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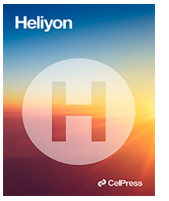

Argan: Phytochemical profiling and evaluation of the antioxidant, hypoglycemic, and antibacterial properties of its fruit pulp extracts

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ABSTRACT

Herein, we isolated three triterpenoid saponins from the methanol extract of the fruit pulp of argan. The structures of the identified compounds were determined using comprehensive NMR spectroscopy analyses (^{1}H, ^{13}C NMR, COSY, TOCSY, ROESY, and HSQC), combined with mass spectroscopy. Gas chromatography (GC) was utilized to determine the monosaccharide contents after the samples underwent methanolysis and their glycoside configuration was proved *via* their trimethylsilyl derivatives. Furthermore, the methanol extract of the fruit pulp and its n-butanol fraction were evaluated for their antioxidant properties *via* DPPH, ABTS, and FRAP assays, antidiabetic activity using α-amylase and α-glucosidase inhibition activities, and antibacterial properties utilizing microdilution and antibiofilm assays. Compared to the crude methanol extract, our results showed that the n-butanol fraction exhibited more potent antioxidant activity and antibacterial potential against *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Enterococcus faecalis,* and *Pseudomonas aeruginosa* (MIC = 12.5–50 mg/mL); while no effect on the bacterial biofilm was observed. The methanol extract was more effective in inhibiting α-glucosidase (EC_{50} = 0.15 mg/mL), however, the n-butanol fraction elicited strong α-amylase inhibition (EC_{50} = 0.49 mg/mL). These findings suggest that the fruit pulp of argan could serve as a potential source of phytochemicals suitable for the treatment of diabetes and its related complications.

1. Introduction

Sideroxylon spinosum, known as Argan (*Argania spinosa* (L.), Skeels), an indigenous tree species of Morocco, is primarily found in the western region of the High Atlas Mountains. The fruit of this plant is formed of a pulp that covers a shell representing half of the dry fruit. The nut contains one to three oily kernels. The versatile applications of argan-based products encompass folk medicine, cosmetics, and animal feed [1,2]. Argan oil has been utilized for centuries in traditional medicine and cosmetics for its potential health

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List of abbreviations

ABTS	2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid radical scavenging assay
DEPT	Distortion enhancement by polarization transfer
DPPH	2 2-diphenyl-1-picrylhydrazylfree radical scavenging assays
DQF-COSY	Double quantum filtered correlated spectroscopy
DU145	Human prostate cancer cell line
EC_{50}	Half maximal effective concentration
ESI	electrospray ionization
FRAP	Ferric reducing antioxidant power
GA	Gallic acid
GC-MS	Gas chromatography-mass spectrometry
HPLC	High performance liquid chromatography
HSQC	Hetero-nuclear multiple-bond correlation spectroscopy
LNCaP	Lymph node metastasis-derived prostate cancer cell line
MH	Mueller Hinton
MIC	Minimum inhibitory concentration
NMR	Nuclear magnetic resonance
PC3	Bone metastasis-derived cell line
QE	Quercetin equivalent
ROESY	Rotating frame nuclear overhauser effect spectroscopy
TFC	Total flavonoid compounds
TOCSY	Total correlation spectroscopy
TFC	Total flavonoids content
TPC	Total phenolic content

benefits [3]. Additionally, the glucose-rich pulp obtained from Argan nuts is employed as cattle feed, whether fresh or dry, in the Argan grove [4].

The Argan tree demonstrates significant resilience and harbors a diverse array of biologically active compounds, including polyphenols, flavonoids, and triterpenoid saponins that have been previously isolated and characterized [5,6]. Triterpenoid saponins, in particular, have garnered considerable attention, with the isolation of ubiquitous triterpenes from the tree [7]. Among these compounds, oleanolic and ursolic acids have been extracted from the leaves [8], while betulinic and maslinic acids have been obtained from the fruit pulp [8]. When combined, the saponins derived from the press cake of the Argan tree exhibit noteworthy anti-acne properties [9] and possess antioxidant characteristics [10]. Furthermore, this mixture demonstrates peripheral analgesic activity in mice and rats, exhibits cytotoxic effects and inhibits the proliferation of three human prostatic cell lines, namely PC3, DU145, and LNCaP [11]. Within *A. spinosa*, a range of bioactive saponins can be found, encompassing arganine A, B, C, D, E, F, and Mi-Saponin A [7]. Out of which, arganine C stands out as a compound of remarkable interest with respect to its pharmacological properties. Nevertheless, it is crucial to acknowledge that the combination of these compounds may yield enhanced potential owing to the synergistic effects that may arise from their combined activity [12].

