



Quercetin Derivatives from *Bidens pilosa* Suppressed Cell Proliferation via Inhibition of RSK2 Kinase and Aldose Reductase Enzymes: UPLC-MS/MS, GC-MS, In Vitro, and Computational Studies

Doaa S. Ali¹ · Alaadin E. El-Haddad² · Hussein S. Mohamed¹ · Ashraf A. El-Bassuony³ · Momtaz M. Hegab⁴ · Gehad AbdElgayed⁵ · Hossam Ebaid⁶ · Shima A. Ahmed³ · Emadeldin M. Kamel³

Accepted: 5 December 2024 / Published online: 6 January 2025
© The Author(s) 2025

Abstract

Traditionally, *Bidens pilosa* L. is an edible herb utilized for various ailments. The study accomplished a complete analysis of *B. pilosa* extract including UPLC/T-TOF-MS/MS, GC-MS, and in vitro antiproliferative activity, in addition to molecular docking on kinase and aldose reductase enzymes. From GC-MS analysis, the percentage of identified unsaturated fatty acids (FAs) (11.38%) was greater than saturated FAs (8.69%), while the sterols percent (39.92%) was higher than the hydrocarbons percent (6.6%). Oleic and palmitic acids are the major FAs (9.48% and 6.14%, respectively). Phytochemical profile uncovered the presence of quercetin, kaempferol, myricetin, and isorhamnetin aglycones and/or glycoside derivatives alongside apigenin, acacetin, and luteolin derivatives. *B. pilosa* extract suppressed cell proliferation in a concentration-dependent manner against SNB-19 and SK-MEL-5 cell lines (IC_{50} 1.66 ± 0.06 and 4.04 ± 0.14 mg/mL, respectively). These potentials aligned with the molecular docking results on aldose reductase and kinase enzymes with promising binding affinities (-5.3 to -8.89 kcal mol⁻¹). *B. pilosa* metabolites were found as kinases and aldose reductase inhibitors, which rationalize their antiproliferative activity. Unfortunately, toxicity assessments were not performed to assess the safety of *B. pilosa* extract. Assessment of the therapeutic efficiency via in vivo and clinical studies is required.

✉ Hussein S. Mohamed
Hussein.shaban@rmp.bsu.edu.eg

¹ Chemistry of Medicinal and Aromatic Plants Department, Research Institute of Medicinal and Aromatic Plants, Beni-Suef University, Beni-Suef 62514, Egypt

² Pharmacognosy Department, Faculty of Pharmacy, October 6 University, Giza, Egypt

³ Chemistry Department, Faculty of Science, Beni-Suef University, Beni-Suef 62514, Egypt

⁴ Botany and Microbiology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt

⁵ Integrated Molecular Plant Physiology Research, Department of Biology, University of Antwerp, 2020 Antwerp, Belgium

⁶ Department of Zoology, College of Science, King Saud University, P.O. Bo2455, 11451 Riyadh, Saudi Arabia

Keywords *Bidens pilosa* · Antiproliferative · Docking · Flavonoids · GC–MS · LC–MS/MS

Introduction

The *Bidens* genus (Asteraceae) comprises approximately 280 species [1]. *Bidens pilosa* L. is an annual and ruderal herb that grows in tropical and subtropical regions because of its outstanding resistance to unfavorable environmental circumstances [2]. *B. pilosa* grows with numerous ridged branches (1.5–2 m). Leaves are petioled oppositely pinnate arranged, with hairy serrately ovate leaflets (3–5) [3]. *B. pilosa* has capitulum inflorescence with white ray petals and yellow centers [3]. The seeds are brown to black and widely spread by wind, adhering to clothes and animal hair allowing its fast growth worldwide.

In the folk medicine, *B. pilosa* has been used with variable indications between countries. *B. pilosa* herb was acceptable in Europe for its astringent, diaphoretic, and diuretic effects [3]. In traditional Chinese medicine, *B. pilosa* is used to manage diabetes, inflammation, dysentery, and pharyngitis [4]. Moreover, *B. pilosa* is commonly known as Picaõ preto in Brazil, where it is widely used for treating diabetes, ulcers, inflammation, and infections [5, 6]. In addition, the roots are regarded as applicable in treating malaria [5] and even tumors [7]. In Martinique, the herb decoction is used to manage inflammation and diabetes [1, 8]. In South Africa, the herbal tea of the aerial parts is thought to have anti-allergic and anti-inflammatory effects [9]. In Cuba, *B. pilosa* is known as an antitumor agent [6]. As dietary supplements, the dried herbs are sold in Taiwan; approximately 4 million dollars per year are marketed for diabetes management [10]. Studies of *B. pilosa* have shown its antihyperglycemic [11], antihypertensive [12], antiulcer [13], hepatoprotective [14], antipyretic [15], anti-inflammatory [9, 16], anti-leukemic [17], antimalarial [5], antibacterial [18], antioxidant [19], and antitumor effects [1, 15]. It has cytotoxic activities against several types of cancer cells [2]. Based on the reported biological activities, some countries like Brazil include *B. pilosa* as an official medicinal plant for public use [20]. The use of *B. pilosa* as a medicinal plant is feasible but further clinical trials and toxicity assessments are still rare.

The major phytoconstituents detected in *B. pilosa* are polyacetylenes [4, 11, 16], terpenes [18, 21], and flavonoids [2]. Polyacetylenes derivatives, in particular, phenylheptatriyne, are the important bioactive phytoconstituent found in *B. pilosa* essential oils [21]. Flavones, flavanones, and flavonols aglycones and/or glycosides were identified from *B. pilosa*, especially quercetin, and kaempferol glycosides [20]. Many scientific reports were proceeded on *B. pilosa*, owing to its traditional use and known bioactive constituents. Further studies are needed to highlight the action mechanism of its phytoconstituents. Hence, metabolomic profiling of *B. pilosa* is a basic step for offering undiscovered medicinal uses. In this respect, gas chromatography (GC) and high-resolution ultra-performance liquid chromatography (HR-UPLC) coupled with mass spectrometry (MS) represent prospective analytical analysis for untargeted metabolome report, providing efficient separation asides from the highly sensitive recognition of phytoconstituents. Diverse kinase enzymes activate transcription factors that transcribe numerous carcinogenic markers; consequently, kinase inhibitors may rationalize the antiproliferative activity [22]. Hence, the current study aimed to execute chemical profiling, in vitro antiproliferative assessment of *B. pilosa*, and verification with computational study on kinase and aldose reductase enzymes.

Material and Methods

Plant Material and Extraction

B. Pilosa L. was collected from Beni-Sueif, Egypt in May 2022. The plant identity was performed in the Faculty of Science, Beni-Sueif University, Beni-Sueif, Egypt. The plant aerial part was washed with tap water, dried under shade (20 days), and ground into a fine powder. The powdered materials (1 kg) were extracted in Soxhlet with aqueous ethanol (70%, 4 × 2 L, 2 h) and then filtered. The filtrate was evaporated (Rotavapor®, BÜCHI, Switzerland) [23].

In Vitro Antiproliferative Assay

The American Type Culture Collection (Manassas, VA, USA) provided SNB-19 (brain cancer cells) and SK-MEL-5 (skin cancer cells). Cell lines were cultured and maintained in RPMI-1640 medium with fetal bovine serum and penicillin/streptomycin (10% and 1%, respectively). The antiproliferation assay of *B. Pilosa* extract was proceeded by MTT assay [24]. Serial dilutions (100–1.56 mg mL⁻¹) of *B. Pilosa* extract in DMSO were added to the cells (1 × 10³ cells mL⁻¹) which were placed in 96-well plates. Furthermore, MTT (10 µL) was added to each well and incubated at 37 °C for 4 h. Using a microplate ELISA reader (FLUO star Omega, Labtech, Germany), the absorbance (Abs) of the produced formazan was measured (490 nm). Each concentration was examined in triplicates and the statistical analysis was done using GraphPad Prism 6 (La Jolla, CA, USA). The cell viability % = [Abs of tested cells/ Abs of control cells] × 100. The values of cell viability % were plotted versus the concentrations using non-linear regression analysis of Sigmoidal dose–response curve [25].

