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Original research paper

Introductory phytochemical analysis and bioactivity screening of *Aaronsohnia factorovskyi* aerial parts: Antioxidant, anti-inflammatory and antidiabetic insights

ELHAM AMIN[1]
AHLAM ELWEKEEL[2]
REEMA I. ALJASIR[3]
NUJUD H. ALHARBI[3]
RAZAN A. ALKHAMIS[3]
GHADEER L. ALFUHAYDI[3]
DALIA F. ALHABEEB[3]
ENAS I. A. MOHAMED[2]
MARWA H. A. HASSAN[2,*]

[1] *Department of Pharmaceutical Chemistry and Pharmacognosy College of Pharmacy, Qassim University, Buraidah 52571 Saudi Arabia*

[2] *Department of Pharmacognosy Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514 Egypt*

[3] *College of Pharmacy, Qassim University, Buraidah 52571 Saudi Arabia*

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ABSTRACT

The current research brings introductory data to phytochemical composition and biological potential of the methanolic extract derived from the aerial parts of *Aaronsohnia factorovskyi*. *In vitro* testing was conducted to evaluate its antioxidant, anti-inflammatory and antidiabetic activities. The total phenolics and total flavonoids contents of the extract were estimated as 52.46 ± 5.93 mg GAE g^{-1} and 19.01 ± 2.50 mg QE g^{-1}, resp. UPLC-ESI-MS analysis disclosed 14 chromatographic peaks corresponding to 19 putatively identified compounds, including flavonoids, sesquiterpenes, lignans, saponins and fatty acids. The antioxidant efficacy was evaluated using DPPH and phosphomolybdenum assays, as total antioxidant capacity equals to 12.31 ± 2.33 mg g^{-1} and 17.40 ± 0.96 mg g^{-1}, resp. *In vitro* testing of the anti-inflammatory activity demonstrated characteristic concentrations for 50 % inhibition of cyclooxygenase enzymes of 20.85 ± 0.73 µg mL^{-1} and 8.25 ± 0.29 µg mL^{-1} against COX-1 and COX-2, resp. Moreover, the extract displayed strong inhibition of α-glucosidase and α-amylase enzymes with concentration for 50 % inhibition of 0.243 ± 0.009 mg mL^{-1} and 0.275 ± 0.01 mg mL^{-1}, resp. Molecular docking studies further supported these findings highlighting the strong binding of yamogenin 3-*O*-neohesperidoside, convallasaponin A and baicalin to α-glucosidase and α-amylase active sites, as evidenced by their high binding affinities that are comparable to that of the co-crystallized ligands. Altogether, these findings recommend *A. factorovskyi* as a promising source for bioactive constituents.

Keywords: Aaronsohnia factorovskyi, UPLC-ESI-MS, antidiabetic, antioxidant, anti-inflammatory, molecular docking simulation

INTRODUCTION

Asteraceae is a large family of flowering plants with worldwide distribution, especially in the Mediterranean, Eastern Europe, and Asia Minor. Several Asteraceae plants were reported to possess significant medicinal importance, being used for various ailments, and

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[*] Correspondence; e-mail: marwa.hassan@pharm.bsu.edu.eg

contain beneficial compounds like flavonoids and phenolic acids, contributing to their therapeutic properties (1). Asteraceae plants exhibit significant pharmacological activities, likely due to their rich content of flavonoids, phenolic acids, and other polyphenolic metabolites. These properties suggest their therapeutic potential in managing inflammatory conditions (2) and diabetes (3, 4). *Aaronsohnia* is a small genus under the family Asteraceae native to North Africa and the Middle East. Little research has previously documented only two *Aaronsohnia* species: *A. factorovskyi* and *A. pubescens,* for some medicinal and commercial applications. *A. factorovskyi* was reported for antimicrobial and anthelmintic efficacy as well as for the biosynthesis of silver nanoparticles (5, 6). Other studies have explored the phytochemical composition, antioxidant, antidiabetic, and antimicrobial activities of *A. pubescens* (7, 8). *A. pubescens* was also reported for its ability to inhibit steel corrosion in an acidic environment (9). Hence, *Aaronsohnia* looks like an interesting candidate for pharmaceutical research.

The LC-MS analysis enabled the phytochemical profiling of several Asteraceae plant extracts in which various metabolites, *e.g.*, flavonoids, phenolic acids, terpenoids and saponins have been detected (10, 11).

Based upon the previous statements, the current research aims to explore the phytochemical composition of *A. factorovskyi* extract, using both qualitative and quantitative methods. It will screen the antioxidant, antidiabetic, and anti-inflammatory activity of *A. factorovskyi* using *in vitro* testing while also conducting *in silico* studies to correlate the observed bioactivity to the detected phytoconstituents.

EXPERIMENTAL

Plant material

A. factorovskyi (Asteraceae) was collected from the Qassim area during March 2023. A specimen labeled QPP-119 was kept at the College of Pharmacy, Qassim University, Saudi Arabia. The aerial parts of the plant were cleaned, dried, and then powdered. The powdered plant material (500 g) was extracted using aqueous methanol (80 %, 4 × 500 mL). The filtered extracts were pooled and dried under vacuum at a temperature not exceeding 50 °C using a rotary evaporator.