There has been an increasing interest in exploring plant-based metabolites as eventual therapeutic agents for managing several health disorders, among them diabetes and its related complications [13]. Secondary metabolites, such as polyphenols, flavonoids, and triterpenoid saponins, found in plants, exhibit diverse biological activities, including antimicrobial and antidiabetic effects. Several preclinical and clinical studies have demonstrated the potential of these compounds as therapeutic agents for managing diabetes complications [14,15].

The aim of this investigation was to isolate and elucidate the structures of three triterpenoid saponins found in the pulp of the argan fruit. Additionally, the evaluation of various *in vitro* bioactivities of the methanolic extract as a crude extract containing different classes of polyphenols as well as the n-butanol fraction considering it a saponin-rich fraction. By testing the whole extract and the saponin-rich fraction, valuable insights into the responsible components for the activities could be obtained. These bioactivities, encompassing antioxidant, antibacterial, and anti-diabetic properties, play roles in diabetic conditions, particularly in the wound healing of individuals with diabetes. This leads to the second aim, which is uncovering the potential of this indigenous plant as a potential treatment for diabetes and its associated complications.

2. Materials and methods

2.1. Plant material, extraction, isolation, and purification

Argan pulp was collected from Essaouira, Morocco. A sample was kept at the scientific institute Mohammed V University (Rabat, Morocco) under RAB114142. Dried pulp (500 g) was ground and defatted by hexane and then extracted with methanol (MeOH) in the Soxhlet apparatus at 80 °C for 8 h. The MeOH extract was concentrated by a rotary evaporator at 50 °C. This residue (120 g) was

dissolved in water (H_2O) and then subjected to column chromatography on Diaion HP20 (80 g) as a stationary phase. The column was eluted with 2 L of H_2O, 2 L of MeOH 50 %, and 1 L of MeOH 100 %. The extract was subjected to purification using Sephadex LH 20 column (30 × 2 cm) and MeOH was utilized as an eluent. Fractions were collected and screened using thin layer chromatography (TLC) [Silica gel plates, *n*-BuOH/AcOH/H_2O (1: 3: 5)]. Three combined fractions comprising the crude glycosidic saponins were purified using HPLC equipped with a Low Dispersion Cooled (LDC) variable-wavelength detector connected to an integrator. Repeated injections of the crude saponins (0.5 mg each) into an octadecylsilane (ODS) Zorbax column (25 cm × 9.4 mm) yielded three compounds: 2 mg of compound **1**, 3 mg of compound **2**, and 3 mg of compound **3** after freeze-drying.

To obtain the n-butanol fraction (saponin-rich fraction), the methanol extract (100 g) was divided between water and *n*-hexane; subsequently, the water-soluble portion had sequential extractions using ethyl acetate and n-butanol.

2.2. Molar carbohydrate composition

GC was utilized to determine the monosaccharides contents after the samples underwent methanolysis (0.5 M HCl, 24 h, 80 °C) according to Kamerling et al. (1975) [16] modified by Montreuil et al. (1986) [17] and their glycosides configuration was proved *via* their trimethylsilyl (−)-2-butylglycosides [18].

2.3. Electrospray mass spectrometry (MS)

A triple quadrupole equipment (Micromass Ltd, Altrincham, UK) coupled with an atmospheric pressure ionization electrospray source was utilized with the following conditions: scan from 600 to 1600 Da with a scan duration of 3.5 s and a scan delay of 0.1 s, collision energy was set to values ranging from 40 to 80 eV. The samples were injected *via* a Harvard syringe pump at a flow rate of 3 μL/min and sprayed using a 3.4 KV needle voltage and the delustering (cone) was set at 70 V.

2.3.1. Gas chromatography-mass spectrometry (GC-MS)

Carlo Erba 8000 Top gas chromatograph (Finnigan, Argenteuil France) coupled with automass II 30 quadripolar mass spectrometer was utilized in a positive ion mode with ionization energy of 70 eV. A column (*C*P-Sil 5CB/MS capillary 25 m × 0.32 mm, Chrompack) gas vector was used, and helium was, as a carrier gas, set at the flow rate of 2 mL/min and the column temperature was raised from 100 to 240 °C at 5 °C/min.

