Progress in Microbes and Molecular Biology

HH PUBLISHER

Original Research Article

Evaluation of Antioxidant and Antimicrobial Activity of Saponin Extracts from Different Parts of *Argania spinosa* L. Skeels

Yousra El Idrissi[1], Youssef Elouafy[1], Hamza El Moudden[2], Najoua Mghazli[3], Chakir El Guezzane[1], Adil El Yadini[1], Hicham Harhar[1], Abdelkader Zarrouk[1], Khang Wen Goh[4], Long Chiau Ming[5,*], Abdelhakim Bouyahya[6,*], Mohammed Tabyaoui[1]

Article History

Received: 14 February 2023;

Received in Revised Form: 11 June 2023;

Accepted: 22 June 2023;

Available Online: 5 July 2023

[1]Laboratory of Materials, Nanotechnology and Environment LMNE, Faculty of Sciences, Mohammed V University in Rabat, Rabat BP 1014, Morocco; yousra.elidrissi93@gmail.com (YEI); youssef.elouafy@um5r.ac.ma (YE); chakir.elguezzane@gmail.com (CEG); a.elyadini@um5r.ac.ma (AEY); h.harhar@um5r.ac.ma (HH); azarrouk@gmail.com (ZA); h.tabyaoui@um5r.ac.ma (MT)

[2]Higher School of Technology of El Kelaa Des Sraghna, Cadi Ayyad University, El Kelaa Des Sraghna BP 104, Morocco; hamzaelm802@gmail.com (HEM)

[3]Team of Microbiology and Molecular Biology, Faculty of Sciences, Mohammed V University in Rabat, BP 1014- Rabat, Morocco; najoua_mghazli@um5.ac.ma (NM).

[4]Faculty of Data Science and Information Technology, INTI International University, 71800 Nilai, Malaysia; khangwen.goh@newinti.edu.my (KWG)

[5]School of Medical and Life Sciences, Sunway University, 47500 Sunway City, Malaysia

[6]Laboratory of Human Pathologies Biology, Faculty of Sciences, Mohammed V University in Rabat, BP 1014, Rabat, Morocco

*Corresponding author: Long Chiau Ming; School of Medical and Life Sciences, Sunway University, 47500 Sunway City, Malaysia; longchiauming@gmail.com (LCM); Abdelhakim Bouyahya; Laboratory of Human Pathologies Biology, Faculty of Sciences, Mohammed V University in Rabat, BP 1014, Rabat, Morocco; a.bouyahya@um5r.ac.ma (AB)

Abstract: The argan tree is a versatile forest tree (silviculture-fruit-forestry) of great importance for the country both in biological, phytogenetic and ecological biodiversity as well as in economic and social aspects. It has significant medicinal and therapeutic potential. The present study concentrated on saccharide-containing molecules, particularly saponins, for their medicinal properties. Butanol was used to fractionate phytochemical groups from different parts of *Argania spinosa* (branch, leaf, pulp, shell and seed), which were subsequently precipitated in ether. It was found that solvent fractionation increased the total saponin amount according to the colorimetric test using vanillin and sulfuric acid. The butanol fraction of the argan shell and its precipitate showed the highest levels of triterpene saponins compared to the rest of the plant parts studied. The samples showed antioxidant capacity by DPPH versus ascorbic acid (IC_{50} = 1.92 µg/mL) and by ABTS using the similar

standard (IC_{50} = 11.31 µg/mL). The antioxidant activity of the samples was significantly improved from the crude shell extract (IC_{50} = 5.85 µg/mL) to its butanolic fraction (IC_{50} = 2.94 µg/mL) and its precipitate (IC_{50} = 1.57 µg/mL) using 2,2-diphenyl-picrylhydrazyl. Indeed, the ABTS test, showed similarity to the core results. Also, the solid disc diffusion method was used to highlight the antimicrobial activity of extracts from different parts of the *Argania spinosa* plant. The EtOH 70% and Aqueous leaf extracts exhibited the highest antibacterial activity, resulting in an inhibition zone of approximately 13 mm against the growth of *Staphylococcus saprophyticus*. On the other hand, the extract of saponin filtrate from the pulp part of the plant displayed the lowest antibacterial activity, with an inhibition zone of 9 mm. All studied types of saponin extract from different parts of the plant do not contribute to any antifungal activity against *Botrytis cinerea*.

Keywords: *Argania spinosa*; antioxidant activity; saponins; antibacterial activity; antifungal activity

1. Introduction

Throughout the world, natural resources are facing a concerning degradation due to the excessive exploitation and mismanagement practices[1]. This not only poses a threat to the overall ecosystem but also has the potential to lead to the loss of valuable natural resources that possess significant pharmacological and biological properties. Many natural resources harbor compounds and substances utilized for centuries in traditional medicine and have shown promising therapeutic potential[2–6]. Morocco is well-known for its diverse and rich forestry, with several species of trees that possess significant medicinal properties[7–14]. Among these trees, the Argan tree (*Argania spinosa* L. Skeels) stands out as a highly endemic species, ranking as the second most abundant forest species in the country, following the Holm oak (*Quercus ilex*) and preceding the Cedar (*Cedrus atlantica*)[15]. It is estimated that the tree will live between 150 and 200 years. Drought and heat are not a problem for it. The Argan tree grows wild and abundant in southwest Morocco's arid and semi-arid areas. In this geographical area, the Argan tree plays an irreplaceable role in the ecological balance and in preserving biodiversity. In addition to this environmental role, the Argan tree has a very important economic interest; The Argan tree produces argan oil as its main product[16].

There are two categories of Argan oil, cosmetic oil from unroasted almonds and edible oil extracted from roasted almonds. The physicochemical properties of these two types of Argan oil have been well studied[17,18], as well as their production in a controlled and reproducible manner[19,20], and their pharmacological properties[21–23]. Indeed, the nutritional properties of Argan oil are due to its chemical composition. According to the Moroccan Standards Institute (IMANOR), Argan oil is composed of 80% unsaturated fatty acids[24], and is particularly rich in tocopherols, molecules with powerful antioxidant properties. The high content of tocopherols and the low content of linolenic acid are responsible for its oxidation resistance. The phospholipids present in Argan oil contribute to oil preservation[25].

Unfortunately, the current worldwide fame of the Argan oil has partially overshadowed the first intensive work carried out on the triterpenic saponins of the Argan tree. For some time, this family of molecules has represented the most likely result to successfully save the Argan tree from various threats it faced. In fact, saponins possess many pharmacological and biological properties[26], and even if these compounds are no longer studied as intensively as they were a few years ago, the need to diversify the *A. spinosa* resource is sufficient to warrant further research.

Literature reports indicate that other Sapotaceae species contain glucuronidic triterpenes[27–35]. Originally, pure ethanol was believed insufficient as a solvent to extract saponins from different parts of the *A. spinosa* plant. This paper investigated the total saponin content in the crude extract of the different parts of *A. spinosa* (branch, leaf, pulp, shell, and seed) as well as its fraction and precipitate. Due to the complexity of phytochemicals contained in plant extracts, liquid-liquid extraction was used to separate the target compounds, utilizing butanol as an organic solvent. The organic fraction was then precipitated in diethyl ether to further recover the chemicals. Saponins can be precipitated by utilizing ether to reduce the medium's dielectric constant. Therefore, this paper aimed to compare the total saponins estimated in the extracts of different parts of the *A. spinosa* plant.

In this work, we investigated the antioxidant power of DPPH (2,2-diphenyl-picrylhydrazyl) and ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) of the saponin extracts from the plant. However, the antimicrobial activity of the different extracts was carried out using four bacterial strains for the antibacterial activity, and two fungi for the antifungal activity. In addition, principal component analysis (PCA) was employed to evaluate the correlation between antioxidant activity (DPPH, ABTS), secondary metabolites (polyphenols, flavonoids, tannins), total sugars, and triterpene saponins in all extracts derived from the *A. spinosa* saponin extraction process.

2. Materials and Methods

2.1. Preparation of Argania spinosa extracts

The characterization of the extracts of *A. spinosa* focused on the different parts of the tree, namely: branch, leaf, pulp, shell, and seed. The different parts of *A. spinosa* from Lakhssas (29° 24′ 43″ north, 9° 43′ 34″ west), Guelmim-Oued Noun region were harvested in the summer of 2020 for the study of saponins. The different parts of *A. spinosa*, freshly harvested, were washed with tap water and left to dry in the shade for two weeks to preserve as much integrity of their chemical composition as possible. After drying, the different parts of *A. spinosa* were finely crushed with a professional grinder and then sieved on sieves.

