant, anti-diabetic, anti-cancer, antibacterial, and enzyme-inhibiting activities (Prasad Pandey *et al.*, 2020; Sarfraz *et al.*, 2022).

O. corymbosa DC. and *O. latifolia* Kunth had not been subjected to any chemical study prior to work on the nonpolar fraction by Draz *et al.* (2021). Limited studies have been reported about the other *Oxalis* species. Some of those works concerned with their nonpolar chemical profiles

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(Draz *et al.*, 2021) while others provided their chemical profiles using polar fractions (Güçlütürk *et al.*, 2012; Gaspar *et al.*, 2018; Prasad Pandey *et al.*, 2020). In addition, the separation, and identification of flavonoids and phenolic acids from *O. pes-caprae* were carried out by Della Greca *et al.* (2009) and Güçlütürk *et al.* (2012).

Lourteig (2000) proposed a system for classifying *Oxalis* L. based on its morphological characters and concluded *O. corymbosa* and *O. latifolia* under section Ionoxalis, while *O. pescaprae* was placed under section Cernuae. There weren't any classifying systems to concern with the chemical characters of this genus before, so the present study aims to evaluate the chemotaxonomic relationship between the studied species.

2. MATERIAL and METHODS

2.1. Plant Materials

Three *Oxalis* plants were collected from different localities in Egypt by Amal A. Draz and were identified and deposited in the Herbaria of the Faculty of Science, Cairo University (CAI) and National Research Centre (CAIRC) by Dr. Eman M. Shamso. *O. corymbosa* DC. (voucher No. A303) was collected from the National Research Centre gardens, Dokki, Giza in March, 2019, while *O. pes-caprae* L. (voucher No. A304) was collected from Bramly's grotto, Burg El-Arab in February, 2019 as well as *O. latifolia* Kunth (voucher No. A305) was collected from Faculty of Science gardens, Cairo University, Giza in April, 2019.

2.2. LC/QTOF/MS Analysis

The LC-QTOF-MS analysis was performed using plant materials that had been cleaned, dried, and ground into a fine powder. Fifty grams of each plant were soaked in 70% MeOH three times, 2 days/each time; the extracts were filtered and evaporated under reduced pressure and temperature till full dryness using Rota-vapour apparatus. Next, 30 mg of each prepared crude extract was analyzed using the -ve mode (Triple TOF 5600+ Sciex) in accordance with the method of Eissa *et al.* (2020). The profiling and annotation of their chemical constituents were described (Table 1).

2.3. Isolation of Pure Compounds

Air-dried and powdered 1.6 kg of *O. pes-caprae* was extracted using 70% MeOH, filtered, and then dried using Rota-vapour apparatus. The crude extract was then suspended in distilled water (500 ml) and fractionated with ethyl acetate (65 g) and n-butanol (98 g). For further separation, each fraction was subjected to column and paper chromatographyaccording to the method of Hussein *et al.* (2018). The identification of the separated compounds was achieved through: NMR experiments that were carried out by using Jeol EX-500 spectrometer: 500 MHz (¹H NMR); UV absorption spectra were recorded on a Shimadzu model-2401CP spectrophotometer, and HR-ESI-MS on a Triple TOF 5600⁺Sciex. Authentic samples were obtained from the department of phytochemistry and plant systematics, NRC. Five compounds were separated, two of which were identified via ¹H-NMR, UV/vis, and HR-ESI-MS (compounds 29 & 37), while the other three compounds were identified through co-chromatography with authentic standards (compounds 40, 38 & 33) (Table 1).

2.4. Statistical Analysis

Multivariate statistical analysis of 50 character states was performed using Minitab 21 software for detecting the affinities among the variable chemical profiles of the investigated plants and a dendrogram was plotted.

3. RESULTS and DICUSSION

3.1. LC/QTOF/MS Analysis

The LC/QTOF/MS analysis revealed the identification and characterization of 50 compounds belonging to different chemical classes from the methanolic extracts of three *Oxalis* L. plants (*O. corymbosa*, *O. latifolia* & *O. pes-caprae*) (Table 1; Figure 1).

3.1.1. Phenolic acid derivatives

They were recorded in different *Oxalis* species. Compounds (1), (2), (3), and (4) were identified in all of the studied taxa, whereas the rest of compounds showed a selective occurrence in certain species and were absent in others.

Compound (5) was only detected in the extract of *O. pes-caprae* at m/z 153.0188 and R_t 4.94 min It produced a major daughter fragment ion at m/z 109 due to loss of CO₂, that supported its identification as 3,4-Dihydroxybenzoic acid (Ruan *et al.*, 2019).

Compound (6) was detected at $R_t 4.96$ min with m/z 359.0782 in all of the studied taxa except for *O. latifolia*. It gave fragmentation pattern of m/z 197, 179, 161 that was in compliance with that of rosmarinic acid (Wang *et al.*, 2021).

Compound (7) was characteristic of *O. latifolia*. This compound appeared at m/z 137.0247 (at R_t 6.97 min.) and produced a daughter fragment ion at m/z 93, suggesting its identification as "salicylic acid" (Lin *et al.*, 2020).

