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Comparison and correlation of phytochemical content with antioxidant potential of different parts of Argan tree, *Argania spinosa* L.

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ABSTRACT

The Argan tree or *Argania spinosa* L. is a plant in the Sapotaceae family. It is an endemic tree from Morocco. This study aims to evaluate the phytochemical and antiradical potential of different parts of Argan for the further valorization of this organic material. Seeds, kernels, pulps, leaves, and branches were targeted for this purpose and were soxhlet extracted with methanol. Amongst the different parts, leaves displayed the greatest DPPH radical scavenging ability with an IC_{50} value (4.37 µg mL^{-1}) close to that of ascorbic acid (1.97 µg mL^{-1}). This observation was true for the ABTS assay as well. Accordingly, leaf extract was also the highest in polyphenols content (TPC) content, while seeds recorded the lowest value. The results were not so different for the flavonoid content, where leaf extract recorded the highest content. The leaves and branch of Agran seem to be the richest in antioxidant agents, as shown by the gathered data and results. Hence, it could be used as an important source of natural antioxidants.

Keywords: Antioxidant activity, phenolic compounds, DPPH; ABTS, *Argania spinosa* L. skeels.

INTRODUCTION

An antioxidant is considered as a chemical that prevents a substrate's oxidation in the presence of an oxidizable compound. Polyphenols, carotenoids, and conventional antioxidant vitamins, such as C and E, are considered as major phytochemicals capable of having antioxidant activity in plant materials (Rice-evans *et al.* 1997). Phenolic substances were hugely examined for their benefits on human health and are considered to be the most bioactive phytochemicals present in particular, medicinal and aromatic plants (Arts & Hollman 2005; Pandey & Rizvi 2009). The Argan tree is a very precious tree that plays an unequaled role in the cultural, socio-economic, and ecological life of the inhabitants of south-west Morocco (M'hirit *et al.* 1998). From this tree, which has many applications, the inhabitants draw their edible oil, firewood, and tools, as well as remedies against their illnesses (Moukal 2004). By its great presence in the landscape and the collective memory of the populations of South Morocco, the derivatives of Argan tree are used for several therapeutic and cosmetic purposes. The most present metabolites in *Argania spinosa* L. skeels are phenolic substances (Charrouf & Guillaume 2002; Khallouki *et al.* 2005).

Several metabolites, namely flavonoids, tannins, and others have shown great pharmacological properties. These metabolites are prevalent in plants where they act as antioxidants and scavengers of free radicals. To our best knowledge, there is no study comparing the antioxidant activity of different parts of Argan tree (seed, kernel, pulp, leaf, and branch). In this study, we aim to relate the phenolic, flavonoid and tannin content of different parts of the plant studied with their possible antioxidant activity measured by the DPPH (2,2-DiPhenyl-PicrylHydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) test.

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MATERIALS AND METHODS

Plant material

The different parts of the Argan tree (seed, kernel, pulp, leaf, and branch) were harvested from Essaouira region, particularly, in "Mejji" villages, at the end of August 2018. "Mejji situated 138 km in the Southwest of Marrakesh at 292 m above the sea level (3132054.000 N and 00922050.200 W) and the mean annual precipitation is 198.7 mm. Depending on this Emberger's card, this area is located in the semi-continental climate (25 C\m\35 C); the geologic nature of the ground is a limestone of the Cretaceous Cenomanian (Zhar *et al.* 2016).

Preparation of the extract

The five parts were washed with water and air-dried for a week in the dark. The flour of each part was placed in soxhlet extractor. The extract was carried out with methanol at 65 °C for 6 h until total exhaustion. Thereafter, the methanolic extract was stored in brown glass bottles at 4 °C until use. All assays were performed in triplicates to guarantee reproducibility.

