Secondary Metabolites And Antioxidant Activity Of Argania Spinosa (L.) Almonds Cultivated In The Three Region Of Morrocco

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Abstract

Argania spinosa (L.) almond from tree region of Morocco have been analyzed for their secondary metabolites and antioxidant activities. The aim of this study was to determine the evolution of biometric parameters, antioxidant activity by DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and hydrogen peroxide scavenging assay as well as total phenolic and flavonoid content of the argan almonds from three Moroccon regions (three argan plantation in the region of Casablanca, Dar Bouazza and Rhamna). Three characters were measured : weight, length and width of the argan almonds. The results showed that the almonds of the Dar Bouazza region presented the higher average width and weight and almonds of Rhamna had the highest average lenght. Total phenolic content extracted from almond using aqueous ethanol (40%, 60% and 80% ethanol) ranged from 12.18 to 48.27 mg GAE/g extract. The total flavonoid contents varied from 1.22 to 5.61 mg QE/g extract. Moreover, Antioxidant activity of different almond extracts from the three regions ranged from 48.35 and 77.43 µg/mL. The results showed that the ethanol extract of 40% from Dar Bouazza region, showed the highest phenolic (42.27mg GAE/g) and flavonoid (5.61 mg GAE/g) content and strong antioxidant and hydrogen peroxide scavenging activity.

These results suggest that Argania spinosa (L.) almond extracts from the three regions could be used as natural plant sources of antioxidants.

Keywords: Argan plantation; Argan almonds; Biometeric parameters; extraction; DPPH; phenolics; flavonoids.

Introduction

Argania spinosa L was known as Argan tree that belongs to the Sapotaceae family which is endemic tree from Morocco [1]. The different parts of Argan tree have been used in therapeutic and cosmetic purposes in South Morocco [2].

Many studies indicate that Argania spinosa L. contain significant amounts of phenols which are class of secondary metabolites known for their high antioxidant activity [3,4]. Antioxidants are compounds that inhibit and/or reduce the oxidation process caused by an oxidant. Flavonoids and phenolics are considered to be the most bioactive phytochemicals where they act as antioxidants and scavengers of free radicals. Theses secondary metabolites have multiple pharmacological effects such as protection against chronic disease and cancer. There is no study in the literature demonstrating the antioxidant activity f Argania spinosa (L.) almond.

The aim of this study is to assess the evolution of biometric parameters as well as the study of the effect of morrocan regions ((three argan plantation in the region of Casablanca, Dar Bouazza and Rhamna) on the quantity of secondary metabolites (polyphenols and flavonoid) and on the in vitro antioxiodant activity evaluated by DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and hydrogen peroxide scavenging assay.

Materials and Methods

Plant Material

The collection of Argania spinosa (L.) almonds was carried out in three different Moroccan ecosystems (Table 1). The first argan trees originated from “Rhamna”, a small rural commune in Marrakech Province of the Marrakech-Safi region (Morocco). The planted argan trees in Casablanca were planted in 2008. The argan plants used were grown in the “Ounagha forest nursery” in Essaouira in polyethylene bags until they were suitable for transplantation (1 year).

The argan tree plantation is located in an urban area of Casablanca, in “Hay Laymoune”, and in the vicinity of the city in “Dar Bouazza". The almonds were harvested in June 2014. After harvest, the almonds were stored at 4°C until their processing.

Table 1: Geographical data of Argania spinosa (L.) almond collection sites.

	Casablanca	Dar Bouazza	Rhamna
Latitudes	33.5731°N	33.4906°N	31.4616°N
Longitude	7.5898°W	7.7911°W	8.0978°W
Average temperature	19°C	17.3°C	18.4°C
Rainfall	324 mm	317 mm	600.2 mm
Climate	mild Mediterranean	mild Mediterranean	Mediterranean temperate

Phytochemical screening

Phytochemical tests were carried out for tannins, saponosides, anthroquinone, quinone, polyphenol and flavonoids. They were carried out according to standard methods [15].

Ultrasound extraction

The sample was ground to a homogeneous dough form using a blender. Analytical grade absolute ethanol was purchased from ThermoFisher Scientific. The extracts were prepared by soaking 2 g of almond dough in 100 mL of different percentages of ethanol (40%, 60%, and 80%) in an ultrasonic bath and sonicated for 15min. The sonication frequency and power were fixed at 20 kHz and 5000 W, respectively, according to previous studies [5]. The filtrate was separated from the residue using Whatmann filter paper and evaporated using a rotary evaporator at 40 °C **[**6,7**]**. Thereafter, the dried extract was stored at 4 °C until use.