2.4. Nuclear magnetic resonance (NMR)

^{1}H and ^{13}C NMR experiments were performed on a Bruker® ASX400 spectrometer at 400.13 and 100.61 MHz respectively, and the UXNMR software package was used for NMR measurement in deuterated methanol. Two dimensional homonuclear (COSY 90, one relayed COSY, double relayed COSY and ROESY) and heteronuclear (HMQC) experiments were performed by using standard Bruker® pulse programs (COSY, COSY1, COSYR2, and ROESY respectively). Typically, homonuclear experiments were performed with spectral widths of 4000 Hz in both frequency domains. 2024 data points were averaged for each free induction decay, and 512 increments were collected in the f1 domain. The heteronuclear experiment (HMQC) was performed with a spectral width of 12074 Hz for f1 and 3200 Hz in the f2 domain.

2.5. Phytocontents composition

The quantification of TPC in the extracts was conducted by employing the Folin–Ciocalteu reagent, utilizing gallic acid as a reference standard, as previously described [19]. The determination of TFC was performed following established protocols as previously described, with quercetin serving as the reference standard [20].

2.6. In vitro assays

2.6.1. Antioxidant activities

The assessment of antioxidant activity was conducted through the utilization of DPPH and ABTS assays, employing ascorbic acid as standard as previously described [21,22]. Furthermore, the FRAP assay was employed in accordance with established protocols, utilizing quercetin as a reference standard [23].

2.6.2. Antibacterial activity

2.6.2.1. Minimum inhibitory concentrations (MICs). The experimental procedures in this study were conducted following previously published protocols [24]. Briefly, the minimum inhibitory concentrations (MICs) of the methanolic and n-butanol extracts were determined using the broth microdilution assay in 96-well microtiter plates, using Ampicillin as a reference standard. Serial dilutions of the extracts and the standard were prepared, and bacterial cultures were incubated with the extracts for 24 h. The MIC was determined as the lowest concentration inhibiting visible microbial growth.

2.7. Bacterial biofilm inhibition assay

The antibiofilm potential of the extracts was evaluated using a colorimetric assay [24,25]. The extracts were prepared at 1/8 MIC and 1/4 MIC doses, incubated with bacterial cultures, and biofilms were assessed by staining with crystal violet.

2.8. Antidiabetic activity

2.8.1. α-Amylase inhibition assay

The inhibitory effects of the extracts were determined using the method described by Telagari and Hullatti with minor modifications [26]. Briefly, 100 μL of varying concentrations of the methanolic extract and n-butanol fraction (10, 8, 6, 4, 2, 1, 0.5, 0.25, and 0.12 mg/mL) were combined with 100 μL of the enzyme α-amylase solution (1 U/mL) and incubated for 10 min at 37 °C. Next, 100 μL of starch 1 % solution was added as a substrate to each tube and incubated further for 30 min at 37 °C. Afterward, 200 μL of 1 M 3, 5-dinitrosali-cylic acid (DNS) color reagent was added. To terminate the colorimetric reaction, the mixture was boiled for 10 min. A control group was established in parallel without the extracts, and each experiment was performed in triplicate. The resulting mixture was diluted with 1.5 mL of distilled water after cooling to ambient temperature, and then the absorbance measurements were performed using a Microplate reader (Costar, 96) at 540 nm. The blank with 100 % enzyme activity was prepared 100 μL of the phosphate buffer instead of the extracts. A blank reaction was also performed utilizing the extract for each concentration without the enzyme solution. Acarbose was used as a positive control and the reaction was performed as mentioned above. The percentage of α-amylase inhibition was calculated using the following formula:

$$\alpha\text{-amylase inhibition } (\%) = [(\mathrm{Ac} - \mathrm{As})/\mathrm{Ac}] \times 100$$

Where Ac is the absorbance of the control (100 % enzyme activity) and As is the absorbance of the sample.

2.8.2. α-Glucosidase inhibition assay

The inhibitory activities of the extracts were executed according to the original protocol [26] with some modifications. An initial 110 μL of α-glucosidase (0.1 U/mL) was mixed with 165 μL of each concentration of the sample (10, 8, 6, 4, 2, 1, 0.5, 0.25, and 0.12 mg/mL). The reaction mixture was incubated at 37 °C for 10 min. Then, 220 μL P-NPG (1 mM) was added as a substrate and maintained for 30 min at 37 °C. The reaction was stopped by adding 605 μL of sodium carbonate Na_2CO_3 (0.1 M). Using a Microplate reader (Costar, 96), the absorbance of released *p*-nitrophenol was determined at 405 nm. Acarbose was used as a standard. Each experiment was performed in triplicates. The α-glucosidase inhibition percentage was calculated using the following equation:

Table 1
^{1}H NMR and the^{13}C NMR Data of the aglycon of compound **1** in CD_3OD.

