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Phytochemical Profiling, Cytotoxic Effect, and *In Vitro* Anti-diabetic Assessment of *Asparagus horridus* L. with *In Silico* Insights of Brassicin

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Abstract

Asparagus L., a traditional medicinal genus, exhibits remarkable biological activities, yet *Asparagus horridus* L. has received little attention in chemical and biological assessments. Isolation procedures on *A. horridus* extract yielded two phenolic acids and nine flavonols. Further chemical profiling through LC-ESI-MS/MS characterized 104 metabolites with a wealth of phenolic acids, flavonoids, and steroids. Moreover, the extract showed moderate inhibition for HepG2 (48.3%) and HCT116 (45.6%) at 100 ppm. Furthermore, it recorded the highest α-glucosidase and α-amylase inhibition at concentrations, ppm, of 182 (62.02±0.06 %) and 285 (87.3±4.6 %), respectively, compared to acarbose (100 ± 1.6 %). To predict the hyperglycemia inhibition of the isolates, molecular docking was established, in which the flavonols showed remarkable values of binding energy in Kcal/mol, among which, (tiliroside and nicotiflorin) exhibited significant values (-8.0 and -8.4) for α-glucosidase whereas (quercetin and brassicin) with (-9.1 and -9.2) for α-amylase compared to the standard (-7.7 and -9.6), respectively. Moreover, various isolates fall within the acceptable bioavailability zone through ADME and pass Lipinski's law and Ghose filter of the drug-likeness test. The findings highlight the potential of *A. horridus* constituents as inhibitors of diabetes-related enzymes and offer the first *in silico* analysis of brassicin, underscoring its uniqueness and potential therapeutic applications.

Keywords: *Asparagus stipularis*, Asparagaceae, Flavonoids, MCF7, PACA2.

1. Introduction

Wild edible food sources, especially wild vegetables, have disappeared as their traditional uses have faded over generations. Promoting these wild herbs as potential sources of functional food ingredients and/or their inclusion in modern diets could help preserve their culinary applications and medicinal properties [1].

Asparagus L. was classified before as a genus from the family Liliaceae, recently categorized within Asparagaceae [1-3]. Different studies on *Asparagus* species have demonstrated anti-cancer [4], anti-inflammatory, antidiabetic [5], antimicrobial [6], and antioxidant activities [7-9]. Different species of *Asparagus* are rich sources of various bioactive chemical constituents [5]; such as steroids, saponins, flavonoids, ascorbic acid, minerals, and polysaccharides [10-12]. In Egypt, three *Asparagus* species were recorded and represented as *A. horridus* L., *A. africanus* Lam., and *A. aphyllus* L. [13]. *A. horridus* (Syn. *A. stipularis* Forssk.), grey asparagus, is an edible wild annual or perennial plant with branching stems and short rootstocks [14]. Rural populations in Egypt, Tunisia, Algeria, Cyprus, Greece, and Rhodes have traditionally consumed their young shoots [15]. Infusions of its tuberous roots have been reported to relieve headaches, prevent kidney stone formation, and cure syphilis. A decoction of the entire plant is used to stimulate appetite and ease stomach pain [14]. In addition, the shoots and roots are used to prevent urinary retention and treat jaundice, liver problems, hemorrhoids, rheumatism, and schistosomiasis [16]. Some studies reported the phenolic profile of *A. horridus* extracts, which is characterized by the presence of flavonols and phenolic acid derivatives that exhibit *in vitro* antioxidant, anti-inflammatory, and anti-schistosomal activities [1,17]. Also, these compounds could be a potential anticancer agent for both liver and thyroid cancer [18].

Recently, researchers have frequently used molecular docking to better comprehend drug-target interactions, more accurately anticipate possible drug possibilities, and accelerate the drug discovery process [19]. It involves predicting the interaction between a small molecule like phytochemicals and the binding site of a target protein at the atomic level to identify potential therapeutic compounds [20].

Diabetes is a heterogeneous condition characterized by hyperglycemia, insulin resistance, or a combination of these factors [21]. Since ancient times, traditional medicinal plants and herbs have proved remarkable cures for diabetes and its complications. Many important classes of natural compounds, such as phenolic compounds found in various species of plants, contain a wide variety of biological functions, many of which are employed in the pharmaceutical industry as therapeutic compounds to treat illness [22]. Moreover, natural dietary sources like *A. horridus* could be crucial for effective diabetes management and public health strategies, as reported before on some *Asparagus* species [5]. This plant may offer advantages for various health issues, including diabetes, necessitating further exploration of its impact on glucose metabolism. Its

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bioactive compounds may interact positively to enhance insulin sensitivity and glucose regulation, complementing existing research.

Although *A. horridus* holds significant medicinal value, it has not been researched as extensively as other commercially valuable species. Accordingly, the main purpose of this study is to conduct the first comprehensive phytochemical analysis of *A. horridus* aerial parts, evaluate the anti-cancer activity, and assess the *in vitro* and *in silico* antidiabetic enzyme inhibitory potential.

2. Experimental

2.1. Plant material and extraction procedure

A. horridus aerial parts (320 g) were gathered from Alexandria-Borg El Arab, in March 2020, and identified by Dr. Mona El Shabrawy (co-author). The voucher specimen (MO 320) was deposited in the NRC herbarium. The dried powdered plant material (285 g) was extracted with 70% MeOH/H_2O yielding a hydromethanol extract which was further dried (49 g) and stored for chemical and biological investigation.

2.2. Phytochemical analysis of hydromethanol extract of *A. horridus*

2.2.1. Acid hydrolysis and paper chromatography of the hydromethanol extract

The hydro-methanolic extract (5 g) was hydrolysed with 10 mL 2N HCl for 2h at 100°C according to the method described by Farid et al. [23].

2.2.2. Isolation and structural elucidation

The hydromethanol extract (35g) was exposed to chromatographic investigation to separate pure compounds including PPC (preparative paper chromatography) (Whatmann 3MM; 15%HOAc, BAW, and/or H_2O), and CC (column chromatography) (SephadexLH-20; 80×3.0 cm; MeOH: H_2O, 1:1). The separated compounds were further purified on Sephadex CC (45 ×1.5 cm; MeOH-HPLC grade), yielding eleven pure compounds (purity 96-99%); **1** (57 mg), **2** (48 mg), **3** (9 mg), **4** (28 mg), **5** (11 mg), **6** (32 mg), **7** (25 mg), **8** (30 mg), **9** (16 mg), **10** (24 mg), and **11** (27 mg). The chemical structures were interpreted based on chromatographic assessment (*Rf* values, color reactions, and Co-PC with reference samples); chemical investigation (acid hydrolysis), and physical investigations (UV, NMR, and ESI–MS). Further confirmation was performed by comparing their spectral data with previously reported values. The spectral data of the isolated compounds, including the values, splitting, and coupling constants of NMR, are accessible as supplementary data (Tables S1 & S2). The investigated extract was further analysed using LC-ESI-MS/MS analysis to detect the compounds that could not be isolated due to their low abundance.

2.2.3. LC-ESI-MS/MS investigation of A. horridus

The hydromethanol extract of *A. horridus* (5 mg), the isolated compounds (1 mg, each), and two standard mixtures (5 mg, each) [40] including various phenolic acids as well as certain flavonoid groups were subjected to LC-ESI-MS/MS investigation using Exion LC AC system for separation through C18 Column (Ascentis® Express 90 Å: 2.1×150 mm, 2.7 µm) and SCIEX Triple Quad 5500+ MS/MS system outfitted with ESI for detection according to Marzouk et al. [24] with some modifications. For negative and positive ionization modes, two eluents, ammonium formate (NH_4HCO_2, 5 Mm, pH 8) and acetonitrile (CH_3CN, HPLC grade), were considered as the mobile phases A and B, respectively as the following gradient (A: B); (95:5) at 0-1 min, (95-0: 5-100) from1.01 to 20 min, (0:100) from 20.01 to 25 min, and (95: 5) from 25.01 to 30 min using. 5 µl injected volume was used with a flow rate (0.3 ml/min). A scan (EMS-IDA-EPI) was performed for MS/MS ionization modes from 100 to 1000 Da for MS1 and 50 to 1000 Da for MS2. The curtain gas (25 psi), ion source gas (45 psi), source temperature (500°C), and collision energy spread (15) were the common parameters for both ionization modes. Collision energy (-35 and 35), IonSpray voltage (-4500 and 5500), and declustering potential (-80 and 80) were achieved for (negative and positive) ionization modes, respectively.