2.2. Extraction of saponins

Samples from different parts of the plant were delipidated by soxhlet for 6 hours using hexane as an apolar solvent. The residue obtained was left in the oven at a temperature of 25 overnight to completely eliminate hexane. The residue was extracted with a 70:30

EtOH/water hydroalcoholic solution in a two-hour heat-reflux system. After extraction, a rotary evaporator was used to chill and filter the solution until it was completely dry[36–38].

Secondly, the liquid-liquid extraction technique was employed. The crude 70% ethanol extract was reconstituted in water and extracted vigorously with butanol in a separatory funnel. For phase separation, the organic phase was removed, and more butanol was added to the residual aqueous solution for further liquid-liquid extraction. This fractionation process was further repeated in triplicate [36–38]. The organic butanol fraction was merged and dried by a rotary evaporator. The aqueous phase was coated and concentrated to dryness by a freeze-dryer.

The butanol fractions of the five parts were reconstituted in absolute methanol. Moreover, adding diethyl ether to the organic fraction was done in a very slow manner and a precipitate was formed at the end[39]. The phytochemicals that were not very soluble in diethyl ether were precipitated. The precipitate was filtered using sintered glass and separated from the solvent using a rotary evaporator (Rotavapor R-100, BUCHI).

2.3. Determination of Secondary Metabolites of Argania Spinosa Extracts

2.3.1. Determination of polyphenols

The content of phenolic compounds in the different extracts of *Argania spinosa* was estimated by the Folin-Ciocalteu method. Briefly, 2.5 mL of Folin reagent (10-fold diluted) is added to 500 µL of sample or standard (prepared in ethanol) with suitable dilutions, 2 mL of Na_2CO_3 sodium carbonate solution (7.5%). After 15 min of incubation in a water bath at 45 °C, the absorbance was measured at 765 nm against a blank without extract, and the calibration range obtained with gallic acid (1-200 g/mL) was used to calculate the quantification of total polyphenols under the identical circumstances as the sample. Results are given as milligrams of gallic acid equivalent per gram of extract (mg GAE/g)[40].

2.3.2. Determination of flavonoids

The determination of total flavonoids was performed by the aluminum chloride colorimetric method. 6.4 mL of distilled water and 1 mL of an extract at a specific concentration were combined in a tube. Then 0.3 mL of sodium nitrite ($NaNO_2$) solution (5% concentration) was added. 0.3 mL of 10% aluminum chloride ($AlCl_3$) was added to the mixture after 5 minutes. It was left for six minutes. The tubes were then filled with 2 mL of sodium hydroxide (1M), thoroughly mixed, and agitated before being permitted to stand for 30 minutes. At 510 nm, the absorbances were measured. Flavonoid concentration was deduced from a calibration range established with quercetin (2–200 µg/mL) and was expressed as milligram quercetin equivalent per gram of extract (mg QE/g)[41].

2.3.3. Determination of condensed tannins

We adopted the vanillin method with hydrochloric acid. This method depends on the property of tannins to transform into red-colored anthocyanidols by reaction with vanillin[42].

3 mL of an ethanolic solution of 4% vanillin is added to 0.5 mL of the sample. The final step is to add 1.5 mL of concentrated hydrochloric acid. The resulting combination is given 15 minutes to react at room temperature. At 500 nm, the absorbance is calculated in comparison to a blank. The calibration curve was generated using various concentrations between 1 and 800 g/mL made from a stock solution of catechin. While the final result was expressed as mg catechin equivalent (CE) per gram of extract (mg CE/g)[43].

2.3.4. Analysis of triterpene saponins by UV spectrophotometer

2.5 mL of sulfuric acid (72% concentration) was added to a 250 µL sample that included 1 mg/mL of sulfuric acid and 250 µL of vanillin (8 g/100 mL ethanol). The combination was heated for 10 minutes at 60 degrees Celsius, then cooled for 5 minutes in an ice-water bath. The mixture's absorbance was measured using a UV/vis spectrophotometer. (LLG-uniSPEC 2) at 544 nm. Different concentrations between 5 and 350 µg/mL prepared from a stock solution of oleanolic acid were used to plot the calibration curve. While the results were expressed as mg oleanolic acid equivalent per gram of extract (mg OAE/g)[36].

2.4. Quantification of Sugar

In summary, 5 mL of concentrated sulfuric acid and 1 mL of phenol (5%) were combined with 1 mL of sample. Stir and allow it to stand at room temperature for 10 minutes. After 20 minutes, the mixture was incubated at 30 °C in a water bath to measure the yellow-orange color at 488 nm. Quantification of the total score was calculated from the regression equation of the calibration range established with glucose (5–100 µg/mL) under the same conditions as the sample. Results are expressed via mg glucose equivalent per gram dry matter (mg DE-Glu/g DM)[44].

2.5. Study of Biological Activity

2.5.1. Antioxidant potentials of Argan extracts

2.5.1.1. Antioxidant test by DPPH

The DPPH solution is solubilized in absolute ethanol (0.2 mM). 2.5 mL of the test extract was added to 0.5 mL of the DPPH solution. After vortexing the mixture, the tubes were left at room temperature and dark for 30 minutes. The reading is made using a spectrophotometer to measure the absorbance at 517 nm[45].

The negative control (NC) contains only the DPPH solution. The DPPH concentration is deduced from a calibration range established with ascorbic acid (0.5–20 µg/mL)[45].

2.5.1.2. Cation-radical reduction test $ABTS^{\circ+}$

The 7 mM ABTS stock solution was mixed with 2.45 mM potassium persulfate (1:1, v/v), and the mixture was allowed to stand for 4 to 8 hours to allow the reaction to finish, and

the absorbance was stable. The $ABTS^{\circ+}$ solution was diluted in ethanol to an absorbance of (0.70 ± 0.05) at 734 nm for measurements. The photometric assay was performed on 0.9 mL of $ABTS^{\circ+}$ solution and 0.1 mL of extracts dissolved in ethanol solution and mixed for 45s; measurements were made at 734 nm after 30 min in the dark[46].

2.5.2. Evaluation of antimicrobial properties

From the saponin extraction, extracts of different parts of the Argan tree of mass 10 mg were solubilized in 5 µL of dimethyl sulfoxide (DMSO) and 995 µL of distilled water. They were filtered before any further use on Millipore filters (0.22 µm).

The antifungal activity was evaluated on the following fungi: *Fusarium oxysporum* and *Botrytis cinerea*. These fungi were provided by the Team of Microbiology and Molecular Biology (EMBM-FSR).

The bacteria used are the following: *Raoultella ornithinolytica*, (MZ853484.2), *Microbacterium resistens* (MZ853466.2), and *Staphylococcus saprophyticus* (OP750262.1), all isolated from a mine highly contaminated with metals and posing major health problems [47]. *Escherichia coli* (DH5α) was also tested. These bacteria were provided by the Team of Microbiology and Molecular Biology (EMBM-FSR).

2.5.2.1. Antifungal test

The fungi were grown on PDA (Potato Dextrose Agar) medium. After seven days of growth, these fungi were used to test the antifungal activity of the saponin extracts. To study the effect of the extracts on the growth of the fungi, petri dishes of 55 mm diameter were used. One hundred microliters of each extract were well spread on the plates containing PDA medium, the plates were left open under a laminar flow hood for a maximum of 30 minutes allowing the extracts to dry on agar. Finally, 0.6 cm discs of fungi were placed in the center of each plate and incubated at 25 °C for 4–5 days in the dark. A plate containing only the fungus was used as a control[47].

To evaluate the effect of the extracts on the growth of the fungi, the percentage of growth inhibition was calculated according to the following formula:

$$Inhibition\ \% = \frac{(\mathrm{dc} - d0) - (dg - d0)}{(dc - d0)} \times 100 \qquad (1)$$

Where dg is the diameter of the fungus growth in the presence of the extract; d0 is the diameter of the fungus disc deposited at T0; and dc is the diameter of the fungus growth in the control box.

2.5.2.2. Antibacterial test

Muller-Hinton agar was prepared and sterilized by autoclaving for 20 minutes at 120 °C. Then the medium was poured onto 90 mm petri dishes and left for 24 hours at room temperature to ensure no contamination.

