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CHEMICAL AND BIOLOGICAL CHARACTERIZE OF SOME *SILENE* SPECIES GROWING IN EGYPT

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ABSTRACT

The present study aims to detect the phenolics and flavonoids constituents of three selected *silene* species belonging to caryophyllaceae family as well as screen their antioxidant and cytotoxic activities. These are *Silene oliveriana* Otth, *Silene rubella* L *and Silene schimperiana* Bioss. The phenolic and flavonoid profiles were carried out using colourmetric and HPLC technique. In addition, invetro antioxidant activity was performed using DPPH assay while the cytotoxicity was summarized by MTT test. Total phenolic and flavonoids (µg%) content in aerial parts of *S. oliveriana*, *S. schimperiana* and *S. rubella* were detected using colorimetric methods were found 3.7 , 15.2 ,7.2 and 32.09, 81.98, 50.70 respectively. Fourty compounds were detected using HPLC technique and comparison with standards. They were detected as 18 phenolic acids and 22 flavonoids. Ellagic acid was amajor content in phenolic acids (32.09, 81.98, 50.70 mg%) for *S. oliveriana*, *S. rubella S schimperiana* L respectively. The flavonoids were represented as eight- *C*-glycosyl flavones, two flavones, five flavonol glycosides, two flavones, four flavonol and two 2,3 dehydro flavone. Luteolin-6-arabinose-8-glucose was amajor componant of *S. oliveriana*(296.26), while Hesperdin was amajor componant of *S. rubella* and *S schimperiana*, (573.68 and 768.70 **(µg** %) respectively. The DPPH assay showed moderate activities with % activity 13.67±0.8, 45.23±0.34 and 23. 55± 0.8 and for *S. oliveriana*, *S schimperiana*, and *S. rubella* L respectively. Cytotoxicity of diffent and extracts have weak activity for the rest investigated plants on M-NFS-60, M-NFS-60, MCF-7. HepG-2 cells, while n- hexane and ethylacetate extract of *S.schimperiana* showed significant cytotoxicity against colon carcinoma (HCT-116) and liver carcinoma(HPG-2) cells, with IC_{50} 5.82,13.9 and 15.6, 20.5 respectively. Among the studied

species, *S,schimperiana* showed the highest content of phenolic acids and flavonoids as well as a highly antioxidant and cytotoxic activities.

INTRODUCTION

Medicinal plants have a promising future because there are approximately half million plants around the world, and most of the medicinal activities has not been investigated yet. In recent times, focus on plant research has increased all over the world, and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems.[1] The genus *Silene* (family Caryophyllaceae) comprises more than 700 species of annuals, biennials, and perennials which are mainly distributed in temperate zones of the Northern Hemisphere of Eurasia and America, but also in Africa.[2,4] 40% of the species located in the Mediterranean Region are found to be endemic.[5] The genus consists mainly of herbaceous plants and, more rarely, small shrubs or subshrubs. The flowers have free petals, with each petal consisting of a usually visible limb that can be divided or entire and a claw that is included within the synsepalous calyx. *Silene* has been placed in the tribe *Sileneae* and the subfamily *Caryophylloideae*. *Silene* also includes a number of cultivated species and widespread weeds.[4,8] *S. acaulis,S. multifida* and *S. regia* have been cultivated as ornamental plants because they produce beautiful flowers.[9] It Represented in Egypt by twenty eight species under the different local names.[10] The roots of several species are rich in saponins with detergent properties, have been traditionally used as a soap substitute for washing clothes similar to other plants of the Caryophyllaceae.[11,16] Few species of genus *Silene* are used as ornamental plants and in folk medicine to treat inflammations, bronchitis, cold and infections or as a diuretic, antipyretic, analgesic and emetic.[17,24] *Silene* species and their isolated compounds also exhibit antibacterial, antifungal, cytotoxic, antioxidant, antitumor, hepatoprotection and antidiabetic activities due to the above mentioned.[25,26] Phytochemical investigations of the genus *Silene* have led to the isolation of several phytoecdysteroids[27], triterpene saponins[28], terpenoids, benzenoids, flavonoids[29], anthocyanidins, *N*-containing compounds[30], sterols and vitamins.[31,32] It woth noting that no thing was reported about phytochemical and biological investigation of *Silene oliveriana* Otth, *Silene schimperiana* Bioss and *Silene rubella* L. Therefore, phytochemical and biological investigations of the different extracts are very important.