2.3. Biological investigation of the hydromethanol extract of *A. horridus*

2.3.1. Cytotoxic effect of A. horridus on human cell lines

The cytotoxicity was considered by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay (MTT) against four human carcinoma cell lines (HepG2, MCF7, PACA2, and HCT116), according to Marzouk et al. [24]. Doxorubicin (100µg/ml) was used as a positive control. DMSO (the vehicle) was used to dissolve the extract; its final concentration in the cells was less than 0.2%. At a wavelength of 595 nm, the absorbance of samples was measured *via* a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA). The percentage of viability change was estimated as % of inhibition = (Reading of tested sample / Reading of negative control) -1 x 100.

2.3.2. In vitro hypoglycemic activity

2.3.2.1. α-Glucosidase inhibitory activity

The α-glucosidase of concentration 0.2 U/ml was obtained from *saccharomyces cerevisiae* (SIGMA G5003-100UN) and prepared in phosphate buffer saline (pH 6.8). The hydromethanol extract (10 µl) at varying concentrations (1.43 to 182 ppm) was separately mixed with 0.2 U/ml α-glucosidase enzyme (60 µl) and incubated in a 96-well plate (20 min, 37°C). 150 µl of the colorless *p*-NPG (1.25 mM p-nitrophenyl α-D-glucopyranoside) (Sigma N1377) was added and then incubated (20 min, 37°C). 2 g/L NaOH (50 µl) was added to terminate the reaction. At a wavelength of 405 nm, the α-glucosidase inhibition was measured by assessing the quantity of bright yellow *p*-NP released. The positive control is acarbose (Sigma-Aldrich PHR1253) while the negative control was a buffer solution (10 µl) in place of the test entity. For blank, buffer solution included *p*-NPG was used as an alternative to the enzyme [25].

2.3.2.2. α-Amylase inhibitory activity

The α-amylase enzyme of concentration 4 U/ml was obtained from the porcine pancreas (Sigma A3176) and prepared in phosphate buffer saline (pH 6.8). The hydromethanol extract (15 µl) at varying concentrations (2.2 to 285 ppm) was mixed separately with α-amylase enzyme (60 µl) and incubated in a 96-well plate (15 min, 37°C). 0.2% soluble starch solution (dissolved in buffer by heating in a microwave and then filtered) (60 µl) was added and then incubated (10 min, 37°C). 1M HCl (30 µl) was added to terminate the reaction, and then KI/I2 aqueous solution (150 µl) was added. At a wavelength of 595 nm, α-amylase activity was established by assessing the quantity of blue color released. The positive control was acarbose, while the negative control was a buffer solution (15 µl) in place of the test entity [26].

2.3.2.3. Statistical analysis

The results were statistically analyzed using the statistical analysis software package (SPSS 11 for Windows®, Version 11, 2001, SPSS Inc., Chicago, USA) to obtain the IC_{50} values.

2.4. Molecular docking studies of isolated compounds from *A. horridus*

2.4.1. The protein structure preparation

The required protein structures for the docking studies were downloaded from the PDB (Protein Data Bank, http:// www.rcsb.org.pdb). The 3-D structures (with 1.9 A^o resolution) of human pancreatic α-amylase in complex with acarbose ligand (PDB ID: 2QV4 file) and α-glucosidase (maltase) in complex with acarbose ligand (PDB ID: 2QMJ). The structure was prepared according to Ragab et al. [27].

2.4.2. Ligands preparation

The eleven compound structures were attained from the PubChem compound database (https://pubchem.ncbi.nlm.nih.gov). The compounds' Structure Data Files (SDF) and native standard files (*.pdb*) were prepared according to Ragab et al. [27].

2.4.3. Docking

After the target enzymes (α-amylase and α-glucosidase) and ligands (acarbose and isolated compounds) were prepared, docking was performed in AutoDock Vina according to Ragab et al. [27]. The grid parameters are provided in Table S3.

2.4.4. Authentication of Docking Protocol

The docking protocol's precision was validated by redocking the co-crystallized ligand back into the binding site of pancreatic α-amylase and α-glucosidase. The docking methodology was reliable as the re-docked pose overlapped almost totally with the experimental orientation. This point towards that AutoDock Vina re-docked the co-crystallized ligand, with very superior sincerity, reversed into the binding pocket of α-amylase and α-glucosidase.

2.5. ADME and Drug-Likeness Assessment

The SwissADME submission page (http://www.swissadme.ch) was accessed, where the ADME evaluation requires the canonical SMILES of the isolated phytoconstituents, retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The SwissADME server is configured to prioritize the rapid processing, robustness and clear result presentation. Furthermore, the isolated compounds were analyzed for drug-likeness using MolSoft software (MolSoft, 2007) [24].

3. Results and Discussion

3.1. Phytochemical Analysis of *A. horridus*

3.1.1. Compounds identification

The phytochemical analysis of the hydromethanol extract of *A. horridus* aerial parts led to the isolation of two phenolic acids and nine flavonoids (Fig.1). They were identified as gentisic acid (2, 5-dihydroxy benzoic acid) (**1**) [28], *trans*-ferulic acid (**2**) [29], quercetin (**3**) [25], isoquercetin (quercetin 3-*O*-glucoside) (**4**) [30], kaempferol (**5**) [31], astragalin (kaempferol 3-*O*-glucoside) (**6**) [31], *trans*-tiliroside (kaempferol 3-*O*-(6''-*p*-coumaroyl)-glucoside) (**7**) [32], nicotiflorin (kaempferol 3-*O*-rutinoside) (**8**) [31], isorhamnetin (**9**) [33], isorhamnetin 3-*O*-glucoside (**10**) [34], and brassicin (isorhamnetin 7-*O*-glucoside)(**11**) [34]. The isolated compounds, except for **2**, **3**, and **5**, were reported for the first time in *A. horridus*.

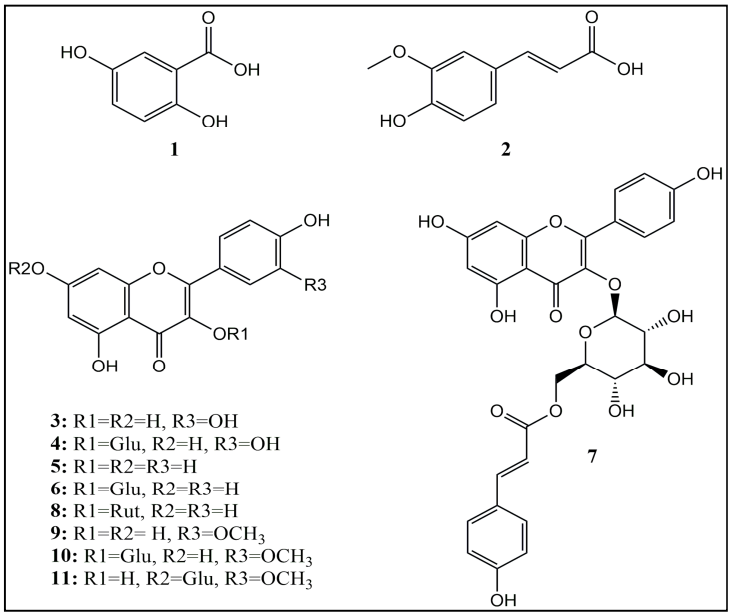

Figure 1: Chemical structures of the isolated compounds

3.1.2. LC-ESI-MS-MS analysis

The hydromethanol extract of *A. horridus* was executed by LC–ESI–MS in both negative and positive ionization modes to interpret the available phytochemicals (Fig. 2). Table (1) summarizes all the annotated compounds for *A. horridus*, including retention times, putative compounds, MS/MS fragments, and *m/z* negative and positive ions. Compounds were recognised by interpreting the mass spectra persevering by their MS1 and MS2, considering data afforded by the literature. The aglycones and the sugar moieties were confirmed guided by the complete acid hydrolysis of the hydromethanol extract [23].