Chemicals

Aluminum chloride, ammonium molybdate, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, potassium acetate, quercetin, sodium carbonate, sodium phosphate, methanol, acetonitrile, formic acid, ascorbic acid, ibuprofen, and acarbose were purchased from Sigma-Aldrich Chemical Co. (USA). All used chemicals were of analytical grade.

Total phenolic content (TPC) and total flavonoid content (TFC)

TPC was evaluated using the Folin-Ciocalteu method according to the reported method (12). The results were expressed as mg gallic acid equivalents (GAE) per gram dry extract. TFC was assessed using aluminum chloride according to the reported method (12). The results were expressed as mg quercetin equivalents (QE) per gram dry extract.

UPLC-ESI-MS analysis

Metabolite profiling of the methanolic extract of *A. factorovskyi* aerial parts was performed using UPLC-ESI-MS analysis, following a previously established protocol (13). The extract was analyzed utilizing CQUITY UPLC, equipped with a BEH C18 column (1.7 μm, 2.1 × 50 mm) connected to an XEVO TQD triple quadruple mass spectrometer (Waters Corporation, USA). The sample was solubilized in methanol of HPLC grade and filtered using a PTFE (polytetrafluoroethylene) membrane with a 0.2-μm pore size. The solvent system is composed of 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B), with the flow rate of 0.2 mL min^{-1}. Gradient elution was as follows: 90 % A (0–2 min), from 90 % A to 70 % A (2–5 min), from 70 % A to 30 % A (5–15 min), from 30 % A to 10 % A (15–22 min), then isocratic at 10 % A (22–25 min), from 10 % A to 0 % A (25–26 min), and finally, isocratic at 100 % B (26–29 min).

Mass spectrometric analyses were performed in both positive and negative electrospray ionization (ESI) modes. The source temperature was maintained at 150 °C, with a desolvation temperature of 400 °C. Nitrogen was used as desolvation and cone gas at (600 and 50 L h^{-1}) flow rates, resp. The spray voltage used was 4.48 kV, the tube lens voltage was 10.00 V, cone voltage was 30.00 V, while the capillary voltage was 3.0 kV. Spectra were acquired over an *m*/*z* range of 100–1200.

Metabolites were tentatively identified by comparing their obtained mass and spectral data with previously reported data for the genus and family, as well as with online public databases (see Table SI). Data acquisition and analysis were managed using MetFrag (https://msbi ipb-halle de/MetFrag/).

Antioxidant activity

DPPH assay (12) and phosphomolybdenum assay (14) were carried out according to the reported methods. The total antioxidant capacity (TAC) was expressed as mg ascorbic acid equivalents (AAE) per gram dry extract.

Anti-inflammatory activity

The inhibitory potentials of *A. factorovskyi* crude extract, as well as the reference compound ibuprofen, against cyclooxygenase enzymes were assessed utilizing an enzyme immunoassay (EIA) human COX-2 and COX-1 inhibitor screening kits (Cayman Chemical, USA).

Antidiabetic activity

The antidiabetic potential of *A. factorovskyi* extract was evaluated *in vitro* by assessing the extract's inhibitory activity against digestive enzymes, including α-glucosidase and α-amylase.

The α-glucosidase inhibitory assay was carried out in compliance with the kit’s protocol instructions (Biovision, USA). For the assay, 10 μL of the extract (at various concentrations), or the reference drug (acarbose), was combined with 10 μL of a diluted α-glucosidase enzyme solution (2 μL enzyme with 38 μL α-glucosidase assay buffer) and 10 μL of assay buffer in a 96-well clear plate. The total volume was adjusted to 80 μL with the buffer, mixed thoroughly, and incubated at room temperature for 15–20 minutes. Finally, 20 μL of the enzyme substrate (*p*-nitrophenyl-α-ᴅ-glucopyranoside, PNPG) was mixed, and the absorbance was measured at 410 nm; the concentration for 50 % inhibition for the extract and acarbose was calculated.

The α-amylase inhibitory capacity of the extract was determined *in vitro* according to the directions in the kit protocol (Biovision). The extract or the standard (50 μL each) was mixed with 50 μL of the enzyme solution, mixed well, and incubated for ten minutes at room temperature. Then the starch solution (50 μL) was added and incubated for 3 min, followed by the addition of 50 μL 3,5-dinitrosalicylic acid (DNS) reagent to terminate the reaction. Then the reaction mixture was boiled at 85–90 °C in a water bath for ten minutes, cooled to room temperature, and the absorbance was read at 405 nm.

Molecular docking

Virtual screening software Pyrx (version 0.8), https://pyrx sourceforge io (15) that uses Autodock Vina, has been utilized for the molecular docking study (Molecular Graphics Laboratory, The Scripps Research Institute, USA). Metabolite structures were downloaded from PubChem (16) [February, 2024], and Chem Bio 3D (Chem Bio Office Ultra 12.0 suite) was used to minimize their energies. PDB entries for human lysosomal acid α-glucosidase enzyme (5NN8) (17) and human pancreatic α-amylase enzyme (4W93) (18) were retrieved from Protein Data Bank (https://www rcsb org/). Grid centers were set to be as follows: $X = -14.650$, $Y = -28.868$, $Z = 94.112$ for α-glucosidase, and $X = -9.872$, $Y = 8.881$ and $Z = -21.291$ for α-amylase. The binding scores for the best poses were tabulated, and the interactions were visualized. Pymol software (https://pymol.org/) was used to convert pdbqt files to pdb format, followed by visualization of the interactions in 3D and 2D views using Biovia Discovery Studio visualizer v21.1.0.20298 (Dassault Systèmes Biovia Corp., USA).