Although compound (8) was only absent from *O. Latifolia*, it was reported at m/z 167.0356 (at R_t 7.14 min.) and produced a fragment ion at m/z 152 due to the loss of CH₃ group in the remaining studied taxa. Thus, it was suggested to be "5-Methoxysalicylic acid" (Massbank Record: PS104208).

3.1.2. Carboxylic acids

A wide variation of carboxylic acids have been detected in the studied taxa, of which the compounds (9), (10), (12), (13), (14), and (15) were detected in all of the studied taxa, while the remaining compounds showed a selective occurrence among the studied taxa that developed a level of variation.

Compound (11) was identified as succinic acid as it appeared at m/z 117.0176 (R_t 1.19) and produced a series of fragments at m/z 99 [M-H-H₂O]⁻ and m/z 73 [M-H-CO₂]⁻ (Eissa *et al.*, 2020). This compound was detected in all of the investigated extracts except *O. latifolia*.

Compound (16) at R_t 1.25 min and m/z 195.0516 was identified as gluconic acid as it produced a fragmentation pattern of m/z 129, 177 and 159 (Lucas, 2021).

Compound (17) was exclusively detected in extracts of *O. corymbosa* and *O. latifolia*. Its fragment ion peak at R_t 1.26 min and m/z 160.0615 produced the fragment ions m/z 142 (due to loss of H₂O molecule) and m/z 116 (due to loss of CO₂ molecule) on the MS/MS fragmentation. This fragmentation spectrum was mostly compatible with that of "1H-indole-3-carboxylic acid" (Gamir *et al.*, 2012).

Compound (18) at $R_t 1.27$ min and m/z 105.0199 was absent only in *O. pes-caprae*. The fragmentation pattern of this compound was mostly compatible with that of glyceric acid, as it produced fragment ions at m/z 75, 73, 59, 57, 47 (Lucas, 2021).

Compound (19) that appeared at R_t 1.28 min and m/z 147.0288 was identified as citramalate as it showed the fragmentation pattern of m/z 129, 103, 87, 85 (Wu *et al.*, 2010). This compound was only present in *O. latifolia*.

Compound (20) was only detected in *O. corymbosa* extract at m/z 149.0451 (Rt 1.29 min.), and on MS/MS fragmentation, it produced a major fragment ion at m/z 73 in a fragmentation pattern being characteristic of tartaric acid (Brent *et al.*, 2014).

Compound (21) appeared at R_t 1.38 min and m/z 145.0613 was only recorded in the extracts of section Ionoxalis representatives (*O. corymbosa* and *O. latifolia*). It gave fragment ions at m/z 127, 101 in a MS/MS fragmentation pattern that was characteristic of 2-Methylglutaric acid (Lucas, 2021).

Compounds (22), (23), and (25) were detected only in *O. pes-caprae*. Those compounds had molecular ion peaks at m/z 175.0616 (at R_t 1.46 min), 138.0556 (at R_t 1.51 min), and 147.0459 (at Rt 4.83 min), respectively. Compound (22) gave a fragmentation pattern of m/z 157 (due to the loss of an H₂O molecule), 131 (due to the loss of a CO₂ molecule), 129, 115, 113 and 85 being characteristic of 2-Isopropylmalic acid (Ricciutelli *et al.*, 2019). Compound (23) gave a fragment ion at m/z 94, so it was characterized as 6-Hydroxynicotinic acid (Llorach *et al.*, 2009). Compound (25) gave fragment ions at m/z 119 and 103 (due to the loss of a CO₂ molecule) that confirmed its identification as cinnamic acid (Zhang *et al.*, 2010).

Compound (24) appeared at $R_t 2.15$ min and m/z 353.0878 was characterized as chlorogenic acid. Its spectrum showed fragment ions at m/z 191, 179, 173 (Zhang *et al.*, 2010). This compound was recorded in the extracts of all studied taxa except *O. corymbosa*.

3.1.3. Flavonoids

Seventeen flavonoid compounds were identified from the plants under investigation, of which compounds (29 & 37) were separated from *O. pes-caprae* and identified using UV/vis and ¹H-NMR techniques, and for further accuracy they were identified along with the compounds (38, 33 & 40) from the same species via co-chromatography with authentic standard samples (Figure 2).

Compounds (26) (Rt 4.36 min., m/z 449.1074) and (36) (Rt 7.95 min., m/z 433.10678) were identified as eriodictyol-7-O-glucoside and naringenin-7-O-glucoside, respectively, as confirmed by comparing their fragmentation patterns (m/z 287, 269) and (271, 151), respectively with the literature (Krasteva & Nikolov, 2008; Kang *et al.*, 2016). Those two compounds were detected in all of the studied taxa except for O. latifolia.

Compound (27) has molecular ion peak at m/z 289.0729 that appeared at Rt 4.93 min and gave fragment ions at m/z 245, 205, 179, 149, 123, and 109 being characteristic of "Catechin" (Kang *et al.*, 2016). Catechin was detected only in *O. corymbosa*.