The yield is expressed as a percentage and is given by the following formula:

$$\text{Yield } (\%) = \frac{\text{Weight of dried extract}}{\text{Dry weight of almonds dough}} \text{x } 100$$

2.3. Biometric parameters

The fruits of different origins were collected for almond biometric evaluations. We selected 40 fruits at random from each origin, then the almonds were evaluated per length, width and weight. A ratio of width to length of the almond (almond ratio, AR) was calculated.

The data obtained were subjected to the variance analysis (F test), at 5% level. When relevant, the averages were compared by the Tukey test, also at 5%.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

The free radical scavenging assay of different extracts were determined using the DPPH method following the procedure of Khattak et al.with modifications **[**8**]**. The extract of various concentrations was mixed with 1ml of 0.1 mM DPPH in methanol. The reaction mixture was incubated for 30 min at a room temperature in dark conditions. The absorbance of the reaction mixture was measured at 517 nm. Ascorbic acid was used as the positive control. DPPH scavenging capacity was calculated by using the following formula:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{ x } 100$$

Hydrogen peroxide scavenging assay

The ability of the extract to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of Boussayoud et al. [9]. A solution of H_2O_2 (2 mM) was prepared in phosphate buffer (pH 7.4). The extract of various concentrations was mixed with 0.6 mL of H_2O_2 solution. The reaction mixture was incubated for 10 min and its absorbance was measured at 230 nm against a blank solution containing the phosphate buffer without hydrogen peroxide. scavenging capacity was calculated by using the following formula:

$$\text{H2O2 scavenging activity percetage} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \text{ x } 100$$

Determination of total flavonoid content

The total flavonoid content was determined by the aluminum chloride colorimetric method as described by

Raaman et al. [10]. The extract of various concentrations was mixed with 0.3 mL of 10% AlCl3, followed by the addition of 0.3 mL of NaNO2. The reaction mixture was kept at room temperature for 5 min. Absorbance of the reaction mixture was recorded at 510 nm. The total flavonoids content was calculated from the linear regression equation of the calibration curve, using quercetin as standards. The total flavonoids was expressed in mg which is equivalent to quercetin per gram of extract [11-13]. Each extract was tested with 3 repetitions and reported as mean ± SD.

Determination of total phenolic content

The amount of total phenolic content was determined according to the Berrani method using the Folin– Ciocalteu reagent [14]. The extract of various concentrations was mixed with 100 µl of 50% Folin–Ciocalteu reagent. The mixure was incubated for 3 minutes, then 2 mL of 2% Na2CO3 solution was added. The reaction mixture was incubated again 1h at a room temperature. Absorbance of the reaction mixture was recorded at 750 nm. The standard calibration was made using gallic acid. The total phenolics were expressed as mg gallic acid equivalent/gram dry weight. Each extract was tested with 3 repetitions and reported as mean ± SD.

Statistical Analyses

Results were expressed as average ± SEM (Standard Error Mean) and statistically analyzed using Minitab.16. A statistical analysis was performed by using one-way analysis of variance of (ANOVA) followed by Tukey's Multiple Comparison Test. Significant differences were considered when $p< 0.05$.

Results

Study of the almond biometric parameters

Table 2 presents biometric indicators of Argania spinosa (L.) seeds from different locations in the region of Morocco. The analyses showed three distinct shapes and also three different colours of almonds obtained from different localities (Casablanca, Rhamna and Dar Bouazza) (Figure 1). Almonds from Dar Bouazza were characterized by oval apiculate shape with yellowish brown colour, while the almonds from Rhamna have oval shape of orange-brown

colour. The fusiform shapes of russet brown were caracterized by almonds from Casablanca

Figure 1. Seeds of Argania spinosa from fruits of different shape. A: oval, B: oval apiculate, C: fusiform

Table 2. Biometric parameters (length, width, and weight) of Argania spinosa (L.) almonds from different locations in the region of Morocco