Ten milliliters of nutrient broth were introduced into each test tube and then sterilized for 20 min at 120 °C by autoclaving. After 24H, the bacterial strains were plated on a liquid medium and left to grow for 24H. To prepare the working suspensions, the bacterial cultures were adjusted by nutrient broth to have an optical density of 0.1.

The solid disk diffusion method demonstrated antimicrobial activity[4]. One hundred microliters of bacterial suspension with OD of 0.1 of each microbial strain were well spread on a solid medium. Then discs were placed on top of the dried bacterial layer, each disc was soaked with 10 µL of extract in such a way as to have different concentrations (a total of 5) of the same extract per petri dish. The control petri dish contained tetracycline (TET 30 µg) antibiotic disc (Sigma).

All tests were repeated three times. Finally, the plates were incubated at 37 °C for 18 to 24 hours, depending on the bacterial growth. The diameter of the bacterial growth inhibition zone (a transparent halo around the discs) was measured (mm)[48], to evaluate the antibacterial activity.

2.6. Statistical Analysis

2.6.1. Principal Component Analysis (PCA)

The PCA is intended to establish the correlation between antioxidant activity, using DPPH and ABTS as much as free radicals and the chemical composition in polyphenols, flavonoids, and tannins for the crude extracts of different parts of the plant as well as the quantification of total sugars and tritepenic saponins of the set of extracts from the process of extraction of saponins from *A. spinosa*. PCA was performed on the results of (TPT, TFT, TTT, TsuT, TsapT, and TTC) and antioxidant capacity by two tests, ABTS and DPPH, which expressed the 8 variables for the five parts of *A. spinosa*.

2.6.2. Correlation matrix

Pearson correlations between TPT, TFT, TTT, TsuT, TsapT, TTC, ABTS, and DPPH were performed by PCA. Which PCA expressed the 5 samples according to their response values in a graph to facilitate the understanding of the variations of the data according to the nature of the Argan parts[49].

2.6.3. Analysis of the data

Pearson correlation was used to investigate the correlation between all parameters used for the Argan parts in this paper. PCA was performed by XLSTAT 2014 software. Data

were represented as mean ± standard error of the mean and were performed using IBM SPSS Statistics 21 software.

3. Results and Discussions

3.1. Yield of Extracts of Different Parts of Argania Spinosa

The yields obtained from the different extracts of each plant part of *A. spinosa* are shown in Figure 1. In the current study, water-ethanol was selected as the extraction solvent for its low toxicity and great solubility for both terpenes and saponins[50]. Most of the plant extract's saponins had low solubility, as evidenced by the fact that decreasing the solvent's polarity by adding more ethanol[36]. Indeed, a hydroalcoholic solution (ethanol/water 70/30) was used to extract different parts of the plant.

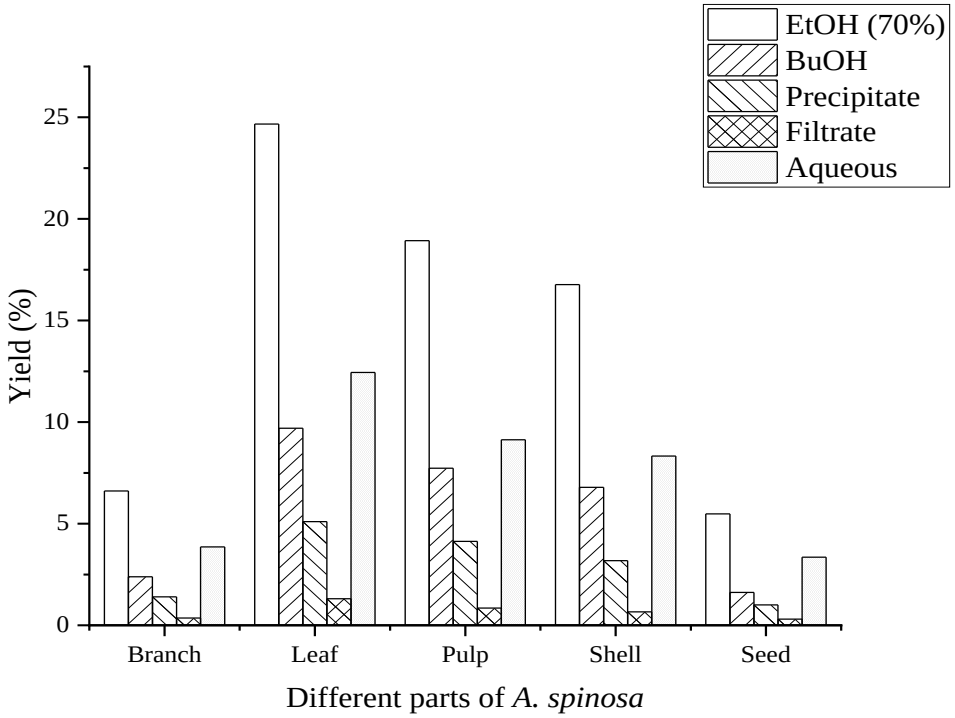

Figure 1. Histogram of yields of the different extracts of the parts of the *A. spinosa*.

The yields of the Argan tree plant differ from one part to another. However, the leaves of the Argan tree present very high yields, followed by the pulp, the shell, the branch, and finally the seed. Generally, the ethanolic extracts of the different parts of *A. spinosa* showed a significantly high percentage, followed by that of the aqueous phase, then the organic phase (butanol) then the precipitate, and finally the filtrate. The saponin content of fresh Argan grains is 0.5%[51], while the wood of the Argan tree is also particularly rich in saponins, these being found at a concentration of 5% to 6%[51,52], or a concentration twelve times higher than that of saponins in the cake. The crude content of saponin in the shell of the fruits of Argan can be 0.01% to 1%[51,52]. However, the pulp of the fruits of Argan is poor in saponins, whose concentration is only 0.02%[51].

3.2. Quantitative evaluation of total secondary metabolites

3.2.1. Quantitative evaluation of total polyphenols, flavonoids, and condensed tannins

In this work, we were interested in analyzing these results, focusing on the composition of polyphenols of 70% ethanolic extracts of different plant parts. The content of phenolic compounds of different parts of the Argan tree was evaluated by the colorimetric method of Folin-Ciocalteau.

These results show that the contents of total polyphenols, are higher at the level of the raw extract of the shell's value of 406.94 mg GAE/g extract followed by that of branch of 207.52 mg GAE/g extract, then leaf of 145.34 mg GAE/g extract then pulp 73.58 mg GAE/g extract, and finally the seed of 16.68 mg GAE/g extract (Table 1).

The results show that the flavonoid dosage follows the same logic as those of polyphenols. The dosage of total flavonoids was carried out again on the 70% ethanolic extracts of different parts of *A. spinosa*.

As for the polyphenols, the content of total flavonoids differs from one part to another of the Argania plant. The shell is the part of the plant that has the highest content of total flavonoids compared to other parts of the plant value of 50.67 mg QE/g extract, followed by a branch of 32.36 mg QE/g extract, then a leaf of 13.27 mg QE/g extract then pulp 7.74 mg QE/g extract, and finally, the seed of 2.03 mg QE/g extract (Table 1) The aerial part of the Argan tree is particularly rich in flavonoids. This metabolic fraction can reach 17% of the mixed leaves and branches[53,54]. The flavonoid content of the fruit pulp varies according to the degree of maturity of the fruit and according to more complex criteria (genotypic) whose impact would also be reflected in the shape of the fruit[55].

Tannins represent an exceptional class of polyphenols. Condensed tannins were determined only on the 70% ethanolic extracts of different parts of *A. spinosa*. The tannin molecules showed their presence only in two ethanolic extracts of the *A. spinosa* plant. However, the leaves present a significant value in condensed tannins at 182.66 mg CE/g of extract, compared to the shell, a value of 61.55 mg CE/g of extract (Table 1).

Table 1. Secondary metabolite content of 70% ethanolic extracts from different parts of *A. spinosa.*

Parts	TPT (mg GAE/g)	TFT (mg QE/g)	TTT (mg CE/g)
Branch	207.52 ± 0.79^{a}	32.36 ± 0.26^{a}	NR
Leaf	145.34 ± 0.66^{b}	13.27 ± 0.65^{b}	182.66 ± 0.55^{a}
Pulp	73.58 ± 0.72^{c}	7.74 ± 0.58^{c}	NR
Shell	406.94 ± 1.05^{d}	50.67 ± 0.26^{d}	61.55 ± 0.55^{b}
Seed	16.68 ± 0.26^{e}	2.03 ± 0.065^{e}	NR

Data are presented as the mean of three individual triplicates (n = 3 ± SEM); means followed by similar letters exposed in the same column are not different ($P < 0.05$). NR: No Results.