Statistical analysis

All the data were expressed as mean ± standard deviation (SD) from three experiments. The data of TPC, TFC, and antioxidant activity were calculated from linear calibration curves.

RESULTS AND DISCUSSION

Chemical composition of A. factorovskyi *aerial parts*

As shown in Table I, the total phenolic content was determined to be 52.46 ± 5.93 mg GAE g^{-1}, while the total flavonoid content was calculated as 19.01 ± 2.50 mg QE g^{-1}. Previous investigations of TPC and TFC in *A. pubescens* aerial parts reported higher phenolic and flavonoid contents (121.5 ± 2.0 GAE g^{-1} and 79.3 ± 5.5 QE g^{-1}, resp.) in the methanolic extract (7).

Table I. Total phenolic and flavonoid contents of A. factorovskyi *aerial parts*

	TPC (mg GAE g^{-1})[a]	TFC (mg QE g^{-1})[b]
*A. factorovsky*i methanolic extract	52.46 ± 5.93	19.01 ± 2.50

Mean ± SD, $n = 3$.

[a] mg gallic acid equivalent in 1 g of dry sample

[b] mg quercetin equivalent in 1 g of dry sample

TFC – total flavonoid content, TPC – total phenolic content

Table II. Tentative metabolite profiling of A. factorovskyi *aerial parts methanolic extract using UPLC-ESI-MS*

Peak No.	Mode of ionization	$t_{R\ (min.)}$	*m*/*z*	Compound name	Formula	Compound class
1	P	0.84	381.1144	Diphyllin	$C_{21}H_{16}O_7$	Lignan
	N	0.85	379.1395			
2	N	1.09		NI		
3	P	1.15		NI		
4	P	1.24		NI		
5	N	5.95	431.2256	Kaempferol-7-*O*-rhamnoside/vitexin/isovitexin	$C_{21}H_{20}O_{10}$	Flavonoid
6	N	6.26	447.2209	Quercitrin	$C_{21}H_{20}O_{11}$	Flavonoid
7	P	6.79	447.1292	Baicalin	$C_{21}H_{18}O_{11}$	Flavonoid
	N	6.78				
8	N	7.88		NI		
9	P	11.69		NI		
10	P	12.05	251.1711	Arbusculin A/warburganal	$C_{15}H_{22}O_3$	Sesquiterpene lactone
11	N	12.29	181.0433	Syringaldehyde/homovanillic acid	$C_9H_{10}O_4$	Phenolic
12	N	14.08	721.4780	Yamogenin 3-*O*-neohesperidoside	$C_{39}H_{62}O_{12}$	Saponin
13	N	14.23	579.3938	Convallasaponin A	$C_{32}H_{52}O_9$	Saponin
14	P	14.48	277.2374	Stearidonic acid	$C_{18}H_{28}O_2$	Fatty acid
15	P	14.62	317.3847	Isorhamnetin	$C_{16}H_{12}O_7$	Flavonoid
16	P	15.40	235.1724	Valerenic acid	$C_{15}H_{22}O_2$	Sesquiterpenoid
17	N	17.32	293.1759	Phytuberin	$C_{17}H_{26}O_4$	Sesquiterpenoid
18	N	20.52	339.3374	Docosanoic acid	$C_{22}H_{44}O_2$	Fatty acid
19	P	20.61		NI		
20	P	22.07		NI		
21	P	31.20		NI		
	N	31.21				
22	P	31.37		NI		
	N	31.40				
23	P	31.72	287.9257	Kaempferol/luteolin	$C_{15}H_{10}O_6$	Flavonoid
24	P	31.76				

N – negative mode of ionization, NI – not identified, P – positive mode of ionization

Analysis of the UPLC-ESI-MS positive and negative modes spectra allowed tentative identification of 19 compounds from 14 chromatographic peaks (Table II, Figs. 1 and 2). The