Collection site	Almonds Parameters	Mean	Minimum	Maximum	Variance	Standard deviation	CV (%)
Casablanca	Length (cm)	3.04^{b**}	2.60	3.60	0.06	0.25	8.23
	Width (cm)	1.84^{b***}	1.30	2.30	0.05	0.22	12.14
	Weight (g)	2.57^{b*}	1.50	4.49	0.45	0.67	25.99
Dar Bouazza	Length (cm)	2.87^{b**}	2.50	3.50	0.08	0.28	9.80
	Width (cm)	2.05^{a***}	1.60	2.50	0.09	0.29	14.32
	Weight (g)	3.89^{a*}	1.20	5.63	1.84	1.36	34.83
Rahamna	Length (cm)	3.31^{a**}	2.40	3.80	0.07	0.27	8.24
	Width (cm)	1.68^{b***}	1.10	2.00	0.05	0.22	13.26
	Weight (g)	2.41^{b*}	1.07	4.47	0.53	0.73	30.13

*,**,***Means that do not share a letter are significantly different by the Tukey test, at 5%.

The results showed that the almonds obtained from different regions demonstrated few changes of biometric aspects evaluated, such as length (ranging from 2.87 to 3.31 cm), width (1.68 to 2.05 cm) and weight (2.41 to 3.89 g) of almonds. Moreover, the almonds of Rhamna had the highest average lenght and almonds of Dar Bouazza presented the higher average width and weight.

In general, the standard deviation values of all variables were low. Thus, the standard deviation of the almonds weignt from Dar Bouazza presented the highest value, and the almond width from Casablanca and Rhamna stood out with the lowest value. Furthermore, the almonds weight value from Rhamna showed highest coefficient of variation, whereas the almonds from Casablanca had the lowest

lenght value. The width of almonds of different origins showed lower variance and standard deviation than length and weight, which may characterize little variability, possibly caused by environmental effects during almonds development. The analysis of variance srealed differences very highly significant differences between the two regions for all measured variables ($p<0.005$). This shows that the characters related to the dimensions of the seeds (length, width and weight) vary significantly depending on the almond origin.

Effect of solvent-solid ratio and ethanol concentration on extraction yield

In this study, three different solvent-solid ratios (w:v) (1:10, 1:15 and 1:20) and Ethanol concentrations of 40%, 60% and 80% (v/v) were investigated (Figure 2). The highest extraction yield observed in 80% ethanol extract of Casa. Ethanol/water concentrations were highly correlated with extraction yields (r = 0.93, p=0.22). Also, The result analysis indicates that the value are significantly affected by solvent-solid ratio (r=0,91), and all three locations had a high degree of acceptance ($P < 0.05$). In addition, extraction yield at solid-to-solvent ratio 1:20 (w:v) were significantly higher than those extracted at 1:10 (w:v) and 1:15 (w:v).

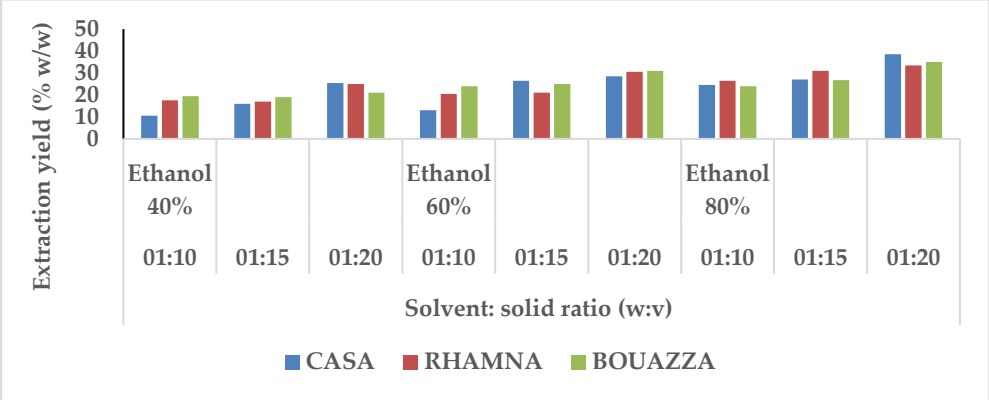

Figure 2. Variation of yield vs. Solvent: solid ratio and ethanol concentration

Total phenolic content and total flavonoids content

The results of phytochemical screening of the ethanol extracts of arhan almonds showed the presence of alkaloids, phenolics, flavonoids, tannins and saponins (Table 3). Although the ethanol solvent used for extraction was from

different regions, it was still able to attract the same type of compound class to the argan almonds.

