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ORIGINAL ARTICLE

HPLC-DAD-ESI-MS/MS Characterization of Bioactive Secondary Metabolites from *Strelitzia nicolai* Leaf Extracts and Their Antioxidant and Anticancer Activities *In vitro*

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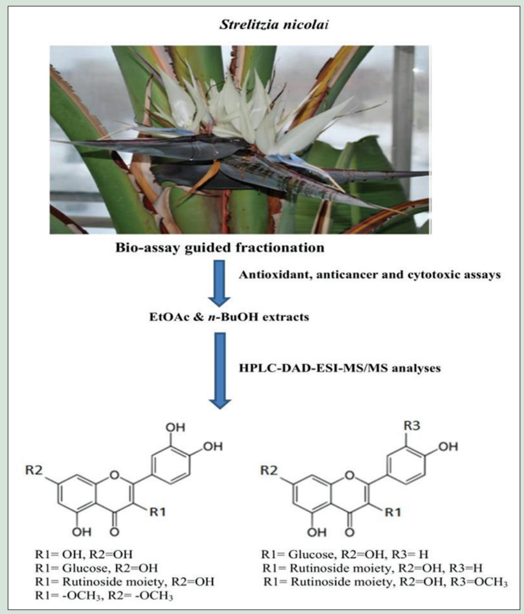
ABSTRACT

Background: Strelitzia nicolai Regel and Körn (Strelitziaceae) is native to Southern Africa whose phytochemistry and pharmacology were slightly investigated. **Materials and Methods:** In the current work, different solvent extracts of *S. nicolai* were screened for their chemical profiles through high-performance liquid chromatography coupled with diode array detection and electrospray ionization mass spectrometry (HPLC-DAD-ESI-MS/MS) analyses. Furthermore, their *in vitro* antioxidant, cytotoxic, and anticancer activities were evaluated using 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) & ferric reducing antioxidant power (FRAP) and crystal violet staining (CVS) colorimetric assays, respectively. **Results:** HPLC-DAD-ESI-MS/MS analyses led to the identification of nineteen and eleven phenolic compounds from the ethyl acetate and *n*-butanol extracts, respectively including flavonoids (e.g., quercetin 3-(2 G-rhamnosylrutinoside, quercetin, quercetin-3-O-glucoside, kaempferol-3,7-O-dirhamnoside, isorhamnetin-3-O-rutinoside and kaempferol-3-O-glucoside), phenolic acids derivatives (e.g., chlorogenic acid glycoside, protocatechuic acid-O-glucoside and caftaric acid), chalcones (e.g., xanthoangelol), and phenylethanoids (e.g., ligstroside glucoside). Moreover, in the DPPH assay the IC_{50} value of the most active ethyl acetate extract was 20.49 µg/mL, relative to 2.92 µg/mL of ascorbic acid. ABTS and FRAP results reinforced the results of DPPH assay. According to the National Cancer Institute criteria, the tested extracts showed weak to moderate cytotoxic activities with IC_{50} values ranged from 65.23 to 451.29 µg/mL. Furthermore, the EtOAc and n-BuOH extracts showed a noticeable anticancer activity with CVS spectroscopic readings for liver hepatocellular carcinoma growth 0.806 and 0.684 at a concentration (125 µg/mL), as well as 0.730 and 0.618 at concentration (500 µg/mL), respectively against control at 1.022. **Conclusion:** The obtained results reveal the high efficacy of the phenolic-rich extracts from *S. nicolai* as naturally occurring antioxidant and anti-tumor agents.

Key words: Anticancer activity, antioxidant activity, cytotoxicity, HPLC-DAD-ESI-MS/MS, polyphenolics, *Strelitzia nicolai*

SUMMARY

- The current research work evaluated the biological activities of different solvent extracts of *Strelitzia nicolai* including antioxidant, anticancer, and cytotoxic activities
- Among the tested extracts, the ethyl acetate and *n*-butanol extracts are the most promising extracts
- High-performance liquid chromatography coupled with diode array detection and electrospray ionization mass spectrometry analyses of the most active extracts led to the characterization of certain polyphenolic compounds, the majority are flavonoids and phenolic acids.



Abbreviations Used: HPLC-DAD-ESI-MS/MS: High-performance liquid chromatography-diode array detection-electrospray ionization-mass/mass; DPPH: 2,2′-Diphenyl-1-picrylhydrazyl radical; ABTS: 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); FRAP: Ferric reducing antioxidant power; TPTZ: Tripyridyl-s-triazine; FE: Ferrous equivalents; DMEM: Dulbecco's modified eagle's medium; DMSO: Dimethyl sulfoxide; EDTA: Ethylenediaminetetraacetic acid; PBS: Phosphate buffered saline; HepG-2: Liver hepatocellular carcinoma; CVS: Crystal violet stain; NCI: National cancer institute; Glu: Glucose; Rha: Rhamnose.

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INTRODUCTION

Strelitziaceae is a tropical monocotyledonous ornamental family famous by its bioactive compounds namely phenalenones.[1] *Strelitzia nicolai* Regel and Körn are usually known as the white bird of paradise tree.[2] This plant is native to Southern Africa and broadly growing in many tropical regions around the world.[3] To the best of our knowledge, there is limited information available in the literature about the phytochemical and biological investigations were reported on the plant. Bilirubin-IX is orange pigment with cyclic tetrapyrrole nucleus was isolated from the arils of *S. nicolai*.[4-7] Moreover, it was reported that this pigment showed potent antioxidant and anticancer activities.[7] In addition, the main chemical ingredients of the essential oil isolated from the seed arils of the plant were categorized as follows: amine, ethers, ketones, hydrocarbons, aromatic compounds, alcohols, amides, and esters.[3] Moreover, accumulation of the reactive species in our bodies led to oxidative stress, which is associated with several disorders like cancer and cardiovascular diseases.[8] Cancer is a well-known global problem which represents the second cause of death and accounts for "7–8 million deaths" worldwide.[9] The treatment of cancer is based on the use of synthetized chemotherapeutic drugs, using this drugs as chemopreventive agents accompanied by a series of health problems due to their side effects and low safety, this concept encourage scientists to discover the role of medicinal plants in cancer therapeutic as natural sources of naturally occurring anticancer agents.[9,10] Recently, there is increasing interest in the chemical investigation and characterization of the different class of secondary metabolites especially polyphenolic compounds to establish their structure-activity relationship.[11] Since there no adequate information has been documented on the chemical and biological profile of the plant, therefore, the aim of the current study is to characterize the polyphenolic compounds of the different solvent extracts from *S. nicolai* leaves via high-performance liquid chromatography coupled with diode array detection and electrospray ionization mass spectrometry (HPLC-DAD-ESI-MS/MS) analysis as well as their antioxidant and anticancer activities.

MATERIALS AND METHODS

Plant material

The fresh leaves *S. nicolai* were collected from Zoo Garden, Giza, Egypt in June 2014. The identity of the plant was established by Dr. Tearse Labib, Botany Specialist, Department of Flora and Taxonomy, El-Orman Botanical Garden, Giza, Egypt. A voucher specimen (No. S25/5/6) was kept at the herbarium of the garden.

Extraction and fractionation

Dry powdered leaves of *S. nicolai* (1.5 kg), were extracted with MeOH in room temperature with shaking day by day followed by filtration and again extraction for 4 times. The extract was filtered using Whatman filter paper No. 1 and concentrated using Rotatory evaporator (Buchi, Switzerland) at (40°C ± 2°C). The crude extract was collected and stored at room temperature in the dark for the further process. The methanolic crude extract (230 g) was defatted by washing several times with petroleum ether (60–80°C), then undergoing fractionation process using organic solvents such as CH_2Cl_2; EtOAc; and *n*-BuOH (5 mL × 150 mL solvent).

High-performance liquid chromatography coupled with diode array detection and electrospray ionization mass spectrometry conditions

The phytochemical analysis of polyphenolic compounds was done using HPLC-photodiode array (PDA)-MS/MS. The liquid chromatography system was Thermofingan (Thermo Electron Corporation, USA) coupled with an LCQ Duo ion trap MS with an ESI source (ThermoQuest). The separation was achieved by using a C18 reversed-phase column (Zorbax Eclipse XDB-C18, Rapid resolution, 4.6 mm × 150 mm, 3.5 µm, Agilent, USA). A gradient of water and acetonitrile (with 1% formic acid each in the positive mode) was applied from 5% to 50% ACN in 60 min with flow rate 1 mL/min throughout the whole run. The samples were injected automatically using autosampler surveyor ThermoQuest. The instrument was controlled by Xcalibur software (Thermo Fisher Scientific Inc., USA) to collect the ultraviolet (UV) chromatogram using PDA mode. The MS operated in the negative mode with a capillary voltage of – 10 V, a source temperature of 200°C, and high-purity nitrogen as a sheath and auxiliary gas at a flow rate of 80 and 40 (arbitrary units), respectively. The ions were detected in a full scan mode and mass range of 50–2000 *m*/*z*.