Aglycon	$\delta_{H\ (ppm)}$	δ_C (ppm)
1	1.06; 2.29	46.8
2	4.34	71.7
3	3.59	83.4
4	–	43.9
5	1.33	49.2
6	4.47	68.9
7	1.56; 1.82	41.7
8	–	37.7
9	–	49.0
10	–	37.7
11	1.20; 2.11	24.8
12	5.42	124.8
13	–	144.7
14	–	43.9
15	–	36.5
16	4.49	74.9
17	-	41.7
18	3.09	42.4
19	2.29; 1.06	46.9
20	–	31.9
21	–	36.9
22	–	31.9
23	3.73 ; 3.42	64.6
24	1.31	16.4
25	1.63	19.2
26	1.09	19.4
27	1.33	27.5
28	–	178.3
29	0.89	33.5
30	0.98	25.4

$$\alpha\text{-glucosidase inhibition } (\%) = [(\text{Ac - As})/\text{Ac}] \times 100$$

Where Ac is the absorbance of the control (100 % enzyme activity) and As is the absorbance of the sample.

2.9. Statistical analysis

The data obtained from the experiment were subjected to statistical analysis using GraphPad Prism 8.0.1 software. The significance of the observed differences was determined using the One-Way Analysis of Variance (ANOVA) followed by Tukey's post hoc test, with a predetermined significance level of $p \leq 0.05$. The results were presented as the mean ± standard deviation (SD) based on three independent replicates (N = 3).

3. Results and discussion

Three triterpenoid saponins (1–3) were isolated from argan fruit pulp. GC analysis confirmed the presence of D-glucose, L-rhamnose, D-xylose, and L-arabinose. Methylation analysis yielded 2-linked arabinopyranose, 3-linked xylopyranose, 4-linked rhamnopyranose (rham inter), terminal rhamnopyranose (rham term), terminal glucopyranose (Glc term) for compound 3 and additional 3-linked glucopyranose (Glc inter) for compounds 1 and 2. For compound 1: the positive electrospray ionization (ESI) displayed a quasi-molecular ion peak at *m/z* 1423 $[M+Na]^+$ indicating a molecular weight of 1400 Da which is in agreement with the molecular formula $C_{64}H_{104}O_{33}$. Signals at 1261 [1423-162], 1277 [1423-146], and 1145 [1423-146-132] indicated the loss of one terminal hexose, one terminal deoxyhexose, and a disaccharide constituted by deoxyhexose and pentose respectively. The MS-MS investigation of the molecular ion at 1423 gave a prominent fragment at *m/z* 867 [M-132-132-146-146] corresponding to the loss of a tetrasaccharide constituted with 2 deoxyhexoses and 2 pentoses. The signal at 579 was attributed to a tetrasaccharide chain. These data suggested a

Table 2
The ^1H NMR, and^{13}C data of the sugar portion of compound 1 in CD_3OD.

28-*O*-sugar	$\delta_{H\text{-(ppm)}}$	$\delta_{C\text{(ppm)}}$
Arabinose		
1	5.61	94.3
2	3.80	75.9
3	3.80	67.5
4	3.84	72.7
5	3.51; 3.91	64.3
Rhamnose inter		
1	5.08	101.7
2	3.84	72.7
3	3.87	72
4	3.59	83.4
5	3.48	69.9
6	1.24	18
Xylose		
1	4.54	106.9
2	3.34	76.5
3	3.47	84.3
4	3.48	69.9
5	3.22; 3.88	67.5
Rhamnose term		
1	5.14	102.9
2	3.95	72.7
3	3.75	72.5
4	3.4	74.2
5	4	70.2
6	1.3	18.2
3-*O*-sugar		
Glucose inter		
1	4.5	105.3
2	3.2	74.9
3	3.54	88.3
4	3.48	69.9
5	3.18	77.6
6	3.71; 3.88	62.4
Glucose term		
1	4.57	105.6
2	3.28	75.8
3	3.39	78.1
4	3.29	71.9
5	3.31	78.5
6	3.63; 3.89	64.9

tetrasaccharide constituted by 2d+2p linked to the aglycone.