Compounds (28), (29), and (37) are luteolin-*C*-glycoside derivatives that appeared at R_t 6, 6.42 & 8.11 with molecular ion peaks at m/z 447.0924, 461.1070 & 447.0887, respectively. They were detected in all of the studied taxa except compound (29) which was absent from *O. corymbosa*. Compounds (28) & (37) have the same fragment ions being m/z 429 [M-H-H₂O]⁻, 357 [M-H-90]⁻, and 327 [M-H-120]⁻. The intensities of the fragment ions of the two compounds were different, being 327 (100%), 357 (55%) for compound (28) and 327 (100%), 357 (19%) for compound (37) that suggested their identification as luteolin-6-*C*-glucoside and luteolin-8-*C*-glucoside, respectively (Hassan *et al.*, 2019) with further confirmation of compound (37) identification through UV/vis and ¹H-NMR (Almahy & Fouda, 2013). Compound (29) produced daughter fragment ions at m/z 446, 313, 298 that suggested its identification as luteolin-7-OCH₃-6-*C*-glucoside "swertiajaponin" (Wang *et al.*, 2008), an identification that was further confirmed using UV/vis and ¹HNMR (Kumarasamy *et al.*, 2004).

Compound (31) was only detected in *O. latifolia* at R_t 6.78 min and m/z 463.0924 with a major fragment ion at m/z 301, suggesting its identification as "quercetin 3-hexoside" (Marzouk *et al.*, 2018).

Compound (38) at R_t 9.52 min was identified as luteolin. It gave molecular ion peak at m/z 285.0387 with production of fragments 241, 217, 151, and 133 that corresponds to the luteolin aglycon (Marzouk *et al.*, 2018), an identification that was more confirmed via cochromatography with an authentic standard. While compound (33) was identified as luteolin-7-*O*-glucoside that appeared at R_t 7.21 and gave a molecular ion peak at m/z 447.0934 with producing the characteristic fragment ion of luteolin aglycon at m/z 285 that corresponds to the loss of the glucoside moiety [M-H-162]⁻ in addition to producing m/z 179, 151 (Marzouk *et al.*, 2018), an identification that was more confirmed via co-chromatography with an authentic standard.

Compound (34) was detected only in *O. pes-caprae* and *O. corymbosa* at R_t 7.64 min with molecular ion peak m/z 447.0917 and gave fragment ions at m/z 285, 133, so it was identified as maritimetin-6-*O*-hexoside (Cao-Ngoc *et al.*, 2020).

Compound (40) at R_t 10.45 and m/z 269.0469 was identified as being apigenin by comparing its fragmentation product ions (225, 151) with the literature and its co-chromatography with an authentic standard (Brito *et al.*, 2014). Apigenin was detected only in *O. pes-caprae* and *O. latifolia*. The fragmentation pattern of compound (32) at R_t 6.87 min and m/z 577.1547 showed a daughter fragment ion at m/z 269 that was characteristic of the apigenin aglycon (due to losing neohesperidoside moiety) and another fragment ion at m/z 413 that indicated the loss of a rhaminose moiety. This fragmentation pattern supported its identification as apigenin 7-Oneohesperidoside (Brito *et al.*, 2014). In the same manner, compound (35) at R_t 7.84 min and m/z 431.1192, it was found that it produced a fragment ion at m/z 269 which is characteristic of the apigenin aglycon after losing the glucose moiety from the original compound and its isotopic fragment also appeared at m/z 268 in addition to another fragment ion at m/z 311 and this supported its identification as apigenin-7-*O*-glucoside (Marzouk *et al.*, 2018).

Compound (39) was identified as naringenin (at R_t 10.13 min and m/z 271.0635) as it gave fragment ions at m/z 151, 125, 107 (Kang *et al.*, 2016).

Compound (41) was recorded only in the extract of *O. pes-caprae* (section: Cernuae) at R_t 12.17 min and *m/z* 267.0652. It gave fragment ions at *m/z* 252, 224 that were characteristic of formononetin (Zhao *et al.*, 2020).

Compound (42) appeared at R_t 14.67 min with a molecular ion peak at m/z 283.0672 and fragment ions at m/z 268, 240, 211, a fragmentation pattern being characteristic of acacetin (Ben Salah *et al.*, 2019). This compound was present only in *O. corymbosa* extract.

One coumarin was identified from the taxa under study being compound (43) that was detected only in *O. pes-caprae* at R_t 8.33 min and m/z 177.0925. It produced fragment ions at m/z 149, 133 that suggested its identification as esculetin (6,7-Dihydroxycoumarin) (Ruan *et al.*, 2019).

Compound (45) was characterized as a stilbene. It was recorded only in the extract of *O. corymbosa*. Its molecular ion peaks appeared at m/z 227.0718 and R_t 9.1 min with producing daughter fragment ions at m/z 185, 183, 159, 157, and 143 that was characteristic of resveratrol (Guerrero *et al.*, 2020).

Compound (50) belonging to the class of sugar alcohols was detected at $R_t 1.39$ min and m/z 181.0717. It gave fragmentation pattern of m/z 163, 119, 89, and therefore it was identified as mannitol (Gervasoni *et al.*, 2016). This compound was detected in *O. pes-caprae* and *O. corymbosa*.