GC–MS Analysis

The lipoidal composition of *B. pilosa* was analyzed using a Trace GC-TSQ-MS (Thermo Scientific, TX, USA) over a capillary column TG-5MS. The column temperature was set at 50 °C (2 min), followed elevated by 5 °C/min to 250 °C and then by 30 °C/min to 300 °C (2 min). The injector and MS transfer line were maintained at 270 and 260 °C, respectively. Samples (1 µL) were injected and carried on Helium (1 mL/min) using an Autosampler AS1300 connected to a GC in split mode. Mass spectra covering *m/z* 50–650 were obtained and furthermore were compared with databases: NIST 14 and WILEY 09 [26].

HR-UPLC/T-TOF–MS/MS Analysis

The *B. pilosa* extract was analyzed at the Proteomics and Metabolomics unit of the Children's Cancer Hospital, Cairo, Egypt. An Exion LC Triple TOF 5600+ system (SCIEX, Framingham, MA, USA) equipped with an X select HSS T3 C₁₈ column (Waters Corporation, CT, USA) was used in negative and positive modes. Extract (50 mg) was dissolved in a working solvent: MilliQ water:methanol:acetonitrile (50:25:25, 50 µL) and then was diluted with the working solvent [23]. Samples (1 µg/µL, 10 µL) were injected using the following: solvent A was ammonium formate buffer (5 mM, pH 8) containing

1% methanol, for the negative mode, while in the positive mode, solvent A was ammonium formate buffer (5 mM, pH 3) containing 1% methanol, and in both modes, solvent B was 100% acetonitrile. The gradient elution (0.3 mL/min) was performed as follows: isocratic 90%:10% (0–1 min), linear from 90%:10% to 10% and 90% (1.1–20.9 min), isocratic 10%:90% (21–25 min), and finally isocratic 90% and 10% (25.1–28 min) of solvents A and B, respectively. The metabolites were recorded by Analyst TF 1.7.1, Peak view 2.2 (SCIEX, Framingham, MA, USA), and MS-DIAL 3.70 softwares [23]. MS (50–1100 m/z) was done on a Triple TOF 5600+ system equipped with a Duo-Spray source operating in the electron spray ionization mode (AB SCIEX, Framingham, MA, USA). The metabolites were characterized by generating the formula with an error limit of 20 ppm and considering R_t , MS2 data, compared to databases and literature [27].

Molecular Docking Study

MOE software (version 2016.10, Chemical Computing Group Inc., Montreal, Canada) was used. The structures of aldose reductase (AKR1B1; PDB: 2IKI) and ribosomal S6 kinase (RSK2 kinase; PDB: 3UBD) were obtained from the RCSB protein data bank. The standard preparation of target protein was applied. The target was validated by redocking the co-crystallized ligands (IDD388 and SL0101 to AKR1B1 and RSK2, respectively) offering low binding energy score (S) and small RMSD value. The essential amino acids were defined. The validated target was used to predict metabolite-target interactions [28].

Results and Discussion

In Vitro Antiproliferative Assay

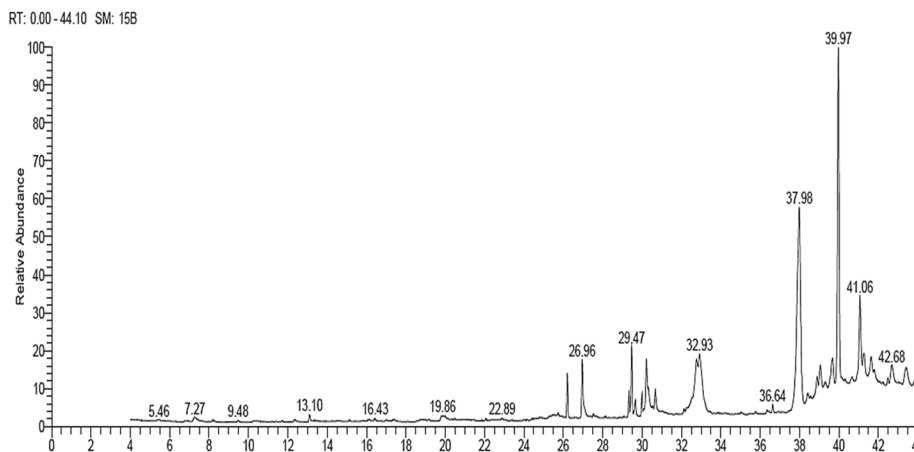
Recent studies informed that *B. pilosa* has a promising in vitro and in vivo anticancer activity [29, 17]. However, its action mechanism has not been fully understood. The antiproliferation activity was evaluated for *B. pilosa* ethanol extract on SNB-19 and SK-MEL-5. The *B. pilosa* extract produced a decrease in cell viability of SNB-19 and SK-MEL-5 cells (IC_{50} 1.66 ± 0.06 and 4.04 ± 0.14 mg/mL, respectively) comparable to doxorubicin (IC_{50} 2.42 ± 0.08 and 11.82 ± 0.41 mg/mL, respectively).

GC–MS Analysis of the Lipoidal Matter

Quantitation was based on separated compounds' relative peak area, R_t , and their area percentages. The GC–MS chromatogram revealed the detection of 19 major metabolites (66.59%) (Table 1, Fig. 1). The percentage of identified unsaturated FAs (11.38%) was higher than the percentage of saturated FAs (8.69%). At the same time, sterols were found to be higher than hydrocarbons percent (39.92% and 6.6%, respectively). Oleic acid is the major unsaturated FA (6.14%). However, palmitic and stearic acids are the major saturated FAs (6.14% and 2.55%, respectively). The percentage of identified sterols was higher than that of hydrocarbons (39.92% and 6.6%, respectively). Lupene-3,28-diol/botulin is the major sterol, followed by sitostenone (24.95% and 6.17%, respectively) [30].

Table 1 Identified metabolites detected in *B. pilosa* lipoidal matter using GC–MS analysis

#	Rt	Name	Formula	M.Wt	Area %
1	26.20	Palmitic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	2.55%
2	26.96	Palmitic acid	C ₁₆ H ₃₂ O ₂	256	3.59%
3	29.33	Linoleic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294	1.32%
4	29.47	Oleic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296	4.45%
5	29.66	Tetramethyl-hexadecenol	C ₂₀ H ₄₀ O	296	1.14%
6	29.66	Methyl-octadecadienol	C ₁₉ H ₃₆ O	280	1.14%
7	29.99	Stearic acid, methyl ester	C ₁₉ H ₃₈ O ₂	298	1.11%
8	30.22	Oleic acid	C ₁₈ H ₃₄ O ₂	282	5.03%
9	30.67	Stearic acid	C ₁₈ H ₃₆ O ₂	284	1.44%
10	32.77	Stigmasterol	C ₂₉ H ₄₈ O	412	3.34%
11	38.41	Stigmastanol	C ₂₉ H ₅₂ O	416	0.67%
12	38.89	Heptatriacotanol	C ₃₇ H ₇₆ O	536	1.03%
13	39.68	Stigmastadiene-3-one	C ₂₉ H ₄₆ O	410	3.23%
14	39.97	lupene-3,28-diol/betulin	C ₃₀ H ₅₀ O ₂	442	24.95%
15	40.65	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	308	0.58%
16	41.06	Sitostenone	C ₂₉ H ₄₈ O	412	6.17%
17	41.28	Cyclolanostan-3-ol, acetate	C ₃₂ H ₅₄ O ₂	470	1.56%
18	41.63	Tocopherol	C ₂₉ H ₅₀ O ₂	430	1.48%
19	42.68	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436	1.81%
% Identified saturated fatty acids					8.69%
% Identified unsaturated fatty acids					11.38%
% Identified sterols					39.92%
% Identified hydrocarbons					6.6%
% of total identified compounds					66.59%

**Fig. 1** GC–MS chromatogram of *B. pilosa* lipoidal matter

UPLC/T-TOF–MS/MS Analysis

LC–MS can analyze a wide range of metabolites; they offer tools for dissecting immense plant biodiversity. The metabolomic profile of *B. pilosa* was studied using ESI–MS/MS in positive and negative modes. Flavones, flavanones, and flavonols aglycones with their glycosides or glucuronic derivatives were identified, with a high abundance of quercetin derivatives, alongside phenolic and organic acids (Table 2, Fig. 2). The major content of flavonoids reflects the plant's diverse biological activities.