Antioxidant assays

2,2'-Diphenyl-1-picrylhydrazyl radical free radical-scavenging assay

2,2'-Diphenyl-1-picrylhydrazyl radical (DPPH) assay was performed according to the method described by Ghareeb *et al*. (2018). Briefly, 200 µl of plant extract, diluted appropriately in methanol in a concentration range from 0.24 to 500 µg/mL, was mixed with 100 µl of 0.2 mM DPPH in methanol in wells of 96-well plates. The plates were kept in the dark for 15 min; thereafter, the absorbance of the solution was measured at 515 nm in a Biochrom Asys UVM 340 Microplate Reader. Appropriate blanks, methanol, and standards (ascorbic acid solutions in methanol) were analyzed simultaneously. The scavenging activity (in %) was calculated using the following equation:

DPPH scavenging (%) =100 × [(Abs sample + DPPH) - (Abs sample blank)]/[(Abs DPPH) - (Abs methanol)]

The IC_{50} value is defined as the amount of extract needed to scavenge 50% of DPPH radicals. All analyses were performed in triplicate.[12]

2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay

The samples were dissolved in water to prepare the stock solutions (1 mg/mL) from which a radical-scavenging activity was determined by the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) $(ABTS)^+$ radical cation decolorization assay,[13] over a concentration range of 0.24–500 µg/mL. The ABTS cation radical was prepared by reacting 7 mM aqueous solution of ABTS (15 mL) with 140 mM potassium persulfate (264 µl). The mixture was allowed to stand in dark at room temperature for 16 h before use. Before assay, the ABTS working reagent was diluted with methanol to give an absorbance of 0.70 ± 0.02 at 734 nm and was equilibrated at room temperature. The reaction mixtures in the 96-well plates consisted of sample (50 µl) and the ABTS methanol working solution (100 µl). The mixture was stirred and left to stand for 10 min in dark, and then the absorbance was determined at 734 nm against a blank. All determinations were performed in triplicate. The scavenging activity (in %) was calculated as follows:

% scavenging rate = $[1-(A_1-A_2)/A_0] \times 100$.

Where A_0 is the absorbance of the control (without sample), and A_1 is the absorbance in the presence of the sample, A_2 is the absorbance of the sample without ABTS working solution. The scavenging activity of the samples was expressed as IC_{50} value, which is the effective concentration, at which 50% of ABTS radicals were scavenged. Trolox was used as a standard.

Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay was carried out according to the previously reported procedure,[14] with minor modifications. Each sample was dissolved in methanol to prepare the stock solution (1 mg/mL). Briefly, the working FRAP reagent was prepared freshly by mixing 300 mM acetate buffer (pH 3.6), a solution of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM hydrochloric acid and 20 mM ferric chloride at 10:1:1 (v/v/v). 20 µl of each extract was mixed with 180 µl FRAP reagent in wells of 96-well plates. The mixture was then incubated for 6 min at 37°C, and the absorbance was measured at 595 nm in a microplate reader (Biochrom Asys UVM 340).

Appropriate blanks of plant extract and FRAP reagent lacking TPTZ (to correct the colors of the extracts) were run, together with quercetin (in methanol), and ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) was used as a standard. FRAP activity was calculated as ferrous equivalents, the concentration of extract/quercetin which produced an absorbance value equal to that of 1 mM $FeSO_4$.

Evaluation of cytotoxic activities

Splenocytes were isolated from normal albino mouse according to Goodman *et al*.[15] beginning with washing thoroughly with 70% alcohol whole body and cervical dislocation done after anesthesia, and then the abdominal cavity was incised, and the spleen was transferred to sterile petri dish and slice into small pieces.[16] Fragments were placed onto a strainer attached to a 50-mL conical tube. Excised spleen pieces were pressed through a strainer using a plunger end of a syringe and cells have been washed with phosphate buffered saline (PBS) (Dulbecco's PBS, pH = 7.4). Cell suspension centrifuged at 1600 rpm for 5 min. Cells pellets were resuspended in 2 mL lysing buffer (0.15 M NH_4Cl, 1 mM $KHCO_3$, and 0.1 mM ethylenediaminetetraacetic acid). The cells have been incubated in a 37°C water bath for 2 min, and 30 mL of PBS were added and cells centrifuged at 1600 rpm for 5 min. Pellet cells were resuspended in 3 mL growth Dulbecco's Modified Eagle's Medium, Lonza, Belgium), 30% FBS (Hyclone, USA), 1% Penicillin/Streptomycin (Lonza, Belgium), and 1% L-glutamine (Lonza, Belgium)[17,18] at final concentration of 2000 × 10000 cells per mL, the cells were cultured in 96 well plates. Cell count was performed and viability checked using trypan blue and a hemacytometer. Cells were incubated with serial dilutions of eleven fractions dissolved in dimethyl sulfoxide (DMSO) as follow (1000 μg/mL, 500 μg/mL, 250 μg/mL, 125 μg/mL, 62.5 μg/mL, and 31.2 μg/mL) at 37°C in 5% CO_2 and 90% humidified atmosphere for 36 h.

Evaluation of anticancer activities

Liver hepatocellular carcinoma (HepG-2) cell line obtained from the holding company for biological products and vaccines, Egypt (VACSERA) passage number 80 ≈ 85 has been cultured in a T25 flask with Roswell Park Memorial Institute medium contains 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin and incubated in a humidified 5% CO_2 incubator at 37°C.[19] Culture medium was removed after reaching 90% confluency and 0.25% trypsin (Gibco/Invitrogen) was added and flask observed under an inverted microscope until all cells were detached. Cells were cultured in 96-well plates at a density of 10000 cells per well. Incubation has been done for 48 h until sheet was observed. Serial dilutions of fractions in DMSO were added to cultured cells at concentrations of 1000 μg/mL, 500 μg/mL, 250 μg/mL, 125 μg/mL, 62.5 μg/mL, and 31.2 μg/mL and incubated for 24 h. Media were decanted and plates were washed carefully with PBS (Dulbecco's PBS, pH = 7.4) and cells were stained with 20 μl of 0.5% crystal violet (Sigma-Aldrich Corp., St. Louis, MO, USA) in 30% ethanol for 10 min at room temperature. Plate wells were washed 3 times using distilled water[20] and the absorbance was measured at optical density = 490 nm.

Statistical analysis and dose-response curve

A dose-response curve has been traced to find the equation and estimate IC_{50} using unpaired Student's *t*-test excel, referring to different concentrations, more than three to draw the curve which presented as mean standard deviation (SD) taking in consideration $P < 0.05$.

RESULTS AND DISCUSSION

Antioxidant activity

Different solvent extracts of *S. nicolai* were evaluated for their antioxidant activities using three techniques including; DPPH, ABTS, and FRAP. In DPPH assay, the IC_{50} values for the tested extracts ranged from 20.49 to 118.17 μg/mL compared to ascorbic acid as standard with IC_{50} equal to 2.92 μg/mL. The results are in the order: EtOAc > n-BuOH> MeOH> H_2O > CH_2Cl_2> Pet. ether. In addition, all tested extracts showed similar activity using ABTS assay with IC_{50} values arranged in the following order: EtOAc (9.18) > n-BuOH (12.43) > MeOH (15.73) > H_2O (23.18) > CH_2Cl_2 (29.89) > Pet. ether extracts (52.25) (μg/mL) compared to Trolox (IC_{50} = 1.63 μg/mL). In FRAP assay, the EtOAc fraction showed high reducing power activity with 22.19 mM FeSO4 equivalent/mg extract, followed by n-BuOH (18.34), MeOH (15.46), H_2O (11.39), CH_2Cl_2 (7.92), and Pet. ether extracts (1.58), respectively, compared to quercetin (21.45). Results are documented in Table 1. In conclusion, the results of the three assays agree with each other and the high antioxidant activity of the EtOAc and n-BuOH extracts may be due to the presence of the tentatively identified polyphenolic compounds which are broadly reported in the literature by their antioxidant potential for instance; flavonoids (e.g. quercetin 3-(2 G-rhamnosylrutinoside), rutin, quercetin-3-*O*-glucoside, kaempferol-3,7-*O*-dirhamnoside, isorhamnetin-3-*O*-rutinoside, kaempferol-3-*O*-rutinoside, and kaempferol-3-*O*-glucoside),[21,22] and phenolic acids (e.g. chlorogenic acid glycoside, sinapaldehyde, and caftaric acid).[23,24]