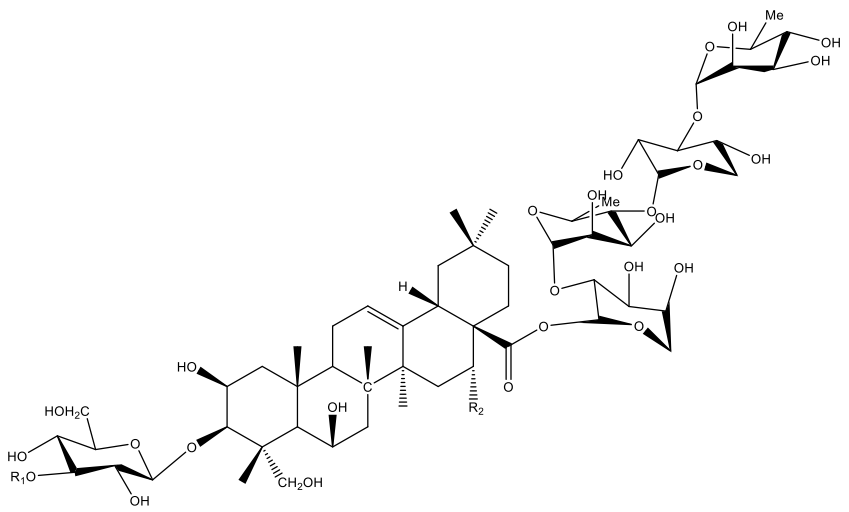
The ^{1}H NMR spectrum of the aglycon moiety of **1** displayed a signal for six tertiary methyl groups (δ 0.89, 0.98, 1.09, 1.31, 1.33, and 1.63). Also, other confirmative signals (Table 1) of Δ-12 oleanane saponin with a trisubstituted olefinic proton at 5.42 (t-like, H-12) and an allylic proton at 3.09 (dd, J = 14.7, 4.7 Hz, H-18) were detected. Signals of H-3-ax at δ 3.59 (d, J = 4.5 Hz) due to the presence of a β-OH group at C-3 and H-2 at δ 4.34 (m) due to the presence of a β-OH group at C-2 were also found. In the ^{1}H NMR spectrum (Table 2), the sugar portion of compound 1 contained six anomeric protons at δ 5.61 (d, J = 3.91 Hz, H-1 Ara); 5.14 (d, J = 1.71 Hz, H-1 Rha term); 5.08 (d, J = 0.73 Hz, H-1 Rha inter); 4.57 (d, J = 7.57 Hz, H-1 Glc term); 4.54 (d, J = 7.81 Hz, H-1 Xyl); 4.50 (d, J = 7.57 Hz, H-1 Glc inter). The two secondary methyls at δ 1.25 ppm (d, J = 6.1 Hz]; and 1.30 ppm (d, J = 6.1 Hz] suggested the occurrence of two deoxyhexose units (Table 2).

The spectra of 1D TOCSY and DQF-COSY experiments made it possible to sequentially assign all proton resonances to the individual monosaccharides (Table 2). Hence, the shifts of the sugar resonances were attributed to α-L-arabinopyranosyl (δ 5.61 J = 3.91 Hz, H-1 Ara), α-L-rhamnopyranosyl (δ 5.14, J = 1.71 Hz, H-1 Rha term), α-L-rhamnopyranosyl (δ 5.08 J = 0.73 Hz, H-1 Rha inter), β-D-glucopyranosyl (δ 4.57, J = 7.57 Hz, H-1 Glc term), β-D-xylopyranosyl (δ 4.54, J = 7.81 Hz, H-1 Xyl) and β-D-glucopyranosyl (δ 4.50, J = 7.57 Hz, H-1 Glc inter) (Table 2).

The ^{13}C NMR spectrum and ^{13}C DEPT of compound 1 displayed 64 signals. Out of which, 30 signals were attributed to the triterpenoid moiety and the other 34 were assigned to the saccharide part. A pair of olefinic carbon at 124.2 ppm (C-12) and 144.2 ppm (C-13); six anomeric carbons at 94.3 (C-1 Ara), 105.3 (C-1 Glc inter), 106.9 (C-1 Xyl), 101.7 (C-1 Rham inter), 102.9 (C-1 Rham term), 105.6 (C-1 Glc t) and a carbonyl carbon at 178.3 (C-28). Five carbons each of which is bonded to 2 hydrogens and one oxygen (CH_2O groups) at δ: 67.51, 64.34, 62.87, 62.43, and 65.93 ppm were revealed by ^{13}C. Altogether, ^{1}H and ^{13}C NMR results (Table 1) and compared to the literature, the aglycon structure was elucidated as: 2β, 3β, 6β, 16 α, 23-pentahydroxyolean-12-en-28-oic acid, known as 16 α-hydroxyprotobassic acid [5,6,27,28].