The results measured for total phenolic content and total flavonoid content were represented in Table 4. Thus, we noted that our extracted samples contain significantly important contents of total phenolics and flavonoids The results showed that the total phenol content extracted from almond using aqueous ethanol (40%, 60% and 80% ethanol) varied from 12.18 to 48.27 mg GAE/g extract. Among the studied areas, the Dar Bouazza had the highest phenolic content for almond extracts using an ethanol concentration of 40%, 42.5±1.56 mg GAE/g, while the lowest total phenolic content was observed in 40% ethanol extract of Casablanca, 12.18±0.57 mg GAE/g.

Table 3. Results of Phytochemical Screening of Ethanol Extract of argan almond

Compound Group	**Collection site**		
	Casablanca	**Rhamna**	**Dar Bouazza**
Alkaloids	**+**	**+**	**+**
Phenolic	**+**	**+**	**+**
Flavonoids	**+**	**+**	**+**
Tannin	**+**	**+**	**+**
Saponins	**+**	**+**	**+**

Description: (+) = detected compound identified; (-) = no identified compound was detected

Table 4. The extraction yield, total phenolic and flavonoid contents in the ethanol extract of argan almond from the three regions studied

Region	**Alomond extract**	**Total phenolic content (mg GAE/g extract)**	**Total flavonoid content (mg QE/g extract)**
Casablanca	Ethanol 40%	12.18±0.57	5.61±1.36
	Ethanol 60 %	15,4±1.64	6.26±0.24
	Ethanol 80%	29.92±0.36	4.56±1.24
Rhamna	Ethanol 40 %	31.9±1.32	5.55±0.86
	Ethanol 60%	39.01±0.29	3.51±1.61
	Ethanol 80%	40.27±0.60	1.22±0.74
Dar Bouazza	Ethanol 40%	42.5±1.56	6.61±0.99
	Ethanol 60%	20.81±0.25	4.21±1.35
	Ethanol 80%	13.31±1.28	0.17±0.73

The data are the mean of three replicates (n = 3e ± SEM); means followed by similar superscript letters in the same row are not different (P < 0.05).

The total flavonoid content ranged from 1.22 to 5.61 mg QE/g extract. The extract of almond cultived in Dar Bouazza gave the highest flavonoid content, 5.61 mg GAE/g extract (40%), while the lowest value was founded in the extract of Rhamna region, 1.22 mg GAE/g extract (80%).

Antioxidant Activity

In this study, the tests DPPH free radical scavenging and Hydrogen peroxide scavenging were used to assess the antioxidant activity of different extracts. The results of these tests are presented in Table 5.

The ethanol extract of 40% from Dar Bouazza region showed strong H_2O_2 scavenging activity (IC_{50}= 43.53 µg/mL µg/mL). The no significant difference in percentage inhibition of H_2O_2 of all extracts in different region studies (p >0.05dx).

Table 5. IC50 and EC50 values of the effective concentration of the DPPH, ABTS, and FRAP free radical scavenging assays for EtOH extracts from of Argania spinosa (L.) almonds from the three regions

Collection site	Proportion EtOH - H2O (%)	DPPH (IC50 µg/mL)	Hydrogen peroxide scavenging assay
Casablanca	40	144.91± 1.06	83.78±0,58
	60	163.07± 0,51	94.59±1,29
	80	166.21±0.32	95.67±1,60
Rhamna	40	158.69±1.34	89.18±1,50
	60	86.77±1.65	82.16±0,12
	80	92.03±0.98	80.32±0,65
Dar Bouazza	40	58.84±0.21	77.83±1,00
	60	411.23±0.21	90.27±0,55
	80	565.25±1.51	95.13±0,99
Ascorbic A	92		

The lower the value of the IC_{50}, the higher the antioxidant activity. The DPPH IC50 values were compared to the standard ascorbic acid IC50 value. The IC_{50} values for DPPH trapping activities of different almond extracts from the three regions varied in the range of 58.84 and 565.25 µg/mL. The ethanol extract of 40% from Dar Bouazza region, showed the best anti-free radical capacities, with an IC_{50}

value = 58.84 ± 0.21 µg/mL, while the highest IC50 was observed in the ethanol extract of 80% IC_{50} = 565.25 ± 1.51 µg/mL. The values recorded are significantly different from the reference used IC_{50} = 92.00 ± 0.7 µg/mL.