In vitro cytotoxic and anticancer activities of different solvent extracts

Different solvent extracts of *S. nicolai* have been evaluated for their *in vitro* cytotoxic potential to murine spleen cells by visual counting for the vital cells after growing under the effect of serial dilutions of these extracts. The results revealed that methanol (1) and *n*-butanol (5) extracts showed very rare cytotoxic activities with IC_{50} values of 451.29 and 382.38μg/mL, respectively. While pet. ether (2), dichloromethane (3), and ethyl acetate (4) extracts showed moderate cytotoxic effects with IC_{50} values of 68.03, 90.29, and 65.23 μg/mL, respectively [Table 2 and Figure 1], indicating that flagging cytotoxic effects against murine spleen cell have been observed. Moreover, the National Cancer Institute instructions demonstrate the criteria and the conditions of cytotoxic activity for a chemical complexes as the concentration of an inhibitor that is required for 50% inhibition of cells growth.[25] IC_{50} where the values ≤ 20 μg/mL, is considered to be potentially cytotoxic, while IC_{50} values 21-100 μg/mL = moderately cytotoxic, IC_{50} 101–200 μg/mL = weakly cytotoxic and IC_{50} > 501 μg/mL = no cytotoxic effect.[26,27] On the other hand, significant anticancer effects against

Table 1: Antioxidant activities of different extracts of *S. nicolai*

Sample	DPPH (IC_{50} μg/mL)	ABTS (IC_{50} μg/mL)	FRAP (mM $FeSO_4$ equivalent/mg extract)
MeOH	24.89±2.73	15.73±1.22	15.46±0.81
Petroleum ether	118.17±3.39	52.25±1.35	1.58±1.47
CH_2Cl_2	65.28±5.14	29.89±0.56	7.92±0.49
EtOAc	20.49±1.59	9.18±0.73	22.19±1.56
n-BuOH	22.09±1.88	12.43±0.78	18.34±1.37
H_2O	56.70±5.06	23.18±1.18	11.39±0.67
Ascorbic acid	2.92±0.29	-	-
Quercetin	-	-	21.45±2.55
Trolox	-	1.63±0.46	-

Results are average of triplicate measurements (*n*=3) and expressed as mean±SD; IC_{50} values were calculated using a four-parameter logistic curve (SigmaPlot® 11.0). SD: Standard deviation; ABTS: Radical-scavenging activity; DPPH: Free radical-scavenging activity; FRAP: Ferric reducing antioxidant power

Table 2: Different solvent extracts of *S. nicolai* showing the variation in cytotoxic effect (IC_{50}) on murine spleen cells by visual counting under the inverted microscope

Concentration µg/ml	Extracts	1	2	3	4	5
1000	Concentration 1	390				210
500	Concentration 2	420				300
250	Concentration 3	510				450
125	Concentration 4	450	210	240	150	330
62.5	Concentration 5	450	300	330	300	450
31.2	Concentration 6		360	420	390	510
Control		600	600	600	600	600
-	Equation	^{a}y = −0.0832 × + 476.25	y = −1.5765 × + 404.92	y = −1.8507 × + 464.92	y = −2.536 × + 464.88	y = −0.2627 × + 461.19
Results	Cytotoxicity IC_{50} µg/ml	451.29	68.03	90.29	65.23	382.38
-	-	Rare	Moderate	Moderate	Moderate	Rare

^{a}y: Is the concentration equivalent to IC_{50} per µg and (x) is the control IC_{50} (300); 1: MeOH; 2: Petroleum ether; 3: CH_2Cl_2; 4: EtOAc and 5: *n*-BuOH

Table 3: Crystal violet staining and colorimetric assay at optical density=490 nm for human hepatoma cell line under the effect of *S. nicolai* extracts showing cytotoxicity profile

Extract	CVS spectroscopic reading for HepG2 growth	
	Complex concentration (125 µg/mL)	Complex concentration (500 µg/mL)
1	0.883	0.684
2	0.931	0.611
3	0.753	0.702
4	0.806	0.730
5	0.684	0.618
Control	1.022	

Control: No added fraction. CVS: Crystal violet stain; HepG-2: Human hepatoma

HepG-2 cell line after 24 h exposure were obvious using colorimetric assay. The results revealed that the crystal violet stain spectroscopic readings for HepG-2 growth at complex concentration (125 µg/mL) were 0.883, 0.931, 0.753, 0.806, and 0.684, while at complex concentration (500 µg/mL) were 0.684, 0.611, 0.702, 0.730, and 0.618, respectively for MeOH, pet. ether, dichloromethane, ethyl acetate and *n*-butanol extracts against control at 1.022 [Table 3]. Results showed an intimate relationship between anticancer activity and concentration. In general, the anticancer activity of the polyphenolic-rich extracts may be due to the presence of such phenolic compounds.[28-32] Moreover, these polyphenolic compounds are capable of inhibiting cancer cells through several modes of actions.[26]

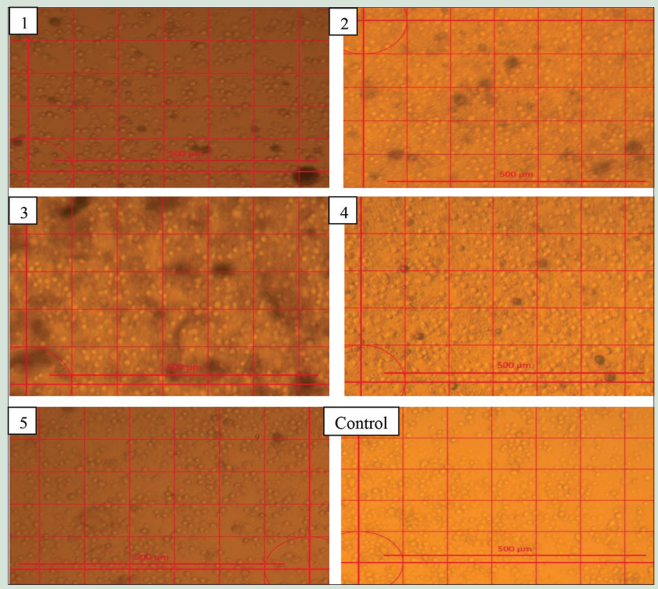

Figure 1: Cytotoxic effect of different solvent extracts of *S. nicolai* on murine splenocytes after 36 h incubation

Characterization of the phenolic compounds in *Strelitzia nicolai*

The ethyl acetate and *n*-butanol extracts of *S. nicolai* were investigated for their polyphenolic constituents using HPLC-DAD-ESI-MS/MS technique. Nineteen compounds were detected and tentatively identified in the ethyl acetate extract were categorized as phenolic acids, flavonoids (glycosides and aglycones), chalcones, and other nucleus. On the other hand, eleven compounds were detected and tentatively identified in the *n*-butanol extract were categorized as phenolic acids and flavonoids (glycosides and aglycones). The identification of the phenolic compounds was based on comparing their fragmentation pattern using negative ion ionization mode with the available data in the literature. The chemical structures of some selected compounds and their full MS/MS^n pattern will be mentioned below Tables 4, 5 and Figures 2-7.

Phenolic compounds were detected in the EtOAc extract

Phenolic acids and their derivatives

Compound 1 was detected at *Rt* = 1.43 min, it showed a deprotonated ion $[M-H]^-$ at *m/z* 515, it also generated a fragment ion as a base peak at *m/z* 341 corresponding to the loss of quinic acid moiety (-*m/z* 174 u) $[M-H\text{-quinic acid moiety}]^-$, further loss of glucosyl moiety (-*m/z* 162 u) was confirmed by the appearance of a fragment ion at *m/z* 179 $[M-H\text{-quinic acid-glucose moiety}]^-$. Therefore, compound 1 could be identified as chlorogenic acid glucoside as previously described.[33] Compound 16 was detected at *Rt* = 28.92 min, it showed a deprotonated ion as a base peak $[M-H]^-$ at *m/z* 177, it also generated a fragment ion at *m/z* 162 corresponding to the loss of methyl moiety (-*m/z* 15) $[M-H-CH_3]^-$, further loss of hydroxyl moiety was confirmed by the appearance of a fragment ion at *m/z* 145 $[M-H-CH_3-OH]^-$, further neutral loss of CO moiety of the aldehyde group was confirmed by the appearance of a fragment ion at *m/z* 117 (-*m/z* 28 u) $[M-H-CH_3-OH-CO]^-$. Therefore, compound 16 could be identified as 3-(4-hydroxy-3-methoxyphenyl) prop-2-enal (coniferyl aldehyde).[34]