Flavone Identification

Hesperidin (1) showed a deprotonated molecular ion peak at m/z 609.1324 and a subsequent loss of 146 and 308 Da, indicating the loss of deoxyhexosyl and deoxyhexosyl-hexosyl moieties yielding fragments at m/z 463 and 301 (hesperetin aglycone), respectively, with the characteristic fragments for flavonoids (177 and 151). Moreover, hesperetin (35) showed a deprotonated molecule at m/z 301.0724, besides the fragments of hesperetin aglycone (m/z 151,134). Luteolin-di-*O*-hexoside (4) showed a deprotonated molecule at m/z 609.1437 and subsequent losses of 162 and 324 Da at m/z 447 and 285 of hexosyl and di-hexosyl moieties, respectively. Luteolin-*C*-hexoside (5) showed a deprotonated molecule at m/z 447.0947 and the daughter peaks at m/z 357 and 327 representing $[M-H-90]^-$ and $[M-H-120]^-$, respectively, indicating C-linkage glycoside; the luteolin aglycone moiety was confirmed at m/z 285 (Fig. 3). Luteolin-*O*-hexoside (17) showed a deprotonated signal at m/z 447.0921 and a neutral loss of hexosyl moiety at m/z 285. The molecular ion peak $[M-H]^-$ of luteolin (36) was noticed at m/z 285.0397, besides the characteristic fragments of luteolin aglycone (m/z 257, 177, 151, 133, and 107) (Table 2) [23].

Apigenin-*C*-hexoside (9) displayed a protonated molecule at m/z 433.1357 and the characteristic peak at m/z 313 $[M+H-120]^+$ indicating C-linkage hexoside; moreover, base peak signal at m/z 271 $[M+H-hexosyl]^+$ signifies apigenin. The MS2 spectrum of the deprotonated molecule at m/z 431.0989 showed a daughter peak at m/z 269 $[M-H-sugar]^-$, with the major fragments of apigenin, which was identified as apigenin-*O*-hexoside (13). The MS2 spectrum of the deprotonated molecule at m/z 269.0447 showed the loss of B-ring- H_2O moiety yielding the daughter ion at m/z 159, besides the fragments at m/z 151, 133, and 117 characteristics for apigenin (34) (Table 2) [23].

Baicalein-*O*-hexuronide (6) showed a deprotonated molecule at m/z 445.0770 and the loss of 176 amu equivalent to hexuronyl moiety resulting in the base peak at m/z 269, representing baicalein aglycone. The MS2 spectrum of a deprotonated molecule at m/z 577.1575 showed two subsequent losses of 146 and 308 amu, representing the deoxyhexosyl and deoxyhexosyl-hexosyl moieties yielding the product ions at m/z 431 and 269, respectively, with the typical fragmentation pattern of vitexin, which was proposed as vitexin-*O*-deoxyhexoside (21). The deprotonated molecular ion peak of acacetin-*O*-rutinoside (31) was observed at m/z 591.1729 and the daughter ion at m/z 283, representing losses of 308 amu (rutinosyl moiety). Acacetin (38) showed a deprotonated molecule at m/z 283.0606, besides signals at m/z 151 and 131 representing $^{1,3}A^-$ and $^{1,3}B^-$, respectively, for acacetin aglycone (Table 2) [27].

Table 2 Identified metabolites in *Bidens pilosa* extract via UPLC-MS/MS using negative and positive ionization modes

#	Rt	Metabolites	Formula	[M-H] ⁻	[M+H] ⁺	Error PPM	MS ²
Flavonoids							
1	4.80	Hesperidin	C ₂₈ H ₃₄ O ₁₅	609.1324		0.3	463, 301, 177, 151
2	4.86	Kaempferol- <i>O</i> -hexuronide	C ₂₁ H ₁₈ O ₁₂	461.077		0.1	285, 257, 135
3	5.09	Quercetin- <i>O</i> -hexuronide	C ₂₁ H ₁₈ O ₁₃	477.0676	479.1381	-6.6	301, 179, 151
4	5.33	Luteolin-di- <i>O</i> -hexoside	C ₂₇ H ₃₀ O ₁₆	609.1437	611.18	8.6	447, 285, 151
5	5.38	Luteolin- <i>C</i> -hexoside	C ₂₁ H ₂₀ O ₁₁	447.0947		-2.9	357, 327, 285, 151, 135
6	5.52	Baicalein- <i>O</i> -hexuronide	C ₂₁ H ₁₈ O ₁₁	445.077	447.1028	2.2	269, 117
7	5.64	Quercetin- <i>O</i> -di-hexoside	C ₂₇ H ₃₀ O ₁₇	625.1737	627.2105	6.1	463, 301, 283
8	6.18	Isoquercitrin	C ₂₁ H ₂₀ O ₁₂	463.0918		0.5	301, 283, 255, 151
9	6.41	Apigenin- <i>C</i> -hexoside	C ₂₁ H ₂₀ O ₁₀		433.1357	0.2	343, 313, 271
10	6.48	Rutin	C ₂₇ H ₃₀ O ₁₆		611.1806	2.2	465, 303
11	6.50	Eriodictyol- <i>O</i> -hexoside	C ₂₁ H ₂₂ O ₁₁	449.1194		0.7	287, 151, 135
12	6.52	Hyperoside	C ₂₁ H ₂₀ O ₁₂	463.0938	465.1223	0.8	301, 271, 255, 151
13	6.57	Apigenin- <i>O</i> -hexoside	C ₂₁ H ₂₀ O ₁₀	431.0989	433.1263	-9.5	269,
14	6.59	Maritinetin- <i>O</i> -hexoside	C ₂₁ H ₂₀ O ₁₁	447.0909	449.1236	4.2	285
15	6.59	Kaempferol- <i>O</i> -neohesperidoside	C ₂₇ H ₃₀ O ₁₅	593.1467		0.3	285
16	6.62	Kaempferol- <i>O</i> -bis-deoxyhexoside	C ₂₇ H ₃₀ O ₁₄	577.1507	579.1816	6.7	431, 285
17	6.66	Luteolin- <i>O</i> -hexoside	C ₂₁ H ₂₀ O ₁₁	447.0921		2.7	285
18	6.66	Isorhamnetin- <i>O</i> -deoxyhexosyl-hexoside	C ₂₈ H ₃₂ O ₁₆	623.159	625.1852	-0.2	315, 300
19	6.79	Okanin- <i>O</i> -hexoside	C ₂₁ H ₂₂ O ₁₁		451.1404	-0.7	289, 271, 179, 163, 153
20	6.91	Naringenin- <i>O</i> -hexoside	C ₂₁ H ₂₂ O ₁₀	433.1115	435.1358	4.5	271, 151, 119
21	7.10	Vitexin- <i>O</i> -deoxyhexoside	C ₂₇ H ₃₀ O ₁₄	577.1575		-3.8	431, 269
22	7.16	Gossypin	C ₂₁ H ₂₀ O ₁₃	479.1038		3.8	317
23	7.27	Syringetin- <i>O</i> -hexoside	C ₂₃ H ₂₄ O ₁₃	507.1129	509.1445	-15.4	492, 345
24	7.30	Quercetin- <i>O</i> -hexoside	C ₂₁ H ₂₀ O ₁₂	463.1217	465.1522	0.4	301, 283, 135
25	7.46	Kaempferol- <i>O</i> -pentoside	C ₂₀ H ₁₈ O ₁₀		419.1226	-2.5	287, 259, 231
26	7.46	Quercetin- <i>O</i> -hexosyl-pentoside	C ₂₆ H ₂₈ O ₁₆		597.1898	-10.6	435, 303

Table 2 (continued)