On the other hand, the Dar Bouazza ethanol extracts of 40% have the highest phenolic content and the lowest IC_{50} in the free radical scavenging activity test, therefore a strong antioxidant activity. Also, a significant negative correlation between the IC_{50} and total phenolic content ($r = -0.958$ and $P < 0.05$) prove that the high antioxidant activity could be related to the high amount of polyphenols in the extracts.

Discussion

The fruits of different origins (three argan plantation in the region of Casablanca, Dar Bouazza and Rhamna) were evaluated per length, width and weight. In this study, the highest values were recorded on argan almonds of Dar Bouazza region and almonds of Rhamna which had the highest average lenght. Those results showed that the weight of almonds were coming from different localities (Casablanca, Dar Bouazza and Rhamna) were higher compared to what Chargui at al. [15] have found in argan of Agadir (southwest Morocco).

The Argania spinosa (L.) almonds were extracted by cold maceration method. The solvent used for extraction was ethanol with 3 variations in concentration: 40%, 60%, and 80% (absolute ethanol). Thus, according to the results, water and 40 wt.% ethanol/water solvent seemed to be less effective in extracting phenolics than those ethanol/water extraction solvents with high concentrations. The ethanol solvent polarity of the three concentrations (40%, 60% and 80% (v/v) used in this study was influenced by the high concentration of water contained in ethanol, which resulted in increased extraction yields. Therefore, ethanol has the ability to attract polyphenols, flavonols, tannins, terpenoids, and alkaloids [16,17]. The choice of ethanol (less toxic) as the extraction solvent with solid-to-solvent ratio (w:v) 1:20 in this study was able to extract more metabolites from the argan almonds.

Phytochemical screening of the ethanol extract almonds indicated the presence of alkaloids, phenolics, flavonoids, tannins and saponins. Although the ethanol solvent that is used for extraction was from different regions, it was still able to attract the same type of compound class to the argan almonds. Thus, we noted that

our extracted samples from three region (Casablanca, Dar Bouazza and Rhamna) contain significantly important contents of total phenolics and flavonoids. Moreover, all values were lower than those found by Lfitat et al. Réf 04. The considerable difference in phenolic content and flavonoid content might be due to environmental factors such as location, climate [18,19]. The phenolic and flavonoid contents in argan almonds have biological activities such as antithrombotic, antiallergic, anticarcinogenic, antimicrobial, hepatoprotective, and antihypertensive activities [20-22]. In addition, the phenolic and flavonoid contents in argan almonds play an important role in its activity as a source of antioxidants [23].

Several studies have evaluated the antioxidant power of Argan trees, by using several parts of this plant [24,25]. In this study, the tests DPPH free radical scavenging and Hydrogen peroxide scavenging were used to assess the antioxidant activity of different extracts. Therefore, the results showed that the ethanol extracts samples have a high antioxidant activity, that varies depending on origin of Argania spinosa (L.). The ethanol extract of 40% originating from Dar Bouazza gave the highest phenolic, flavonoid content, strong antioxidant and hydrogen peroxide scavenging activity. This result is in agreement with the data reported by Lfitat et al. [26]. Argan may be considered as an accessible and sustainable source of bioactive compounds of pertinent antioxidant functionality.

Conclusions

The Secondary metabolites and Antioxidant activity of Argania spinosa (L.) almonds from three regions of Morocco (two argan plantation in the region of Casablanca and in Dar Bouazza and one spontaneou culture) were analyzed and compared. Our study showed that Argania spinosa (L.) almonds constitutes a significant source bioactive compounds with higher antioxidant activities although they were grown outside their biotope in the region of Casablanca and Dar Bouazza. Those bioactive compounds with fascinating biological and pharmacological properties can be used for multiples pharmacological effects such as protection against chronic disease and cancer.

Author Contributions

Conceptualization, S.R.; methodology, S.R; software, F.B. and M.B.; validation, S.R. and F.B.; writing—original draft

preparation, S.R and F.B.; writing—review and editing,S.R. and F.B.; visualization, C.S., A.D. and M.B.; supervision, S.R. and A.D. All authors have read and agreed to the published version of the manuscript.

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Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

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