Compound 17 was detected at *Rt* = 30.57 min, it showed a deprotonated ion $[M-H]^-$ at *m/z* 207, it also generated a fragment ion as a base peak at *m/z* 192 corresponding to the presence of sinapoyl moiety and due to the loss of methyl moiety (-*m/z* 15 u) $[M-H-CH_3]^-$, further loss of methyl moiety (-*m/z* 15 u) was confirmed through the fragment ion at *m/z* 177 $[M-H-2CH_3]^-$. Thus, compound 17 could be identified as 3,5-dimethoxy-4-hydroxycinnamaldehyde (sinapaldehyde).[35] Compound 19 was detected at *Rt* = 40.70 min, it showed a deprotonated molecular ion [M-H]⁻ at *m/z* 311, it also generated a fragment ion at *m/z* 179 corresponding to the loss of tartaric moiety (-*m/z* 132 u) [M-H-tartaric]⁻, in addition to a fragment ion was appeared at *m/z* 149 corresponding to the loss of caffeoyl moiety (-*m/z* 162 u) [M-H-caffeoyl]⁻. This fragmentation pattern was typically assigned to caftaric acid (*cis*-caffeoyl tartaric acid).[35,36]

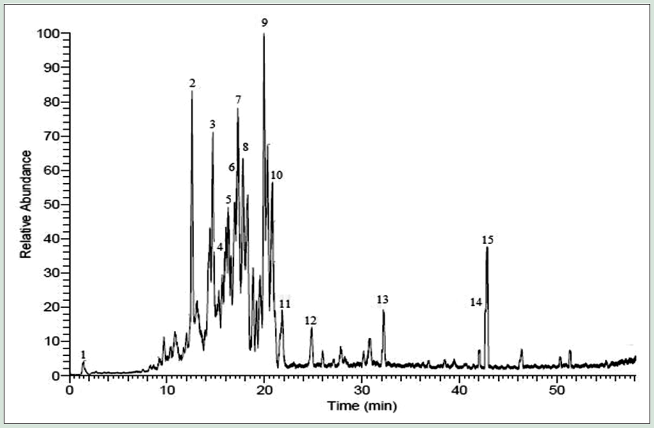

Figure 2: Negative HPLC-DAD-ESI-MS/MS profiles of phenolic compounds were detected in the EtOAc extract of *S. nicolai* leaves

Flavonoids

Compound 3 was detected at *Rt* = 14.66 min, it showed a deprotonated molecule [M-H]⁻ at *m/z* 755, a MS/MS fragment was observed at *m/z* 609 was assigned to rutin molecule after the neutral loss of one rhamnosyl moiety (-*m/z* 146 u) [M-H-rhamnose moiety]⁻, a fragment ion was detected at m/z 591 due to further loss of water molecule (-*m/z* 18 u) [M-H-rhamnose moiety-H_2O]⁻, another fragment ion was also appeared at *m/z* 445 due to the loss of the second rhamnosyl moiety (-*m/z* 146 u) [M-H-2rhamnose moiety-H_2O]⁻, a diagnostic fragment ion was observed at *m/z* 301 was assigned to quercetin a glycone and can be explained by the release of the glucose moiety (-*m/z* 162 u) [M-H-2rhamnose moieties-glucose moiety]⁻, another key fragment ions of quercetin a glycone were observed at *m/z* 271 and 255. Therefore, compound 3 was identified as quercetin 3-(2 G-rhamnosylrutinoside) in comparison with the previously published data.[37] Compound 5 was detected at *Rt* = 16.18 min, it displayed a deprotonated molecule [M-H]⁻ at *m/z* 609, the neutral loss of rhamnosyl moiety (-*m/z* 146 u) afford a fragment ion at *m/z* 463 [M-H-rhamnose moiety]⁻, further loss of glucosyl moiety (-*m/z* 162 u) was confirmed through a diagnostic fragment ion at *m/z* 301 [M-H-rhamnosyl-glucosyl]⁻, which was assigned to quercetin a glycone and other key fragment ions of quercetin a glycone were detected at *m/z* 271, 255 and 179. Therefore, compound 5 was identified as quer cetin-3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranose (rutin).[36]

Compound 6 was detected at *Rt* = 17.18 min; it displayed a deprotonated molecule as a base peak at *m/z* 301, which was assigned to quercetin a

Table 4: Phenolic compounds tentatively identified in the EtOAc extract of *S. nicolai* leaves by HPLC-DAD-ESI-MS/MS in negative ion mode

Peak number	R_t	MW	[M-H]⁻ (*m/z*)	MS/MS fragments*	Proposed compounds	References
1	1.43	516	515	**341**, 179, 173	Chlorogenic acid glycoside	Abu-Reidah *et al*. (2013a)
2	8.57	454	453	**407**	Unknown	-
3	14.66	756	755	737, 609, 591, 489, 445, 301, **300**, 271, 255	Quercetin 3-(2 G-rhamnosylrutinoside)	Abu-Reidah *et al*. (2013b)
4	15.78	612	611	491, 447, 303, 302, **300**, 273, 255	Unknown	-
5	16.18	610	609	463, 300, 301, 271, 255, 179	Rutin	Abu-Reidah *et al*. (2015)
6	17.18	301	300	301, 300, 271, 269, 255, 229, 179, 169, 151	Quercetin	Abu-Reidah *et al*. (2014)
7	18.12	464	463	**301**, 271, 255, 179, 151	Quercetin-3-*O*-glucoside	Bravo *et al*. (2007); Abu-Reidah *et al*. (2012)
8	19.29	578	577	**431**, 285	Kaempferol-3,7-*O*-dirhamnoside (Kaempferitrin)	Abu-Reidah *et al*. (2012)
9	19.66	624	623	459, **315**, 300, 271, 255, 179	Isorhamnetin-3-*O*-rutinoside	Abu-Reidah *et al*. (2012)
10	20.18	594	593	473, 447, 429, **285**, 255, 227, 151		Al-Rawahi *et al*. (2014)
11	20.75	448	447	285, **284**, 255, 227, 151	Kaempferol-3-*O*-glucoside	Bravo *et al*. (2007); Chen *et al*. (2014)
12	21.19	564	563	473, 443, 413, 383, 353, 285, **284**	kaempferol-3-O-β-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl	NA
13	21.98	392	391	**376**, 359, 239, 161, 151, 135	Xanthoangelol	Kim *et al*. (2014)
14	24.35	462	461	446, 341, 299	Isoorientin-3′-*O*-methyl ether (Isoscoparin)	Abu-Reidah *et al*. (2012)
15	28.85	446	445	269, 193, 176, **175**, 160	Unknown	-
16	28.92	178	177	162, 145, 117	3-(4-hydroxy-3-methoxyphenyl) prop-2-enal (coniferyl aldehyde)	Sanz *et al*. (2012)
17	30.57	208	207	**192**	3,5-dimethoxy-4 hydroxycinnamaldehyde (Sinapaldehyde)	Sanz *et al*. (2012)
18	32.25	328	327	309, 291, 239, **229**, 221, 211, 171	1,7-bis-(3,4-dihydroxyphenyl)-4-hepten-3-one (hirsutenone)	Riethmller *et al*. (2013)
19	38.57	312	311	179, 149	caftaric acid	Chen *et al*. (2012); Abu-Reidah *et al*. (2015)
20	41.29	330	329	**314**, 300, 299, 285, 243	Quercetin-dimethyl-ether	Pellati *et al*. (2011)
21	42.05	315	314	300, **299**, 285, 271	Unknown	-
22	44.84	314	313	**298**, 283	4′,7′ -dimethoxy luteolin (4′,5-dihydroxy-3′,7-dimethoxyflavone)	Simirgiotis *et al*. (2015)
23	49.38	344	343	**328**, 313, 300, 283, 181	Dihydroxy trimethoxy flavonol isomer	Abdel-Hameed *et al*. (2014)
24	51.05	522	521	505, 493, **453**, 433, 398, 372, 303	Unknown	-

*Bold items referred to the main aglycones fragments. NA: Not available; MS: Mass spectrometer

Table 5: Phenolic compounds tentatively identified in the *n*-butanol extract of *S. nicolai* leaves by HPLC-DAD-ESI-MS/MS in negative ion mode