3.2.2. Evaluation of triterpene saponins present in extracts of different parts of the Argan tree

Saponin assay showed that the total triterpene saponins of Argan parts increased proportionally from the 70% ethanolic extract, followed by the butanolic extract, and finally, the precipitate (Table 2). Total saponin concentration in the organic fraction appears to increase modestly with cold ether precipitation. Again, the precipitate provided the highest total saponin content. Ether, thus contributing to higher saponin content. Also, since highly polar molecules like organic acids, polysaccharides, and proteins can stay in the aqueous phase, their higher solubility in ethanol than in water can be explained. While other relatively less polar substances, such as terpenoids and saponins, are dispersed in the organic phase, the butanolic phase has a higher total saponin content as a result.

Table 2. Tritepene saponin content (mg OAE/g) of extracts from different parts of *A. spinosa.*

	EtOH70%	**BuOH**	**Precipitate**	**Filtrate**	**Aqueous**
Branch	99.74±0.71^{a}	117.42±0.53^{a}	137.42±0.53^{a}	54.74±0.35^{a}	65.46±0.36^{a}
Leaf	98.14±0.89^{a}	104.56±0.53^{b}	160.10±0.26^{b}	50.81±0.71^{b}	64.92±0.53^{a}
Pulp	78.85±0.53^{b}	84.21±0.54^{c}	130.81±0.71^{a}	9.21±0.54^{c}	24.93±0.18^{b}
Shell	246.28±0.39^{c}	297.47±0.26^{d}	342.60±0.39^{c}	121.17±0.35^{d}	126.89±0.36^{c}
Seed	43.31±0.35^{d}	53.31±0.35^{e}	61.71±0.54^{d}	9.21±0.54^{c}	14.21±0.54^{d}

Data are presented as the mean of three individual triplicates (n = 3 ± SEM), means followed by similar letters exposed in the same column are not different ($P < 0.05$).

We were also interested in analyzing these results, focusing on the composition of triterpenic saponins of different parts of *Argania spinosa*. For any type of extract, the triterpene saponin content is best in the shell, followed by the leaf, branch, pulp, and the seed.

The shell precipitate presented the best content of triterpenic saponins with a value of 342.60 mg OAE/g of extract compared to the other precipitates of the plant. On the other hand, the seed precipitate of *A. spinosa* presented the lowest triterpene saponin value of 61.71 mg OAE/g of extract.

3.3. Quantification of total sugars

Sugars constitute the hydrophilic part of saponins. They can comprise one or more osidic chains (linear or branched) at different positions on the aglycone[51]. The contents of soluble sugars vary according to the plant species presented in Table 3. Generally, the amount of sugar in the 70% ethanolic extract is remarkably high in the pulp part of 477.50 mg D-GluE/g extract, followed by the branch part of 447.28 mg D-GluE/g extract, the leaf part of 379.42 mg D-GluE/g extract, the shell part of 291.86 mg D-GluE/g extract, and finally the seed part of 216.97 mg D-GluE/g extract.

Table 3. Total sugar content (mg D-GluE/g) of extracts from different parts of *A. spinosa.*

	EtOH70%	BuOH	Precipitate	Filtrate	Aqueous
Branch	447.28±1.77[a]	254.75±0.44[a]	300.97±0.89[a]	58.51±0.16[a]	160.08±0.44[a]
Leaf	379.42±1.11[b]	268.97±0.44[b]	340.97±0.89[b]	70.24±0.11[b]	193.48±0.11[b]
Pulp	477.50±1.77[c]	261.95±0.8[c]	270.31±0.89[c]	44.35±0.22[c]	147.86±0.66[c]
Shell	291.86±1.55[d]	143.59±0.44[d]	408.31±1.11[d]	119.70±0.55[d]	127.26±0.33[d]
Seed	216.97±0.44[e]	175.86±0.66[e]	317.86±0.44[e]	22.79±0.22[e]	336.30±0.22[e]

Data are presented as the mean of three individual triplicates (n = 3 ± SEM); means followed by similar letters exposed in the same column are not different ($P < 0.05$).

The part of the branch, leaf, and pulp have kept the total sugar classification of extracts as: Ethanol 70% ; Precipitate ; Butanol ; Aqueous ; and Filtrate. However, the seed and shell precipitate extract showed better total sugar contents than the ethanolic extract with values of 408.31 mg D-GluE/g extract and 317.86 mg D-GluE/g extract, respectively. Also, the aqueous phase of Argan seed presented a total sugar content of 336.30 mg D-GluE/g extract higher than its precipitate. The sugar content is strongly influenced by the plant parts and the choice of solvent.

3.4. Evaluation of the antioxidant activity

3.4.1. Determination of the scavenger effect of the DPPH radical

The data on the antioxidant activity of the extracts of different parts of Argania towards the free radical DPPH are summarized in Table 4. The anti-free radical activity by DPPH was measurably better in the precipitate of each part of the Argan tree compared to the other extracts studied. The precipitate of the shell shows the best IC_{50} value of 1.57 µg/mL, followed by the leaves (2.63 µg/mL), the branch (11.23 µg/mL), the pulp (90.74 µg/mL), and finally, the seed (372.87 µg/mL). The IC_{50} values of the shell as well as leaf and branch are very close to that of ascorbic acid (1.92 µg/mL) compared to those of pulp and seed. The shell, leaf, and branch present better antioxidant activity than the pulp and seed. With the exception of the seed of the Argan tree, the other extracts demonstrated significant antioxidant power as assessed by DPPH. According to the results obtained in Table 4, we noticed a chronological order in IC_{50} of the DPPH of the extracts of the Argan tree as follows: precipitate ; butanol ; ethanolic 70% ; aqueous, and filtrate. While the Argan tree seeds showed no antioxidant activity by DPPH in the butanolic, ethanolic, filtrate, and aqueous extracts for a concentration range of up to 2000 µg/mL. This result of antioxidant activity can be translated by the content of secondary metabolites. The free radical scavenging activity of the saponins in the cake was determined against DPPH, and IC_{25} = 85 mM was observed, while an IC_{25} = 560 mM was established against hydroxy radicals[56].

Table 4. Evaluation of the antioxidant activity of extracts of different parts of *A. spinosa* by DPPH.

IC_{50} (µg/mL)	EtOH70%	BuOH	Precipitate	Filtrate	Aqueous
Branch	15.77±0.11[a]	12.57±0.04[a]	11.23±0.16[a]	25.27±0.37[a]	17.03±0.03[a]
Leaf	7.52±0.17[b]	3.00±0.01[b]	2.63±0.02[b]	9.74±0.13[b]	8.51±0.065[b]
Pulp	164.27±0.8[c]	149.12±1.7[c]	90.74±0.79[c]	180.35±0.70[c]	177.34±0.85[c]
Shell	5.85±0.16[b]	2.94±0.02[b]	1.57±0.2[b]	8.20±0.02[b]	6.48±0.085[b]
Seed	NA	NA	372.87±0.05[d]	NA	NA
Ascorbic Acid			1.92 ± 0.04[d]		

Data are presented as the mean of three individual triplicates (n = 3 ± SEM); means followed by similar letters exposed in the same column are not different ($P < 0.05$). NA: No Activity.

3.4.2. Determination of the scavenger effect of the ABTS radical.

In our work, we opted for ABTS to test extracts of different parts of *A. spinosa*. The results obtained illustrated in Table 5 showed that the inhibitory action of the samples was improved from the crude extract of 70% ethanol to the butanolic fraction as well as its precipitate for the different parts of the plant except the seeds. The precipitate of the latter part showed low antioxidant activity in a concentration range of up to 2000 µg/mL.

Table 5. Evaluating the antioxidant activity of extracts of different parts of *A. spinosa* by ABTS.