Figure 2. Chemical structures of isolated compounds from Oxalis pes-caprae.



							Section	Reference	
	P				MS/MS	Cernuae	Ionox	alis	
NO.	(Min.) \mathbf{K}_t	M.Wt	[M-H] ⁻	Compound name	spectrum	Oxalis	Oxalis	Oxalis	
						pes- caprae	corymbosa	Latifolia	
				phenolic a	cid derivativ	res			
1	1.98	180	179.0341	Caffeic acid #	135, 161	+	+	+	Marzouk <i>et al.</i> , 2018
2	2	164	163.03873	<i>P</i> -Coumaric acid #	93, 119	+	+	+	Zhong <i>et al.</i> , 2020
3	2.25	194	193.04855	ferulic acid #	178, 149	+	+	+	Zhong <i>et al.</i> , 2020
4	3.31	138	137.0238	<i>P</i> -Hydroxybenzoic acid #	93	+	+	+	Zhong <i>et al.</i> , 2020
5	4.94	154	153.0188	3,4- Dihydroxybenzoic acid	109	+	-	-	Ruan <i>et al</i> ., 2019
6	4.96	360	359.07828	Rosmarinic acid	161, 179, 197	+	+	-	Wang <i>et al.</i> , 2021
7	6.97	138	137.02477	Salicylic acid	93	-	-	+	Lin <i>et al.</i> 2020
8	7.14	168	167.0356	5-Methoxysalicylic acid	152	+	+	-	(Massbank Record: PS104208)
				Carbo	xylic acids				
9	1.16	134	133.01486	Malic acid #	70, 71, 115	+	+	+	Brent <i>et al.</i> , 2014
10	1.17	210	208.9362	Galactaric acid	71, 85, 191	+	+	+	Lucas, 2021
11	1.19	118	117.01762	Succinic acid	73, 99	+	+	-	Eissa <i>et al.</i> , 2020
12	1.21	116	115.00307	Maleic acid	71	+	+	+	Brent <i>et al</i> ., 2014
13	1.22	174	173.00958	Cis-Aconitate	85, 111, 129	+	+	+	Lucas, 2021
14	1.23	192	190.97238	Citric acid #	111, 129	+	+	+	Marzouk <i>et al.</i> , 2018
15	1.24	192	191.05583	Quinic acid	85, 111, 127, 173	+	+	+	Marzouk <i>et al.</i> , 2018
16	1.25	196	195.05165	Gluconic acid	129, 159, 177	+	+	-	Lucas, 2021
17	1.265	161	160.06158	1H-indole-3- carboxylic acid	142, 116	-	+	+	Gamir <i>et al.</i> , 2012
18	1.27	106	105.01991	Glyceric acid	73, 75, 57, 59, 47	-	+	+	Lucas, 2021
19	1.28	148	147.02885	Citramalate	85, 87, 129, 103	-	-	+	Wu <i>et al.</i> , 2010
20	1.29	150	149.04518	Tartaric acid #	73	-	+	-	Brent <i>et al.</i> , 2014
21	1.38	146	145.06139	2-Methylglutaric acid	101, 127	-	+	+	Lucas, 2021

 Table 1. Different chemical classes detected in the studied Oxalis taxa by using LC-QTOF-MS.

22	1.46	176	175.06165	2-Isopropylmalic acid	157, 129, 131, 115, 113, 85	+	-	-	Ricciutelli et al., 2019
23	1.51	139	138.0556	6-Hydroxynicotinic acid	94	+	-	-	Llorach <i>et al.</i> , 2009
24	2.15	354	353.0878	Chlorogenic acid #	173, 179, 191	+	-	+	Zhang <i>et al.</i> , 2010
25	4.83	148	147.04594	Cinnamic acid	103, 119	+	-	-	Zhang <i>et al.</i> , 2010
				Flav	ronoids				
26	4.36	500	449.10742	Eriodictyol-7- <i>O</i> - glucoside	269, 287	+	+	-	Krasteva & Nikolov, 2008
27	4.93	290	289.07294	Catechin	109, 123, 149, 179, 205, 245	-	+	-	Kang <i>et al.</i> , 2016
28	6.00	448	447.09247	Luteolin-6- <i>C</i> - glucoside [#]	285, 297, 327, 357, 429	+	+	+	Hassan <i>et al.</i> , 2019
29	6.42	462	461.10702	Luteolin-7-OCH ₃ -6- <i>C</i> -glucoside [§]	298, 313, 415, 446	+	-	+	Wang <i>et al.</i> , 2008; Kumarasamy <i>et al.</i> , 2004
30	6.45	578	577.2334	Vitexin-2"-O- rhamnoside	269, 293, 311, 341, 413	+	+	+	Hassan <i>et al.</i> , 2019
31	6.78	464	463.09241	Quercetin 3- hexoside	301	-	-	+	Marzouk <i>et al.</i> , 2018
32	6.87	578	577.15472	Apigenin 7- <i>O</i> - neohesperidoside	413, 269	+	+	+	Brito <i>et al.</i> , 2014
33	7.21	448	447.09341	Luteolin-7- <i>O</i> - glucoside *#	285, 179, 151	+	+	+	Marzouk <i>et al.</i> , 2018
34	7.64	448	447.09177	Maritimetin-6-O- hexoside	285, 133	+	+	-	Cao-Ngoc <i>et</i> <i>al.</i> , 2020
35	7.84	432	431.11929	Apigenin-7- <i>O</i> - glucoside [#]	268, 269, 311	+	+	+	Marzouk <i>et al.</i> , 2018
36	7.95	434	433.10678	Naringenin-7- <i>O</i> - glucoside	271, 151	+	+	-	Kang <i>et al.</i> , 2016
37	8.11	448	447.08871	Luteolin-8- <i>C</i> - glucoside ^{#\$}	327, 357, 429	+	+	+	Hassan <i>et al.</i> , 2019; Almahy & Fouda, 2013
38	9.52	286	285.03873	Luteolin *#	133, 217, 241, 151	+	+	+	Marzouk <i>et al.</i> , 2018
39	10.13	272	271.06351	Naringenin	107, 125, 151	-	+	+	Kang <i>et al.</i> , 2016
40	10.45	270	269.04691	Apigenin *#	151, 225	+	-	+	Brito <i>et al.</i> , 2014
41	12.17	268	267.06525	Formononetin	224, 252	+	-	-	Zhao <i>et al.</i> , 2020