Peak number	R_t	MW	$[M-H]^-$ (*m/z*)	MS/MS fragments*	Proposed compounds	References
1	1.47	316	315	225, 163, **153**, 152, 109, 108	Protocatechuic acid-*O*-glucoside	Abu-Reidah *et al.* (2015)
2	12.58	576	577	503, 474, **473**, 383, 353	Unknown	-
3	14.70	624	623	608, 477, **315**, 300, 271, 255, 279	Isorhamnetin 3-*O*-rutinoside	Abu-Reidah *et al.* (2012)
4	15.04	756	755	737, 609, 591, 489, 445, 301, **300**, 271, 255	Quercetin 3-(2 G-rhamnosylrutinoside)	Abu-Reidah *et al.* (2013a)
5	15.80	740	739	593, **575**, 473, 429, 393, 285, 284, 257, 255, 227	Kaempferol 3-*O*-rutinoside-7-*O*-rhamnoside	Ágnes, 2013
6	16.61	770	769	**605**	Unknown	-
7	17.2	612	611	593, 374, 344, **301**, 271, 255, 179	Unknown	-
8	18.18	464	463	**301**, 271, 255, 179, 151	Quercetin-3-*O*-glucoside	Bravo *et al.* (2007); Abu-Reidah *et al.* (2012); Al-Rawahi *et al.* (2014)
9	19.95	594	593	447, 429, **285**, 255, 227, 179, 169	Kaempferol 3-*O*-rutinoside	Al-Rawahi *et al.* (2014)
10	20.83	564	563	**473**, 443, 413, 383, 353, 285, 284	Unknown	-
11	21.80	448	447	285, **284**, 255, 227, 151	Kaempferol-3-*O*-glucoside	Ágnes, 2013; Bravo *et al.* (2007); Chen *et al.* (2014)
12	24.84	610	609	463, 445, **300**, 301, 271, 255, 179	Quercetin-3-*O*-rutinoside (Rutin)	Abu-Reidah *et al.* (2015)
13	32.20	686	685	**523**, 505, 477, 343	Ligstroside glucoside	Sanz *et al.*, 2012
14	42.70	312	311	179, 149	Monocaffeyltartaric acid	Chen *et al.* (2012); Abu-Reidah *et al.* (2015)
15	42.90	330	329	**314**, 285	Quercetin-dimethyl-ether	Pellati *et al.* (2011)

*Bold items referred to the main aglycones fragments. NA: Not available; MS: Mass spectrometer

glycone and other characteristic key fragment ions were appeared at *m/z* 271, 269, 255, 229, 179, and 151. Therefore, compound 6 was identified as 5,7,3′,4′-flavon-3-ol (quercetin).[38] Compound 7 was detected at *Rt* = 18.12 min, it displayed a deprotonated molecule $[M-H]^-$ at *m/z* 463, the neutral loss of glucosyl moiety (-*m/z* 162 u) was confirmed through a fragment ion as a base peak at *m/z* 301 $[M-H-glucose]^-$ was assigned to quercetin a glycone. Other key fragment ions were also observed at *m/z* 271, 255, 179, and 151. This fragmentation pattern was typically assigned to quercetin-3-*O*-β-D-glucoside (isoquercetin).[39-41] Compound 8 was detected at *Rt* = 19.29 min, it displayed a deprotonated molecule $[M-H]^-$ at *m/z* 577, the neutral loss of rhamnosyl moiety (-*m/z* 146 u) give a fragment ion as a base peak at *m/z* 431 [M-H-rhamnosyl moiety]$^-$ was assigned to kaempferol-*O*-rhamnoside, further neutral loss of the second rhamnosyl moiety (-*m/z* 146 u) was confirmed through the appearance of a fragment ion at *m/z* 285 which was attributed to kaempferol a glycone. Accordingly, compound 8 was identified as kaempferol-3,7-*O*-dirhamnoside (kaempferitrin).[40] Compound 9 was detected at *Rt* = 19.66 min, it displayed a deprotonated molecule $[M-H]^-$ at *m/z* 623, the neutral loss of methyl moiety (-*m/z* 15 u) give a fragment ion at *m/z* 608 $[M-H-CH_3]^-$, while the neutral loss of rhamnosyl moiety (-*m/z* 146 u) give a fragment ion at *m/z* 477 $[M-H-Rha]^-$, the loss of glucose moiety (-*m/z* 162 u) was confirmed through a diagnostic fragment ion as a base peak at *m/z* 315 $[M-H-rutinoside]^-$, which was assigned to isorhamnetin a glycone. Therefore, compound 9 was identified as isorhamnetin-3-*O*-rutinoside.[40]

Compound 10 was detected at *Rt* = 20.18 min, it displayed a deprotonated molecule $[M-H]^-$ at *m/z* 593, the neutral loss of rhamnosyl moiety (-*m/z* 146 u) give a fragment ion $[M-H-Rha]^-$ at *m/z* 447 was assigned to kaempferol glucoside, further loss of the glucosyl moiety (-*m/z* 162 u) was confirmed through the appearance of a fragment ion as a base peak at *m/z* 285 $[M-H-rhamnosyl-glucosyl]^-$ was assigned to kaempferol a glycone and due to total loss of rutinoside moiety. Therefore, compound 10 was identified as kaempferol-3-*O*-rutinoside.[41] Compound 11 was detected at *Rt* = 20.75 min, it displayed a deprotonated molecule $[M-H]^-$ at *m/z* 447 was assigned to kaempferol glucoside, the neutral loss of glucosyl moiety (-*m/z* 162 u) give a fragment ion at *m/z* 285 $[M-H-glucosyl]^-$ was assigned to kaempferol a glycone, and other key fragment ions were detected at *m/z* 255, 227, 179 and 151. Compound 11 could be identified as kaempferol-3-*O*-glucoside.[32,39]

Compound 12 was detected at *Rt* = 21.19 min, it displayed a deprotonated molecule $[M-H]^-$ at *m/z* 563, the neutral loss of rhamnosyl moiety (-*m/z* 146 u) give a fragment ion at *m/z* 417 $[M-H-rhamnosyl]^-$, further loss of xylosyl moiety (-*m/z* 132) [M-H- rhamnosyl-xylosyl]$^-$ was confirmed through the appearance of a diagnostic fragment ion at *m/z* 285 was assigned to kaempferol a glycone. Thus, compound 12 was tentatively identified as kaempferol-3-O-β-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl. Compound 14 was detected at *Rt* = 22.67 min, it displayed a deprotonated molecule $[M-H]^-$ at *m/z* 461, the loss of methyl moiety (-*m/z* 15 u) give a fragment ion at *m/z* 446 $[M-H-CH_3]^-$, while neutral loss of glucosyl moiety (-*m/z* 162 u) give a fragment ion as a base peak at *m/z* 299 $[M-H-glu]^-$ was assigned to tri-hydroxy flavone nucleus. This fragmentation pattern was typically assigned to isoorientin-3′-*O*-methyl ether (isoscoparin).[42] Compound 20 was detected at *Rt* = 42.05 min, it displayed a deprotonated molecule $[M-H]^-$ at *m/z* 329, the loss of methyl moiety (-*m/z* 15 u) give a fragment ion as a base peak at *m/z* 314 $[M-H-CH_3]^-$, further neutral loss of methyl moiety (-*m/z* 15 u) give a fragment ion at *m/z* 299 $[M-H-2CH_3]^-$, while the neutral loss of CO_2 moiety (-*m/z* 44 u) give a fragment ion at *m/z* 285 $[M-H-CO_2]^-$, and another ion (-*m/z* 42 u) at *m/z* 243 $[M-H-C_2H_2O-CO_2]^-$, thus compound 20 could be identified as quercetin-dimethyl-ether.[43]

Compound 22 was detected at *Rt* = 49.38 min, the MS/Ms spectrum displayed a deprotonated molecular ion $[M-H]^-$at *m/z* 313, while the loss of methyl moiety (-*m/z* 15 u) give a fragment ion as a base peak at *m/z* 298 $[M-H-CH_3]^-$, further neutral loss of methyl moiety (-*m/z* 15 u) give a fragment ion at *m/z* 283 $[M-H-2CH_3]^-$, therefore, compound 22 was identified as 3′,7-dimethoxyluteolin (4′,5-dihydroxy-3′,7-dimethoxyflavone).[44] Compound 23 was detected at *Rt* = 51.05 min, the MS/Ms spectrum displayed a deprotonated molecular ion $[M-H]^-$ at *m/z* 343, the loss of methyl moiety (-*m/z* 15 u) give a fragment ion as a base peak at *m/z* 328 $[M-H-CH_3]^-$, further neutral loss of methyl moiety (-*m/z* 15 u) give a fragment ion at *m/z* 313 $[M-H-2CH_3]^-$, other fragment ions were observed at *m/z* 297 and 285. Therefore, compound 23 was identified as dihydroxy-trimethoxy flavonol.[45]