#	Rt	Metabolites	Formula	[M-H] ⁻	[M+H] ⁺	Error PPM	MS ²
27	7.54	Isorhamnetin- <i>O</i> -hexoside	C ₂₅ H ₂₂ O ₁₂	477.1333		4.9	315, 300, 151
28	7.89	Phlorizin	C ₂₁ H ₂₄ O ₁₀	435.1261		6.6	273, 151, 119
29	8.63	Quercetin- <i>O</i> -pentoside	C ₂₀ H ₁₈ O ₁₁	433.1016	435.0974	8.7	301, 193, 161, 151,
30	8.66	Eriodictyol	C ₁₅ H ₁₂ O ₆	287.0629	289.0796	0.5	213, 151, 135, 107
31	8.83	Acacetin- <i>O</i> -rutinoside	C ₂₈ H ₃₂ O ₁₄	591.1729		-1.2	283, 268
32	8.95	Kaempferol- <i>O</i> -(<i>p</i> -coumaroyl)-hexoside	C ₃₀ H ₂₆ O ₁₃	593.1296		-0.5	447, 285
33	9.22	Quercetin	C ₁₅ H ₁₀ O ₇	301.0233	303.0576	1.5	255, 193, 151, 135, 121
34	10.13	Apigenin	C ₁₅ H ₁₀ O ₅	269.0447	271.0641	-3.2	159, 151, 133, 117
35	10.31	Hesperetin	C ₁₆ H ₁₄ O ₆	301.0724	303.0969	-0.4	286, 151, 134
36	10.34	Luteolin	C ₁₅ H ₁₀ O ₆	285.0397	287.0589	3.3	257, 177, 151, 133, 107
37	10.56	Trihydroxy-methoxyflavone	C ₁₆ H ₁₂ O ₆	299.0556		-1.7	284, 256, 151
38	13.16	Acacetin	C ₁₆ H ₁₂ O ₅	283.0606	285.0812	0.4	268, 151, 131
39	13.43	Isorhamnetin	C ₁₆ H ₁₂ O ₇	315.0881	317.076	6.8	300, 269, 151, 107
40	14.35	Naringenin	C ₁₅ H ₁₂ O ₅	271.0979	273.086	12.4	225, 136, 122
41	19.81	Rhamnetin	C ₁₆ H ₁₂ O ₇		317.122	6.1	299
42	19.82	Kaempferide	C ₁₆ H ₁₂ O ₆		301.1492	0.8	
Phenolic acids							
43	1.24	Gentisic acid	C ₇ H ₆ O ₄	153.0193		0.8	109, 91
44	1.24	Caffeic acid	C ₉ H ₈ O ₄	179.055		-0.6	161, 135,
45	1.73	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	353.0866	355.1069	3.1	191, 179, 161, 135
46	2.52	Homogenetic acid	C ₈ H ₈ O ₄	167.0331		0.5	149, 123, 108
47	2.77	Hydroxybenzoic acid	C ₇ H ₆ O ₃	137.0242		4.4	93, 75
48	3.37	Coumaric acid	C ₉ H ₈ O ₃	163.0233	165.093	10.2	119, 101
49	4.26	Protocatechuic acid	C ₇ H ₆ O ₄	153.0183		-4.3	135, 109, 91
50	4.82	Dihydroxymandelate	C ₈ H ₈ O ₅	183.0079	184.9872	24.8	139, 109

Table 2 (continued)

#	Rt	Metabolites	Formula	[M-H] ⁻	[M+H] ⁺	Error PPM	MS ²
51	5.24	Sinapic acid- <i>O</i> -hexoside	C ₁₇ H ₂₂ O ₁₀	385.1803		8.4	223, 205
52	6.50	Methoxysalicylic acid	C ₈ H ₈ O ₄	167.0341		2	152, 123, 108
53	8.40	Ferulic acid	C ₁₀ H ₁₀ O ₄	193.0501	195.1168	1.4	178, 161, 149, 133
		Coumarin					
54	1.27	Hydroxy-Methylcoumarin	C ₁₀ H ₈ O ₃	175.0423		7.9	157, 131, 113
55	2.68	Esculin	C ₁₃ H ₁₆ O ₉	339.0741	341.0936	-3	177, 133
56	4.68	Dihydroxycoumarin	C ₉ H ₆ O ₄	177.0187	179.0312	0.9	149, 133, 105
57	7.09	Scopoletin	C ₁₀ H ₈ O ₄		193.0547	-7.1	178
		Acids					
58	1.01	Malic acid	C ₄ H ₆ O ₅	133.0145		-0.9	115, 89, 71
59	1.04	Maleic acid	C ₄ H ₄ O ₄	115.0022		9.3	71
60	1.06	Hydroxy-butyric acid	C ₄ H ₈ O ₃	103.002		9.4	59
61	1.07	Lactic acid	C ₃ H ₆ O ₃	89.02383		-0.6	71
62	1.11	Succinic acid	C ₄ H ₆ O ₄	117.0177		9	99, 73
63	1.15	Citrate	C ₆ H ₈ O ₇	191.0556		-0.3	173, 129, 111, 85
64	1.19	Tartrate	C ₄ H ₆ O ₆	149.0448		3.2	131, 89, 87
65	1.25	Citramalate	C ₅ H ₈ O ₅	146.9487		3.6	129, 85
66	1.28	Isopropylmalic acid	C ₇ H ₁₂ O ₅	175.0605		-1	157, 131, 113, 69
67	1.35	Methylglutaric acid	C ₆ H ₁₀ O ₄	145.0258		13.1	127, 109, 101
68	2.27	Phenyllactic acid	C ₉ H ₁₀ O ₃	165.0562		1	147, 121, 103
69	4.90	Quinic acid	C ₇ H ₁₂ O ₆	191.0559		0.9	173, 127, 93
70	5.09	Shikimic acid	C ₇ H ₁₀ O ₅	173.0432		-4.3	155, 137, 131, 111, 93
		Amino acids					
71	1.11	Arginine	C ₆ H ₁₄ N ₄ O ₂		175.121	-4.3	
72	1.55	Oxoproline	C ₅ H ₇ NO ₃		130.0488	3.9	

Table 2 (continued)

#	R _t	Metabolites	Formula	[M-H] ⁻	[M+H] ⁺	Error PPM	MS ²
73	1.71	Hydroxyproline	C ₅ H ₉ NO ₃	130.0862		4.2	
74	2.15	Phenylalanine	C ₉ H ₉ NO ₂		166.0876	-0.6	
75	2.80	Tryptophan	C ₁₁ H ₁₂ N ₂ O ₂	203.0821		1.3	
76	3.94	Homoisoleucine	C ₇ H ₁₅ NO ₂	144.0459		0.8	
		Fatty acids					
77	12.82	Hydroxy-hexadecanoic acid	C ₁₆ H ₃₂ O ₃	271.2264		9.1	253, 212
78	18.48	Linolenic acid	C ₁₈ H ₃₀ O ₂	277.2177		0.5	233
		Sugar derivatives					
79	1.36	Mannitol	C ₆ H ₁₄ O ₆	181.0724		-0.8	
80	2.23	Maltitol	C ₁₂ H ₂₄ O ₁₁	343.1402		-0.5	
81	2.46	Maltotriose	C ₁₈ H ₃₂ O ₁₆	503.1371		3.8	341, 179
82	3.82	Melibiose	C ₁₂ H ₂₂ O ₁₁	341.0872		7.6	

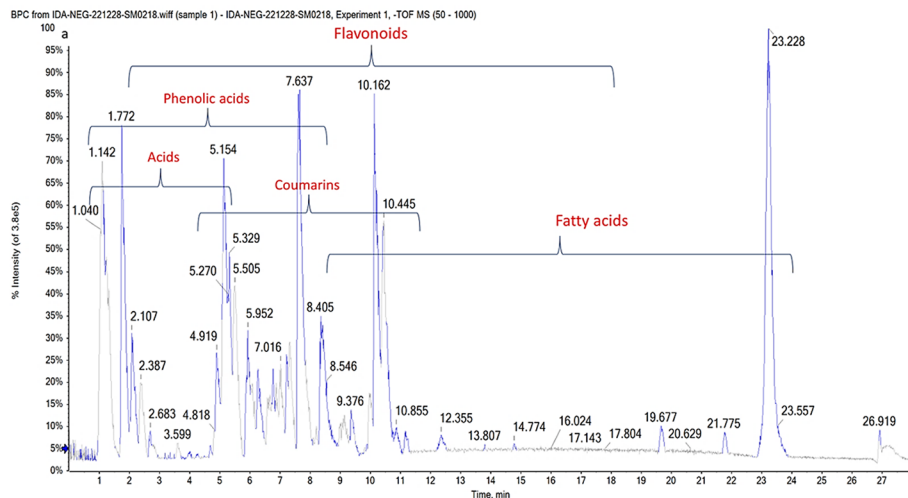


Fig. 2 Base peak chromatogram obtained from UPLC/T-TOF-MS/MS analysis of *B. pilosa* extract in negative ionization mode