IC_{50} (µg/mL)	EtOH70%	BuOH	Precipitate	Filtrate	Aqueous
Branch	61.25±0.49[a]	59.4±0.33[a]	56.53±0.57[a]	73.82±0.06[a]	68.68±0.39[a]
Leaf	33.85±0.28[b]	29.6±0.19[b]	24.07±0.12[b]	46.05±0.22[b]	41.01±0.06[b]
Pulp	210.64±1.55[c]	179.13±1.03[c]	174.24±0.78[c]	270.16±2.91[c]	255.34±1.26[c]
Shell	31.48±0.17[b]	27.25±0.47[b]	21.77±0.02[b]	44.16±0.41[b]	39.37±0.24[b]
Seed	NA	NA	516.86±4.03[d]	NA	NA
Ascorbic Acid			11.31±0.05[e]		

Data are presented as the mean of three individual triplicates (n = 3 ± SEM); means followed by similar letters exposed in the same column are not different ($P < 0.05$). NA: No Activity.

However, the other extracts of *A. spinosa* seed showed no activity against the ABTS radical, which means that a high concentration of the samples is necessary to present its antioxidant power. As for the DPPH test, the precipitate of the shell has the best IC_{50} value (21.77 µg/mL), followed by leaves (24.07 µg/mL), the branch (56.53 µg/mL), the pulp (174.24 µg/mL), and finally the seed (516.86 µg/mL). The precipitate of the shell and leaf was very close to the value of ascorbic acid (11.31 µg/mL). Saponins were associated with various biological activities, such as antioxidant activity[57]. It was shown that the total flavonoid extract of the leaves has antiradical and antioxidant activity. The antioxidant effect was confirmed by observing a reduction in the effects of oxidative stress produced on UV-

irradiated human skin cells[58]. The extract of flavonoids from the Argan tree could thus be used in cosmetology as a skin protector[58]. The confirmation of these effects *in vivo* would allow great valorization of the leaves of the Argan tree.

3.5. Evaluation of the Antimicrobial Activity of Argan Extracts

The study of the antimicrobial activity of the extracts of different parts of Argania prepared at a concentration of 10 mg/mL was examined on microorganisms by the disc diffusion method.

3.5.1. Antibacterial activity

The antibacterial activity of the extracts of different parts of *Argania spinosa* was evaluated on different bacterial strains: *Escherichia coli*, *Microbacterium resistens*, *Raoultella ornithinolytica*, and *Staphylococcus saprophyticus*. The inhibition results generated by the extracts from different parts of *Argania spinosa* after 24 hours at 37 °C incubation are recorded in Table 6.

Based on the results in Table 6, the extracts of different parts of the *Argania spinosa* plant showed no bactericidal activity on *Escherichia coli* and *Raoultella ornithinolytica* strains. However, saponin precipitates obtained from the leaf and shell of *Argania spinosa* had inhibitory effects on the growth of *Microbacterium resistens*, with inhibition zone measurements of 10 mm and 11 mm, respectively. As for the extracts EtOH 70%, aqueous and filtrate of the leaf of the Argan tree showed an inhibition of the growth of the bacterium *Staphylococcus saprophyticus*, yielding inhibitory zones of 13 mm, 13 mm, and 12 mm, respectively. The aqueous phase of the leaves was the only one that showed antibacterial activity against both *Microbacterium resistens* and *Staphylococcus saprophyticus*. The filtrate of the *Argania spinosa* pulp exhibited sensitivity against *Microbacterium resistens* bacteria, resulting in an inhibition zone of 9 mm. Generally, the leaves of *Argania spinosa* were the only ones that exhibited stronger antibacterial activity against *Microbacterium resistens* and *Staphylococcus saprophyticus* compared to other parts of the plant studied. Saponin extract from Argan oilcake showed antibacterial activity at a concentration of 500 µg/mL on *Cutibacterium acne* bacteria, while no inhibition of bacterial growth was observed against *Prevotella intermedia* up to the highest concentration (12500 µg/mL)[59].

Table 6. Inhibition zone diameter (mm) of different extracts from different parts of *Argania spinosa* compared to Tetracycline (TET) as reference.

Inhibition zone diameter (mm)		***Escherichia coli***	***Microbacterium resistens***	***Raoultella ornithinolytica***	***Staphylococcus saprophyticus***
Branch	EtOH 70%	-	-	-	-
	BuOH	-	-	-	-
	Aqueous	-	-	-	-
	Precipitate	-	-	-	-
	Filtrate	-	-	-	-
Leaf	EtOH 70%	-	-	-	13 ± 0.05^{a}
	BuOH	-	-	-	-
	Aqueous	-	15 ± 0.02^{a}	-	13 ± 0.07^{a}

Inhibition zone diameter (mm)		*Escherichia coli*	*Microbacterium resistens*	*Raoultella ornithinolytica*	*Staphylococcus saprophyticus*
	Precipitate	-	10 ± 0.03[a]	-	-
	Filtrate	-	-	-	12 ± 0.03[a]
Pulp	EtOH 70%	-	-	-	-
	BuOH	-	-	-	-
	Aqueous	-	-	-	-
	Precipitate	-	-	-	-
	Filtrate	-	9 ± 0.03[a]	-	-
Shell	EtOH 70%	-	-	-	-
	BuOH	-	-	-	-
	Aqueous	-	-	-	-
	Precipitate	-	11 ± 0.05[a]	-	-
	Filtrate	-	-	-	-
Seed	EtOH 70%	-	-	-	-
	BuOH	-	-	-	-
	Aqueous	-	-	-	-
	Precipitate	-	-	-	-
	Filtrate	-	-	-	-
TET 30 µg		23	33	15	25

Data are presented as the mean of three individual triplicates (n = 3 ± SEM), means followed by similar letters exposed in the same column are not different ($P < 0.05$).

3.5.2. Antifungal activity

The antifungal activity of different parts of Argania spinosa extracts was investigated against two fungi *Botrytis cinerea* and *Fusarium oxysporum*. The results obtained on *Botrytis cinerea*, after incubation in the dark at 25 °C for five days, show that our prepared samples do not contribute to any antifungal activity (Table 7).

Unlike *Fusarium oxysporum* which showed inhibitions ranging from 1.92% to 32.69% for some extracts from the five parts of *A. spinosa*. The 70% of leaf, pulp, shell, and seed ethanolic extracts showed various inhibitions of 1.92%, 13.46%, 26.92%, and 1.92 %, respectively. While the butanolic fraction showed antifungal activity only at the branch, shell, and seed parts. The latter part of the organic fraction showed good antifungal activity with a value of 32.69% compared to the branch and shell of 5.77% and 17.31%, respectively. As for the saponin precipitates, the different parts of the plant revealed their ineffectiveness towards the two studied fungi, which means that a high concentration of the saponin precipitates samples is necessary to present its antifungal power and/or the range of chosen fungi or yeasts to prove this effect. Also, the aqueous phase of the branch, pulp, and seed showed inhibitions towards *Fusarium oxysporum* fungus of a significant value of 32.69% for seed and pulp, while the branch presented a low value of 1.92%. In addition, the seed is the only part of the Argan tree that showed antifungal activity in its different extracts except for its saponin precipitate. This result confirms then that the seed part of the Argan tree fruit has antifungal potential rather than antibacterial activity. Saponins are natural substances with a wide spectrum of biological activity; some are already used in therapeutics as hepatoprotective, anti-inflammatory, and anticancer activities[37]. Antifungal studies have revealed an action against *Cladosporium cucumerinum* (phytopathogenic fungus of the Cucurbitaceae family), *Polysticus versicolor*, and a fungus pathogenic to humans: *Candida*

albicans. The minimum inhibitory concentrations are 12.5 and 50 µg/mL for *C. cucumerinum* and *P. versicolor*, respectively. On the other hand, the mixture of saponins does not show any activity against *Candida albicans*[60].

Table 7. Evaluation of the antifungal power of extracts of different parts of *Argania spinosa* by the disc diffusion method.

Inhibition %		*Botrytis cinerea*	*Fusarium oxysporum*
Branch	EtOH 70%	-	-
	BuOH	-	5.77 ± 0.05[a]
	Aqueous	-	1.92 ± 0.06[b]
	Precipitate	-	-
	Filtrate	-	-
Leaf	EtOH 70%	-	1.92 ± 0.02[b]
	BuOH	-	-
	Aqueous	-	-
	Precipitate	-	-
	Filtrate	-	-
Pulp	EtOH 70%	-	13.46 ± 0.08[c]
	BuOH	-	-
	Aqueous	-	32.69 ± 0.08[d]
	Precipitate	-	-
	Filtrate	-	-
Shell	EtOH 70%	-	26.92 ± 0.07[e]
	BuOH	-	17.32 ± 0.04[f]
	Aqueous	-	-
	Precipitate	-	-
	Filtrate	-	-
Seed	EtOH70%	-	1.92 ± 0.02[b]
	BuOH	-	32.69 ± 0.07[d]
	Aqueous	-	32.69 ± 0.09[d]
	Precipitate	-	-
	Filtrate	-	9.62 ± 0.04[g]

Data are presented as the mean of three individual triplicates (n = 3 ± SEM); means followed by similar letters exposed in the same column are not different ($P < 0.05$).