Chalcones

Compound 13 was detected at *Rt* = 21.98 min, it displayed a deprotonated molecule $[M-H]^-$ at *m/z* 391. The neutral loss of methyl moiety (-*m/z* 15 u) give a fragment ion as a base peak at *m/z* 376 $[M-H-CH_3]^-$ was assigned

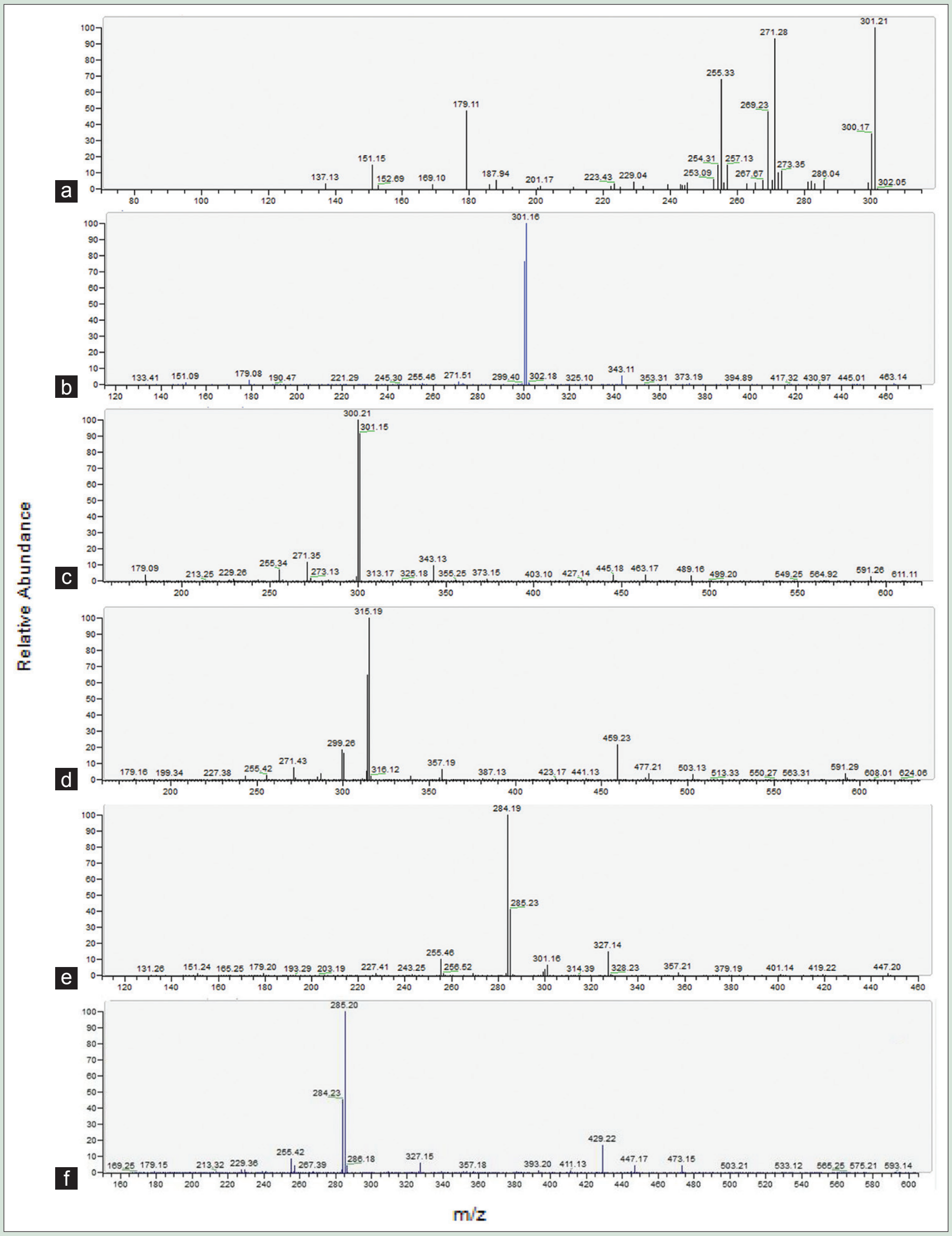

Figure 3: Mass spectrometry spectra and postulated fragmentation pattern of some selected phenolic compounds detected in the EtOAc extract of *S. nicolai* leaves using HPLC-DAD-ESI-MS/MS in negative ionization mode; (a) quercetin $[M-H]^-$ *m/z* = 301; (b) quercetin-3-*O*-glucoside $[M-H]^-$ *m/z* = 463; (c) rutin $[M-H]^-$ *m/z* = 609; (d) isohammetin-3-*O*-rutinoside $[M-H]^-$ *m/z* = 623; (e) kaempferol-3-*O*-glucoside $[M-H]^-$ *m/z* = 447; and (f) kaempferol-3-*O*-rutinoside $[M-H]^-$ *m/z* = 593

to methylated chalcone, the appearance of a fragment ion at *m/z* 135 was assigned to *p*-hydroxycinnamic acid moiety and due to the loss of 2,6-octa-dienyll-4-hydroxyphenyl moiety. Therefore, compound 13 was tentativelyidentifiedas3'-(3,7-dimethyl-2,6-Octadienyl)-2'-4,4'-trihydroxy chalcone.[46]

Other detected compounds

Compound 18 was detected at *Rt* = 34.23 min, it displayed a deprotonated molecular ion $[M-H]^-$ at *m/z* 327, the neutral loss of water moiety (-*m/z* 18 u) give a fragment ion at *m/z* 309 $[M-H-H_2O]^-$, the appearance of another fragment ion at *m/z* 291 due to further loss of another water moiety $[M-H-2H_2O]^-$, other fragment ions were observed at *m/z* 239, 229, 221, 211 and 171. Therefore, compound 18 was tentatively identified as 1,7-bis-(3,4-dihydroxyphenyl)-4-hepten-3-one (hirsutenone) in comparison with the previously published data.[47]

Phenolic compounds were detected in the n-BuOH extract

Phenolic acids and their derivatives

Compound 1 was detected at *Rt* = 2.76 min, it showed a deprotonated molecular ion $[M-H]^-$ at *m/z* 315, a diagnostic fragment ion as a base peak was detected at *m/z* 153 due to the loss of glucosyl moiety (-*m/z* 162 u) and corresponding to protocatechuic acid $[M-H\text{-glucosyl moiety}]^-$, further loss of CO_2 moiety (-*m/z* 44 u) was confirmed through the appearance of fragment ion at *m/z* 109 $[M-H\text{-glucosyl moiety-}CO_2]^-$.