MATERIALS AND METHODS

Plant material

The plant materials (*Silene schimperiana* Bioss, *Silene oliveriana* and *Silene rubella* L.) were collected from Snti Cathrein (South Sinia), Mediterranean costal strip and from midel Delta (Tanta-Ekhnawy) in flowering stage at april 2015 and were kindly established by Prof. Dr. Abdo Marey, Prof. of Botany, Faculty of Science, Al-Azhar University. Avoucher specimen (C.S. # 0912-914) were deposited in a herbarium in Pharmacognosy Department, Faculty of Pharmacy, Al Azhar University, Cairo, Egypt. The plant leaves were separated, air-dried, powdered (2mm mesh) and kept in tightly closed amber coloured glass containers protected from light at low temperature.

Material for determination of total phenol content

Folin-Ciocalteu reagent (Sigma Chemical Co., St. Louis, MO, USA) and Gallic acid (E. Merck, Darmstadt, Germany).

Material for determination of total flavonoid content

Quercetin (Merck Co. Darmstadt, Germany) and Aluminium chloride (E. Merck, Darmstadt, Germany).

Material for determination of antioxidant effect

DPPH (Sigma-Aldrich Quimica, South Madrid, Spain), Silica gel 60 F254 (Merck, Darmstadt, Germany), Mobile phase [butanol: acetic acid: water (40: 10: 50)] and Butylated hydroxyl toluene (BHT): Sigma-Aldrich, Quimica, South Madrid, Spain.

Material for determination of anticancer effect

Hepatocellular carcinoma cells (HEP-G2), Colon carcinoma cells (HCT-116) and Breast carcinoma cells (MCF-7) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA), they were grown on Roswell Park Memorial Institute (RPMI) 1640 medium (Nissui Pharm. Co., Ltd., Tokyo, Japan) and were supplied through The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Apparatus

Soxhlet, vacuum oven (Vacucell, Einrichtungen GmbH), Genesys Spectrophotometer (Milton Roy, INC., Rochester, NY) for UV/Vis. Investigation of nanoparticles, field emission scanning electron microscope (SEM, JSM 6490A, Jeol, Tokyo, Japan), bench-top

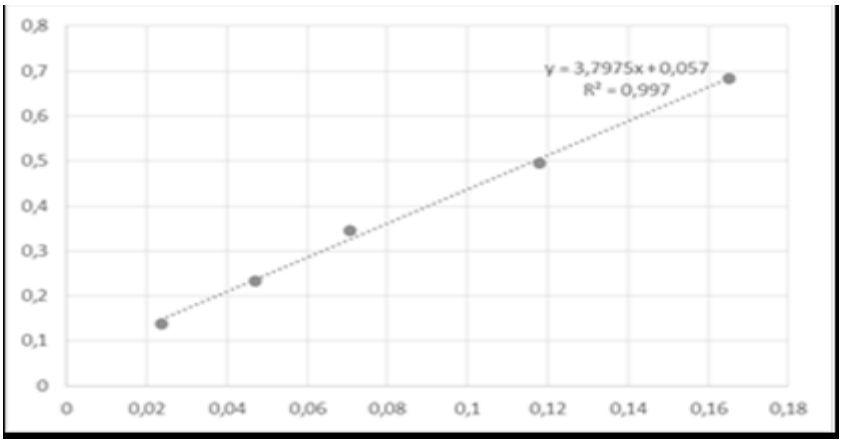
Spectrum™ 65 FT-IR spectrometer equipped with universal diamond Attenuated Total Reflectance (ATR) accessory (Perkin Elmer Inc., USA), Chromatographic glass jars, 96 Micro-well™ Plates, Conical Wells, Spectrophotometer (Perkin-Elmer Lambada 3) for quantitative determination of antioxidant effect, Rotatory evaporator (BUCHI Rotavapor® R-210/R-215, Germany), Genesys Spectrophotometer (Milton Roy, INC., Rochester, NY) for quantitative estimation of total phenolics, flavonoids.