3.6. Statistical Analysis

3.6.1. Correlation matrix

The correlation coefficients for TPT, TFT, TTT, TsuT, TsapT, ABTS, and DPPH are presented in Table 8. Also, DPPH (1/DPPH IC_{50}) and ABTS (1/ABTS IC_{50}) represent the free radical inhibition power, DPPH, and ABTS, respectively. However, Table 9 shows the p-values of the correlation matrix coefficient between all variables.

Table 8. Coefficient of the Pearson correlation matrix between the antioxidant capacity and antioxidant compounds in the different parts of the Argan tree.

Variables	TPT	TFT	TTT	TsuT	TsapT	1/DPPH	1/ABTS
TPT	1						
TFT	0.980	1					
TTT	0.210	0.057	1				
TsuT	-0.042	-0.003	-0.037	1			

Variables	TPT	TFT	TTT	TsuT	TsapT	1/DPPH	1/ABTS
TsapT	0.963	0.925	0.369	-0.226	1		
1/DPPH	0.861	0.769	0.673	-0.113	0.930	1	
1/ABTS	0.801	0.717	0.730	0.059	0.863	0.977	1

Values in bold differ from 0 at a significance level of alpha = 0.05.

Table 9. *p*-values of the correlation matrix coefficient between all variables.

Variables	TPT	TFT	TTT	TsuT	TsapT	1/DPPH	1/ABTS
TPT	0						
TFT	0.004	0					
TTT	0.734	0.928	0				
TsuT	0.946	0.996	0.952	0			
TsapT	0.009	0.025	0.541	0.714	0		
1/DPPH	0.061	0.128	0.213	0.857	0.022	0	
1/ABTS	0.104	0.173	0.161	0.925	0.060	0.004	0

Values in bold differ from 0 at a significance level of alpha = 0.05.

According to Table 8 and Table 9, Pearson's correlation analysis between antioxidant activity and phytochemical amounts revealed that TsapT had a strong positive correlation (*p*-value < 0.05) with antioxidant activity. The correlation data of TsapT was 0.930 with the scavenger effect of DPPH radical. The linear positive correlation between polyphenols (TPT) and flavonoids (TFT) was also observed (r^2 = 0.980). However, tannins (TTT) did not contribute much to antioxidant activities. In addition, the correlation matrix showed a strong correlation between ABTS and DPPH (r^2 = 0.977).

3.6.2. Analysis of the data by PCA

The results of the testing on phytochemicals are regarded as variables. The PCA in Figure 2 (F1-F2) factorial design projects them. 67.84% of the information is presented in the first component (F1), and 17.11% is explained in the second component (F2). 84.94% is the combined proportion of the first two elements. However, because their linear combination is more than 50%, it already adequately represents the variables. As a result, the first two axes can adequately explain all of the data. The plane created by the axes F1 and F2 in Figure 2 depicts the correlation between the variables. The positive correlation between the DPPH and ABTS tests and the TPC, TFC, and TsapT tests substantially determines the shape of the F1 axis (Figure 2).

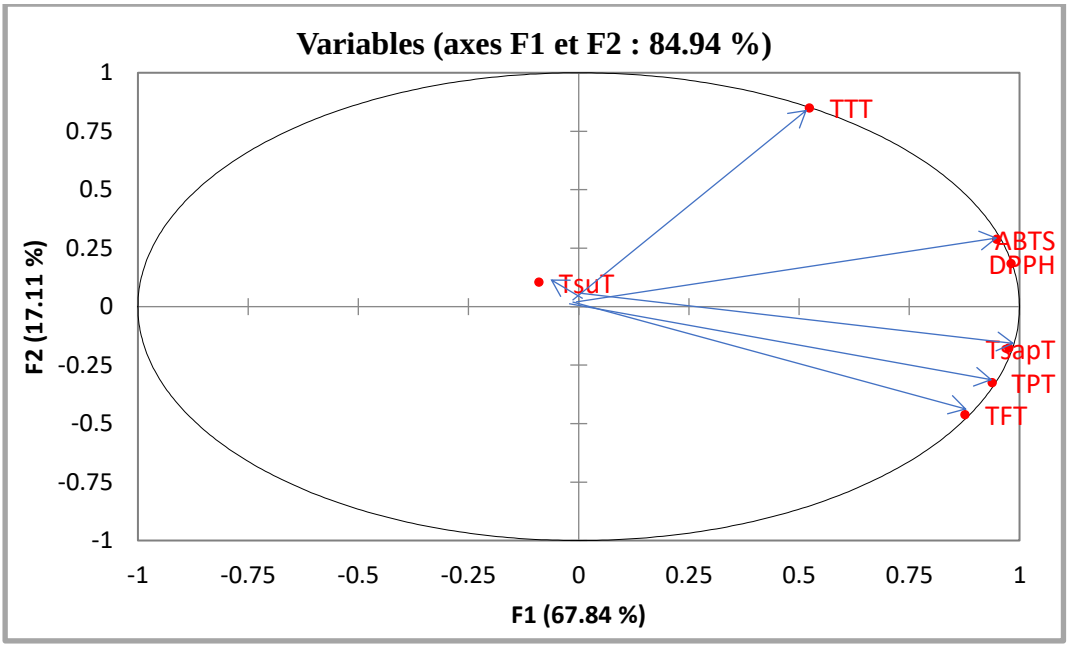

Figure 2. Factorial plan of principal component analysis carried out on the values (TPT, TFC, TTC, TsapT, TsuT, DPPH and ABTS) of different parts of the Argan tree.

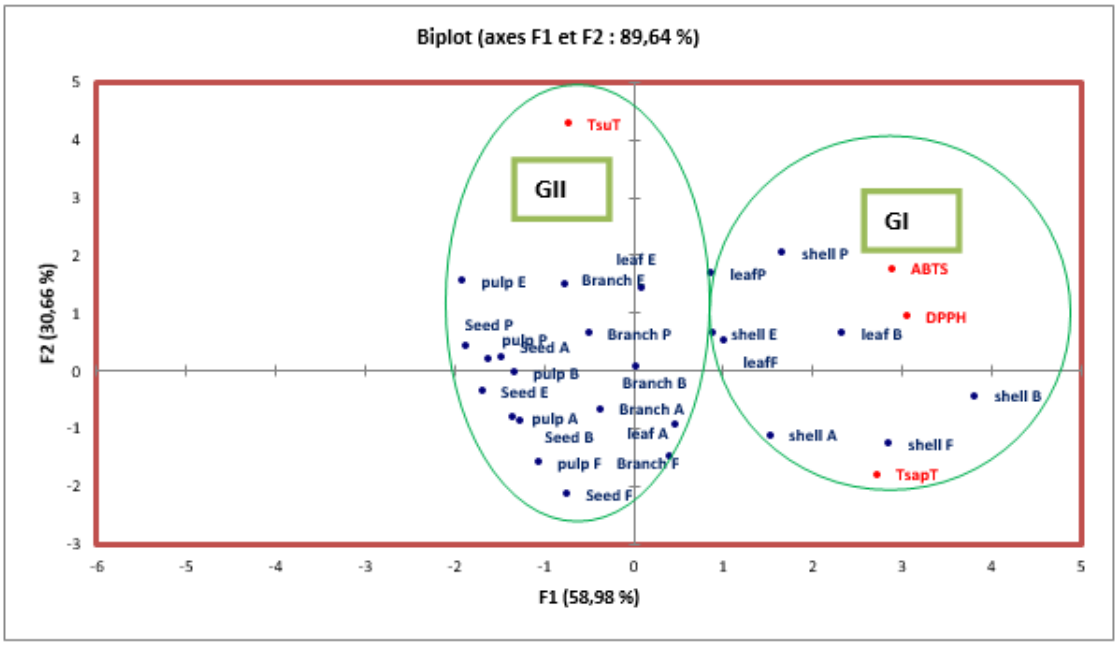

Figure 3. Projection on the factorial plane (F1×F2) of the variables of different parts of *A. spinosa*. GI: Group I; GII: Group II DPPH(1/DPPH IC_{50};ABTS(1/ABTS IC_{50}).