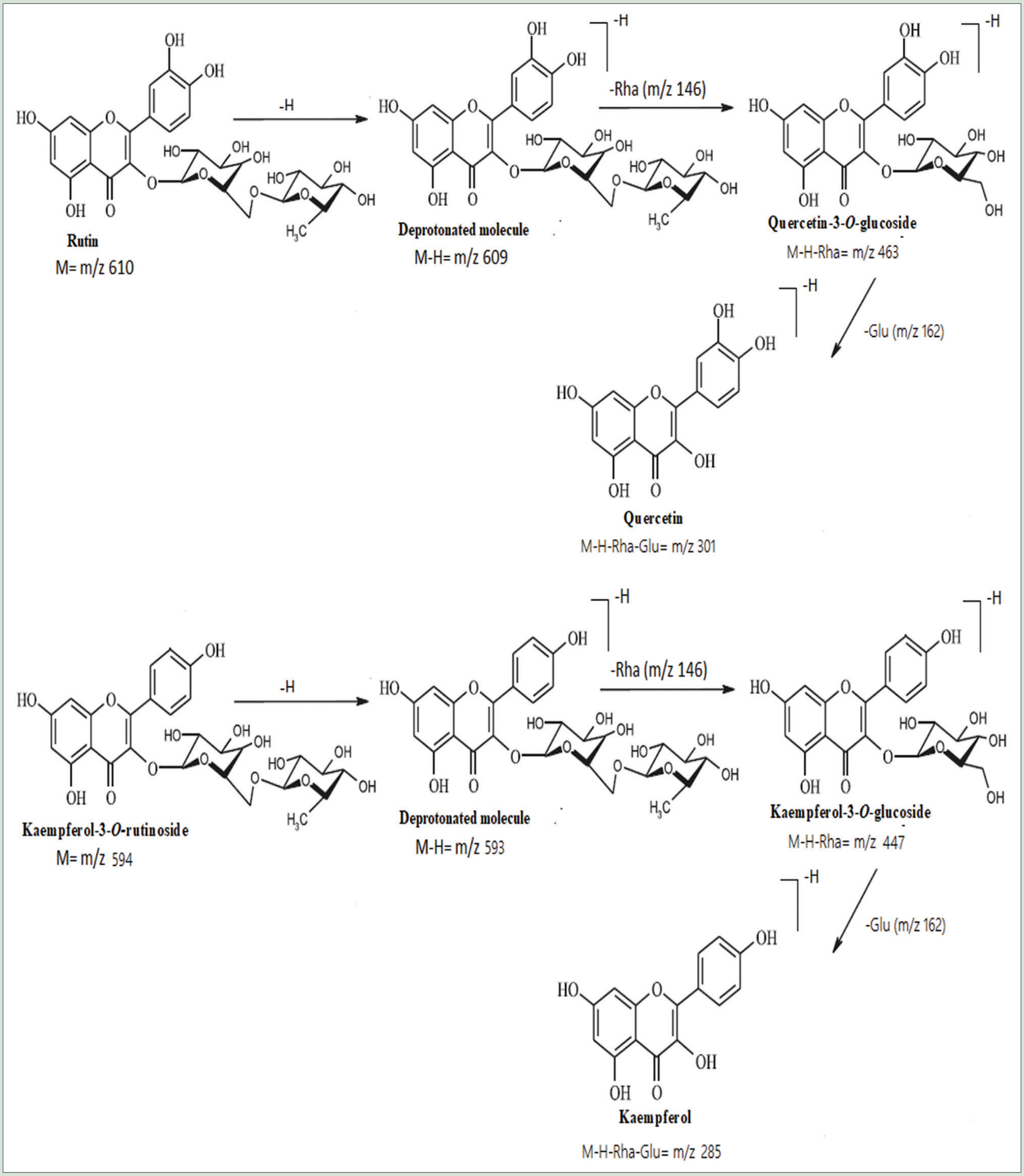

Figure 4: A proposed fragmentation pattern of compound 5 (rutin) and compound 10 (Kaempferol-3-O-rutinoside)

Therefore, compound 1 could be identified as protocatechuic acid glucoside.[36] Compound 14 was detected at *Rt* = 42.08 min, it showed a deprotonated molecular ion $[M-H]^-$ at *m/z* 311, it also generated a fragment ion at *m/z* 179 corresponding to the loss of tartaric moiety (-*m/z* 132) $[M-H\text{-tartaric moiety}]^-$, another fragment ion was appeared at *m/z* 149 due to the loss of caffeoyl moiety (-*m/z* 162) $[M-H\text{-caffeoyl moiety}]^-$, this fragmentation profile was assigned to monocaffeyltartaric acid (caftaric).[35,36]

Flavonoids

Compound 3 was detected at *Rt* = 14.70 min, it showed a deprotonated molecular ion $[M-H]^-$ at *m/z* 623, it also generated a fragment ion at *m/z* 608 corresponding to the loss of methyl moiety (-m/z 15 u) $[M-H-CH_3]^-$, another fragment ion was observed at *m/z* 477 refer to the loss of rhamnosyl moiety (-*m/z* 146 u) $[M-H\text{-rhamnosyl moiety}]^-$, it also generated a diagnostic fragment ion as a base peak at *m/z* 315 refer to further loss of glucosyl moiety (-*m/z* 162 u), which may represent isorhamnetin a glycone [M-H-rhamnosyl moiety-glucosyl moiety]$^-$, another fragment ion was detected at *m/z* 300 due to the loss of methyl moiety from the a glycone nucleus [M-H-rhamnosyl moiety-glucosyl moiety-CH_3]$^-$, this fragmentation was typically assigned to isorhamnetin 3-*O*-rutinoside.[40] Compound 4 was detected at *Rt* = 15.04 min, it showed a deprotonated molecule [M-H]$^-$ at *m/z* 755, a MS/MS fragment was appeared at *m/z* 609 corresponding to rutin molecule after the neutral loss of one rhamnosyl moiety (-*m/z* 146 u) [M-H-rhamnose moiety]$^-$, a fragment ion was observed at *m/z* 445 due to the loss of another rhamnosyl and water moieties (-*m/z* 146-18 u) [M-H-2 rhamnose moiety-H_2O]$^-$, a diagnostic fragment ion was detected at *m/z* 301 was assigned to quercetin a glycone and can be explained by the release of the glucose moiety (-*m/z* 162 u) [M-H-2 rhamnose moieties-glucose moiety]$^-$, in addition to the appearance of key fragment ions of quercetin a glycone at *m/z* 271, and 255. Therefore, compound 4 was identified as quercetin 3-(2 G-rhamnosylrutinoside).[37]

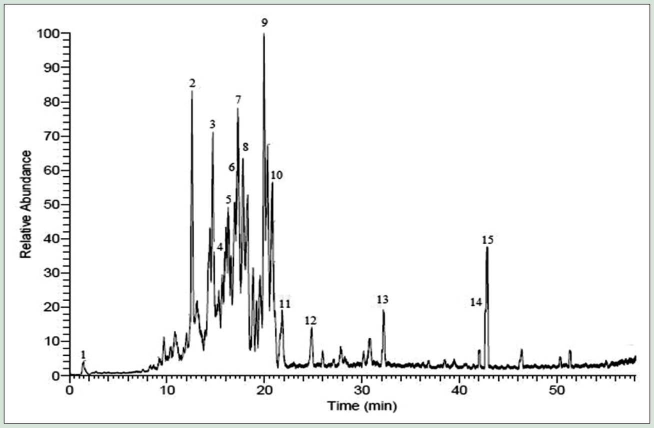

Figure 5: Negative HPLC-DAD-ESI-MS/MS profiles of phenolic compounds were detected in the EtOAc extract of *S. nicolai* leaves

Compound 5 was detected at *Rt* = 15.80 min, it showed a deprotonated molecule [M-H]$^-$ at *m/z* 739, a MS/MS fragment ion was observed at *m/z* 593 due to the loss of rhamnosyl moiety (-*m/z* 146 u) [M-H-rhamnose moiety]$^-$, further neutral loss of water molecule (-*m/z* 18 u) led to generation of a fragment ion at *m/z* 575 [M-H-rhamnose moiety-H_2O]$^-$, further neutral loss of another rhamnosyl moiety (-*m/z* 146 u) led to generation of a fragment ion at *m/z* 429 [M-H-2rhamnose moiety-H_2O]$^-$, the appearance of fragment ion at *m/z* 285 was accounted for neutral loss of glucosyl moiety (-*m/z* 162 u) [M-H-2 rhamnose moiety-glucosyl moiety]$^-$ it was assigned to kaempferol a glycone with key fragment ions at *m/z* 257, 255, and 227. Therefore, compound 5 was identified as kaempferol 3-*O*-rutinoside-7-*O*-rhamnoside.[41] Compound 8 was detected at *Rt* = 18.18 min, it showed a deprotonated molecule [M-H]$^-$ at *m/z* 463, a diagnostic MS/MS fragment ion as a base peak was detected at *m/z* 301 due to the loss of glucosyl moiety (-*m/z* 162 u) [M-H-glucosyl moiety]$^-$ it was assigned to quercetin a glycone, another key a glycone fragments were appeared at *m/z* 271, 255, 179 and 151. Thus compound