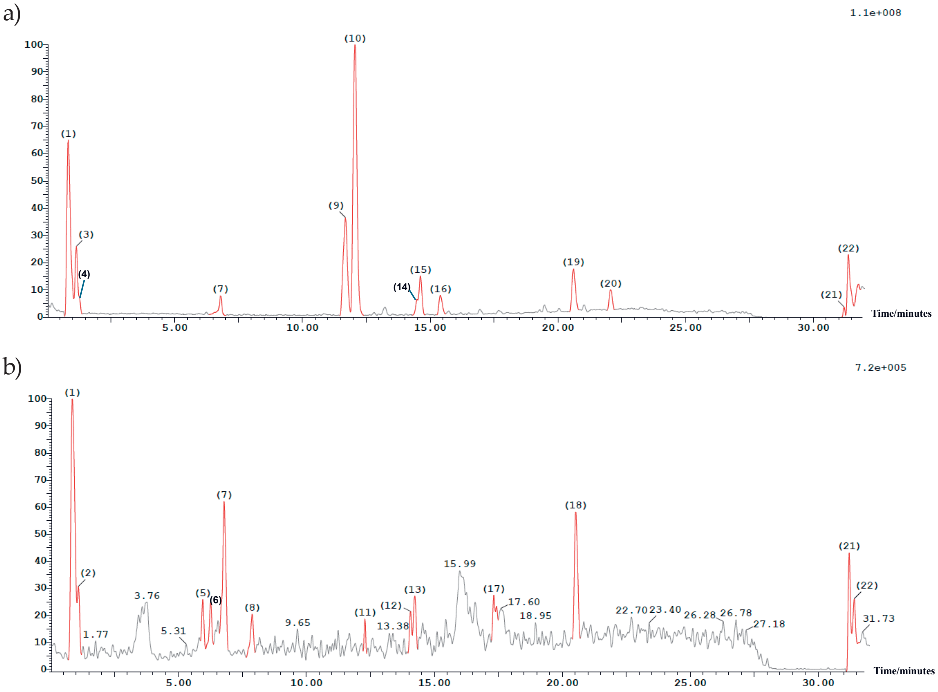

Fig. 1. Total ion chromatograms of the methanolic extract of the *A. factorovskyi* aerial parts using UPLC-ESI-MS: a) positive mode; b) negative mode.

identified compounds were classified as eight flavonoids, four sesquiterpenes, one lignan, two fatty acids, two saponins, and two phenolics.

The eight flavonoids were identified from the mass ion peaks at *m/z* 431.2256, 447.2209, 447.1292, 317.3847, and 287.9257. The mass ion peak at 431.2256 $[M-H]^-$ was in agreement with the molecular formula $C_{21}H_{20}O_{10}$ and could be annotated as kaempferol-7-*O*-rhamnoside, vitexin, or isovitexin. As for Asteraceae, kaempferol-7-*O*-rhamnoside (19) was previously isolated from *Carduus nutans* L., while vitexin and isovitexin (20) from North American *Trugopogon* species. The mass ion peak at 447.2209 $[M-H]^-$ is in agreement with the molecular formula $C_{21}H_{20}O_{11}$ and was annotated as quercitrin, whereas the peaks at 447.1292 $[M+H]^+$ and 445.1909 $[M-H]^-$ matched with $C_{21}H_{18}O_{11}$ that could be annotated as baicalin. Quercitrin (21) was previously detected in *Solidago chilensis* Meyen (Asteraceae), and baicalin (22) was isolated from *Scutellaria baicalensis* (Lamiaceae) using high-speed counter current chromatography. Isorhamnetin was annotated from the mass ion peak at 317.3847 $[M+H]^+$ ($C_{16}H_{12}O_7$), isorhamentin (23, 24) was previously isolated from *Cichorium* species and marigold flowers (Asteraceae). Finally, the mass ion peak at 287.9257 $[M+H]^+$ matched with the molecular formula $C_{15}H_{10}O_6$ which could be annotated as kaempferol or luteolin. Kaempferol (25, 26) was previously characterized in *Parthenium* and *Clibadium* species (Asteraceae), while luteolin (27) was formerly detected using LC-MS in the flower of *Chrysanthemum morifolium* (Asteraceae).

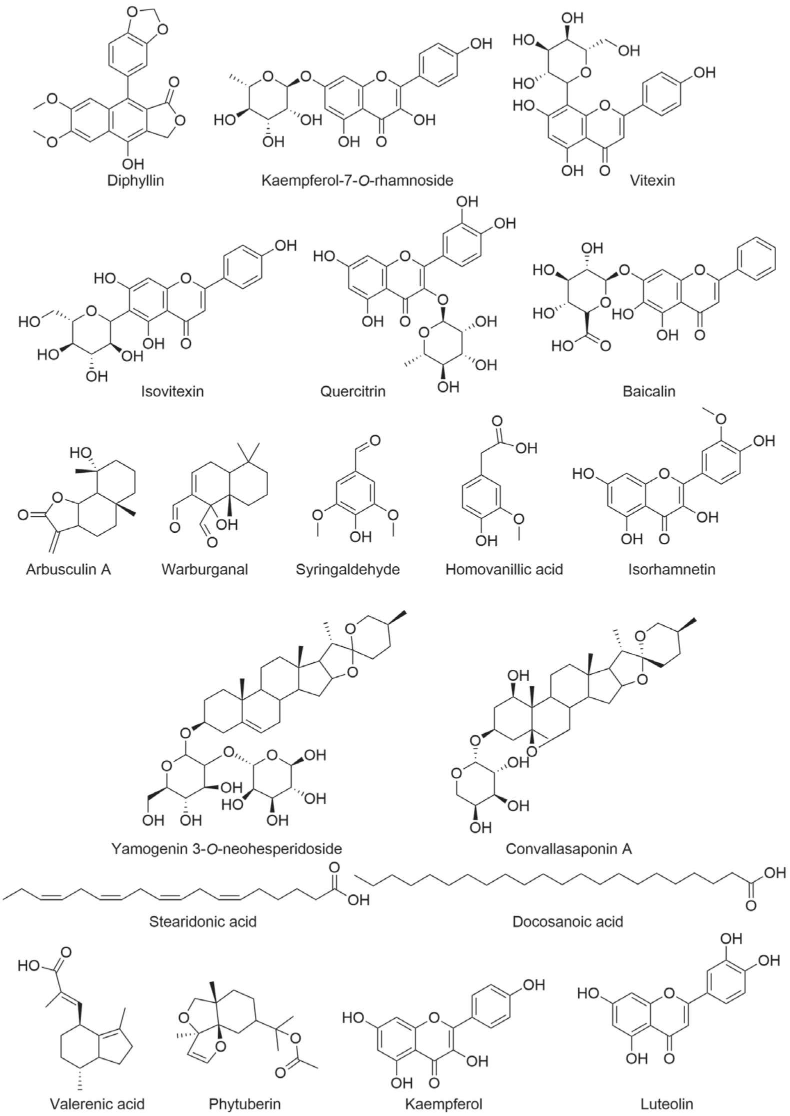

Fig. 2. Chemical structures of possible metabolites in *A. factorovskyi* aerial parts.