Determination of total phenolic, total flavonoid and tannin content

The Folin-Ciocalteu spectrophotometric method was used to quantify the polyphenols content (TPC). The extract obtained upon extraction was diluted by ten folds. The aliquot (0.5 mL) was then transferred into a test tube and thoroughly mixed with FCR (2.5 mL) earlier diluted ten folds with distilled water. 2 mL of 7.5% sodium carbonate was added. After 30 min of standing in the dark, the measurement was performed using a double beam UV-visible spectrophotometer (VWR UV-6300PC, China) at 765 nm against a blank. The result was expressed as an mg gallic acid equivalent per gram of extract (singleton *et al.* 1999).

The aluminum chloride colorimetric method was used to evaluate the flavonoid content (TFC), with minor modifications (Jia *et al.* 1999). 2 mL of extract was fitted into a tube with 2 mL 10% $NaNO_2$, to which 2 mL of 10% $AlCl_3$ solution was added. The mixture was left to stand for 30 min, centrifuged for 5min at 4000 rpm, and then measured at 415 nm. The result was expressed as an mg Quercetin equivalent per gram of extract. The total tannin content (TTC) extraction was performed as described by Sun *et al*. (1998). Thus, 3 mL of 4% vanillin-methanol solution was added to 500 mL of the extract with the addition of 1.5 mL hydrochloric acid afterward. The mixture was left to stand for 15 min. Thereafter, the absorbance was measured at a wavelength of 500 nm, and the results were reported in mg catechin equivalent (CE)/g of extract.

Antioxidant activity

The 2,2-diphényl-1-picrylhydrazyl antiradical assay (DPPH) was performed according to Huang *et al.* (2011), with slight modifications. Briefly, 0.5 mL of a 0.2 mM DPPH solution was added to 2.5 mL of sample, mixed and left to stand for 30 min. The measurement was then performed at 517 nm and the results expressed as a percentage according to the equation 1:

$$\%RSA = \frac{A - A_S}{A} \times 100 \quad \textbf{(1)}$$

where: A is the recorded absorbance of the blank sample, A_S is the absorbance value of the sample solution.

The ABTS assay was performed as described by Arnao *et al.* (2001). The 2 mM ABTS solution was added to $K_2S_2O_8$ in equal quantities and allowed to stand for 12 to 16 h before use. The solution was then diluted with methanol until reaching an absorbance of 0.70 ± 0.02 at 734 nm. The solution was added to 200 µL of different concentrations of the extract. The absorbance of 2 mL of the resulting solution was then measured at 734 nm after 30 min of incubation.

Statistical processing and data analysis

Results obtained were reported as mean ± SEM. Significant differences for multiple comparisons were determined by One-Way ANOVA followed by post-hoc Tukey HSD test using SPSS (version 20). The *p-value* of ≤ 0.05 level was considered significant. Correlations between the total phenolic, flavonoid, tannins content, and antioxidant activity were conducted using the Spearman's rho method.

Values (mean ± ESM) are the average of each plant material. Superscript letters within the different row indicate significant ($p < 0.05$) differences of means within the plant materials according to ANOVA, Tukey HSD test.

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RESULTS AND DISCUSSION

Yield of extracts

Table 1 shows the yield of different parts of the plant. The highest yield of methanolic extract was obtained from pulp followed by the leaf. Branch and seed achieved the same extraction yield. The lowest yield value was obtained from the extract of kernels. Strong significant differences ($p < 0.001$) were observed among extracts, except those between seed and branch yield. The estimation of Argan yields varies greatly depending on the region and on tree specimens (Aithammou *et al*. 2019). Additionally, the hydroalcoholic extract represents 24% of the cake, which is rich in saccharose and contains 4% in saponins (Charrouf 1998).

Table 1. Extraction yield of methanolic extract from different part of Argan tree.