Table 1. Continues.

42	14.67	284	283.06726	Acacetin #	268, 240, 211	-	+	-	Ben Salah <i>et</i> <i>al.</i> , 2019
Coumarins									
43	8.33	178	177.0925	Esculetin	133, 149	+	-	-	Ruan <i>et al.</i> , 2019
Stilbenes									
44	4.81	406	405.17596	Astringin	225, 243	+	+	+	Guerrero <i>et</i> <i>al.</i> , 2020
45	9.10	228	227.07181	Resveratrol	143, 157, 159, 183, 185	-	+	-	Guerrero <i>et</i> <i>al.</i> , 2020
				Te	rpenes				
46	1.26	136	135.02867	Gamma-terpinene	89, 117	+	+	+	(MassBank Record: PT208863).
				S	ugars				
47	1.21	260	259.02151	Mannose 1- phosphate	79	+	+	+	Cocuron & Alonso 2014
48	1.41	180	179.0554	Hexoside	89, 161	+	+	+	Jin <i>et al.</i> 2018
49	1.47	342	341.10941	Sucrose	89, 119, 161, 179	+	+	+	Jin <i>et al.</i> 2018
Sugar alcohols									
50	1.39	182	181.07173	Mannitol	8 9, 119, 163	+	+	-	Gervasoni <i>et</i> <i>al.</i> 2016

Table 1. Continues.

Notes: #Compounds that were previously identified from genus Oxalis L.

^{\$} Compounds that were separated and identified in the present study from *O. pes-caprae* using ¹H-NMR and UV/vis.

*Compounds that were identified in the present study from *O. pes-caprae* via co-chromatography.

+ =present, - =absent

3.2. Statistical Analysis

The statistical analysis was presented in a dendrogram that showed two clusters (I & II) at similarity level 50.2%. Cluster I ended with *O. pes-caprae* while cluster II included both *O. corymbosa* and *O. latifolia* at 58.19% similarity level, results being in accordance with Lourteig's (2000) findings about the sectional affinity of those species (Figure 3).





3.3. Chemosystematic Evaluation

According to Lourteig (2000), the investigated members of *Oxalis* in this study could be distributed under two sections *viz*. section Cernuae Knuth includes *O. pes-caprae* and section Ionoxalis (small) Knuth comprises *O. corymbosa* and *O. latifolia*.

On comparing the chemical profiles of the studied taxa, significant differences among them were recorded, where *O. pes-caprae* can be recognized by the presence of 3, 4-Dihydroxybenzoic acid, esculetin, formononetin, cinnamic acid, 6-Hydroxynicotinic acid and 2-Isopropylmalic acid in its extract and lacking of naringenin, glyceric acid, 1H-indole-3-carboxylic acid and 2-Methylglutaric acid compounds.

O. corymbosa was characterized by presence of catechin, tartaric acid, acacetin and resveratrol, in addition to the absence of chlorogenic acid, apigenin and luteolin-7-OCH₃-6-C-glucoside in its extract.

O. latifolia can be distinguished from the other species by lacking of maritimetin-6-*O*-hexoside, 5-Methoxysalicylic acid, succinic acid, gluconic acid, eriodictyol-7-*O*-glucoside, mannitol, naringenin-7-*O*-glucoside, and rosmarinic acid in its extract, as well as the presence of salicylic acid, citramalate, and quercetin 3-hexoside. Among the studied plants, *O. latifolia* is the only species being characterized with its ability of forming flavonol compounds (quercetin 3-hexoside), these compounds were previously reported in *O. corniculata* L. (Prasad Pandey *et al.*, 2020).