A total of 104 compounds, belonging to different metabolite classes and their derivatives, were characterized in *A. horridus* extract. The chemical classes were assigned, including flavonoids, phenolic acids and derivatives, coumarins, steroids, terpenes, organic acids, amino acids and derivatives, fatty acids, glycolipids, and others. The present study showed an abundant accumulation of phenolic compounds and steroids.

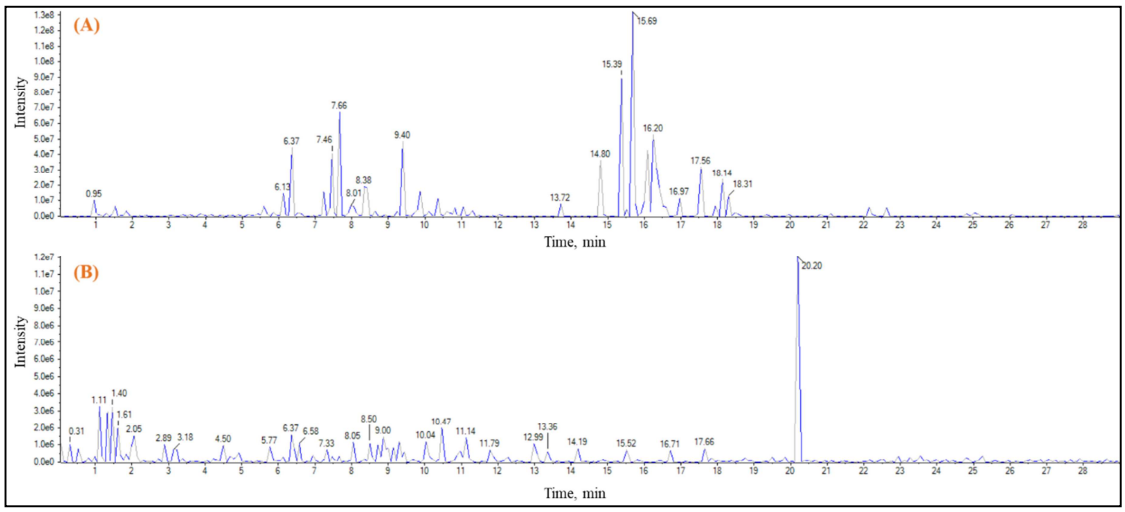

Figure 2: Base Peak Chromatograms of hydromethanol extract of *A. horridus* in A: negative ion mode, B: positive ion mode

Table 1: Tentatively identified metabolites in the hydromethanol extract of *A. horridus*

No.	Class	RT (min)	Compound name	$[M-H]^-$	$[M+H]^+$	MS/MS		Ref
						Neg	Pos	
1	Organic acid	0.927	Citric/isocitric acid	191.01		111		[7]
2	Saccharide	1.14	Disaccharide	341.11		179, 161, 143		[35]
3	Amino acid	1.43	Proline		116.05		97 70	[36]
4	Amino acid	1.46	Phenylalanine	164.03		147, 119		[35]
5	Organic acid	1.478	Tartaric acid	149.01		121, 105		[36]
6	Phenolic acid	1.487	Vanillin-*O*-hexoside	313.09		151, 135, 121, 107		[37]
7	Phenolic acid	1.53	*p*-Coumaric acid [a, b]	163.01		145, 135, 119		[38]
8	Stellbine	1.554	Resveratrol	227.09		183		[36]
9	Amino acid	1.570	Asparagine	131.11		114, 87		[39]
10	Organic acid	1.586	Chelidonic acid	183.06		155, 119		[40]
11	Organic acid	1.603	Citramalate	147.03		129, 119		[36]
12	Phenolic acid	1.66	Protocatechuic acid [a, b]	153.02	155.09	135, 122, 109	137, 129, 119, 114, 109	[36]
13	Phenolic acid	1.68	Coumaric acid isomer	163.01	165.02	145, 135, 119	137, 121	[38]
14	Amino acid	1.747	Valine	116.01	118.01	99	72	[41]
15	Phenolic acid	1.769	Gallic acid [a, b]	169.02	171.05	125, 123, 107	153, 111	[42]

16	Phenolic acid (Benzoic acid)	1.831	*p*-Hydroxybenzoic acid [a, b]	137.02	139.08	119, 109	111	[36]
17	Amino acid	1.92	Tryptophane	203.03	205.05	142, 130, 116	146, 118, 111, 100	[39]
18	Benzaldehyde	2.03	*p*-Hydroxy benzaldehyde [a]	121.01		109		[43]
19	Organic acid	2.431	Malic acid	133.05		115		[39]
20	Coumarin	2.63	Hydroxy methyl coumarin	175.03		160, 147, 132, 117		[36]
21	Benzaldehyde	2.808	Trimethoxy benzaldehyde [a]	195.05		167, 152, 149, 123		[24]
22	Phenolic acid	2.92	Methyl gallate [a]	183.01	185.05	152, 119	153, 139, 167, 125, 111	[44]
23	Phenolic acid	3.021	Vanillic acid [a, b]	167.04	169.02	121, 108	115	[43]
24	Phenolic acid	3.12	Gentisic acid [a, c]	153.01		135, 123, 109		[24]
25	Dipeptide	3.44	Glutamylglutamine		276.05		147, 131	[45]
26	Flavonoid	3.52	Lut 7-*O*-glucoside [a]	446.92		285, 284, 255, 227, 151		[36]
27	Phenolic alcohol	3.741	Coniferin	341.06	343.07	179, 161, 149, 131	181, 137	[46]
28	Phenolic acid	4.326	Syringaldehyde	181.02	183.01	151, 137, 123	167, 155, 121	[47]
29	Flavonoid	4.338	Hexamethoxy flavone	401.03		285, 269, 161, 143		[36]
30	Phenolic acid	4.842	Sinapic acid-*O*-glucoside	385.12		223, 203, 179, 153		[37]
31	Flavonoid	4.97	Trihydroxy flavone-*O*-rhamnoside	415.05		269, 161, 159		[48]
32	Coumarin	5.05	Hydroxy methoxy coumarin	191.01	193.07	176, 148, 120, 104	178, 133, 115	[36]
33	Phenolic acid	5.35	Caffeic acid [a, b]	179.03	181.01	135	137, 121, 109	[34]
34	Coumarin	5.58	Hydroxy coumarin	161.01	163.06	144, 133	148, 135, 122, 119, 107	[36]
35	Flavonoid	5.99	Trihydroxy monomethoxy flavone-*O*-hexoside	461.01		299, 179		[48]
36	Flavonoid	6.15	Hexamethoxy flavone isomer	401.03		285, 327,		[36]

						314, 255, 227, 151		
37	Phenolic acid	6.23	Methoxy-hydroxybenzoic acid	167.07		152, 124, 121		[43]
38	Flavonoid	6.32	Tetrahydroxy-trimethoxy flavone	375.18		359, 343, 329, 315, 175, 167		[49]
39	Flavonoid	6.47	Dihydroxy-hexamethoxy flavone-*O*-glucoside	595.02		433, 403, 373, 251		[48]
40	Flavonoid	6.47	Dihydroxy-hexamethoxy flavone	433.01		403, 373, 359, 343		[48]
41	Flavonoid	6.48	Trihydroxy trimethoxy flavone-*O*-glucoside	521.05		359, 329		[48]
42	Flavonoid	6.66	Lut 4'-*O*-glucoside [a]	446.98	449.01	285	287	[23]
43	Flavonoid	6.67	Km 3-*O*-rutinoside [a, c]	592.92		285		[39]
44	Flavonoid	6.74	Trihydroxy trimethoxy flavone-*O*-glucoside isomer	521.05		359, 329		[48]
45	Phenolic acid	6.83	Coumaroyl quinic acid	337.02		191, 175, 161, 134		[50]
46	Phenolic acid	6.91	Ferulic acid [a,b, c]	193.08	195.07	149, 134, 133	165, 163, 149, 134, 117	[51]
47	Coumarin	6.92	Coumarin		147.01		129, 117, 119, 106	[51]
48	Coumarin	6.98	Coumarin-*O*-hexoside		309.01		147, 119	[52]
49	Flavonoid	6.99	Isn 3-*O*-glucoside [a, c]	476.95	479.01	315, 314, 299, 271	317	[53]
50	Flavonoid	6.97	Qn 3-*O*-glucoside (Isoquercitin) [a,c]		465.01		303, 257, 229	[54]
51	Coumarin	7.06	Dihydroxy coumarin-*O*-hexoside	339.02		177, 145, 117		[36]
52	Flavonoid	7.07	Km [a,b, c]	285.01	287.02	257, 229, 185, 145	269, 241, 153, 121	[55]
53	Flavonoid	7.11	Km-3-*O*-(6''-*p*-coumaroyl)-glucoside [c]	593.04		547, 447, 285, 284, 163, 145		[39]
54	Phenolic acid	7.16	Hydroxyphenyl ethyl ferulate	313.01		295, 285, 193, 175, 161,		[56]