Different part	Seed (Mean ± SEM)	Pulp (Mean ± SEM)	Kernel (Mean ± SEM)	Branch (Mean ± SEM)	Leaf (Mean ± SEM)
Rate (%) Extraction yield	11.12 ± 0.58^{a}	59.36 ± 1.12^{b}	4.02 ± 0.42^{c}	14.63 ± 0.52^{a}	37.35 ± 1.40^{d}

Total phenolic, total flavonoid and tannin content

Table 2 shows the TPC, TFC, and TTC of the various parts of Argan extracted by methanol solvent. Among Argan materials, the leaf methanol extracts had the highest TPC and TFC, followed by the branch, the kernel and the pulp. The lowest TPC and TFC values were obtained from the extract of seed. Statistically, all the differences in means were strongly significant ($p < 0.001$). Results of the present study showed that among all the Argan materials, its leaves offered the highest TTC, followed by branch, kernel, and pulp extracts. These results were strongly significant ($p < 0.005$). However, no TTC was found in seeds. Phenolic compounds in *A. spinosa* (L) gradually increase with maturation, reaching up to five folds in the last month of fruit development (Vela *et al.* 2002). The number of phenolic may vary from one study to another due to several factors, such as the geolocalisation and the maturation stage. A high amount of polyphenols was previously reported for the pulp extract (75.78 mg GAE /g of extract), while it was reported to be 125.24 mg GAE/g of extract using microwave-assisted soxhlet extraction as compared to 105.10 mg GAE/g of the extract with ethanol-water extraction (El Monfalouti *et al.* 2012; Yassine *et al.* 2019). The leaves of this plant are usually used in traditional medicine for its anti-inflammatory properties to treat rheumatism, and articulation-related issues (Moussaoui *et al.* 2019). Those leaves are particularly rich in flavonoids, known for their antioxidant properties. In some cases, isolation and identification of multiple compounds were performed, successfully reporting the presence of quercetin and myricetin which are known for their antibacterial and antifungal properties (Charrouf & Guillaume 1999).

Table 2. Total polyphenol, flavonoid and tannin contents of methanolic extract from different parts of Argan tree.

	Seed	Pulp	Kernel	Branch	Leaf
TPC (mg GAE/g extract)	49.36 ± 0.33a	155.52 ± 0.59b	207.52±0.49c	256.70± 0.73c	643.02± 0.17d
TFC (mg QE/g extract)	18.41± 0.39a	73.34± 0.46b	103.43± 0.59c	150.55± 0.71d	395.68± 0.86e
TTC (mg CE/ g extract)	-	2.15± 0.27a	9.47± 0.52b	21.12± 0.45c	37.46± 0.80d

(TPC = total phenolic content, TFC = total flavonoid contents, TTC = total tannin contents, GAE: gallic acid equivalent, QE: quercetin equivalent and CE: catechin equivalent).

Antioxidant activity

While no scavenging activity was found in Argan seeds (Table 3), the extracts of all other tested materials possessed free radical scavenging properties but to varying degrees, ranging from 4.37 to 17.49 µg mL^{-1} IC_{50} of DPPH and from 39.40 to 81.26 µg mL^{-1} IC_{50} of ABTS. Results show that leaves hold a better antioxidant power compared to the other plant parts. It is well known that the lowest IC_{50} DPPH and ABTS scavenging activities will reveal the highest antioxidant activity. The pulps are the part that has the least antioxidant power, as it presents the highest values. Statistically, all the differences in means were strongly significant ($p < 0.001$). An IC_{50} value of *A. spinosa* hulls of 54.00 µg mL^{-1} instead of 17.1 µg mL^{-1} was previously reported, using ethanol/water as an extraction solvent (El Adib *et al.* 2015).

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Table 3. Antioxidant activity of methanolic extract from different parts of Argan tree.