Four sesquiterpene compounds were annotated from the signals at *m/z*: 251.1711 $[M+H]^+$ ($C_{15}H_{22}O_3$), which matched with arbusculin A (28), earlier detected in *Artemisia arbuscula* (Asteraceae), or warburganal (29) that was previously identified in the ethyl acetate fraction of *Warburgia stuhlmannii* root (Canellaceae). The mass ion peaks at 235.1724 $[M+H]^+$ matched with $C_{15}H_{22}O_2$, identified as valerenic acid (30) previously also reported in *Valeriana officinalis* L. (Caprifoliaceae), and 293.1759 $[M-H]^-$ in agreement with $C_{17}H_{26}O_4$

recognized as phytuberin (31) that was formerly isolated from the leaves of *Nicotiana tabacum* (Solanaceae).

One lignan was detected at *m*/*z* 381.1144 $[M+H]^+$ and 379.1395 $[M-H]^-$, which are in agreement with $C_{21}H_{16}O_7$ and identified as diphyllin. Diphyllin (32, 33) was previously isolated from *Justicia procumbens* (Acanthaceae).

Two phenolic compounds were annotated in the negative mode from *m*/*z* at 181.0433, which matched with $C_9H_{10}O_4$ and were identified as syringaldehyde or homovanillic acid. Syringaldehyde and homovanillic acid (34) were both identified in the Asteraceae plant *Picris rhagadioloides* (L.) Desf.

Also, two saponins, yamogenin 3-*O*-neohesperidoside and convallasaponin A, were identified from the signals at *m*/*z* $[M-H]^-$ 721.4780 ($C_{39}H_{62}O_{12}$) and 579.3938 ($C_{32}H_{52}O_9$), resp. Yamogenin 3-*O*-neohesperidoside (35) was previously identified in *Borassus flabellifer* (Arecaceae), and convallasaponin A (36) was identified in *Convallaria majalis* (Liliaceae).

The two signals at *m*/*z*: 277.2374 $[M+H]^+$ and 399.3374 $[M-H]^-$ were in agreement with $C_{18}H_{28}O_2$ and $C_{22}H_{44}O_2$ and were annotated as fatty acids, stearidonic acid and docosanoic acid, resp. Stearidonic acid (37) and docosanoic acid (38) were previously detected in *Ibicella lutea* and *Proboscidea louisiana* (Martyniaceae).

It is noteworthy that all the above-mentioned metabolites have been identified for the first time from *A. factorovskyi* extract, thereby enriching the phytochemical library of the genus *Aaronsohnia*. Still, this preliminary data needs to be confirmed.

Biological activity of A. factorovskyi *aerial parts*

The antioxidant potential of *A. factorovskyi* extract evaluated by DPPH and phosphomolybdenum assay indicated TAC of 12.31 ± 2.33 and 17.40 ± 0.96 mg AAE g^{-1}, resp. (Table III). It is worth mentioning that this is the first report for the assessment of the antioxidant capacity of *A. factorovskyi* extract. In the previous research on *A. pubescens*, antioxidant activity was attributed to the high content of phenolics and flavonoids (7).

COX enzymes play a key role in prostanoid production, where COX-1 is constitutively expressed to support gastric protection and platelet aggregation, and COX-2, produced in response to inflammation. The current study assessed the anti-inflammatory potential of *A. factorovskyi* extract utilizing the COX inhibition assay. Results in Table IV indicate characteristic activity of the extract against COX-1 and COX-2, with concentration for 50 % inhibition of 20.85 ± 0.73 and 8.252 ± 0.29 μg mL^{-1}, resp. (respective IC_{50} for ibuprofen is 6.195 ± 0.23 and 2.398 ± 0.09 μg mL^{-1}). Metabolic profiling of the extract revealed flavonoids as prevailing metabolites in *A. factorovskyi* aerial parts. Flavonoids are known for their

Table III. Antioxidant activity of A. factorovskyi *aerial parts using DPPH and phosphomolybdenum assay*

	DPPH (mg AAE g^{-1})^a	TAC (mg AAE g^{-1})^a
A. factorovskyi methanolic extract	12.31 ± 2.23	17.40 ± 0.96

Mean ± SD, *n* = 3.

[a] mg ascorbic acid equivalent in 1 g of dry extract.