Preparation of crude extract

About 50 g of dried powdered powdered herb of three studied species were extracted by soxhlet for 24h with 600 ml methanol, four successive times, after filtration, extracts were pooled together and concentrated under vacuum then washed within *n*-hexane until the chlorophyll was completely removed. The defatted aqueous extracts were subjected to the phytochemical investigation as well as the biological screening for antioxidant and anticancer activities.

Spectrophotometric determination of the total phenolics content

The concentration of total phenol compounds in crude methanol and nanoparticulated extracts were determined spectro-photometrically using the Folin-Ciocalteu's reagent where standard curve was done using different concentrations of gallic acid in methanol.[33] The concentrated extracts were dissolved each in least methanol volume then completed to 10ml, 100µl of these extracts were separately diluted with 8 ml distilled water, to each sample 0.5 ml of 50% Folin-Ciocalteu's reagent was added and left 8 min and then 1.5 ml of 5% sodium carbonate was added, mixed and allowed to stand for 60 min protected from light, their absorbance was measured at 725 nm using methanol as blank and concentration of the total phenolic content.



(2): The calibration curve of gallic acid with different concentrations.

Determination of phenolic compounds

Phenolic compounds were determined by HPLC according to the method of (34) as follow: 5 g of sample were mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtrated through a 0.2 µm Millipore membrane filter then 1-3 ml was collected in a vial for injection into HPLC Hewllet Packared (series 1050) equipped with autosamplling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quarter HP pump (series 1050). The column temperature was maintained at 35˚C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml⁄ min. Phenolic acid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis of HE WLLET Packared soft ware.

Spectrophotometric determination of the Total flavonoids in samples

Aluminum chloride colorimetric method is used for flavonoids determination. Two milliliters of 2% AlCl3 in ethanol is added to 2 ml of the test sample. The UV absorption is measured at 425 nm after 1 h at room temperature. Concentration of 0.05 mg/ml sample solution is used while Quercetin concentrations of 0.01 to 0.09 mg/ml are used to obtain a calibration curve. Determinations were performed in triplicates. Total flavonoid contents were obtained from**,** the regression equation of the calibration curve of Quercetin (Y=0.0103+0.0102, r2= 0.9966).[34]

Determination of Flavonoid compounds

Flavonoid compounds were determined by HPLC according to the method of Mattila *et al.* (200)[35] as follow: 5 g of sample were mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtrated through a 0.2 µm Millipore membrane filter then 1-3 ml was collected in a vial for injection into HPLC Hewllet Packared (series 1050) equipped with autosamplling injector, solvent degasser, ultraviolet (UV) detector set at 330 nm and quarter HP pump (series 1050). The column temperature was maintained at 35˚C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml⁄ min. Flavonoid acid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis of HE WLLET Packared soft ware.

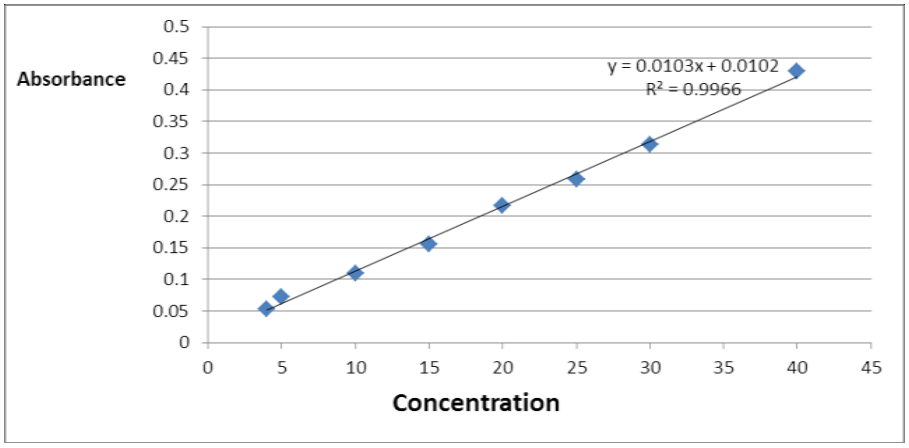


Figure (2): The calibration curve of Quercetin with different concentrations.