Flavonol Identification

Quercetin-*O*-hexuronide (3) was detected by its parent ion at m/z 477.0676 $[M-H]^-$ and a fragment at m/z 301 for losing of hexuronil moiety (176 Da), besides the characteristic fragments of quercetin aglycone m/z 179 and 151 for $^{1,4}B^-$ and $^{1,3}A^-$, respectively (Fig. 3). The MS2 spectrum of the deprotonated molecule at m/z 625.1737 showed two subsequent losses at m/z 463 and 301 representing $[M-H\text{-hexosyl}]^-$ and $[M-H\text{-di-hexosyl}]^-$, respectively, moreover another fragment at m/z 283 $[Ag-H_2O]^-$ with the major fragments of quercetin, which was proposed as quercetin-*O*-di-hexoside (7). Isoquercitrin (8) was detected by its parent ion at m/z 463.0918 $[M-H]^-$ and a fragment at m/z 301 $[M-H\text{-sugar}]^-$, moreover, characteristic flavonoid fragments at m/z 283, 255, and 151. Rutin (10) was identified by its protonated molecule at m/z 611.1806. Moreover, deoxyhexosyl and deoxyhexosyl-hexosyl moieties were confirmed by the two daughter ions at m/z 465 and 303, respectively. Hyperoside (12) showed a deprotonated molecule at m/z 463.0938 and a loss of hexosyl at m/z 301 with $^{1,3}A^-$ fragment which signifies quercetin aglycone. Quercetin-*O*-hexoside (23) was identified by its deprotonated molecule at m/z 463.1217; moreover, hexosyl and $Ag-H_2O$ moieties were confirmed by the two daughter ions at m/z 301 and 283, respectively, with another fragment at m/z 135 representing $^{0,2}A^-$. Quercetin-*O*-hexosyl-pentoside (26) was identified by its protonated molecule at m/z 597.1898; moreover, hexosyl and hexosyl-pentosyl moieties were confirmed by two daughter ions at m/z 435 and 303, respectively. The MS2 spectrum of a deprotonated molecule at m/z 433.1016 showed two product fragments at m/z 301 $[M-H\text{-pentosyl}]^-$ and 193 $[M-H\text{-B-ring}]^-$, besides the characteristic fragments of quercetin, which was suggested as quercetin-*O*-pentoside (29). The quercetin (33) molecular ion peak $[M-H]^-$ was noticed at m/z 301.0233, besides the characteristic fragments of quercetin aglycone (m/z 255, 193, 151, and 135) (Table 2) [27].

Kaempferol-*O*-hexuronide (2) showed a deprotonated molecular ion peak at m/z 461.0770, and a fragment at m/z 285 signifies kaempferol aglycone, moreover its $^{0,3}A^-$ fragment at m/z 135. Kaempferol-*O*-neohesperidoside (15) was identified by a deprotonated molecule at m/z

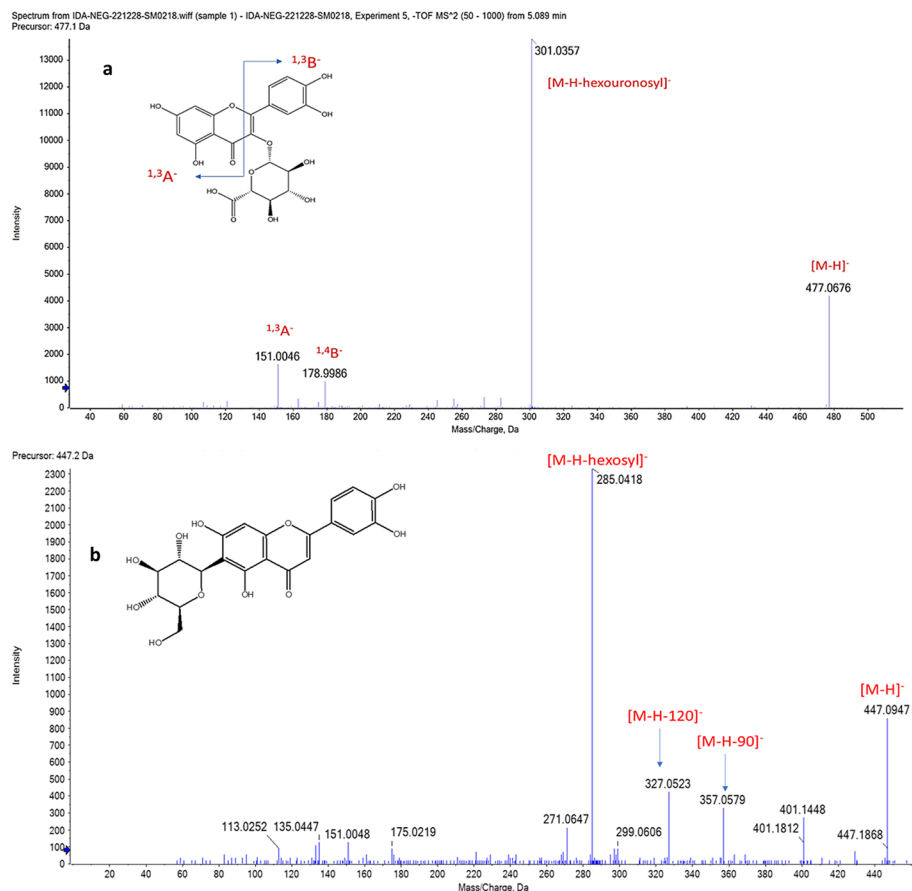


Fig. 3 Mass fragments of major classes identified from *Bidens pilosa* extract: **a** quercetin-*O*-hexuronide (3) and **b** luteolin-*C*-hexoside (5)

593.1467; moreover, neohesperidoside moiety was confirmed by the daughter peak at m/z 285. The MS² spectrum of a deprotonated molecule at m/z 577.1507 showed two product ions at m/z 431 [M-H-deoxyhexosyl][−] and 285 [M-H-di-deoxyhexosyl][−], with the typical fragments of kaempferol, which was suggested as kaempferol-*O*-bis-deoxyhexoside (16). The molecular ion peak [M+H]⁺ of kaempferol-*O*-pentoside (25) was observed at m/z 419.1226, followed by daughter ions at m/z 287 [M+H-pentosyl]⁺, 259 [M+H-pentosyl-CO]⁺, and 231 [M+H-pentosyl-2CO]⁺. Kaempferol-*O*-(coumaroyl)-hexoside (32) showed a deprotonated molecule at m/z 593.1296 and a fragment at m/z 447 [M-H-coumaroyl][−], with another fragment at m/z 285 [M-H-coumaroyl-hexosyl][−] (Table 2) [27].

Isorhamnetin-*O*-deoxyhexosyl-hexoside (18) showed a deprotonated molecule at m/z 623.1590 and the aglycone fragment at m/z 315 [M-H-deoxyhexosyl-hexosyl][−]. Isorhamnetin-*O*-hexoside (27) showed a molecule ion [M-H][−] at m/z 477.1333, besides the aglycone base peak at m/z 315. Gossypin (22) showed a deprotonated molecule at m/z 479.1038, and the loss of hexosyl moiety was confirmed by the peak at m/z 317. Syringetin-*O*-hexoside (23) showed a molecule peak [M-H][−] at m/z 507.1129, followed by the signal of syringetin

aglycone at m/z 345. The protonated ion peaks $[M+H]^+$ of rhamnetin and kaempferide (41, 42) were noticed at m/z 317.1220 and 301.1492, respectively (Table 2).

Flavanone Identification

Eriodictyol-*O*-hexoside (11) showed a deprotonated molecule at m/z 449.1194, besides the eriodictyol aglycone fragment at m/z 287. Eriodictyol (30) showed a deprotonated molecule at m/z 287.0629, moreover, the characteristic flavonoid fragments at m/z 213, 151, 135, and 107. Naringenin-*O*-hexoside (20) showed a signal at m/z 433.1115 $[M-H]^-$ and a loss of hexosyl moiety at m/z 271, besides the naringenin fragments at m/z 151 and 119 for $^{1,3}A^-$ and $^{1,3}B^-$, respectively. Naringenin (40) showed a signal at m/z 271.0979 $[M-H]^-$, besides the fragments at m/z 225, 136, and 122) [30]. Maritimetin-*O*-hexoside (14) showed a signal at m/z 447.0909 $[M-H]^-$ and a base peak at m/z 285 $[M-H\text{-hexosyl}]^-$. In the same behavior, okanin-*O*-hexoside (19) and phlorizin (28) were identified (Table 2).