						137		
55	Flavonoid	7.19	Qn [a,b,c]	300.97	302.99	285, 257, 229, 153		[38]
56	Flavonoid	7.21	Qn-*O*-glucoside isomer		465.01	303, 302, 285, 257		[50]
57	Flavonoid	7.22	Km 7-*O*-feruloyl glucoside	622.98		447, 285, 193, 175, 161		[57]
58	Flavonoid	7.27	Isn 7-*O*-glucoside [c]	476.92	479.01	315, 314, 299, 151	317, 303, 302	[58]
59	Flavonoid	7.31	Ap [a]	269.01		227, 151, 117		[34]
60	Flavonoid	7.33	Km 3-*O*-glucoside [a,c]	446.95	449.01	285, 284, 257, 151	287, 153	[59]
61	Fatty acid	7.39	Trihydroxy-octadecadienoic acid	327.21		291, 229, 211, 183		[59]
62	Flavonoid	7.41	Dihydroxy-trimethoxy flavone		331.05		299, 287, 271, 251	[60]
63	Flavonoid	7.54	Km 7-*O*-glucoside	447.01	449.01	285, 284, 257, 151	287	[39]
64	Phenolic acid	7.72	Hydroxy ferulic acid-*O*-hexosyl pentoside	503.04	505.08	485, 371, 209, 161, 111	211, 343, 211, 193, 175, 135, 119	[60]
65	Phenolic acid	7.8	Hydroxy ferulic acid-*O*-hexoside	371.18		209, 175, 161, 113		[48]
66	Flavonoid	7.96	Monomethoxy-tetrahydroxy flavone		317.09		303, 302, 273, 257	[61]
67	Phenolic acid	8.01	Hydroxy ferulic acid		211.15		193, 151, 135, 109	[61]
68	Flavonoid	8.13	Eriodictyol [a]	287.14		269, 241, 155		[62]
69	Flavonoid	8.14	Liquiritigenin [a]	255.03		135, 227, 119		[54]
70	Flavonoid	8.51	Naringenin-*O*-glucoside	433.01	435.06	271, 227, 151	273, 227	[24]
71	Flavonoid	8.81	Rutin [a,b]		611.08		465, 303	[16]

72	Flavonoid	8.84	Catechin/epicatechin [a]	289.03		261, 245, 163, 145, 119		[42, 54]
73	Flavonoid	8.89	Isn [a,c]	315.1	317.02		303, 302, 301, 194, 177, 135	[42]
74	Phenolic acid amide	8.93	N-Feruloyltyramine		314.05		177, 149, 145, 121	[63]
75	Flavonoid	8.51	Naringenin-*O*-glucuronide	447.01	449.03	271, 227, 151	273	[24]
76	Steroid	8.95	Spirostenol-*O*-glucopyranoside		577.18		415	[10]
77	Steroid	9.01	Spirostanol-*O*-triglucoside		741.13		579, 417	[10]
78	Flavonoid	9.08	Km 3,7-di-*O*-glucoside	609.02		447, 285		[23]
79	Phenolic acid	9.11	Ferulic acid-*O*-hexoside	355.08	357.03	193, 175, 161, 135	177, 137	[1]
80	Flavonoid	9.36	Ap 6,8-di-*C*-glucoside [a]	593.01	595.12	473, 311	475, 313	[12]
81	Steroid	9.45	Asparagoside A= Spirostanol-*O*-glucoside		579.08		417	[10]
82	Steroid	9.45	Spirostanol-*O*-arabinosyl-glucoside		711.14		579, 417, 273, 255	[10]
83	Steroid	9.43	Spirostanol-*O*-arabinosyl-glucosyl-glucoside		873.07		711, 579, 417, 273, 255	[10]
84	Flavonoid	9.46	Tetrahydroxy-trimethoxy flavone isomer	375.18		343, 329, 293, 285		[49]
85	Flavonoid	9.67	Monomethoxy-tetrahydroxy flavone isomer		317.09		302, 193, 177, 167, 145	[36]
86	Steroid	10.13	Sarsasapogenin		417.05		399, 273, 255	[64]
87	Flavonoid	10.18	Qn 3-*O*-glucosyl rutinoside	770.98	773.02	625, 609, 301	627, 303	[16]
88	Flavonoid	10.43	Isn 3-*O*-rhamnosyl rutinoside	769.01	771.03	623, 315	625, 479, 317	[16]
89	Phenolic acid	10.64	Caffeoyl quinic acid [a]	353.11		191, 135		[60]
90	Steroid	10.13	Sarsasapogenin-*O*-glucuronide		593.05		575, 417, 273, 255	[64]
91	Phenolic acid	11.06	Coumaric acid isomer	163.01		145, 135, 119		[38]

92	Fatty acid	11.48	Hydroxy-octadecatrienoic acid	293.07	275, 249, 183	[37]
93	Flavonoid	11.99	Isn 3-*O*-glucoside-7-*O*-arabinoside	609.07	477 447 315	[24]
94	Fatty acid	13.17	Hydroxy-octadecadienoic acid	295.18	277, 183, 171	[39]
95	Terpenoid	13.36	Corosolic acid	471.16	427, 248	[65]
96	Flavonoid	13.57	Dimethoxy apigenin	297.11	269, 251, 227, 119	[66]
97	Fatty acid	14.412	Hydroperoxy-octadecadienoic acid	311.18	197, 183	[39]
98	Fatty acid	14.421	Hydroxy-octadecenoic acid	297.13	279, 171, 155	[23]
99	Flavonoid	14.8	Naringenin [a,b]	271.14	253, 227, 151	[54]
100	Fatty acid	15.26	Hydroxy-octadecanoic acid	299.12	281, 253, 169	[23]
101	Terpenoid	16.33	Glycyrrhetinic acid	469.18	425, 451, 439	[67]
102	Lipid	16.6	Linoleoyl-di-glucosyl glycerol	675.05	397, 415, 277	[39]
103	Fatty acid	17.46	Hydroxyoctadecanoic acid isomer	299.12	253	[23]
104	Terpenoid	21.39	Asiatic acid	487.07	472, 459	[67]

a; standard sample, b; compounds reported before in *A. horridus*, c; isolated compounds, Ap; apigenin, Isn; isorhamnetin, Km; kaempferol, Lut; luteolin, Qn; quercetin.

Flavonoids. In the current study, various flavonoid aglycone classes were counted in positive and/or negative modes aided with reference standards and/or fulfilled by the accurate literature data with the prevalence of polymethylated flavonoid (PMF) aglycone structures that are reported for the first time in *A. horridus* and other *Asparagus* species.