The present study also helps in understanding the chemotaxonomic relationship between the studied *Oxalis* species at the sectional level. Certain identified compounds confirmed the sectional demarcation of *Oxalis* as suggested by Lourteig (2000). Section Cernuae represented by *O. pes-caprae* could be distinguished from section Ionoxalis by the presence of 3,4-dihydroxybenzoic acid, 2-isopropylmalic acid, 6-hydroxynicotinic acid, cinnamic acid, formononetin, and esculetin, and lacking of glyceric acid, 2-methylglutaric acid, 1H-indole-3-carboxylic acid and naringenin. Coumarin and isoflavone compounds (esculetin and formononetin) were recorded in *O. pes-caprae* for the first time, and they have never been detected in any other *Oxalis* species, so it may be a valuable compound in distinguishing between *Oxalis* sections.

Our chemical and statistical findings were in consistence with Lourteig (2000) findings that supported her taxonomic treatment concerning with the sectional affinities of the species under investigation.

As new chemical classes were detected in the present work from *Oxalis* plants (flavanones, stilbenes and catechin) and to have a complete overview concerning the chemotaxonomic evaluation of *Oxalis* sections and their species, more investigations are needed for the rest of the worldwide distributed *Oxalis* species and in particular for section Cernuae.

4.CONCLUSION

This is the first chemical report to shed light on the polar constituents of Egyptian Oxalis that was an informative achievement with respect to both O. corymbosa and O. latifolia that had not been previously subjected to any chemical separation and identification processes. The obtained results suggested significant chemical variations among the three Oxalis sp. that could be a diagnostic character in their identification. All the chemical and statistical data supported the grouping of the studied taxa within two sections under genus Oxalis L. being Ionoxalis and Cernuae.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author(s).

Authorship Contribution Statement

Amal A. Draz: Design, analysis and interpretation. Salwa A. Kawashty: Supervision, data collection and processing. Sameh R. Hussein: Conception, supervision, critical review. Eman M. Shamso: Supervision, writing. Hasnaa A. Hosni:Supervision.

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REFERENCES

- Almahy, H.A., & Fouda, H.A.R. (2013) Isolation of luteolin 8-*C*-β-D-glucopyranoside from the roots of *Salvadora persica* (Rutaceae). *Journal of Current Chemical and Pharmaceutical Sciences*, *3*(1), 49-53.
- Ben Salah, H., Smaoui, S., Abdennabi, R., & Allouche, N. (2019). LC-ESI-MS/MS phenolic profile of *Volutaria lippii* (L.) Cass. extracts and evaluation of their in vitro antioxidant, Antiacetylcholinesterase, antidiabetic, and antibacterial activities. *Evidence-Based Complementary and Alternative Medicine*, 2019. https://doi.org/10.1155/2019/9814537
- Brent, L.C., Reiner, J.L., Dickerson, R.R., & Sander, L.C. (2014). Method for characterization of low molecular weight organic acids in atmospheric aerosols using ion chromatography mass spectrometry. *Journal of Analytical Chemistry*, *86*(15), 7328-7336. https://doi.org/10. 1021/ac403937e
- Brito, A., Ramirez, J.E., Areche, C., Sepúlveda, B., & Simirgiotis, M.J. (2014). HPLC-UV-MS profiles of phenolic compounds and antioxidant activity of fruits from three *citrus* species consumed in Northern Chile. *Molecules*, *19*(11), 17400-17421. https://doi.org/10.3390/mol ecules191117400
- Cao-Ngoc, P., Leclercq, L., Rossi, J.C., Hertzog, J., Tixier, A.S., Chemat, F., Nasreddine, R., Al HamouiDitBanni, G., Nehmé, R., Schmitt-Kopplin, P., & Cottet, H. (2020). Water-based extraction of bioactive principles from black currant leaves and *chrysanthellum americanum*: A comparative study. *Foods*, 9(10), 1478. https://doi.org/10.3390/foods9101 478
- Cocuron, J.C., & Alonso, A.P. (2014). Liquid chromatography tandem mass spectrometry for measuring ¹³C-labeling in intermediates of the glycolysis and pentose phosphate pathway. Plant Metabolic Flux Analysis, Springer. https://doi.org/10.1007/978-1-62703-688-7_9. PMID: 24222414.
- Dellagreca, M., Previtera, L., Purcaro, R., & Zarrelli, A. (2009). Phytotoxic Aromatic Constituents of *Oxalis pes-caprae*. *Chemistry & Biodiversity*, *6(1)*, 459-465. https://doi.org/10.1002/cbdv.200800179
- Draz, A.A., Shamso, E.M., Hosni, H.A., Kawashty, S.A., & Hussein, S.R. (2021). Comparative study of Oxalis L. species growing wild in Egypt: GC-MS analysis and chemosystematic significance. Egyptian Journal of Chemistry, 64(9), 5251-5256. https://doi.org/10.21608/E JCHEM.2021.72328.3592