Phenolic, Organic, and Amino Acid Identification

The expected losses of 18, 44, and 62 Da, equivalent to the loss of H_2O , CO_2 , and both, respectively, were observed in MS2 fragmentations of acids. Gentisic acid (43) was detected by its deprotonated molecule at m/z 153.0193, moreover ions at m/z 109 and 91. Protocatechuic acid (49) showed a deprotonated molecule at m/z 153.0183 and subsequent losses of H_2O , CO_2 , and both moieties. In the same approach, caffeic, chlorogenic, coumaric, and ferulic acids (44, 45, 48, 53) were identified. The common neutral loss of CO_2 (44 Da) was observed in MS2 fragments

Table 3 Docked conformations of quercetin and phenolic derivatives from *Bidens pilosa* on aldose reductase (AKR1B1; PDB: 2IKI) protein

Metabolites	ΔG^a (kcal mol ⁻¹)/ No. of interactions	RMSD	Interactions	Distance/E (kcal/mol)
Quercetin-3- <i>O</i> -glucuronide	− 5.31/3	1.62	HOH1184	2.92/− 0.8
			HOH1050	2.69/− 0.9
			TRP20	4.73/− 1.5
Isoquercitrin	− 5.49/3	2.32	HOH1184	3.08/− 0.9
			HOH1050	2.88/− 1.4
			HOH1056	3.05/− 1.1
Quercetin-4'- <i>O</i> -glucoside	− 5.75/5	2.14	GLN183	3.53/− 0.7
			HOH1218	2.92/− 1.0
			ASN160	2.96/− 2.5
			HOH1056	2.80/− 1.5
			TYR209 (pi-pi)	3.69/− 0.0
Chlorogenic acid	− 7.20/3	1.46	ASP43	2.79/− 3.2
			ILE260	2.97/− 4.6
			SER210	2.92/− 2.9
Ferulic acid	− 5.3/3	1.11	HIS110	2.91/− 1.8
			TRP20 (H-pi)	4.06/− 0.8
			TRP20 (H-pi)	4.03/− 0.8

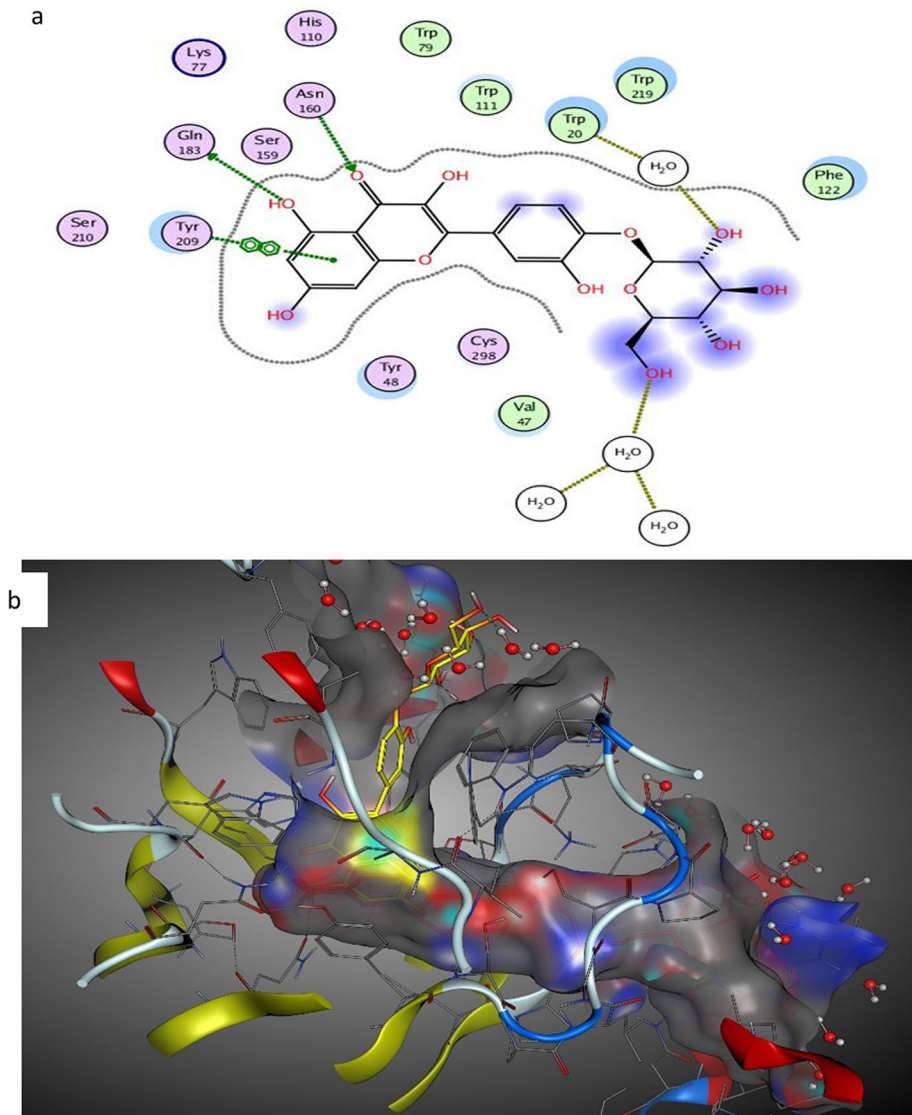


Fig. 4 The interaction of quercetin-4'-O-glucoside (yellow) on aldose reductase protein (PDB ID: 2IKI) in the binding pocket: **a** 2D and **b** 3D diagrams

of organic acids as in succinic and malic acids (58, 62) (Table 2) [27]. Various amino acids were identified such as oxoproline, hydroxyproline, and phenylalanine (Table 2).

Molecular Docking of Quercetin and Phenolic Derivatives on Aldose Reductase Protein

Inhibition of aldose reductase has a role in cancer management by preventing the activation of many transcription factors responsible for producing carcinogenic mediators [31].

The studies informed that the activity of aldose reductase increased in human cancers [32]. Inhibitors of aldose reductase showed a possible increase in the doxorubicin cytotoxic effect with a diminution in its cardiotoxicity [33, 34]. Flavonols mainly quercetin were reported as inhibitors of kinase enzymes [35, 36]. Quercetin derivatives are inhibiting tumors in rats [37]. Thus, molecular docking of *B. pilosa* metabolites on aldose reductase and kinase enzymes should be performed to rationalize its observed antiproliferative activity. The active pocket of AKR1B1 consists mainly of VAL47, TYR48, GLN49, HIS110, GLN183, and TRP111, where its co-crystallized ligand IDD388 was interacted by two H–H bonding with TYR48 and HIS110 ($-9.87 \text{ kcal mol}^{-1}$ binding affinity). Five major metabolites were docked on aldose reductase protein with low energy of binding affinities (-5.3 to $-7.2 \text{ kcal mol}^{-1}$) (Table 3). Quercetin-4'-*O*-glucoside exhibited interactions ($-5.75 \text{ kcal mol}^{-1}$ binding affinities) with four H-bonds between GLN183, HOH1218, ASN160, and HOH1056. The best docking activity was recorded on chlorogenic acid ($-7.20 \text{ kcal mol}^{-1}$) that interacts with three H-bonds, mainly with ASP43, ILE260, and SER210 (Fig. 4).