In addition to the flavonol aglycones; kaempferol (**52**, *m/z* 285.01 $[M-H]^-$ & 287.02 $[M+H]^+$), quercetin (**55**, *m/z* 300.97 $[M-H]^-$ & 302.989 $[M+H]^+$), and isorhamnetin (**73**, *m/z* 315.1$[M-H]^-$ & 317.02 $[M+H]^+$), flavone aglycones; apigenin (**59**, *m/z* 269.01 $[M-H]^-$) and dimethoxy apigenin (**96**, *m/z* 297.11 $[M-H]^-$), flavanone aglycones; eriodictyol (**68**, *m/z* 287.14 $[M-H]^-$), liquiritigenin (**69**, *m/z* 255.03 $[M-H]^-$), and naringenin (**99**, *m/z* 271.14 $[M-H]^-$), as well as dihydroflavonol aglycones; catechin (**72**, *m/z* 289.03 $[M-H]^-$) that were previously reported in other *Asparagus* species [54, 55, 65], eight PMF aglycones were tentatively identified with variable hydroxylation and methylation substitutions, two hexa-methoxy flavone isomers (**29** and **36**, *m/z* 401.03 $[M-H]^-$), two tetrahydroxy trimethoxy flavone isomers (**38** & **84** *m/z* 375.18 $[M-H]^-$), dihydroxy trimethoxy flavone (**62**, *m/z* 331.05 $[M+H]^+$), dihydroxy hexamethoxy flavone (**40** *m/z* 433.01 $[M-H]^-$), and monomethoxy tetrahydroxy flavone isomers (**66** & **85**, *m/z* 317.09 $[M+H]^+$). The PMF aglycones have the most common fragments that indicate loss of water molecules $(-18\ Da)_n$ and one or more CH_2 molecules $(-14\ Da)_n$. Prior chemical research showed that *Asparagus* species contain various flavonol-*O*-glycoside structures as kaempferol 3-*O*-rutinoside (**43**, *m/z* 592.92 $[M-H]^-$), isorhamnetin 3-*O*-glucoside (**49**, *m/z* 476.95 $[M-H]^-$ & 479.01 $[M+H]^+$), and quercetin 3-*O*-glucoside (**50**, *m/z* 465.01 $[M+H]^+$) [51, 54]. Also, quercetin 3-*O*-rutinoside (rutin) (**71**, *m/z* 611.08 $[M+H]^-$), quercetin 3-*O*-glucosyl rutinoside (**87**, *m/z* 770.98 $[M-H]^-$ & 773.02 $[M+H]^+$) and isorhamnetin 3-*O*-rhamnosyl rutinoside (**88**, *m/z* 769.01 $[M-H]^-$ & 771.03 $[M+H]^+$) were reported before in *A. horridus*. [16] In addition to these flavonol glycosides, other flavonol glycosides were genus-first dereplicated compounds and annotated as kaempferide 3-*O*-glucoside (**35**, *m/z* 461.01 $[M-H]^-$), kaempferol-3-*O*-coumaroyl glucoside (**53**, *m/z* 593.04 $[M-H]^-$), quercetin-*O*-glucoside isomer (**56**, *m/z* 465.01 $[M+H]^+$), kaempferol-7-*O*-feruloyl glucoside (**57**, *m/z* 622.98 $[M-H]^-$), kaempferol 3-*O*-glucoside (**60**, *m/z* 446.95 $[M-H]^-$ and 449.01 $[M+H]^+$), kaempferol 7-*O*-glucoside (**63**, *m/z* 447.01 $[M-H]^-$ and 449.01 $[M+H]^+$), isorhamnetin 7-*O*-glucoside (**58**, *m/z* 476.92 $[M-H]^-$ and 479.01 $[M+H]^+$), kaempferol 3,7-di-*O*-glucoside (**78**, *m/z* 609.02 $[M-H]^-$) and isorhamnetin 3-*O*-glucoside-7-*O*-arabinoside (**93**, *m/z* 609.07 $[M-H]^-$). Herein, the flavone-type structures were reported for the first time in *Asparagus* species as *O*-glycoside derivatives, meanwhile, various previous studies described the flavone-type forms as aglycones (apigenin, luteolin, and 5,7 dimethoxy apigenin). [51-52, 65], In this context, apigenin 7-*O*-rhamnoside (**31**, *m/z* 415.05 $[M-H]^-$), luteolin 7-*O*-glucoside (**26**, *m/z* 446.92 $[M-H]^-$) and luteolin 4'-*O*-glucoside (**42**, *m/z* 446.98 $[M-H]^-$ & 449.01 $[M+H]^+$) were identified. PMF glycosides were also annotated based on the loss of 162 Da (glucose unit) and the successive loss of 14 Da (CH_2). They were

tentatively identified as *O*-glucoside derivatives of dihydroxy hexamethoxy flavone (**39**, *m/z* 595.02 $[M-H]^-$) and trihydroxy trimethoxy flavone (**41** & **44**, *m/z* 521.05 $[M-H]^-$). Further, one flavone *C*-glycosides was detected and identified as apigenin 6,8-di-*C*-glucoside (**80**; *m/z* 593.01 $[M-H]^-$ & 595.12 $[M+H]^+$) based on a fragmentation pattern and a reference sample [54]. This compound was reported before from *Asparagus africanus* Lam [12]. Finally, compounds **70** and **75** are flavanone O-glycoside structures. They gave rise to *m/z* 433.01/435.06 and 447.01/449.03 $[M\pm H]^{\pm}$ and were annotated as *O*-glucoside and *O*-glucuronide derivatives of naringenin, respectively. Both compounds shared characteristic fragments at *m/z* 273, 227, and 151 [24], after the loss of dehydrated molecules of glucose and glucuronide (Table 1).

Phenolic acids and derivatives. Several studies showed that several flavonoids and hydroxycinnamic acids are present in green *Asparagus* [54, 68,69]. The derivatives of benzoic and cinnamic acids were among the two separate groupings of phenolic acids that were found. They were extensively dispersed among several family members of *Asparagaceae*. Herein, eleven different phenolics in the form of hydroxybenzoic acid derivatives were found to occur in *Asparagus* extract. The fragment ions at *m/z* 109, *m/z* 119, and *m/z* 151 corresponded to the loss of the carboxylic group (-44 Da), H_2O (-18 Da), and glycosylated additives (-162 Da), respectively. Five methylated benzoic acid derivatives were found **6**, **22**, **23**, **28,** and **37**, they were proposed as vanillin-*O*-hexoside (*m/z* 313.09 $[M-H]^-$), methyl gallate (*m/z* 183.01 $[M-H]^-$ and 185.05 $[M+H]^+$), vanillic acid (*m/z* 167.04 $[M-H]^-$ and 169.02 $[M+H]^+$), syringaldehyde *m/z* 181.02 $[M-H]^-$ and 183.01 $[M+H]^+$) and methoxy-hydroxybenzoic acid (*m/z* 167.07 $[M-H]^-$) respectively; in addition to two benzaldehyde derivatives annotated as *p*-Hydroxy benzaldehyde (**18,** *m/z* 121.01 $[M-H]^-$) and trimethoxy benzaldehyde (**21,** *m/z* 195.05 $[M-H]^-$). The MS signals at *m/z* 153.02 $[M-H]^-$ and 155.09 $[M+H]^+$, *m/z* 153.01 $[M-H]^-$, *m/z* 169.02 $[M-H]^-$ and 171.05 $[M+H]^+$, and *m/z* 137.02 $[M-H]^-$ and 139.08 $[M+H]^+$ were identified as protocatechuic acid (**12**), gentisic acid (**24**), gallic acid (**15**), and *p*-hydroxybenzoic acid (**16**) respectively by associating Rt and MS data with those of authentic samples.

In addition to the reported hydroxybenzoic acid derivatives, eleven compounds characterized as hydroxycinnamic acid derivatives were eluted. Peaks (**7**, **13**, **91**) at *m/z* 163.01 were annotated as three isomers of coumaric acid, based on the product ions at *m/z* 145 ($[M-H-H_2O]^-$) and *m/z* 119 ($[M-H-CO_2]^-$) corresponding to the loss of CO_2 molecule (44 Da). Caffeic acid was also annotated as peak (**33**, *m/z* 179.03 $[M-H]^-$ and 181.01 $[M+H]^+$). Further, hydroxycinnamic acids-*O*-hexoside were annotated as sinapic acid *O*-glucoside (**30**, *m/z* 385.12 $[M-H]^-$), hydroxy ferulic acid *O*-hexosyl pentoside (**64**, *m/z* 503.04 $[M-H]^-$ and 505.08 $[M+H]^+$), hydroxy ferulic acid *O*-hexoside (**65**, *m/z* 371.18 $[M-H]^-$), and ferulic acid *O*-hexoside (**79**, *m/z* 355.08 $[M-H]^-$ and 357.03 $[M+H]^+$) all exhibited fragment ions $[M-H\text{-hexosyl (-Da 162)}]^-$. The lateral compound was reported before in *A. stipularis*. Two chlorogenic acid derivatives were assigned through fragment ion *m/z* 191 $[\text{quinic acid-H}]^-$ representative coumaroyl quinic acid (**45**, *m/z* 337.02) and caffeoyl quinic acid (**89**, *m/z* 353.11). Ferulic acid (**46**, *m/z* 193.08 $[M-H]^-$ and 195.07 $[M+H]^+$) and its two derivatives were identified hydroxyphenyl ethyl ferulate (**54**, *m/z* 313.01 $[M-H]^-$), hydroxy ferulic acid (**67**, *m/z* 211.15 $[M+H]^+$), and N-feruloyltyamine (**74**, *m/z* 314.05 $[M+H]^+$), confirmed by their common fragment ions at *m/z* 193 $[\text{ferulic acid-H}]^-$). Another phenolic derivative is a monoglycoside of phenylpropanoid structure and annotated as coniferin (**27***, m/z* 341.06 $[M-H]^-$ and 343.07 $[M+H]^+$).