An HMQC experiment allowed the identification and determination of the displacement of most of the protons of the molecule. The ratio of these values on the HMQC spectrum allows the identification of the corresponding carbons and the determination of their chemical displacements. The knowledge of the chemical displacements of the carbons allows the identification of the structure of the aglycone on the one hand and the determination of the substitution of a monosaccharide on the other hand by the observation of a deblending of the carbons. For sugars, the C3 carbons of xylose, C4 of rhamnose, C2 of arabinose, and C3 of glucose are unshielded. These results correlate with those observed during methylation and the mass spectra results show the existence of a 3-substituted xylose, 4-substituted rhamnose, 2-substituted arabinose, 3-substituted glucose, in addition to terminal glucose and rhamnose.

In the ROESY experiment, the inter-sugar correlations made it possible to determine their sequence. The spectrum showed a correlation spot between H-3 of (δ 3.59) of the aglycone and H-1 of Glc inter (δ 4.5), a correlation between H-3 of Glc inter (δ 3.54) and H-1 Glc term (δ 4.57), H-2 Ara (δ 3.8) and H-1 Rha inter (δ 5.08), H-3 Xyl (δ 3.47) and H-1 Rha term (δ 5.14). Chemical shifts of H-1 arabinose (δ 5.64) and C-1 arabinose (δ 93.1) indicated that this sugar unit was involved in an ester linkage with the C-28 carboxylic group.



Compounds	R_1	R_2
1	Glc	OH
2	Glc	H
3 (Misaponine A)	H	H

Fig. 1. Structure of triterpenoid saponins 1–3.

Consequently, compound 1 is a bidesmosidic saponin of 16-α hydroxyprotobassic acid. A tetrasaccharide (2d+2p) was linked by ester linkage at C-28 and disaccharide Glc 1 → 3 Glc by ether linkage at C-3. The last problem to be solved on the structure of compound 1 was the sequencing of the tetrasaccharide. This was determined by ROESY. Cross-peaks due to long-range correlation were observed between H-1 terminal rhamnose (5.61 ppm) and H-3 xylose (3.47 ppm). The results correlated to the signal at *m/z* 1145 [1423-146-132] observed at the ESI spectrum in the positive mode indicated that the terminal rhamnose is linked to xylose.

To sum up, the results presented in Table 1, **2** and **3** show that the structure of compound 1 was concluded to be 3-*O*-(*β*-D-glucopyranosyl (1 → 3) *β*-D-glucopyranosyl)-28-*O*-(α-L-rhamnopyranosyl (1 → 3) *β*-D-xylopyranosyl (1 → 4) α-L-rhamnopyranosyl (1 → 2) α-L-arabinopyranosyl)-16-α-hydroxy protobassic acid (Fig. 1). Noteworthy, this structure was previously described in Chafchaouni-Moussaoui et al. [7] without enough spectral data.

Compounds 2 and 3 are known as triterpene saponins and their identities were comprehensively elucidated by 1D and 2D NMR experiments and GC after methanolysis. The structure of compound 2 was assigned to 3-*O*-(*β*-D-glucopyranosyl (1 → 3)–*β* D-glucopyranosyl)-28-*O*-(α-L-rhamnopyranosyl (1 → 3) β-D-xylopyranosyl (1 → 4) α-L rhamnopyranosyl (1 → 2) α-L arabinopyranosyl) protobassic acid. It was previously isolated and characterized from *Mimusops elengi* [27] and manilkara [28]. The structure of compound 3 was established as Mi saponin A: 3-*O*-(*β*-D-glucopyranosyl)-28-*O*-(α-L-rhamnopyranosyl (1 → 3) *β*-D-xylopyranosyl (1 → 4) α-L rhamnopyranosyl (1 → 2) α-L arabinopyranosyl) protobassic acid. This saponin was previously isolated from many plant species belonging to the Sapotaceae family [5,6,29].

3.1. Phytocontents and antioxidant activity

Considering the search for bioactive substances of plant origin, the present study investigated the potential of the methanolic extract and n-butanol fraction of argan pulp *via* three widely used methods, namely DPPH scavenging, ABTS radical scavenging, and FRAP assays, along with the determination of their total phenolics and flavonoids relative to conventionally used standards. The results of antioxidant activity revealed noteworthy differences in the antioxidant capacities (Table 3). When comparing the extracts with the reference compounds, the n-butanol fraction was the closest to the reference used, with EC_{50} values for both DPPH and ABTS being lower than those of the methanolic extract and close to those of ascorbic acid. Conversely, the FRAP value of the n-butanol extract was higher compared to the methanolic extract and comparable to quercetin. The present study also determined the total polyphenol and flavonoid contents in the two extracts namely methanolic and n-butanol. Results showed that the n-butanol fraction exhibited a significantly higher TPC compared to the methanolic extract. Moreover, the methanolic extract contained a greater quantity of flavonoids than the n-butanol fraction. Notably, the n-butanol fraction, aside from its rich polyphenolic content, was also found to be saponin-rich. Therefore, the observed high antioxidant activity of the n-butanol fraction can be attributed to the synergistic effect of its substantial polyphenolic and saponin contents.