On the other hand, the active site of RSK2 consists mainly of PHE79, LYS100, VAL101, LYS103, LEU147, ASP148, GLU197, and LEU200 amino acids. The crystal ligand, SL0101, interacts with three H–H bonds with LYS100, ASP148, and GLU197 ($-9.54 \text{ kcal mol}^{-1}$). The major metabolites were docked on RSK2 protein and demonstrated auspicious binding affinities (-5.6 to $-8.89 \text{ kcal mol}^{-1}$) (Table 4) with a variety of degrees of interactions. Isoquercitrin exhibited interactions with four H-bonds between SER78, GLU197, and LEU200 ($-8.89 \text{ kcal mol}^{-1}$) (Fig. 5). The low binding energy of interactions between metabolites and aldose reductase and kinase proteins may justify its antiproliferative activity [38]. The phenolic and hydroxyl OH groups in metabolites are essential for interaction acting as a hydrogen bond acceptor-donor. Moreover, it was found that quercetin-4'-*O*-glucoside occupies the pocket of aldose reductase better than

Table 4 Docked conformations of quercetin and phenolic derivatives from *Bidens pilosa* on ribosomal S6 kinase (RSK2) (PDB: 3UBD) protein

Metabolites	ΔG^a (kcal mol $^{-1}$)/ No. of interactions	RMSD	Interactions	Distance/E (kcal/mol)
Quercetin-3- <i>O</i> -glucuronide	$-8.29/3$	1.25	GLU197	2.65/ -3.6
			LEU200 (pi-H)	3.89/ -1.0
			PHE79 (pi-pi)	3.85/ -0.0
Isoquercitrin	$-8.89/5$	1.41	SER78	3.18/ -0.7
			GLU197	2.73/ -4.3
			GLU197	2.66/ -2.5
			LEU200 (pi-H)	3.77/ -0.8
			PHE79 (pi-pi)	3.64/ -0.0
Quercetin-4'- <i>O</i> -glucoside	$-7.22/3$	1.10	ASP148	2.82/ -3.4
			LYS216	2.86/ -1.9
			PHE79 (pi-pi)	3.70/ -0.0
Chlorogenic acid	$-6.48/3$	2.76	ASP148	3.44/ -0.8
			ASP148	2.67/ -2.7
			LEU147 (pi-H)	3.83/ -1.3
Ferulic acid	$-5.62/2$	0.81	ASP148	2.64/ -2.4
			LEU147 (pi-H)	4.11/ -0.7

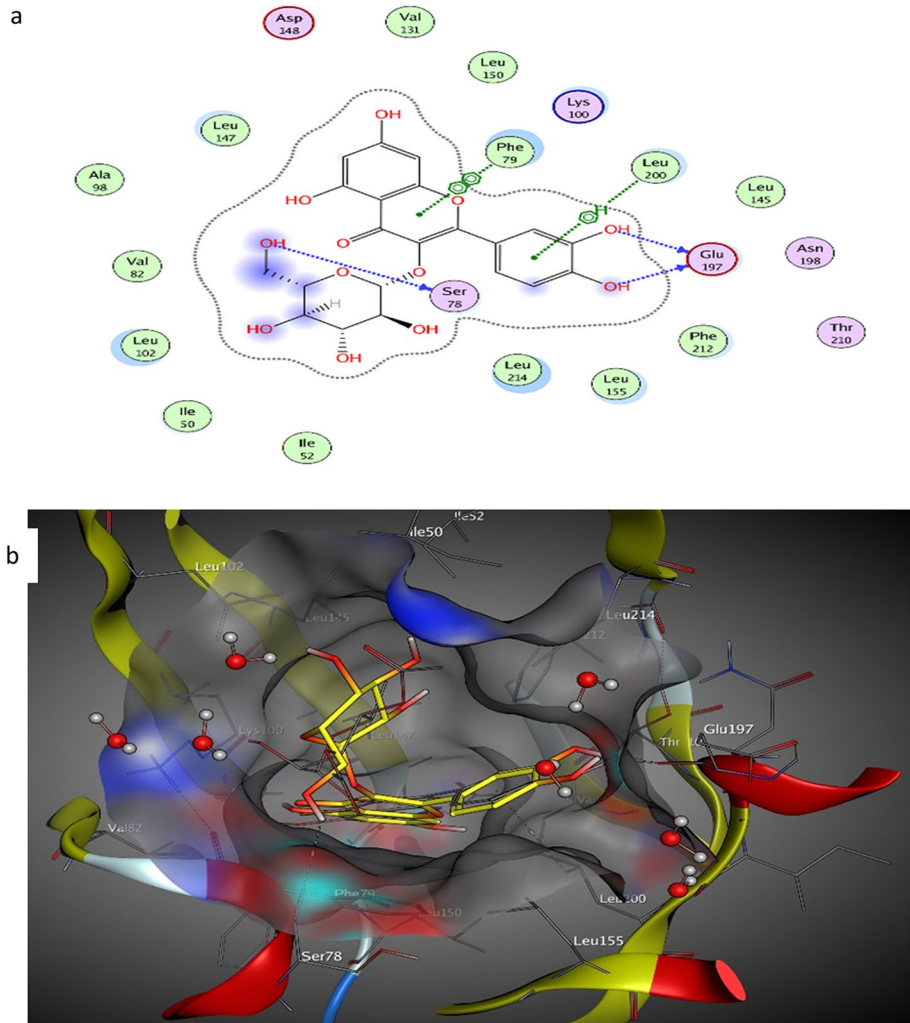


Fig. 5 The interaction of isoquercitrin (yellow) on RSK2 protein (PDB: 3UBD) in the binding pocket: **a** 2D and **b** 3D diagrams

its co-crystallized ligand. Moreover, quercetin-4'-*O*-glucoside and isoquercitrin exhibited binding scores to targeted enzymes relatively equal to the co-crystallized ligands, expected acts as kinases and aldose reductase inhibitors leading to rationalizing its antiproliferative activity.

Conclusion

Metabolomic profiling of the *B. pilosa* revealed its enrichment of flavonoids and phenolic acids besides coumarins, fatty, and amino acids. Moreover, *B. pilosa* extract showed in vitro antiproliferative activity. Based on in silico study, major metabolites act as

inhibitors of kinases and aldose reductase leading to rationalizing its antiproliferative activity. The use of *B. pilosa* as a medicinal plant is feasible but further clinical trials and toxicity assessments are still rare.

Acknowledgements The authors extend their appreciation to the Researchers Supporting Project number (RSP2025R366) King Saud University, Riyadh, Saud Arabia.

Author Contribution Methodology and writing draft manuscript: D.S.A., A.E.E., A.A.E., E.M.K., and S.A.A.; artwork and schemes: D.S.A., H.S.M., S.A.A.; review and editing: D.S.A., A.E.E., A.A.E., E.M.K., and S.A.A.; conceptualization, validation, supervision: A.E.E., E.M.K., S.A.A., and M.M.H. All authors have read and agreed to the published version of the manuscript.

Funding Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Data Availability The data that support the findings of this study are available on request.

Declarations

Ethical Approval Not applicable.

Competing Interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

1. Xuan, T. D., & Khanh, T. D. (2016). Chemistry and pharmacology of *Bidens pilosa*: An overview. *Journal of Pharmaceutical Investigation*, 46, 91–132.
2. Chiang, Y.-M., Chuang, D.-Y., Wang, S.-Y., Kuo, Y.-H., Tsai, P.-W., & Shyur, L.-F. (2004). Metabolite profiling and chemopreventive bioactivity of plant extracts from *Bidens pilosa*. *Journal of Ethnopharmacology*, 95(2–3), 409–419.
3. Mitich, L. W. (1994). Beggarticks. *Weed Technology*, 8(1), 172–175.
4. Chiang, Y.-M., Chang, C.L.-T., Chang, S.-L., Yang, W.-C., & Shyur, L.-F. (2007). Cytopiloyne, a novel polyacetylenic glucoside from *Bidens pilosa*, functions as a T helper cell modulator. *Journal of Ethnopharmacology*, 110(3), 532–538.
5. Brandão, M. G. L., Krettl, A. U., Soares, L. S. R., Nery, C. G. C., & Marinuzzi, H. C. (1997). Anti-malarial activity of extracts and fractions from *Bidens pilosa* and other *Bidens* species (Asteraceae) correlated with the presence of acetylene and flavonoid compounds. *Journal of Ethnopharmacology*, 57(2), 131–138.
6. Valdés, L., Humberto, A., de León Rego, L., et al. (2001). *Bidens pilosa* Linné. *Rev Cuba Plantas Med*, 6(1), 28–33.
7. Alvarez, L., Marquina, S., Villarreal, M. L., Alonso, D., Aranda, E., & Delgado, G. (1996). Bioactive polyacetylenes from *Bidens pilosa*. *Planta Medica*, 62(04), 355–357.
8. Longuefosse, J.-L., & Nossin, E. (1996). Medical ethnobotany survey in Martinique. *Journal of Ethnopharmacology*, 53(3), 117–142.
9. Horiuchi, M., & Seyama, Y. (2006). Antiinflammatory and antiallergic activity of *Bidens pilosa* L. var. *radiata* SCHERFF. *Journal of Health Science*, 52(6), 711–717.