Coumarins. The coumarins are found naturally in various plants as a defence mechanism against herbivores. It has been reported before the occurrence of coumarins in some *Asparagus* species [70]. In our study, six coumarins were characterized including hydroxy methyl coumarin (**20**, *m/z* 175.03 $[M-H]^-$), hydroxy methoxy coumarin (**32**, *m/z* 191.01 $[M-H]^-$ & 193.07 $[M+H]^+$), hydroxy coumarin (**34**, *m/z* 161.01 $[M-H]^-$ & 163.06 $[M+H]^+$), coumarin (**47**, *m/z* 147.01 $[M+H]^+$), coumarin-*O*-hexoside (**48**, *m/z* 309.01 $[M+H]^+$) and dihydroxy coumarin-*O*-hexoside (**51**, *m/z* 339.02 $[M-H]^-$) (Table 1).

Steroids. Seven steroids were annotated and characterized for the positive ion mode. All were reported before in other *Asparagus* species [10]. Compound **86** revealed a protonated molecule at *m/z* 417.05 $[M+H]^+$, conforming to the $[\text{sarsasapogenin+H}]^+$ ion. In addition to the dehydrated fragment ion at *m/z* 399 $[\text{sarsasapogenin-H}_2\text{O+H}]^+$, two characteristic fragments at *m/z* 273 and 255 were also observed. Compound **90** (*m/z* 593.05 $[M+H]^+$) is observed with a mass difference (176 Da) and characterized with the same fragment ions, suggesting that it was the glucuronide derivative of sarsasapogenin. Therefore, 86 and 90 could be annotated as sarsasapogenin and sarsasapogenin-*O*-glucuronide, respectively. Similarly, four spirostanol glycosides were detected at *m/z* 741.13, 579.08, 711.14, and 873.07 $[M+H]^+$ and annotated as *O*-triglucoside (**77**), *O*-glucoside (**81**), *O*-arabinosyl-glucoside (**82**), and *O*-arabinosyl-diglucoside (**83**) derivatives. Finally, spirostenol *O*-glucopyranoside (**76**, *m/z* 577.18 $[M+H]^+$) was also detected.

Triterpenes. Other detected metabolites are triterpenes, including corosolic acid (**95**, *m/z* 471.16 $[M-H]^-$), glycyrrhetinic acid (**101,** *m/z* 469.18 $[M-H]^-$), and asiatic acid (**104**, *m/z* 487.07 $[M-H]^-$), which show the specific fragment *m/z* 427, 425, and 443, respectively, referring to the loss of the carboxylic group (- 44 Da).

Organic acids, amino acids, and derivatives. Five organic acids were detected in the negative mode and annotated as citric/isocitric acid (**1**, *m/z* 191.01 $[M-H]^-$), tartaric acid (**5**, *m/z* 149.01 $[M-H]^-$), chelidonic acid (**10**, *m/z* 183.06 $[M-H]^-$), citramalate (**11**, *m/z* 147.03 $[M-H]^-$), and malic acid (**19**, *m/z* 133.05 $[M-H]^-$). On the other hand, the annotated amino acids and their derivatives ascend from either losing H_2O (-18 Da) or H_2O + CO (-46 Da), yielding their residue mass and immonium ions, respectively. The annotated amino acids were counted as proline (**3**, *m/z* 116.05 $[M+H]^+$), phenylalanine (**4**, *m/z* 164.03 $[M-H]^-$), asparagine (**9**, *m/z* 131.11 $[M-H]^-$), valine (**14**, *m/z* 116.01 $[M-H]^-$ & 118.01 $[M+H]^+$) and tryptophane (**17**, *m/z* 203.03 $[M-H]^-$ and 205.05 $[M+H]^+$). Besides, the compound (**25**, *m/z* 276.05 $[M+H]^+$) was annotated as glutamyl glutamine (Table 1).

Fatty acids and glycolipids. Six fatty acids and one glycolipid were detected in the negative ionization mode. For compounds (**61** & **94**), the product ions at *m/z* 183, 171, and 211 refer to the hydroxyl groups of fatty acids and were identified as trihydroxy-octadecadienoic acid (*m/z* 327.21 $[M-H]^-$) and hydroxy-octadecadienoic acid (*m/z* 295.18 $[M-H]^-$), respectively. Other annotated fatty acids include hydroxy-octadecatrienoic acid (**92**, *m/z* 293.07), hydroperoxy-octadecadienoic acid (**97**, *m/z* 311.18), hydroxy-octadecenoic acid (**98**, *m/z* 297.13 $[M-H]^-$), and the two hydroxy-octadecanoic acid isomers (**100** & **103**, *m/z* 299.12 $[M-H]^-$). Meanwhile, the only annotated glycolipid was linoleoyl-di-glucosyl glycerol (**102**, *m/z* 675.05 $[M-H]^-$).

Others. One disaccharide was assigned (**2**, *m/z* 341.11 $[M-H]^-$) and confirmed by fragment ions *m/z* 179, *m/z* 161 due to loss of hexoside unit and water. Finally, one stellbine compound was identified as resveratrol (**8**, *m/z* 227.09 $[M-H]^-$) and was appointed through a product ion at *m/z* 183 $[M-H-CO_2]^-$.

3.2. Biological investigation

3.2.1. Cytotoxic effect of A. horridus on human cancer cell lines

It has been previously reported that *Asparagus* species can be utilized as a natural anticancer agent against several types of cancer [63, 71-73]. Furthermore, it has been reported that methanol extract of *A. horridus* could be used as an anticancer drug to treat liver (HepG2) and thyroid (B-CPAP) human cancer cell lines [18]. The current study screened the hydromethanol extract of *A. horridus* on four human cancer cell lines. The extract showed moderate inhibition for HepG2 (48.3%) and HCT116 (45.6%) at 100 ppm, compared to doxorubicin (100 %) at 100 µg/ml. No activities were observed for MCF7 and PACA2. In previous studies, isorhamnetin and its glycosides have been proven to possess *in vitro* anticancer effects against HePG2 and HCT116 cell lines [74].

3.2.2. α- Glucosidase and α- Amylase inhibitory activities

The α-glucosidase breaks the disaccharide moieties into simple sugars, easily accessible for absorption in the human gut besides α-amylase catalyzes the hydrolysis of the glucoside linkages of starch, and glycogen into smaller pieces with two or three glucose units, so inhibiting their potential in the digestive system is believed to be operative in controlling diabetes. Various reports have suggested that phytochemicals such as flavonoids and steroids derived from different extracts of several *Asparagus* species are stated to modulate diabetes through α-amylase and α-glucosidase inhibition [5]. In the current study, the extract recorded the highest α-glucosidase and α-amylase inhibitory potential at concentrations of 182 and 285 ppm with % inhibition of 62.02±0.06 and 87.3±4.6, respectively, compared to acarbose by 100% inhibition activity (Fig. 3. Table S4). Moreover, the extract revealed the IC_{50} (ppm) values of 90.24 on α-glucosidase and 135.74 on α-amylase, compared to a reference standard (acarbose) by 21.89 and 67.01, respectively (Table S4).