Determination of free radical scavenging activity (DPPH assay)

Quantitative measurements of radical scavenging properties of extracts were carried out according to.[36,39] Briefly, 0.1 mM solution of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) in methanol was prepared and 1 ml of this solution was added to 3 ml of each methanolic extract at different concentration (50-200 µg/mL). Butylated hydroxytoluene (BHT) was used as a positive control. Discoloration was measured at 517 nm after incubation for 30 min. The capacity to scavenge the DPPH radical was calculated using the following equation: DPPH scavenging effect (%) = ADPPH – AS /ADPPH x100 Where, ADPPH is the absorbance of the DPPH solution and AS is the absorbance I solution when the sample extract is added.

Cytotoxic assay Cell culture

A549 human lung carcinoma and prostate cancer (PC3) were maintained in DMEM medium, HCT-116 (colorectal carcinoma), HepG2 (hepatocellular carcinoma) and MCF-7 (breast adenocarcinoma) were maintained in RPMI. All media was supplemented with 10% fetal bovine serum and incubated at 37C in 5% CO2 and 95% humidity. Cells were sub-cultured using trypsin versene 0.15%. All cell lines were purchased from Vacsera (Giza, Egypt).[40]

Cell viability assay

After 24 h of seeding 20000 cells per well in case of A-549, HCT-116 and PC3, 10000 cells per well in case of HepG2 and MCF-7 cell lines (in 96 well plates), the medium was changed to serum-free medium containing a final concentration of the extracts of 100 µg/ml in triplicates. The cells were treated for 24 h. 100 µg/ml doxorubicin was used as positive control and 0.5 % DMSO was used as negative control. Cell viability was determined using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide).[41] The equation

used for calculation of percentage cytotoxicity: (1- (Av(X) / (Av(NC)))* 100 Where Av: average, X: absorbance of sample well measured at 595 nm with reference 690 nm, NC: absorbance of negative control measured at 595 nm with reference 690.

RESULT AND DISCUSSION

In the present work, among the investigated extracts, the three species of family Fabaceae (*S. oliveriana, S schimperiana* & *S. rubella xandrina*) have been shown the highest concentrations of phenolic acids and flavonoids. They have been showed the highest content of various phenolic acids. The total phenol content was determined using Folin–Ciocalteu reagent[36] in comparison with standard gallic acid, and the results the expressed interms of mg GAE/g dry sample. The total phenol content values for the herb extracts of plants under investigation were *S. oliveriana* (3.7 mg±0.1), *S schimperiana* (15mg mg±0.3) and *S. rubella* (7.2±0.2), all as $GAEg^{-1}$ dry sample respectively (Table 1). HPLC analysis of phenolic acids revealed the presence of 14 phenolic acids; Pyrogallol, gallic acid, protocatechuic salycilic, *p*-Hydroxy benzoic salycilic. Chlorogenic salycilic, vanillic acid, Caffeic acid, P-coumaric acid, Ferulic acid, Iso-ferulic acid, e-vanillic acid, o-coumaric acid, Benzoic acid, ellagic 3,4,5-methoxy cinnamic acid, Coumarin, and salycilic acid. (Table 1). Among the detected phenolic acids; ellagic acid was the major compound in *S. oliveriana*, *S schimperiana*, and *S. rubella* (32.09±0.35, 50.70±0.45 and 81.98±0.65 mg/100g DW). The highest content vanillic acid and e-vanillic acid has been detected in *S. rubella.* (76.94 and 81.98 mg/100g) (Table 2).

The total flavonoids content was determined using $AlCl_3$ reagent[33] in comparison with standard qurectin and the results the expressed in terms of mg Q/g dry sample. The total flavonoids content values for the herb extracts of plants under investigation were *S. rubella* (44.8 mg±0.1), *S. oliveriana* (21.5±0.2) and *S schimperiana* (34.02 mg±0.3) and all as Q/g^{-1} dry sample respectively (Table 3). HPLC analysis of the flavonoid compositions of the investigated species (Table 2) revealed the presence of 23 compounds of different class (eight *C*-glycosyl flavones, two dehydro *C*-glycosyl flavones, two flavones, two dehydro flavones, five flavonols glycosides and four flavonols). The detected *C*-glycosyl flavones seem to be 6,8-di-*C*-glycosyl pattern of apigenin and luteolin; the major compounds were represented as luteolin 6-*C*-arabinose-8-*C*-glucose and luteolin 6-*C*- glucose-8-*C*- arabinose in *S. oliveriana, S. rubella and S schimperiana* (296.26, 449.46 and643.61 mg/100g DW) and (928.4 mg/100g DW) in *S schimperiana*. Luteolin-7- glucoside (356.7±0.27 mg/100g DW) *S. rubella* while Narengin and Hesperdin; the major compounds of *S.rubella* and *S.rubella, S. schimperiana*