The five individuals are depicted in Figure 3 as split into two groups (extracts). Group I contain 8 extracts (shell P, leaf B, leaf P, shell E, leaf F, shell A, shell F, shell B), According to the DPPH and ABTS assays, this group's antioxidant capacity is noticeably stronger than that of group II. This group's antioxidant activity is also distinguished by extremely high TsapT levels.

The 17 remaining extracts comprise Group II, distinguished by its high TsuT level and lower antioxidant activity when compared to extracts from Group I by the DPPH and ABTS assays. This low antioxidant activity is reflected in the low content of TsapT.

4. Conclusions

The technique of butanol fractionation followed by ether precipitation appears to be important for recovering saccharide-containing compounds from the crude extract of *A. spinosa*. Given the calorimetric results of the colorimetric test using vanillin and sulfuric acid, it turned out that the precipitates of the different parts of *A. spinosa* are rich in saponin molecules. This family of molecules seems to be the secondary metabolites with the best potential for industrial use. For this purpose, the biological activities of the extracts of different parts of A. spinosa was evaluated. The study of the antimicrobial activity of the extracts of the Argan tree prepared at a concentration of 10 mg/mL was examined on microorganisms by the disc diffusion method. The extracts of saponin EtOH 70% and aqueous from leaves of *A. spinosa* plant showed significant antibacterial activity against *Microbacterium resistens*, with a remarkable inhibition zone of 13 mm for both extracts. While the saponin extracts did not exhibit any antifungal activity against *Botrytis cinerea*, many of them presented an antifungal activity ranging from 1.92% to 32.69% inhibition of the growth of *Fusarium oxysporum* fungi. In addition, the antioxidant activity of the extracts of different parts of the studied plant was also evaluated by two methods: DPPH free radical test and ABTS free radical test. The different tests showed that the samples' inhibitory action was improved from the crude extract of 70% ethanol to the butanol fraction as well as it's precipitate for the different parts of the plant except the unroasted shells of *A. spinosa*. In addition, the PCA data analysis study demonstrated a positive correlation between the content of saponin compounds and antioxidant activity.

Author Contributions: Conceptualization - NM, CEG, AEY. HH, AB; methodology - YEI, YE, HEM, NM, CEG, AB, MT; software - AZ, KWG, LCM, AB, MT; validation - YEI, YE, HEM, NM, CEG, AEY; analysis - YEI, YE, HEM, NM, CEG, AEY, HH, AZ, AB, MT; investigation - YEI, YE, HEM, AB, MT; resources - HH, AZ, KWG, LCM; data curation - YEI, YE, HEM, NM, CEG, AEY; writing of original draft – YEI, YE, HEM, NM, CEG, AEY; writing, review, and editing – AZ, KWG, LCM, AB, MT.

Funding: No external funding was provided for this research.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Elloumi M. Changements Dpropes Modes de Gouvernance et Tension Sur Les Ressources Naturelles Au Maghreb. Revue des Régions Arides 2013**;** 30(1): 191–205.
2. Ab Mutalib N-S, Wong SH, Ser H-L, *et al.* Bioprospecting of Microbes for Valuable Compounds to Mankind. Prog Microbes Mol Biol 2020; 3(1).
3. Mrehil Elsalami R, Goh KW, Mahadi M, *et al.* The Antibacterial Activities of Secondary Metabolites Derived from Streptomyces Sp. Prog Microbes Mol Biol 2022; 5(1).
4. Battah B, Rajab A, Shbibe L, *et al.* Evaluation of Antibiofilm Activity of Thymus Syriacus Essential Oil against Clinically Isolated MDR Bacteria. Prog Microbes Mol Biol 2022; 5(1).

5. Bouaouda K, Elagdi C, El Hachlafi N, *et al.* HPLC-UV-MS/MS Profiling of Phenolics from Euphorbia Nicaeensis (All.) Leaf and Stem and Its Antioxidant and Anti-Protein Denaturation Activities. Prog Microbes Mol Biol 2023; 5: 6(1).
6. Zouhra D, El Jemli M, Bouyahya A, *et al.* Radical-Scavenging Effect, Ferric Reducing Ability and Phytochemical Analysis of Urtica Urens (L.) and Mercurialis Annua (L.). Prog Microbes Mol Biol 2023; 7:6(1).
7. Taous F, Amenzou N, Marah H, *et al.* Stable Isotope Ratio Analysis as a New Tool to Trace the Geographical Origin of Argan Oils in Morocco. Forensic Chem 2020; 17: 100198.
8. Elouafy Y, el Idrissi ZL, el Yadini A, *et al.* Variations in Antioxidant Capacity, Oxidative Stability, and Physicochemical Quality Parameters of Walnut (Juglans Regia) Oil with Roasting and Accelerated Storage Conditions. Molecules 2022; 27(22).
9. Mrabti HN, Doudach L, Mekkaoui M, *et al.* Profile of Medicinal Plants Traditionally Used for the Treatment of Skin Burns. Evid Based Complement Alternat Med 2022; 2022: 1–10.
10. Melhaoui R, Kodad S, Houmy N, *et al.* Characterization of Sweet Almond Oil Content of Four European Cultivars (Ferragnes, Ferraduel, Fournat, and Marcona) Recently Introduced in Morocco. Scientifica (Cairo) 2021; 2021: 1–10.
11. Elouafy Y, el Yadini A, el Moudden H, *et al.* Influence of the Extraction Method on the Quality and Chemical Composition of Walnut (Juglans Regia L.) Oil. Molecules 2022; 27(22).
12. Drioua S, El-Guourrami O, Ameggouz M, *et al.* Toxicological Review of Anticancer Plants Used in Traditional Medicine in Morocco. Prog Microbes Mol Biol 2023; 6(1).
13. El Yadini A, Elouafy Y, Amiri-Ardekani E, *et al.* A Comprehensive Review of the Pharmacological Properties and Bioactive Components of Retama Monosperma. Molecules 2023; 28(4): 1708.
14. El Hachlafi N, Benkhaira N, Al-Mijalli SH, *et al.* Phytochemical Analysis and Evaluation of Antimicrobial, Antioxidant, and Antidiabetic Activities of Essential Oils from Moroccan Medicinal Plants: Mentha Suaveolens, Lavandula Stoechas, and Ammi Visnaga. Biomed Pharmacother 2023; 164: 114937.
15. El Alaoui N. Paysages, Usages et Voyages d'Argania Spinosa (L.) Skeels (IXe-Xe Siècles). Journal d'agriculture traditionnelle et de botanique appliquée 1999; 41(2): 45–79.
16. Msanda F, El Aboudi A, Peltier J-P. Biodiversité et Biogéographie de l'arganeraie Marocaine. Cahiers Agricultures 2005; 14(4): 364.
17. Hilali M, Charrouf Z, Soulhi A, *et al.* Influence of Origin and Extraction Method on Argan Oil Physico-Chemical Characteristics and Composition. J Agric Food Chem 2005; 53(6): 2081–2087.
18. Matthäus B, Guillaume D, Gharby S, *et al.* Effect of Processing on the Quality of Edible Argan Oil. Food Chem 2010; 120(2): 426–432.
19. Harhar H, Gharby S, Ghanmi M, *et al.* Composition of the Essential Oil of Argania Spinosa (Sapotaceae) Fruit Pulp. Nat Prod Commun 2010; 5(6): 935–936.
20. Gharby S, Harhar H, Guillaume D, *et al.* Oxidative Stability of Edible Argan Oil: A Two-Year Study. LWT - Food Sci Technol 2011; 44(1): 1–8.
21. Charrouf Z, Guillaume D. Argan Oil: Occurrence, Composition and Impact on Human Health. Eur J Lipid Sci Technol 2008; 110(7): 632–636.
22. Charrouf Z, Guillaume D. Should the Amazigh Diet (Regular and Moderate Argan-Oil Consumption) Have a Beneficial Impact on Human Health? Crit Rev Food Sci Nutr 2010; 50(5): 473–477.
23. Adlouni A. Argan Oil: From Nutrition to Health. Phytotherapie 2010; 8(2): 89–97.