- Eissa, M.A., Hashim, Y.Z., El-Kersh, D.M., Abd-Azziz, S.S., Salleh, H.M., Isa, M.L.M., & Abd Warif, N.M. (2020). Metabolite Profiling of *Aquilaria malaccensis* leaf extract using Liquid Chromatography-Q-TOF-Mass spectrometry and investigation of its potential antilipoxygenase activity in-vitro. *Processes*, 8(2), 202. https://doi.org/10.3390/pr8020202
- El-Khanagry, S.S.G. (2005). New records of dicotyledonous taxa to the flora of Egypt. *Bulletin* of Faculty of Agriculture, Cairo University, 56(1), 89-105.
- Gamir, J., Pastor, V., Cerezo, M., & Flors, V. (2012). Identification of indole-3- carboxylic acid as mediator of priming against cucumerina. *Plant Physiology and Biochemistry*, 61, 169-179. https://doi.org/10.1016/j.plaphy.2012.10.004
- Gaspar, M.C., Fonseca, D.A., Antunes, M.J., Frigerio, C., Gomes, N.G., Vieira, M., Santos, A.E., Cruz, M.T., Cotrim, M.D., & Campos, M.G. (2018). Polyphenolic characterisation and bioactivity of an *Oxalis pes*-caprae L. leaf extract. *Natural Product Research*, 32, 732-738. https://doi.org/10.1080/14786419.2017.1335728
- Gervasoni, J., Schiattarella, A., Giorgio, V., Primiano, A., Russo, C., Tesori, V., Scaldaferri, F., Urbani, A., Zuppi, C., & Persichilli, S. (2016). Validation of an LC-MS/MS method for urinary lactulose and mannitol quantification: Results in patients with irritable bowel syndrome. *Disease Markers*, 2016, ID 5340386, 6. https://doi.org/10.1155/2016/5340386
- Güçlütürk, I., Detsi, A., Weiss, E.K., Ioannou, E., Roussis, V., & Kefalas, P. (2012). Evaluation of anti-oxidant activity and identification of major polyphenolics of the invasive weed *Oxalispes*-caprae. *Phytochemical Analysis*, 23(6), 642-646. https://doi.org/10.1002/pca.236 7
- Guerrero, R.F., Valls-Fonayet, J., Richard, T., & Cantos-Villar, E. (2020). A rapid quantification of stilbene content in wine by ultra-high pressure liquid chromatography–Mass spectrometry. *Food Control*, 108, 106821. https://doi.org/10.1016/j.foodcont.2019.10 6821
- Hassan, W.H., Abdelaziz, S., & Al Yousef, H.M. (2019). Chemical composition and biological activities of the aqueous fraction of *Parkinsonea aculeata* L. growing in Saudi Arabia. *Arabian Journal of Chemistry*, 12(3), 377-387. https://doi.org/10.1016/j.arabjc.2018.08.003
- Hussein, S.R., Latif, R.R.A., Marzouk, M.M., Elkhateeb, A., Mohammed, R.S., Soliman, A.A., & Abdel-Hameed, E.S.S. (2018). Spectrometric analysis, phenolics isolation and cytotoxic activity of *Stipagrostis plumosa* (Family Poaceae). *Chemical Papers-Chemicke Zvesti*, 72(1), 29-37. https://doi.org/10.1007/s11696-017-0254-0
- Jin, J., Lao, J., Zhou, R., He, W., Qin, Y., Zhong, C., Xie, J., Liu, H., Wan, D., Zhang, S., & Qin, Y. (2018). Simultaneous identification and dynamic analysis of saccharides during steam processing of rhizomes of *Polygonatum cyrtonema* by HPLC–QTOF–MS/MS. *Molecules*, 23(11), 2855. https://doi.org/10.3390/molecules23112855
- Kang, J., Price, W.E., Ashton, J., Tapsell, L.C., & Johnson, S. (2016). Identification and characterization of phenolic compounds in hydromethanolic extracts of *sorghum* whole grains by LC-ESI-MSⁿ. *Food Chemistry*, 211, 215-226. https://doi.org/10.1016/j.foodchem .2016.05.052
- Krasteva, I., & Nikolov, S. (2008). Flavonoids in Astragalus corniculatus. Quím Nova, 31, 59-60. https://doi.org/10.1590/S0100-40422008000100012
- Kumarasamy, Y., Byres, M., Cox, P.J., Delazar, A., Jaspars, M., Nahar, L., Shoeb, M., & Sarker, S.D. (2004). Isolation, structure elucidation, and biological activity of flavone 6-*C*-glycosides from *Alliaria petiolata*. *Chemistry of Natural Compounds*, *40*(2), 122-128.
- Lin, Y.T., Mao, Y.W., Imtiyaz, Z., Chiou, W.F., & Lee, M.H. (2020). Comprehensive LC-MS/MS-based phytochemical perspectives and osteogenic effects of Uraria crinita. Food Function, 11(6), 5420-5431. https://doi.org/10.1039/D0FO00782J
- Llorach, R., Urpi-Sarda, M., Jauregui, O., Monagas, M., & Andres-Lacueva, C. (2009). An LC-MS-based metabolomics approach for exploring urinary metabolome modifications after

cocoa consumption. *Journal of Proteome Research*, 8(11), 5060-5068. https://doi.org/10.1 021/pr900470a