(310.4 and 573.68, 768.70 mg/100g DW). Kaempferol-3,7-dirahmnoside and Kaempferol-3-(3-*p*-coumaroyl)glucose was concentrated in *S. schimperiana* and in *S. oliveriana*, *S. rubella(*283.87and 31.37;211.8mg/100g DW), respectively. (Table**4).** The *in vitro* radical scavenging activity of the investigated extracts showed moderate activities with % inhibition 13.67±0.3, 23± 0.3 and 45.23±0.34 for S. *oliveriana*, *S schimperiana* and *S. rubella* respectively. The results of Cytotoxic activities indicated that *S schimperiana* extracts (n-hexan, ethylacetate, and n-butanol) showed significance highly activity against HCT116. at 100 mg/mL with IC_{50} (µg/ml) (5.82, 15.5, 15.6) and while n – hexane and ethyl acetate HPG-2 at 100 mg/mL with IC_{50} (µg/ml**)** (13.9 and 15.6) respectively. Other extracts of S. *oliveriana*, *S schimperiana* and *S. rubell* have week effect on M-NFS-60, HCT-116, MCF-7and HepG-2. In the present study, S. *oliveriana*, contains the lowest phenolic and flavonoid constituents as well as the extract showed a weak antioxidant and cytotoxic activities. All plant contains a wide range of bioactive constituents; phenolic acids, flavonoids, terpenoids, saponins and glycosides.[27,32] The genus also has anti-cancer, antihypertensive, antispasmodic, antimutagenic, antioxidant, anti-inflammatory and antimicrobial activities.[17,26] In the present work, all flavonoids were detected for the first time from the plant. Additionally, the phenolic acids were also observed firstly. This species showed the highest antioxidant and cytotoxic activities (Tables 3-4), confirmed its medicinal properties.

Table 1: Total phenolic (mg GAE g-1), flavonoid (mg QE g-1), by Spectrophotometric and HPLC analysis.

Plant name	Total phenolics	Total flavonoids	Antioxidant %
S. rubella	14.2	50.8	23. 55± 0.8
S. oliveriana	30.7	32.09	13.67±0.8
S. schimperiana	32.09	81.98	45.23±0.34

Table 2. Phenolic acids detected by HPLC and their amounts for investigated species (mg/100g DW).

Phenolic compound	Retention time		Test results of phenolic compounds (mg/100gm)		
	Standard	Test	S.O.	S.R.	S.S.
Pyrogallol	6.92	6.9	0.05	17.35	14.92
Gallic	7.05	7.0	0.02	0.42	0.66
Protocatchuic	9.03	9.06	0.10	3.4	7.99
***p*-Hydroxy benzoic**	9.84	9.81	0.99	4.57	7.92
Chlorogenic	10.01	10.1	0.24	6.0	7.97
Vanillic	10.21	10.23	0.36	**76.94**	6.23
Caffeic	10.2	10.31	0.33	2.76	3.07
***p*-Coumaric**	11.58	11.6	0.21	5.09	3.55
Ferulic	11.8	11.8	0.09	7.80	5.37

Iso-ferulic	12.16	12.2	0.06	0.99	2.4
***E*-vanillic**	12.24	12.3	3.86	**81.98**	---
***O*-Coumaric**	13.27	13.1	1.2	1.54	8.22
Benzoic	13.3	13.3	2.27	6.22	---
Ellagic	13.18	13.4	**32.09**	**81.98**	**50.70**
3,4,5-methoxy cinnamic	14.0	14.2	1.10	6.0	16.03
Coumarin	14.44	14.41	0.46	2.84	4.22
Cinnamic	15.29	15.4	0.03	6.2	0.19
Salicylic	16.4	16.5	3.22	6.13	13.60
total			**46.527**	**31.777**	**15.2647**

S.O= S. oliveriana, *S.S =S. schimperiana* *S.r= S. rubella*

All results were expressed as mean ± standard deviations from replications n = 3.

Table 3. Flavonoids detected by HPLC and their amounts for investigated species (mg/100g DW).