3.2. Antibacterial activity

Plant extracts have been utilized in traditional medicine to cure several infectious diseases, and in recent years, there has been a growing interest in the use of plant extracts as natural alternatives to synthetic antimicrobial agents. Plant extracts are considered safe, cost-effective, and environmentally friendly, and therefore, have the potential to play a significant role in the development of new antimicrobial agents [30]. Here, the methanolic extract and its n-butanol fraction from the pulp of the fruit of argan were evaluated for their antibacterial activities against five human pathogenic bacteria. MIC determination revealed that the n-butanol fraction exhibited higher inhibitory effects than the methanol extract (Table 4). Noteworthy, *P. aeruginosa* was shown to be the most sensitive bacterium (MIC = 12.5 mg/mL) while *E. coli*, *S. aureus, S. typhi*, and *E. faecalis* were more resistant (MIC = 50 mg/mL).

Furthermore, as there is a growing interest in targeting bacterial virulence factors as a new strategy to reduce microbial pathogenicity [31], we examined here how the extract affects the ability of the five bacterial species to form biofilms. Biofilms are a critical virulence factor that enables bacteria to form protected bacterial communities and move across biological surfaces [32]. We evaluated the effect of the methanolic extract and its n-butanol fraction from *A. spinosa* fruit pulp at sub-MICs (1/8 and 1/4 MIC) on biofilm production. The results showed that neither the extract nor its n-butanol fraction had a significant effect on the amount of biofilm produced by the five bacteria (table S3).

Table 3
Total phenolic content (TPC), total flavonoids content (TFC), and *in vitro* antioxidant activities of the methanol extract and its n-butanol fraction from argan pulp.

Sample	Extracts	DPPH	ABTS	FRAP	TPC	TFC
		(EC_{50}, μg/mL)		(mM of $FeSO_4$/g Extract)	mg GA/g Extract	mg QE/g Extract
Argan pulp	Methanolic extract	9.73 ± 0.21[ab]	0.338 ± 0.031[ab]	30.74 ± 3.91[ab]	108 ± 0.0014	19.39 ± 3.2
	n-Butanol fraction	5.35 ± 0.25[a]	0.271 ± 0.032[a]	48.03 ± 3.89[a]	131 ± 0.0014	1.0 ± 0.004
Reference compounds	Ascorbic acid	3.47 ± 0.26	0.040 ± 0.003	–		
	Quercetin	–	–	57.48 ± 0.55		

Results are shown as mean ± S.D, n = 3. Significance was estimated for $p \leq 0.05$ (One-Way ANOVA). [a] Vs n-butanol fraction and [b] Vs the reference compounds.

Table 4
MICs values of the methanol extract and its n-butanol fraction from argan pulp on Gram-positive and Gram-negative human pathogenic bacteria.

Bacteria	MIC (mg/mL)		
	Methanolic extract	n-Butanol fraction	Ampicillin (Reference compound)
Pseudomonas aeruginosa	12.5	12.5	0.3125
Escherichia coli	50	25	0.15625
Staphylococcus aureus	50	25	0.3125
Salmonella typhi	50	50	0.15625
Enterococcus faecalis	50	25	0.078125

3.3. Antidiabetic activities

The inhibition potential of the two tested samples (the extract and its butanol fraction) on α-amylase and α-glucosidase activities were determined (Table 5). The findings demonstrated that both extracts exhibited concentration-dependent inhibitory activities against both enzymes. The n-butanol fraction furnished significant α-amylase inhibitory activity compared to the methanolic extract (Table 5). Conversely, there are no significant differences in α-glucosidase inhibitory activity between the extracts and the reference used.