IC_{50} (µg mL^{-1})	Seed	Pulp	Kernel	Branch	Leaf	Standard
DPPH	-	17.49 ± 0.97^{a}	11.69 ± 0.43^{b}	8.11 ± 0.38^{c}	4.37 ± 0.18^{d}	Ascorbic Acid: 1.97 ± 0.02^{e}
ABTS	-	81.26 ± 2.75^{a}	62.51 ± 1.42^{b}	50.20 ± 1.17^{c}	39.40 ± 0.82^{d}	Trolox: 30.84 ± 0.05^{e}

Correlation between total phenolic, flavonoid and tannin contents in each Argan material with their antioxidant activities

In the present study, Spearman's rho correlation (Table 4) revealed that TPC and TTC in the pulp, kernel, branch, and leaf samples have significantly strong negative correlations with their IC_{50} DPPH and ABTS capacities. It means that increases in TPC and TTC cause an elevated antioxidant activities. TFC exhibited also a weak negative and significant correlation with IC_{50} DPPH ($p \leq 0.05$).

Table 4. Spearman's rho correlation coefficient of total phenolic, flavonoid and tannin contents in each Argan material with their antioxidant activities.

		DPPH	ABTS
Pulp	TPC	-0.979****	-0.768****
	TFC	-0.450	-0.024
	TTC	-0.976****	-0.765****
Kernel	TPC	-0.956****	-0.765****
	TFC	-0.408	-0.024
	TTC	-0.956****	-0.765****
Branch	TPC	-0.953****	-0.765****
	TFC	-0.402	-0.024
	TTC	-0.953****	-0.765****
Leaf	TPC	-0.647**	-0.718***
	TFC	-0.592*	-0.450
	TTC	-0.953****	-0.976****

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.005, **** p ≤ 0.001.

CONCLUSION

Nutraceuticals and cosmeceuticals maybe not crucial or as important for the human body, it is still an important way of maintaining it. Argan was proven to be an interesting source of bioactive compounds. The present study emphasizes the potential role that the different parts of the tree may play in this regard. Those compounds can be further valorized to treat different health issues such as diabetes, skin diseases, cardiovascular issues, or even cancer. *Argania spinosa* L. turns out to be a rich source of polyphenolic compounds that should be further studied for incorporation in the nutrition and cosmetic fields. Further studies are needed to further explain the results obtained in this work.

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Conflict of Interest

No conflict of interest.

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مقایسه و تعیین ارتباط محتویات شیمیایی و توان بالقوه ضد اکسایشی بخش های مختلف درخت آرگان (*Argania spinosa* L.)

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چکیده

درخت آرگان، آرگانیا اسپینوزا ال. گیاهی از خانواده ساپوتاسه آ است. این گیاه درخت بومی مراکش است. هدف از این مطالعه ارزیابی مواد شیمیایی وتوان بالقوه ضد رادیکال قسمت‌های مختلف گیاه آرگان برای ارزش گذاری‌های آتی آن است. دانه، هسته، پولپ و برگ آن برای این منظور انتخاب شد و از طریق دستگاه سوکسله با متانول عصاره آن استخراج شد. در بین بخش‌های مختلف، برگ‌ها بالاترین توان مهار رادیکال DPPH را با IC50 ۴/۳۷ میکروگرم در میلی‌لیتر نزدیک به توان اسید اسکوربیک (۱/۹۷ میکرو گرم در میلی‌لیتر) نشان داد. این مشاهده برای سنجش ABTS هم صادق بود. بر همین اساس، عصاره برگ از نظر محتویات پلی فنولی هم در بالاترین حد قرار داشت، در حالی که در مورد دانه این عدد پایین‌ترین مقدار بود. نتایج در مورد محتویات فلاوونوئیدی تفاوتی نداشت به طوری که برگ این درخت بالاترین مقدار را نشان داد. به نظر می‌رسد که برگ و ساقه آرگان از نظر ترکیبات ضد اکسایشی هم بسیار غنی است که بر اساس اطلاعات جمع آوری شده و نتایج به دست آمده است. از این‌رو، می توان آن را به عنوان منبع مهمی از ترکیبات ضد اکسایش طبیعی به حساب آورد.

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