Flavonoids	**Retention time**		**Test results of flavonoids (µg)**		
	Standard	**Test**	**S.O.**	**S.R.**	**S.S.**
Luteolin-6-arabinose-8-glucose	9.48	9.44	**296.26**	**449.46**	**643.61**
Luteolin-6- glucose -8-arabinose	10.81	10.82	9.97	152.80	**928.4**
Apigenin-6-arabinose-8-galactose	11.37	11.36	1.29	274.8	94.45
Apigenin-6- rhamnose-8- glucose	11.81	11.83	5.69	48.39	92.52
Apigenin-6-glucose-8-rhamnose	12.19	12.18	1.71	65.39	150.63
Luteolin-7- glucose	12.30	12.28	4.06	**356.63**	----
Narengin	12.35	12.37	2.10	177.68	**310.40**
Hesperdin	12.48	12.48	21	**573.68**	**768.70**
Rutin	12.61	12.57	12.03	12.01	134.30
Quercetin-3-*O*-glucoside	12.51	12.52	0.71	78.87	----
Apigenin-7-*O*-neohisperoside	13.14	13.41	**42.97**	----	**75.16**
Kaempferol-3,7-dirahmnoside	13.21	13..26	9.70	25.08	**283.87**
Quercetrin	13.45	13.42	14.70	73.11	107.86
Rosmarinic					
Quercetin	14.90	14.91	3.76	----	44.48
Naringenin	15.03	15.05	11.62	6.91	15.52
Kaempferol-3-(3-*p*-coumaroyl)glucose	15.16	15.18	**31.37**	**211.82**	31.37
Hespertin	15.35	15.3	17.49	29.37	17.49
Kaempferol	16.24	16.26	2.44	6.85	2.44
Rhamnetin	16.44	16.49	0.29	8.02	0.29
Apigenin	16.56	16.64	----	9.36	1.29
Apigenin-7-glucose	17.24	17.26	----	3.39	----
Acacetin	18.82	18.86	1.29	169.87	----
Total			**495.47**	**2658.1**	**3702.78**

S.O= S. oliveriana, *S.S = schimperiana* *S.r= S. rubella*

All results were expressed as mean ± standard deviations from replications n=3.

Table 4. LC_{50}(µg/ml) of cytotoxic effect different extracts of investigated *Silene* species.

	IC_{50} (µg/ml)			
Extract/compound	**M-NFS-60**	**HCT-116**	**MCF-7**	**HepG-2**
Total extract (methanolic extract)				
S. S. rubella	242	23.8	38.6	168
S. oliveriana	128	27	48.9	77.9
S. schimperiana	52.1	**15.5**	55.5	25.8
n*-Hexane *extracts				
S. rubella	109	28	61.9	50.7
S. oliveriana	57.7	56.7	28.8	30.7
S. schimperiana	51.8	**5.82**	36.7	**13.9**
Ethylacetate extracts				
S. rubella	61.7	24.8	30.6	45.2
S. oliveriana	69.7	26.2	29.4	50.1
S. schimperiana	48	**15.6**	26.8	**20.5**
n-butanol extracts				
S. S. rubella	175	57.2	42.3	111
S. oliveriana	161	47.5	29.7	101
S. schimperiana	181	96.5	52.4	122

All results were expressed as mean ± standard deviations from replications n=3.

IC50: Inhibition concentration of the sample, which causes the death of 50% of cells in 48 hrs This plant contains a wide range of bioactive constituents; phenolic acids, flavonoids, terpenoids, tannins, alkaloids, saponins and glycosides. The genus also has anti-cancer, antihypertensive, antispasmodic, antimutagenic, antioxidant, anti-inflammatory and antimicrobial activities.[17,26] In the present work, except of kaempferol, quercetin and its glycoside derivative (rutin), all flavonoids were detected for the first time from the plant. Additionally, the phenolic acids (protocatechuic, chlorogenic, salicylic and p-Hydroxybenzoic) were also observed firstly. This species showed the highest antioxidant and cytotoxic activities (Tables 3-4), confirmed its medicinal properties.

CONCLUSION

The present study demonstrated that all studied species collected from Egypt some phenolic acids and flavonoids with different concentrations although they are belonging to same species. It has been reported that the antioxidant activity of plants might be due to their phenolic and flavonoid compounds.

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