24. IMANOR. Corps gras d'origines animale et végétale – Huiles d'argane – Spécifications – Norme Code : NM 08.5.090. Moroccan Standard MS 2003; Available from: https://www.imanor.gov.ma/Norme/nm-08-5-090/
25. Gharby S, Harhar H, el Monfalouti H, *et al.* Chemical and Oxidative Properties of Olive and Argan Oils Sold on the Moroccan Market. A Comparative Study. Med J Nutrition Metab 2012; 5(1): 31–38.
26. Du J-R, Long F-Y, Chen C. Research Progress on Natural Triterpenoid Saponins in the Chemoprevention and Chemotherapy of Cancer. In *Enzymes*; Academic Press, 2014; 95–130.
27. Massiot G, Lavaud C, Delaude C, *et al.* Saponins FromTridesmostemon Claessenssi. Phytochemistry 1990; 29(10): 3291–3298.
28. Nigam SK, Xing-Cong L, De-Zu W, *et al.* Triterpenoidal Saponins from Madhuca Butyracea. Phytochemistry 1992; 31(9): 3169–3172.
29. Jiang Y, Ali AO, Guillaume D, *et al.* Triterpenoid Saponins from the Root of Sideroxylon Cubense. Phytochemistry 1994; 35(4): 1013–1015.
30. Li X, Liu Y, Wang D, *et al.* Triterpenoid Saponins from Madhuca Butyracea. Phytochemistry 1994; 37(3): 827–829.
31. Sahu NP, Koike K, Jia Z, *et al.* Novel Triterpenoid Saponins from Mimusops Elengi. Tetrahedron 1995; 51(48): 13435–13446.
32. Lavaud C, Massiot G, Becchi M, *et al.* Saponins from Three Species of Mimusops. Phytochemistry 1996; 41(3): 887–893.
33. Gosse B, Gnabre J, Bates RB, *et al.* Antiviral Saponins from *Tieghemella h Eckelii*. J Nat Prod 2002; 65(12): 1942–1944.
34. Eskander J, Lavaud C, Pouny I, *et al.* Saponins from the Seeds of Mimusops Laurifolia. Phytochemistry 2006; 67(16): 1793–1799.
35. Ahmed R, Rashid F, Ahmed VU, *et al.* Saponins from the Seeds of Achras Sapota. J Asian Nat Prod Res 2008; 10(1): 7–16.
36. Chua LS, Lau CH, Chew CY, *et al.* Solvent Fractionation and Acetone Precipitation for Crude Saponins from Eurycoma Longifolia Extract. Molecules 2019; 24(7): 1416.
37. Elouafy Y, Mortada S, El Yadini A, *et al.* Bioactivity of Walnut: Investigating the Triterpenoid Saponin Extracts of Juglans Regia Kernels for Antioxidant, Anti-Diabetic, and Antimicrobial Properties. Prog Microbes Mol Biol 2023; 6(1).
38. Elouafy Y, El Yadini A, Mortada S, *et al.* Antioxidant, Antimicrobial, and α-Glucosidase Inhibitory Activities of Saponin Extracts from Walnut (Juglans Regia L.) Leaves. Asian Pac J Trop Biomed 2023; 13(2): 60–69.
39. Charrouf Z, Wieruszeski JM, Fkih-Tetouani S, *et al.* Triterpenoid Saponins from Argania Spinosa. Phytochemistry 1992; 31(6): 2079–2086.
40. El Idrissi Y, El Moudden H, Harhar H, *et al.* Comparison and Correlation of Phytochemical Content with Antioxidant Potential of Different Parts of Argan Tree, Argania Spinosa L. Caspian J Environment Sci 2021; 19(2): 261–266.
41. El Guezzane C, El Moudden H, Harhar H, *et al.* Optimization of Roasting Conditions on the Bioactive Compounds and Their Antioxidant Power from Opuntia Fiscus-Indica Seeds Using Response Surface. Biointerface Res Appl Chem 2021; 11(3): 10510–10532.
42. Sun B, Ricardo-da-Silva JM, Spranger I. Critical Factors of Vanillin Assay for Catechins and Proanthocyanidins. J Agric Food Chem 1998; 46(10): 4267–4274.

43. Belmaghraoui W, Manni A, Harir M, *et al.* Phenolic Compounds Quantification, Antioxidant and Antibacterial Activities of Different Parts of Urtica Dioica and Chenopodium Murale. Res J Pharm Technol 2018; 11(12): 5490–5496.
44. El Moudden H, El Idrissi Y, El Yadini A, *et al.* Effect of Filtration of Olive Mill Wastewater on the Phenolic Composition and Its Influence on Antioxidant Activity. Pharmacologyonline 2019; 2: 161–176.
45. Boujemaa I, El Bernoussi S, Harhar H, *et al.* The Influence of the Species on the Quality, Chemical Composition and Antioxidant Activity of Pumpkin Seed Oil. OCL 20202; 27: 40.
46. Kemung HM, Tan LT-H, Chan K-G, *et al.* Streptomyces Sp. Strain MUSC 5 from Mangrove Forest in Malaysia: Identification, Antioxidant Potential and Chemical Profiling of Its Methanolic Extract. Prog Microbes Mol Biol 2020; 3(1).
47. Mghazli N, Bruneel O, Zouagui R, *et al.* Characterization of Plant Growth Promoting Activities of Indigenous Bacteria of Phosphate Mine Wastes, a First Step toward Revegetation. Front Microbiol 2022; 13.
48. Hajib A, Nounah I, Oubihi A, *et al.* Chemical Composition and Biological Activities of Essential Oils from the Fruits of *Cuminum Cyminum* L. and *Ammodaucus Leucotrichus* L. (Apiaceae). Journal of Essential Oil Bearing Plants 2020; 23(3): 474–483.
49. El Guezzane C, El Moudden H, Harhar H, *et al.* A Comparative Study of the Antioxidant Activity of Two Moroccan Prickly Pear Cultivars Collected in Different Regions. Chemical Data Collections 2021; 31: 100637.
50. Kuo P-C, Damu AG, Lee K-H, *et al.* Cytotoxic and Antimalarial Constituents from the Roots of Eurycoma Longifolia. Bioorg Med Chem 2004; 12(3): 537–544.
51. Guillaume D, Charrouf Z. Saponines et Métabolites Secondaires de l'arganier (Argania Spinosa). Cahiers Agricultures 2005; 14(6): 509-516 (1).
52. Chafchaouni-Moussaoui I, Charrouf Z, Guillaume D. Triterpenoids from Argania Spinosa: 20 Years of Research. Nat Prod Commun 2013; 8(1): 43–46.
53. Tahrouch S, Rapior S, Bessière J-M, *et al.* Les Substances Volatiles de *Argania Spinosa* (*Sapotaceae*). Acta Botanica Gallica 1998; 145(4): 259–263.
54. Charrouf Z, Guillaume D, Driouich A. The Argan Tree, an Asset for Morocco. Biofutur (France) 2002; 220(220): 54-56.
55. Chernane H, Hafidi A, el Hadrami I, *et al.* Phenolic Derivatives from the Pulp of the Fruits of Argan Tree (Argania Spinosa L. Skeels) and Their Relationships with Morphological Features. Agrochimica 1999; 43: 1337–1350.
56. Alaoui K. Toxicité et Action Pharmacologique Des Saponines Du Tourteau d'Argania Spinosa L.; Doctoral dissertation, Université Ain Chock, Casablanca, université de Casablanca 1998.
57. Milugo TK, Omosa LK, Ochanda JO, *et al.* Antagonistic Effect of Alkaloids and Saponins on Bioactivity in the Quinine Tree (Rauvolfia Caffra Sond.): Further Evidence to Support Biotechnology in Traditional Medicinal Plants. BMC Complement Altern Med 2013; 13(1): 285.
58. Pauly G, Henry F, Danoux L, *et al.* Cosmetic and/or Dermopharmaceutical Composition Containing Extracts Obtained from the Leaves of Argania Spinosa. Pat Appl EP 2002; 12: 13025.
59. Lall N, Canha M de, Szuman K, *et al.* The Anti-Proliferative and Anti-Bacterial Activity of Argan Oil and Crude Saponin Extract from Argania Spinosa (L.) Skeels. Pharmacognosy J 2019; 11(1): 26–31.
60. Charrouf Z. Valorisation d'Argania Spinosa (L.) Sapotaceae: Etude de La Composition Chimique et de l'activité Biologique Du Tourteau et de l'extrait Lipidique de La Pulpe, Mohammed V University: Rabat, 1991.