These findings suggested that both the methanolic and n-butanol extracts have the potential to inhibit α-amylase and α-glucosidase, which could be beneficial in the management of hyperglycemia and related complications. Similar results were reported by Kamal et al. [33], who investigated the antidiabetic activity of saponin cake and argan oil from *A. spinosa*, where the saponin fraction exhibited the greatest activity when compared to standards. Similarly, Samane et al. [34], reported that the saponin-rich fraction obtained from the pressed cake (MeOH extract) exhibited a more potent stimulating effect on the activation of protein kinase B (PKB/Akt) induced by insulin. Given the presence of various compounds in the extract, including polyphenols and triterpenoid saponins, particularly arganine A, B, C, D, E, and F, as well as Mi-Saponin A and compounds 1 and 2 [35], it should be considered that the synergistic effect of these bioactive compounds could potentially result in greater activity than that of individual compounds [36]. Although more investigations on the possible activities of the isolated compounds are still needed and considering the safety profile of the argan pulp extract, cell based as well as animal experiments would be necessary to translate these findings into scientifically sound evidence. Moreover, bioformulation of active principles inhibiting the key enzymes (α-amylase and α-glucosidase) in the small bowel should be developed further. In addition, it would be interesting to evaluate the extracts' inhibitory activities against other relevant enzymes involved in carbohydrate metabolism and perform preclinical and clinical studies to determine their therapeutic potential as antidiabetic agents.

Herein, the methanolic extract and n-butanol fraction of argan pulp showed strong antioxidant activity, diabetic related enzymes' inhibition, and antibacterial activity against several pathogenic bacteria. Antioxidants are well-known for their ability to neutralize free radicals and protect cells from oxidative damage, which is implicated in various diseases, including diabetes and microbial infections [37]. The significant antioxidant properties of these extracts, as demonstrated through DPPH, ABTS, and FRAP assays, are in line with the high content of polyphenols and flavonoids in the n-butanol fraction, suggesting a strong correlation between antioxidant capacity and phenolic content. The complex interaction between oxidative stress and diabetes pathogenesis has become increasingly evident, with oxidative stress being implicated in insulin resistance, β-cell dysfunction, and the progression of diabetes-related complications [38]. The n-butanol fraction which exhibited the highest antioxidant activity also has notable antidiabetic activity by inhibiting α-amylase and α-glucosidase. This suggests that the antioxidant activity of the extract may contribute to its antidiabetic potential by reducing oxidative stress associated with hyperglycemia. Furthermore, the complexity of wound healing in individuals with diabetes is well-documented [37]. Diabetes predisposes individuals to an elevated risk of chronic non-healing ulcers and infections due to the distinctive physiological condition characterized by impaired angiogenesis, reduced collagen synthesis, and compromised immune response [37,38]. The prospect of bolstering wound healing while simultaneously countering bacterial pathogens holds promise for improving the quality of life for individuals struggling with diabetes-related concerns. The relationship between antioxidant, antidiabetic, and antimicrobial activities in our study underscores the multifaceted bioactivity of Argan pulp extracts. The observed synergistic effect of polyphenolic and saponin compounds in the n-butanol fraction further highlights the potential for developing therapeutic agents from this natural source against diabetes.

Table 5
EC_{50} values of the methanol extract and its n-butanol fraction from argan on α-glucosidase and α-amylase enzymes.

Sample	α-amylase	α-glucosidase
	EC_{50}, mg/mL	
Methanolic extract	0.68 ± 0.034^{ab}	0.15 ± 0.074
n-Butanol fraction	0.49 ± 0.029	0.23 ± 0.052
Acarbose (Standard)	0.5 ± 0.067	0.17 ± 0.009

Results are shown as mean ± S.D, n = 3. Significance was estimated for $p \leq 0.05$ (One-Way ANOVA). [a] Vs n-butanol fraction and [b] Vs the standard.

4. Conclusion

The present study sheds light on the potential therapeutic applications of the pulp of *A. spinosa* fruit. The potent antibacterial activity against a range of pathogenic bacteria, including *P. aeruginosa, E. coli, S. aureus, S. typhi,* and *E. faecalis* along with the inhibition of α-amylase and β-glucosidase enzymes by the extracts indicate their potential as a natural remedy for diabetic and related complications. Moreover, the extracts' remarkable antioxidant activity against DPPH, ABTS, and FRAP suggests their potential as natural antioxidant agents in the food, cosmeceutical, and pharmaceutical industries. The observed activities may be attributed to the synergistic effect of the extracts' bioactive compounds. However, further studies are needed to determine the contribution of individual isolated compounds to the observed effects.

Data availability statement

The data and supporting information are available within the article. Data associated with the study has not been deposited into a publicly available repository, it will be made available on request from the corresponding author.

CRediT authorship contribution statement

Asmae Alaoui: Writing - review & editing, Writing - original draft, Software, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Nihad Sahri:** Writing - review & editing, Writing - original draft, Software, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ismail Mahdi:** Writing - original draft, Methodology, Funding acquisition, Formal analysis. **Nidal Fahsi:** Writing - original draft, Methodology, Funding acquisition, Formal analysis. **El hassania El herradi:** Writing - review & editing. **Mansour Sobeh:** Writing - review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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