DPPH – 2,2-diphenyl-1-picrylhydrazyl, TAC – total antioxidant capacity

Table IV. Antidiabetic and anti-inflammatory activity of A. factorovskyi *aerial parts*

Sample	α-glucosidase	α-amylase	COX-1	COX-2	SI
	Concentration for 50 % inhibition				
	mg mL^{-1}	mg mL^{-1}	µg mL^{-1}	µg mL^{-1}	
A. factorovskyi methanolic extract	0.243 ± 0.009	0.275 ± 0.01	20.85 ± 0.73	8.252 ± 0.29	2.527[a]
Ibuprofen (IC_{50})	–	–	6.195 ± 0.23	2.398 ± 0.09	2.583[b]
Acarbose (IC_{50})	0.418 ± 0.015	0.168 ± 0.006	–	–	–

Mean ± SD, n = 3.

[a] Selectivity index (SI) = conc. 50 % inhibition$_{\text{COX-1}}$/ conc. 50 % inhibition$_{\text{COX-2}}$

[b] Selectivity index (SI) = $IC_{50\ \text{COX-1}}/IC_{50\ \text{COX-2}}$

anti-inflammatory activity that is attributed to variable mechanisms, including COX enzyme inhibition (39). Consequently, the observed anti-inflammatory effect of the tested extract may likely be attributed to its flavonoid content.

The extract was tested for its inhibition of the metabolic enzymes (α-glucosidase and α-amylase) to assess its antidiabetic potency. The results of α-glucosidase and α-amylase inhibition (Table IV) indicated extract concentration for 50 % inhibition as 0.243 ± 0.009 mg mL^{-1} (IC_{50} for acarbose is 0.418 ± 0.015 mg mL^{-1}) and 0.275 ± 0.01 mg mL^{-1} (IC_{50} for acarbose is 0.168 ± 0.006 mg mL^{-1}), resp.

Molecular docking study

To highlight the possible potential of antidiabetic phytoconstituent(s) in *A. factorovskyi* extract, a molecular docking study was conducted, where the detected metabolites were docked against α-glucosidase and α-amylase enzymes. The comparative values for binding scores of the docked compounds against both enzymes are given in Table V. Results have been compared with those for the co-crystallized ligands (acarbose for α-glucosidase and montbretin A for α-amylase).

The binding score is an estimate of the free energy of binding in kcal mol^{-1} between the ligand and the receptor; the more negative the score, the stronger and more favorable the binding is considered. It is a combination of various terms into a scoring function, such as steric interactions, hydrophobic interactions, and hydrogen bonding, where all contributions are summed to give a final relative score (40). Among the docked compounds yamogenin 3-*O*-neohesperidoside, convallasaponin A and baicalin unveiled notably strong binding affinities for both α-glucosidase and α-amylase binding sites with binding energies of –8.2, –8.0 and –8.0 kcal mol^{-1}, resp., for α-glucosidase, and –9.9, –9.9 and –9.1 kcal mol^{-1}, resp., for α-amylase enzyme, compared to the co-crystallized ligand of α-glucosidase acarbose, –7.0 kcal mol^{-1}, and that of α-amylase montbretin A, –9.3 kcal mol^{-1}.

The sugar moiety in yamogenin 3-*O*-neohesperidoside was accommodated in the active site of α-glucosidase and its hydroxyl groups interacted *via* hydrogen bonds with the acid-base residue Asp616 and the nucleophile Asp518 and *via* sulfur bond with Met519, while its triterpenoid nucleus established hydrophobic interactions with Trp376 and with

Table V. The docking scores of tentatively identified metabolites towards α-glucosidase and α-amylase

Metabolite	Binding score (kcal mol^{-1})	
	α-glucosidase	α-amylase
Diphyllin	–7.2	–8.7
Kaempferol-7-*O*-rhamnoside	–7.4	–9.4
Vitexin	–7.2	–8.2
Isovitexin	–7.2	–8.9
Quercitrin	–7.8	–8.8
Baicalin	–8.0	–9.1
Arbusculin A	–6.4	–7.7
Warburganal	–5.7	–6.8
Syringaldehyde	–5.4	–6.0
Homovanillic acid	–5.8	–4.9
Yamogenin 3-*O*-neohesperidoside	–8.2	–9.9
Convallasaponin A	–8.0	–9.9
Stearidonic acid	–6.0	–5.8
Isorhamentin	–6.9	–8.8
Valerenic acid	–6.8	–7.6
Phytuberin	ND	ND
Docosanoic acid	–5.4	–5.3
Kaempferol	–7.1	–9.2
Luteolin[a]	–7.3	–8.8
Acarbose (co-crystallized ligands for α-glucosidase)	–7.0	–
Montbretin A (co-crystallized ligands for α-amylase)	–	–9.3

[a] Tentative identification.

ND – not docked into the active site

Leu677 and Leu678 positioned at the broad groove that leads to the active site (Fig. 3a). Hydroxyl groups in sugar moiety of the steroidal saponin convallasaponin A were involved in hydrogen bond interactions with Leu283 and Ala284, while the acid-base residue Asp616 and Arg411 formed hydrogen bonds with OH group at C-5 and oxygen of the lactone ring in the steroid nucleus, and further stabilization has been accomplished *via* hydrophobic interactions between Trp376, Trp481 and Leu650, and the steroidal saponin backbone (Fig. 3b). On the other hand, the ring B of the flavonoid baicalin has been positioned close to the entry of the active site *via* π-π stacking with Trp376 and π-alkyl interactions with Leu677 and Leu678 while the sugar moiety was accommodated in the active site and interacted *via* hydrogen bonds with Trp481, Asp518, Asp616 and His674, in addition to π-sulfur bond between ring A and Met519 (Fig. 3c).