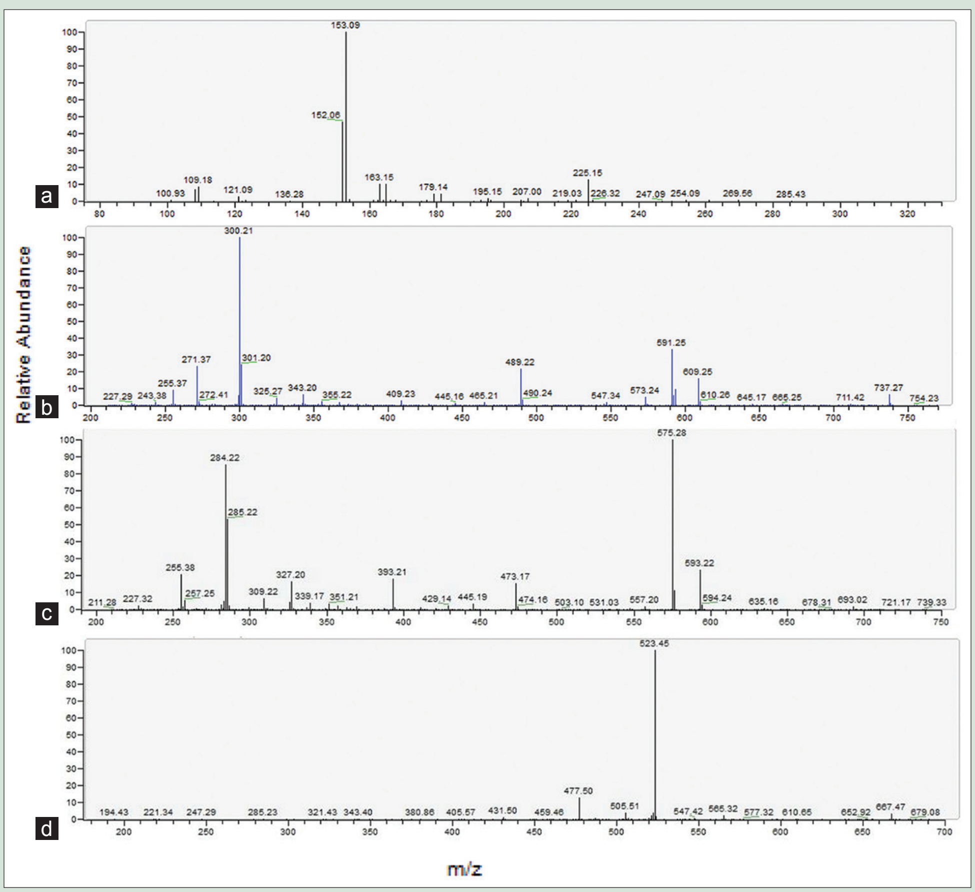

Figure 6: Mass spectrometry spectra and postulated fragmentation pattern of some selected phenolic compounds detected in the *n*-BuOH extract of *S. nicolai* leaves using HPLC-DAD-ESI-MS/MS in negative ionization mode; (a) protocatechuic acid-*O*-glucoside [M-H]$^-$ *m/z* = 315; (b) quercetin 3-(2 G-rhamnosylrutinoside) [M-H]$^-$ *m/z* = 755; (c) kaempferol 3-*O*-rutinoside-7-*O*-rhamnoside [M-H]$^-$ *m/z* = 739, and (d) ligstroside glucoside [M-H]$^-$ *m/z* = 685

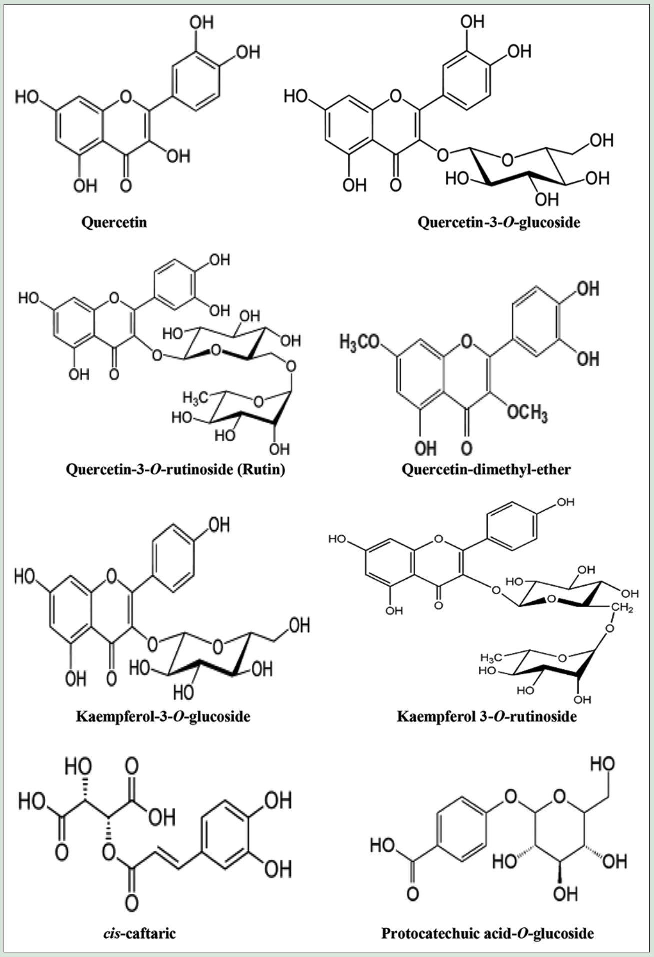

Figure 7: Chemical structures of some identified phenolic compounds in EtOAc and *n*-BuOH extracts of *S. nicolai* leaves

8 could be characterized as quercetin-3-*O*-β-D-glucoside.[39-41]

Compound 9 was detected at *Rt* = 19.95 min, it showed a deprotonated molecule [M-H]⁻ at *m/z* 593, a fragment ion was observed at *m/z* 447 refer to the release of rhamnosyl moiety (-*m/z* 146 u) [M-H-rhamnosyl moiety]⁻, further elimination of water molecule (-*m/z* 18 u) led to generation of fragment ion at *m/z* 429 [M-H-rhamnosyl moiety-H_2O]⁻, while the release of glucosyl moiety (-*m/z* 162 u) led to generation of a diagnostic MS/MS fragment ion as a base peak at *m/z* 285 [M-H-rhamnosyl moiety-glucosyl moiety]⁻ it was assigned to kaempferol a glycone, another key a glycone fragments were detected at *m/z* 255, 227, 179, and 169. Therefore, compound 9 was identified as kaempferol 3-*O*-rutinoside.[41]

Compound 11 was detected at *Rt* = 21.80 min, it showed a deprotonated molecule [M-H]⁻ at *m/z* 447, while the release of glucosyl moiety (-*m/z* 162 u) led to generation of a diagnostic MS/MS fragment ion as a base peak at *m/z* 285 [M-H-glucosyl moiety]⁻ it was assigned to kaempferol a glycone, another key a glycone fragments were detected at *m/z* 255, 227, 179, 169 and 151. Therefore, compound 11 was identified as kaempferol-3-*O*-β-D-glucoside.[39,42,48] Compound 12 was detected at *Rt* 27.82 min, it showed a deprotonated molecule [M-H]⁻ at *m/z* 609, while the release of rhamnosyl moiety (-*m/z* 146 u) led to generation of MS/MS fragment ion at *m/z* 463 [M-H-rhamnosyl moiety]⁻ it was assigned to quercetin-*O*-glucoside, the removal of water molecule (-*m/z* 18 u) produce a fragment ion at *m/z* 445, while the elimination of the glucosyl moiety (-*m/z* 162 u) was confirmed through a diagnostic fragment at *m/z* 301 was assigned to quercetin a glycone, another key a glycone fragments were observed at *m/z* 271, 255, and 179. Therefore, compound 12 was identified as quercetin-3-*O*-rutinoside (rutin).[36] Compound 15 was detected at *Rt* = 42.90 min, it showed a deprotonated molecule [M-H]⁻ at *m/z* 329, while the release of methyl moiety (-*m/z* 15 u) led to generation of MS/MS fragment ion at *m/z* 314 [M-H-CH_3]⁻ it was assigned to quercetin methyl ether, the removal of another methyl moiety (-*m/z* 15 u) produce a fragment ion at *m/z* 299 [M-H-2CH_3]⁻. Therefore, compound 15 was identified as quercetin-dimethyl-ether as previously reported.[43]

Phenylethanoid derivatives

Compound 13 was detected at *Rt* = 32.20 min, it showed a deprotonated molecule [M-H]⁻ at m/z 685, while the release of glucosyl moiety (-*m/z* 162 u) led to generation of a diagnostic MS/MS fragment ion as a base beak at *m/z* 523 [M-H-glucosyl moiety]⁻ it was assigned to ligstroside molecule, further removal of water molecule (-*m/z* 18 u) produce a fragment ion at *m/z* 505 [M-H-glucosyl moiety-H_2O]⁻ and the elimination of another glucosyl moiety (-*m/z* 162 u) was confirmed through the appearance of a diagnostic fragment at *m/z* 343 [M-H-H_2O-2glucosyl moiety]⁻. Therefore, compound 13 was identified as ligstroside glucoside as previously described.[34]

CONCLUSION

In the current study, the HPLC-DAD-ESI-MS/MS analysis led to the tentative identification of a total 30 phenolic compounds in the ethyl acetate and *n*-butanol extracts of *S. nicolai* leaves based on the determination of the precise mass of the deprotonated ions [M-H]⁻, which was obtained from the MS data and MS^n fragmentation pattern. Among the tentatively identified compounds, flavonoids were the major constituents. To the best of our knowledge, this research work is the first comprehensive study on the polyphenolic composition of the Egyptian *S. nicolai* species. Moreover, *S. nicolai* extracts demonstrated considerable antioxidant and anticancer activities. *S. nicolai* could be further studied to isolate its biologically-active constituents and to study their modes of actions.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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