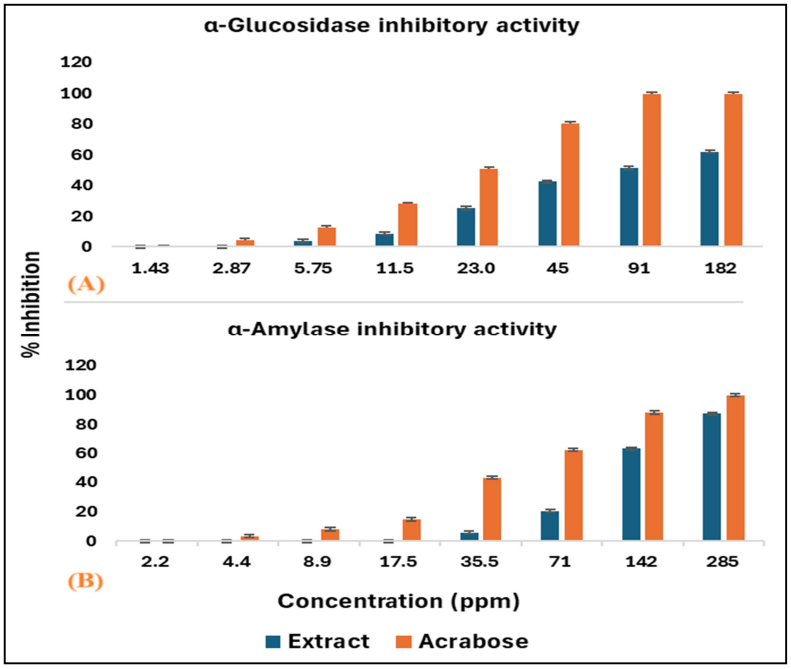

Figure 3: *In vitro* α-glucosidase(A) and α-amylase (B) inhibitory activities of hydromethanol extract of *A. horridus* (Blue) compared to a reference standard (Orange)

3.3. *Docking Study*

The docking results of α-glucosidase show that nicotiflorin (**8**) and tiliroside (**7**) are the leading compounds with the highest binding energy (Kcal/mol) of -8.4 and -8.0. At the same time, the standard acarbose had −7.7 Kcal/mol (Table 2). The stabilization of (**8**) and α-glucosidase is performed through six hydrogen bonds and seven π- interactions. In contrast, compound (**7**) displayed seven hydrogen bond interactions and four π-bonds as well as two unfavourable donar with enzyme (Fig. 4). On the other hand, acarbose had ten hydrogen bonds (Fig. 4). The amino residue of α-glucosidase that is shared to acarbose, nicotiflorin, and tiliroside in providing hydrogen bonds is Asp 542. Herein, this suggests that the binding site of the isolated phytochemicals is around the active site of this enzyme [75-77]. It is familiar that a viable inhibitor will exclude the substrate from entering the active site of α-glucosidase. Unlike the binding of acarbose with the protein residue, which depends mostly on hydrogen bonds, these docking results propose that the driving binding force of the flavonoid derivatives is a mixture of hydrogen bonds and π-bonds. Binding energy depends not solely on the number of hydrogen bonds it establishes, but also on other kinds of interactions [78].

In α-amylase docking analysis, quercetin (**3**) and brassicin (**11**) presented good binding statistics, including the least binding energy with physical contacts in terms of hydrogen-bonded interactions (Table 2). They showed 4 and 8 hydrogen-bonded interactions, respectively, whereas the standard ligand (acarbose) displayed more than 12. Acarbose and brassicin (Fig. 5) had similar interaction patterns as both formed hydrogen-bonded interactions with Asn 105 and His 305 residues of α-amylase. Quercetin (Fig. 5) modelled hydrogen bonds with His 299 and His 305 residues like the native ligand. This is an indication of a promising inhibitor activity of **3** and **11**.

Table 2: The energy of interactions between compounds and enzymes (α-glucosidase and α-amylase)

No.	Compounds	PubChem CID	α-Glucosidase affinity (kcal/mol)	α-amylase affinity (kcal/mol)
1	Gentisic acid	3469	-6.1	-5.7
2	Ferulic acid	445858	-5.9	-6.5
3	Quercetin	5280343	-7.5	-9.1
4	Isoquercetin	25203368	-7.6	-8.3
5	Kaempferol	5280863	-7.4	-8.8
6	Astragalin	25203515	-7.6	-8.4
7	Tiliroside	5320686	-8.0	-8.8
8	Nicotiflorin	122173234	-8.4	-8.2
9	Isorhamnetin	5281654	-7.2	-8.8
10	Isorhamnetin 3-*O*-glucoside	44258009	-7.5	-8.5
11	Brassicin	6455477	-7.6	-9.2
Standard	Acarbose	-	-7.7	-9.6

Unlike prior studies [79-81], residues such as human pancreatic α-amylase in complex with acarbose ligand (PDB ID: 2QV4 file) and α-glucosidase (maltase) in complex with acarbose ligand (PDB ID: 2QMJ), have not been targeted for docking in earlier studies with most of the isolated compounds included in the present research. This allowed us to reveal unique binding interactions that provide the potential mechanism of action. Docking results clearly showed the significant inhibition of phenolic constituents on *α*-glucosidase and α-amylase. Previous studies confirmed the potential of nicotiflorin (**8**) as an α-glucosidase inhibitor by retaining a large number of hydrogen bonds with the targeted *α*-glucosidase enzyme [79]. Furthermore, tiliroside (**7**) showed a potent *α*-glucosidase inhibition activity with an IC_{50} (μM) value of 2128 ± 63 , which is comparable to standard acarbose (6561 ± 207) [80]. Moreover, Zhang et al. [81] reported a high binding score of (**7**) with α-glucosidase and pancreatic lipase enzymes. These reports agree with the current study, where tiliroside (**8**) and nicotiflorin (**7**) showed remarkable binding energy directed at the *α*-glucosidase enzyme. Additionally, isorhamnetin 3-*O*-glycosides exert a suppressive effect on multiple enzymes associated with diabetes management. In the small intestine, isorhamnetin glycosides restrain the activity of α-amylase and α-glucosidase, therefore, the process of dietary saccharides conversion into an easily absorbed monosaccharide is hindered [74]. Quercetin has drawn considerable attention as a potent therapy in diabetes control [82]. Previous findings demonstrated that quercetin has a strong inhibitory effect on α-amylase enzyme (IC_{50} = 0.008 mg/mL), noticeably less than that of a standard reference acarbose (IC_{50} = 0.213 mg/mL). Molecular docking showed that hydrophobic interactions and hydrogen bonds primarily drove the strong complex between α-amylase and quercetin [83]. The current results offer the first investigation into the molecular docking of brassicin (**11**), highlighting its uniqueness and potential inhibition of α-amylase.

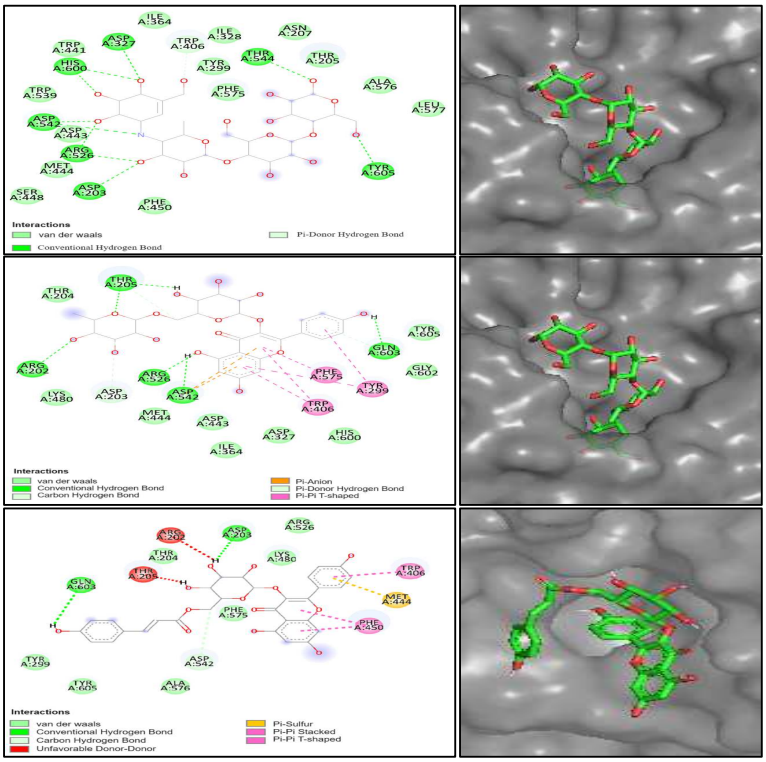

Figure 4: Interactions with the Residues in the Binding site of alpha-glucosidase in 2D and surface view; A: acarbose, B; nicotiflorin (8), C; *trans*-tiliroside (7)