10. Young, P. H., Hsu, Y. J., Yang, W. C., & others. (2010). *Bidens pilosa* and its medicinal use. In *Recent progress in medicinal plants/drug plants II vol 28* (Houston, TX, USA: Stadium Press LLC). Stadium Press LLC.
11. Chien, S.-C., Young, P. H., Hsu, Y.-J., Chen, C.-H., Tien, Y.-J., Shiu, S.-Y., others. (2009). Anti-diabetic properties of three common *Bidens pilosa* variants in Taiwan. *Phytochemistry*, 70(10), 1246–1254.
12. Dimo, T., Rakotonirina, S. V., Tan, P. V., Azay, J., Dongo, E., & Cros, G. (2002). Leaf methanol extract of *Bidens pilosa* prevents and attenuates the hypertension induced by high-fructose diet in Wistar rats. *Journal of Ethnopharmacology*, 83(3), 183–191.
13. Alvarez, A., Pomar, F., Sevilla, M. A., & Montero, M. J. (1999). Gastric antisecretory and antiulcer activities of an ethanolic extract of *Bidens pilosa* L. var. *radiata* Schult. Bip. *Journal of Ethnopharmacology*, 67(3), 333–340.
14. Yuan, L.-P., Chen, F.-H., Ling, L., Dou, P.-F., Bo, H., Zhong, M.-M., & Xia, L.-J. (2008). Protective effects of total flavonoids of *Bidens pilosa* L.(TFB) on animal liver injury and liver fibrosis. *Journal of Ethnopharmacology*, 116(3), 539–546.
15. Sundararajan, P., Dey, A., Smith, A., Doss, A. G., Rajappan, M., & Natarajan, S. (2006). Studies of anticancer and antipyretic activity of *Bidens pilosa* whole plant. *African Health Sciences*, 6(1), 27–30.
16. Pereira, R. L. C., Ibrahim, T., Lucchetti, L., da Silva, A. J. R., & de Moraes, V. L. G. (1999). Immunosuppressive and anti-inflammatory effects of methanolic extract and the polyacetylene isolated from *Bidens pilosa* L. *Immunopharmacology*, 43(1), 31–37.
17. Chang, J.-S., Chiang, L.-C., Chen, C.-C., Liu, L.-T., Wang, K.-C., & Lin, C.-C. (2001). Antileukemic activity of *Bidens pilosa* L. var. *minor* (Blume) Sherff and *Houttuynia cordata* Thunb. *The American Journal of Chinese Medicine*, 29(02), 303–312.
18. Deba, F., Xuan, T. D., Yasuda, M., & Tawata, S. (2008). Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. *Radiata*. *Food Control*, 19(4), 346–352.
19. Yang, H.-L., Chen, S.-C., Chang, N.-W., Chang, J.-M., Lee, M.-L., Tsai, P.-C., & others. (2006). Protection from oxidative damage using *Bidens pilosa* extracts in normal human erythrocytes. *Food and Chemical Toxicology*, 44(9), 1513–1521.
20. Lima Silva, F., Fischer, D. C. H., Fachine Tavares, J., Sobral Silva, M., de Athayde-Filho, P., & Barbosa-Filho, J. M. (2011). Compilation of secondary metabolites from *Bidens pilosa* L. *Molecules*, 16(2), 1070–1102.
21. Priestap, H. A., Bennett, B. C., & Quirke, J. M. E. (2008). Investigation of the essential oils of *Bidens pilosa* var. *minor*, *Bidens alba* and *Flaveria linearis*. *Journal of Essential Oil Research*, 20(5), 396–402.
22. Tammali, R., Srivastava, K. S., & Ramana, V. K. (2011). Targeting aldose reductase for the treatment of cancer. *Current Cancer Drug Targets*, 11(5), 560–571.
23. El Gizawy, H. A., El-Haddad, A. E., Attia, Y. M., Fahim, S. A., Zafer, M. M., & Saadeldeen, A. M. (2022). In vitro cytotoxic activity and phytochemical characterization (UPLC/T-TOF-MS/MS) of the watermelon (*Citrullus lanatus*) rind extract. *Molecules*, 27(8), 2480.
24. Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1–2), 55–63. [https://doi.org/10.1016/0022-1759\(83\)90303-4](https://doi.org/10.1016/0022-1759(83)90303-4)
25. El-Haddad, A. E., Saadeldeen, A. M., & El-Emam, S. Z. (2019). Anti-angiogenic activity of major phenolics in tamarind assessed with molecular docking study on VEGF kinase proteins. *Pakistan Journal of Biological Sciences*, 22(10), 502–509.
26. Abd El-Kareem, M. S. M., Rabbih, M. A. E. F., Selim, E. T. M., Elsherbiny, E. A. E., & El-Khateeb, A. Y. (2016). Application of GC/EIMS in combination with semi-empirical calculations for identification and investigation of some volatile components in basil essential oil. *International Journal of Analytical Mass Spectrometry and Chromatography*, 4(1), 14–25.
27. Mounir, R., Alshareef, W. A., El Gebaly, E. A., El-Haddad, A. E., Ahmed, A. M. S., Mohamed, O. G., & others. (2023). Unlocking the power of onion peel extracts: Antimicrobial and anti-inflammatory effects improve wound healing through repressing notch-1/NLRP3/caspase-1 signaling. *Pharmaceuticals*, 16(10), 1379.
28. Gendy, A., Elnagar, M. R., Soubh, A., Al-Mokaddem, A., El-Haddad, A., & El-Sayed, M. K. (2021). Morin alleviates hepatic ischemia/reperfusion-induced mischief: In vivo and in silico contribution of Nrf2, TLR4, and NLRP3. *Biomedicine & Pharmacotherapy*, 138, 111539.
29. Wu, L.-W., Chiang, Y.-M., Chuang, H.-C., Wang, S.-Y., Yang, G.-W., Chen, Y.-H., & Shyur, L.-F. (2004). Polyacetylenes function as anti-angiogenic agents. *Pharmaceutical Research*, 21, 2112–2119.
30. El Gizawy, H. A., El-Haddad, A. E., Saadeldeen, A. M., & Boshra, S. A. (2022). Tentatively identified (UPLC/T-TOF-MS/MS) compounds in the extract of *saussurea costus* roots exhibit in vivo

- hepatoprotection via modulation of HNF-1 α , sirtuin-1, C/ebp α , miRNA-34a and miRNA-223. *Molecules*, 27(9), 2802.
31. Tanagala, K. K. K., Baba, A. B., Kowshik, J., Reddy, G. B., & Nagini, S. (2018). Gedunin, a neem limonoid in combination with epalrestat inhibits cancer hallmarks by attenuating aldose reductase-driven oncogenic signaling in SCC131 oral cancer cells. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, 18(14), 2042–2052.
 32. Saraswat, M., Mrudula, T., Kumar, P. U., Suneetha, A., TS, R. R., Srinivasulu, M., & Reddy, B. (2006). Overexpression of aldose reductase in human cancer tissues. *Medical Science Monitor: International Medical Journal Of Experimental And Clinical Research*, 12(12), CR525–529.
 33. Sonowal, H., Pal, P., Shukla, K., Saxena, A., Srivastava, S. K., & Ramana, K. V. (2018). Aldose reductase inhibitor, fidarestat prevents doxorubicin-induced endothelial cell death and dysfunction. *Biochemical Pharmacology*, 150, 181–190.
 34. Antony, P., & Vijayan, R. (2015). Identification of novel aldose reductase inhibitors from spices: A molecular docking and simulation study. *PLoS ONE*, 10(9), e0138186.
 35. Boly, R., Gras, T., Lamkami, T., Guissou, P., SerTEYN, D., Kiss, R., & Dubois, J. (2011). Quercetin inhibits a large panel of kinases implicated in cancer cell biology. *International Journal of Oncology*, 38(3), 833–842.
 36. Hou, D.-X., & Kumamoto, T. (2010). Flavonoids as protein kinase inhibitors for cancer chemoprevention: Direct binding and molecular modeling. *Antioxidants & redox signaling*, 13(5), 691–719.
 37. Devipriya, S., Ganapathy, V., & Shyamaladevi, C. S. (2006). Suppression of tumor growth and invasion in 9,10 dimethyl benz (a) anthracene induced mammary carcinoma by the plant bioflavonoid quercetin. *Chemico-biological Interactions*, 162(2), 106–113.
 38. Francis, R., Kalyanaraman, R., Boominathan, V., Parthasarathy, S., Chavaan, A., Ansari, I. A., & Tharumasivam, S. V. (2024). Piperine's potential in treating polycystic ovarian syndrome explored through in-silico docking. *Scientific Reports*, 14(1), 21834.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.