- Lourteig, A. (2000). Oxalis L., subgenera Monoxalis (Small) Lourt., Oxalis y Trifidus Lourt. Bradea, 7(2), 201-629.
- Lucas, A.R. (2021). Viruses as therapeutics: Methods and protocols. Springer.
- Marzouk, M.M., Hussein, S.R., Elkhateeb, A., El-shabrawy, M., Abdel-Hameed, E.S.S., & Kawashty, S.A. (2018). Comparative study of *Mentha* species growing wild in Egypt: LC-ESI-MS analysis and chemosystematic significance. *Journal of Applied Pharmaceutical Science*, 8, 116-122. https://doi.org/10.7324/JAPS.2018.8816
- MassBank Europe, Mass Spectral DataBase. Available online: <u>https://massbank.eu/MassBank/</u> (accessed on 19thJanuary, 2022).
- Minitab 21, Statistical & Data Analysis Software. Available online: https://www.minitab.com/en-us/support/downloads/ (accessed on 17thFebruary, 2022).
- Oberlander, K.C., Dreyer, L.L., & Elser, K.J. (2002). Biogeography of Oxalis (Oxalidaceae) in South Africa: A preliminary study. Bothalia. African Biodiversity & Conservation, 32(1), 97-100. https://doi.org/10.4102/abc.v32i1.472
- Prasad Pandey, B., Prakash Pradhan, S., & Adhikari, K. (2020). LC-ESI-QTOF-MS for the profiling of the metabolites and in vitro enzymes inhibition activity of *Bryophyllum pinnatum* and *Oxalis corniculata* collected from Ramechhap District of Nepal. *Chemistry & Biodiversity*, 17, e2000155. https://doi.org/10.1002/cbdv.202000155
- Ricciutelli, M., Moretti, S., Galarini, R., Sagratini, G., Mari, M., Lucarini, S., Vittori, S., & Caprioli, G. (2019). Identification and quantification of new isomers of isopropyl-malic acid in wine by LC-IT and LC-Q-Orbitrap. *Food Chemistry*, 294, 390-396. https://doi.org/10.10 16/j.foodchem.2019.05.068
- Ruan, J., Yan, J., Zheng, D., Sun, F., Wang, J., Han, L., Zhang, Y., & Wang, T. (2019). Comprehensive chemical profiling in the ethanol extract of *Plucheaindica* aerial parts by liquid chromatography/mass spectrometry analysis of its silica gel column chromatography fractions. *Molecules*, 24(15), 2784. https://doi.org/10.3390/molecules24152784
- Sarfraz, I., Rasul, A., Hussain, G., Shah, M. A., Nageen, B., Jabeen, F., Selamoğlu, Z., Uçak, İ., Asrar, M., & Adem, S. (2022). A review on phyto-pharmacology of *Oxalis corniculata*. Comb. *Combinatorial Chemistry & High Throughput Screening*, 25(7), 1181-1186. https://doi.org/10.2174/1386207324666210813121431
- Täckholm, V. (1974). Students' Flora of Egypt (2nd ed.). Cairo University, Cairo.
- The plant list (2013). Version1.1.. Available online: http://www.theplantlist.org/ (accessed 1st January, 2021).
- Wang, X., Qian, Y., Li, X., Jia, X., Yan, Z., Han, M., Qiao, M., Ma, X., Chu, Y., Zhou, S., & Yang, W. (2021). Rapid determination of rosmarinic acid and its two bioactive metabolites in the plasma of rats by LC–MS/MS and application to a pharmacokinetics study. *Biomedical Chromatography*, 35(2), e4984. https://doi.org/10.1002/bmc.4984
- Wang, Z., Ma, C., Tang, S., Xiao, H., Kakiuchi, N., Kida, H., & Hattori, M. (2008). Qualitative and quantitative analysis of *Swertia* herbs by high performance liquid chromatography-diode array detector-mass spectrometry (HPLC-DAD-MS). *Chemical and Pharmaceutical Bulletin*, 56(4), 485-490. https://doi.org/10.1248/cpb.56.485
- Wu, B., Zhang, B., Feng, X., Rubens, J.R., Huang, R., Hicks, L.M., Pakrasi, H.B., & Tang, Y.J. (2010). Alternative isoleucine synthesis pathway in cyanobacterial species. *Microbiology*, 156(2), 596-602. https://doi.org/10.1099/mic.0.031799-0
- Zhang, J., Chen, M., Ju, W., Liu, S., Xu, M., Chu, J., & Wu, T. (2010). Liquid chromatography/Tandem mass spectrometry assay for the simultaneous determination of chlorogenic acid and cinnamic acid in plasma and its application to a pharmacokinetic study.

Journal of Pharmaceutical and Biomedical Analysis, 51(3), 685-690.-https://doi.org/10.10 16/j.jpba.2009.09.039

- Zhao, X., Zhang, S., Liu, D., Yang, M., & Wei, J. (2020). Analysis of flavonoids in *dalbergia* odorifera by Ultra-Performance Liquid Chromatography with Tandem mass spectrometry. *Molecules*, 25(2), 389. ; https://doi.org/10.3390/molecules25020389
- Zhong, B., Robinson, N.A., Warner, R.D., Barrow, C.J., Dunshea, F.R., & Suleria, H.A. (2020). LC-ESI-QTOF-MS/MS characterization of seaweed phenolics and their antioxidant potential. *Marine Drugs*, 18(6), 331. https://doi.org/10.3390/md18060331