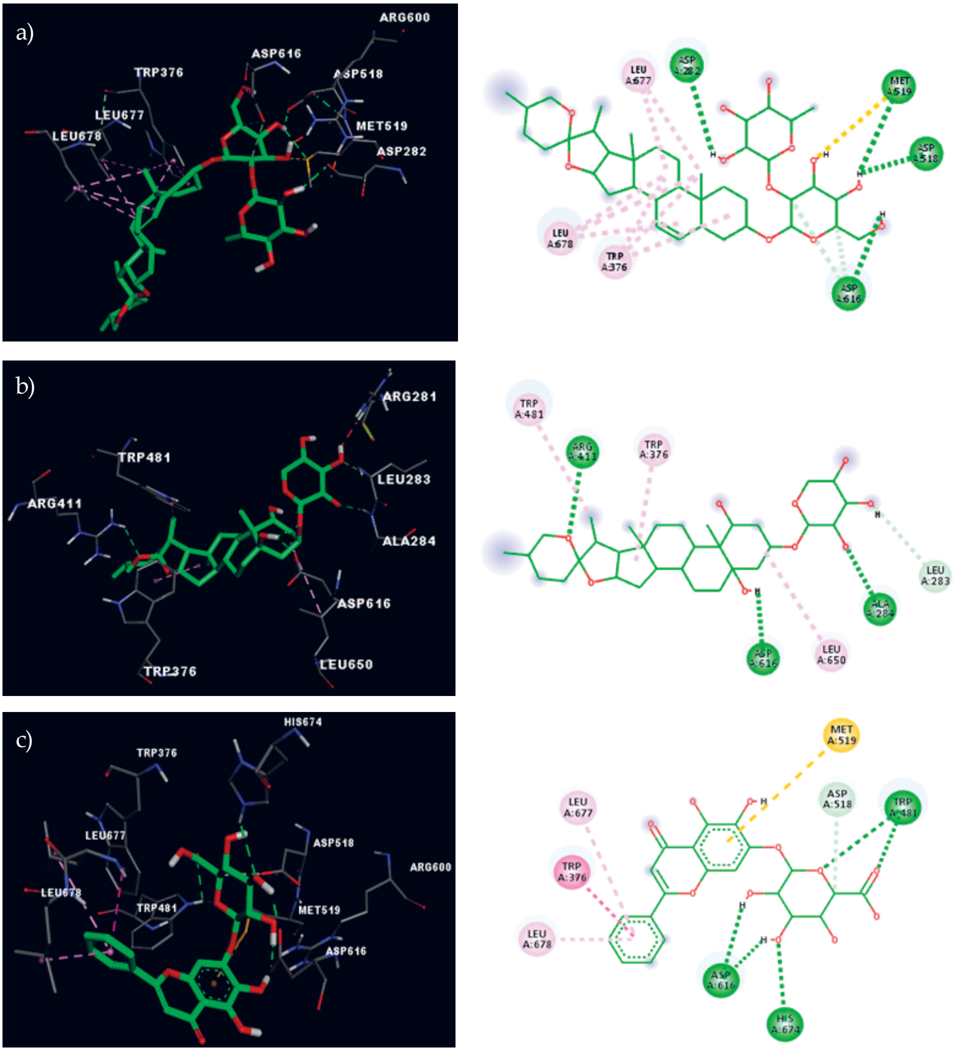

Fig. 3. 2D (right) and 3D (left) depictions of docked poses and interactions of: a) yamogenin 3-*O*-neohesperidoside; b) convallasaponin A; c) baicalin, in the active site of *α*-glucosidase enzyme. Ligands are depicted in green stick model, labeled residues in grey line models, representing hydrogen bonds by green dashed lines, hydrophobic interactions by pink and purple dashed lines, and sulfur bonds by yellow dashed lines.

Regarding *α*-amylase, yamogenin 3-*O*-neohesperidoside bound to *α*-amylase and the interactions involved hydrogen bonding with the catalytic residue Gln63, with further stabilization *via* a number of hydrophobic interactions with Tyr151, Ile235, Leu237, and Ala307 (Fig. 4a). The steroidal saponin convallasaponin A exhibited multiple interactions with the enzyme. Its hydroxyl groups on the nucleus formed hydrogen bonds with Glu233,