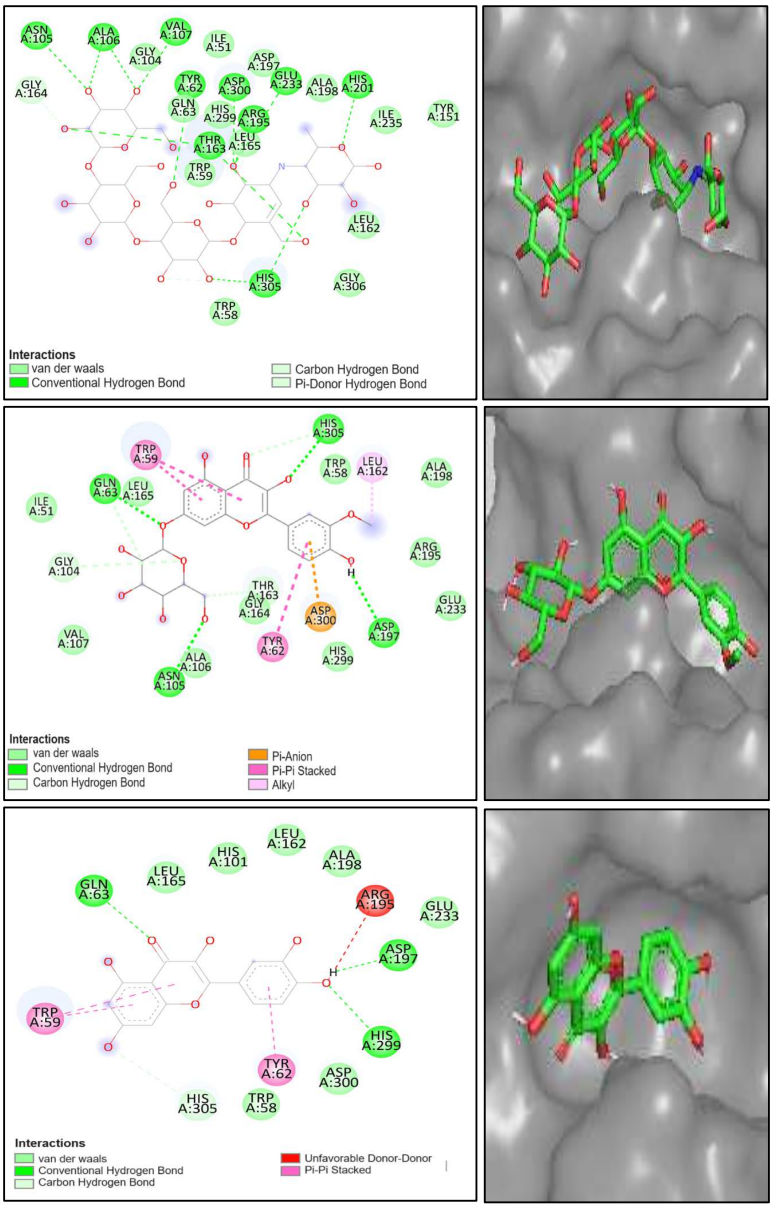

Figure 5: Interactions with the Residues in the Binding site of alpha-amylase in 2D and surface view; A: acarbose, B; isorhamnetin 7-*O*-glucoside (11), C; quercetin (3)

3.4. Assessment of ADMA/T and Drug-Like Properties

A comprehensive analysis of the pharmacokinetic characteristics of the isolated metabolites was conducted. Lipinski established the Rule of Five (RO5) as a guideline to identify potential drug candidates or drug-like compounds [24].

According to the findings presented in Table S5, five of the examined compounds comply with Lipinski's rule, namely, gentisic acid (**1**), trans-ferulic acid (**2**), quercetin (**3**), kaempferol (**5**), and isorhamnetin (**9**). However, other phytoconstituents violated the Lipinski rule by exceeding the limited weights of 500 g mol^{-1}, the combined total of hydrogen bond donors of 5 or/and the number of hydrogen bond acceptors of 10. All isolated compounds fall within the acceptable lipophilicity range of Lipinski (Log P greater than 5) [24]. Additionally, the Ghose filter is a knowledge-driven filter that aims to provide a detailed quantitative and qualitative overview of drug-like chemical space that can facilitate drug discovery. The Ghose filter is derived as follows: 160 < MW < 480, –0.4 < logP < 5.6, 160 < MW < 480, 20 < number of atoms < 70, and 40 < molar refractivity < 130 [84]. Most isolated compounds follow the Ghose filter except for gentisic acid (**1**), *trans*-tiliroside (**2**), and nicotiflorin (**8**). Moreover, the bioavailability radar is a visual overview that provides a glimpse at the potential of the isolated molecules; the pink area within the hexagon marks the optimal pharmacokinetic characteristics for oral bioavailability. Each vertex of the hexagon represents a crucial parameter for oral bioavailability (lipophilicity, size, polarity, solubility, saturation, and flexibility). Most of the *A. horridus* isolates fall within five criteria of the pink area and qualify as drug-like (Fig. S1) [24]. Finally, except for compound **2**, which showed a negative drug-likeness score (-0.61), all isolated compounds represent positive values of drug-likeness scores ranging from 0.3 to 0.9 (Fig. S3). These present results are consistent with our

previous ADME and drug-likeness studies of compounds (**3**-**6**) [40]. As well, other previous findings have analysed the pharmacokinetics and drug-like properties of some isolated constituents (**1**, **2**, and **7**-**10**) [85-89]. Ultimately, this study provides *in silico* profiling of *A. horridus* alongside the inaugural ADME and drug-likeness analysis of brassicin (**11**) (Fig. 6), highlighting its novelty and medicinal potential.

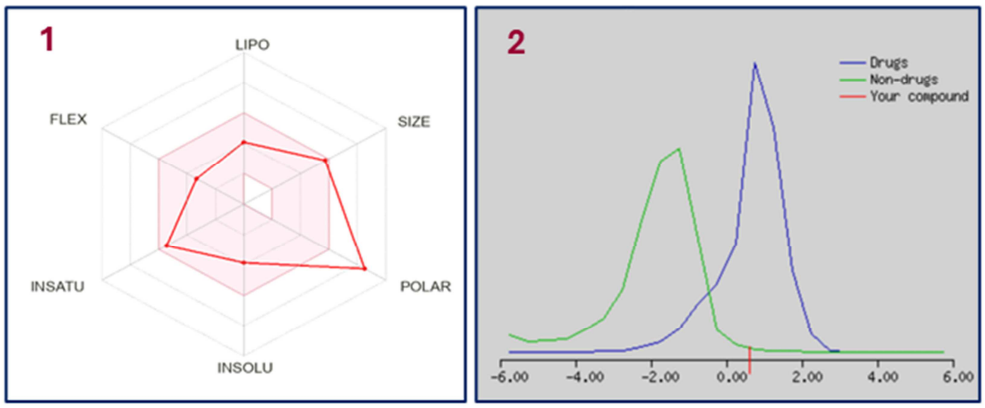

Figure 6: Bioavailability radar using SwissADME (1) and Drug-likeness plot using MolSoft (2) of brassicin

4. Conclusion

This study presents a comprehensive chemical characterization of the hydromethanol extract of *A. horridus* aerial parts, identifying a total of 104 metabolites, including eleven isolated phenolic compounds. Notably, it provides a pioneering *in silico* analysis of the isolated compounds, marking the first exploration of molecular docking, ADME, and drug-likeness properties of brassicin. The results highlight the significant medicinal potential of trans-tiliroside, nicotiflorin, and brassicin, which emerge as promising candidates for further research. These findings lay the groundwork for future investigations, particularly in vivo studies, aimed at elucidating the mechanisms of action of these compounds and their potential applications in developing pharmaceuticals and nutraceutical supplements for diabetes management.

Credit authorship contribution

M.O.S. and M.M.F.; conceptualization, resources, methodology, investigation, data analysis, writing original draft, writing review and editing; N.A.R. and M.M.M.; conceptualization, resources, methodology, visualization, data analysis, formal analysis, writing original draft, designing the figures and tables, writing review and editing. S.A.K. and S.A.E.S.; resources, writing review, and editing.

Conflict of Interest

The authors declare that the present work has not been influenced by any known competing financial interests or personal relationships.

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