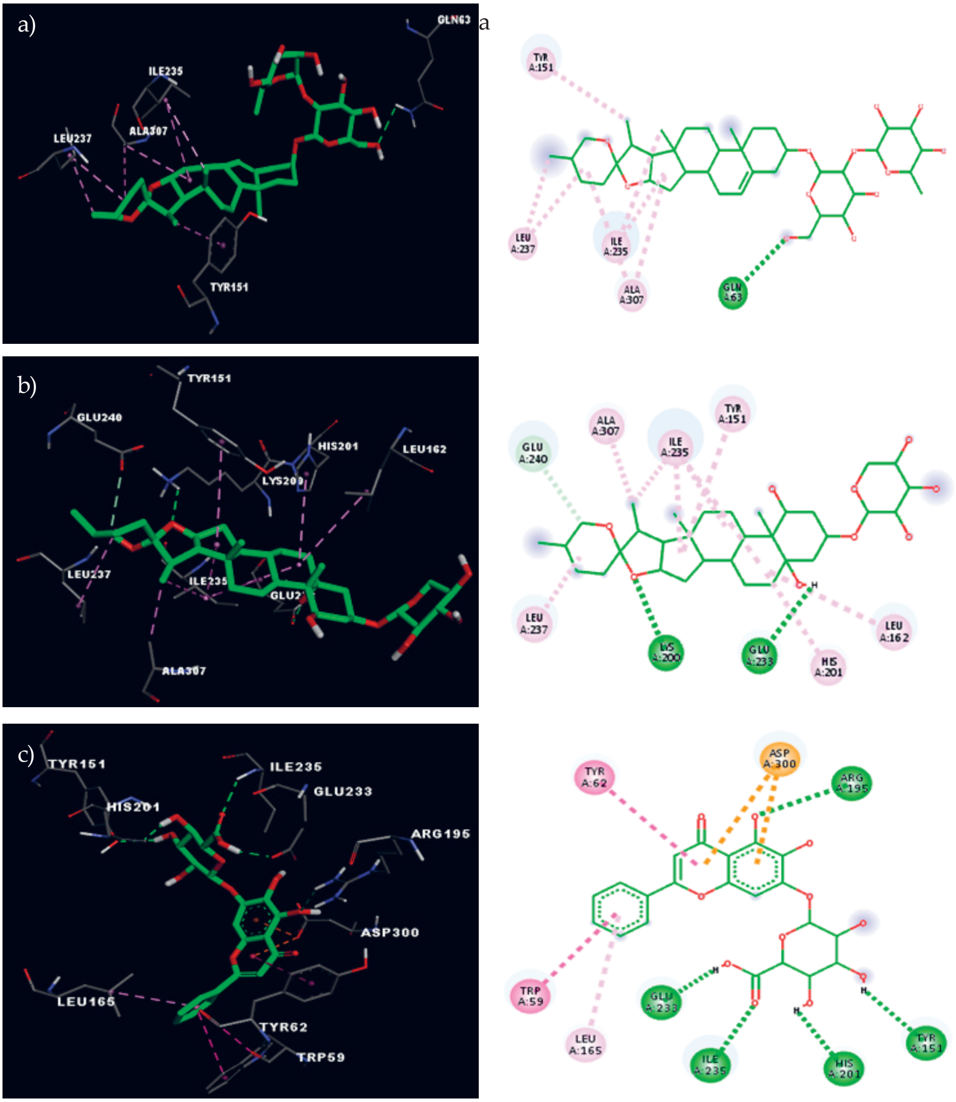

Fig. 4. 2D (right) and 3D (left) depictions of docked poses and interactions of: a) yamogenin 3-*O*-neohesperidoside; b) convallasaponin A; c) baicalin in the active site of α-amylase. Ligands are depicted in green stick model, labeled residues in grey line models, representing hydrogen bonds by green dashed lines, hydrophobic interactions by pink and purple dashed lines, and ionic bonds by orange dashed lines.

critical active site residue involved in catalytic activity, and with Lys200. Additionally, carbon-hydrogen bonding was observed with Glu240. Furthermore, the steroidal saponin backbone was engaged in hydrophobic interactions with a surface comprising Tyr151, Leu162, His201, Ile235, Leu237 and Ala307 (Fig. 4b). The flavonoid baicalin revealed five hydrogen bonds with Tyr151, Arg195, His201, Glu233 and Ile235, two π-anion bonds with Asp300, two π-π stackings with Tyr59 and Tyr62, and π-alkyl interaction with Leu165 (Fig. 4c). It is noteworthy that many natural triterpenoids have been reported to act against diabetes and various *in vitro* investigations unveiled the potential of triterpenoids from plant sources to inhibit α-glucosidase and α-amylase and to form hydrogen bonds with important residues in the active sites of both enzymes (41). Furthermore, plenty of investigations have reported the impact of saponins and flavonoids in the management of diabetes by different mechanisms (42–44).

CONCLUSIONS

The present research provides the introductory phytochemical and biological exploration of the aerial parts of *A. factorovskyi,* adding a contribution to the phytochemistry of this less frequently studied species. Preliminary insight into complex composition and metabolite profiling using UPLC-ESI-MS analysis highlighted some phytoconstituents. *In vitro* testing indicated antioxidant, anti-inflammatory, and antidiabetic activities of the plant extract. Molecular docking further supported these findings by recording strong interactions between the active sites of key enzymes and the three metabolites: yamogenin 3-*O*-neohesperidoside, convallasaponin A, and baicalin. These findings highlight the potential medicinal value of this plant and recommend further detailed phytochemical and *in vivo* testing.

Abbreviations, acronyms, symbols. – AAE – ascorbic acid equivalent, COX – cyclooxygenase enzymes, DPPH – 1,1-diphenyl-2-picrylhydrazyl, GAE – gallic acid equivalent, IC_{50} – half maximal inhibitory concentration, QE – quercetin equivalent, TAC – total antioxidant capacity, TFC – total flavonoid content, TPC – total phenolic content, UPLC-ESI-MS – ultra-performance liquid chromatography-electrospray ionization mass spectrometry.

Availability of data and materials. – Supplementary material is available upon request.

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Conflict of interest. – The authors declare that they have no competing interests.

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