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Research Article

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Antioxidant Activities, Total Polyphenolic Compounds And Hplc/Dad/Ms Phenolic Profile of Argan Oil Derived from Two Differents Methods of Extractions

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ABSTRACT

Objective: In order to validate the traditional use of argan oil, to compare between two different methods of extraction (hand pressed (HP) and mechanical cold-pressed (MP)) and to begin the understanding of their activities, waiting to find the structure-activity relationship, radical scavenging activity, ferric reducing ability and phenolic content were studied.
Methods: Antioxidant potential of phenolic extract of argan oil derived from both extraction methods was evaluated using DPPH radical scavenging assay, Trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power test (FRAP). The chemical composition of phenolic extract was also studied spectrophotometrically.
Results: The both extracts showed a promising scavenging effect and an important reducing activity measured by DPPH, TEAC and FRAP assays, compared to the standard antioxidants (Butylatedhydroxytoluene (BHT), Quercetin and Trolox). Moreover, phenolic extracts of argan oil can be a promising new source of natural compounds such as polyphenols (1.11 to 1.79 eq Gallic acid (mg/g dry)), flavonoids (3.13-3.83 eq rutin (mg/g dry)) and tannins (12.31–18.19 eq catechin (mg/g dry)). In addition, the strong correlation was observed between antioxidant capacities and their total phenolic contents.
Conclusion: From this work, it can be concluded that argan oil could be a promising source of antioxidant metabolites, while the oil devived from hand pressed (HP) was the most effective using DPPH, TEAC and FRAP assays and the highest in terms of total phenol, flavonoid and tannin contents.

Keywords: Argania spinosa; Phenolic extract; Antioxidant activity; Phenolic compound; DPPH; TEAC; FRAP

INTRODUCTION

Because of the possible toxicities of the synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), increasing attention has been directed toward natural antioxidants [1,2] .Several plants extracts of various species were tested for their antioxidant capacities. The Argan tree, called *Argania spinosa* (L.) is endemic to Southwestern Morocco where the Argan forest covers an area of 800,000 ha [3]. It represents the only species of the genus *Argania*. In Morocco, this tree is considered an important forest species from economy and social stand points, but also for medical reasons. Populations of Morocco traditionally use the fruits of *A. spinosa* to prepare edible oil [4].This oil is used for many purposes. In food, argan oil has been known for centuries in Morocco where it constitutes the basic ingredient and sometimes exclusive source of vegetable fat in the “Amazigh diet” [5-7]. In cosmetics, virgin Argan oil is advocated as a moisturizing oil,

acting against juvenile acne and flaky skin flaking of the skin as well as nourishing the hair [6,8]. The Argan oil has also a medicinal uses to fight rheumatism, soothe inflammation and heal scars and burns.

Science has reaffirmed the validity of many of its traditional uses and reported that argan oil contains most of the important sterols tocopherols, saponines and polyphenols compounds [9,10]. Despite the interesting findings across data literature, it seems of interest to explore the phenolic content of argan oil derived from two different methods of extraction hand pressed (HP) and a mechanical cold-pressed (MP), since the content and structure of the plant phenolics compounds may differ depending on the method used for extraction. Therefore, the present study aims to determine and compare the antioxidant potential of argan oil derived from two different methods of extraction (traditional and half-industrialized methods) using Trolox equivalent antioxidant capacity (TEAC), DPPH free radical-scavenging activity (DPPH), and ferric reducing antioxidant power (FRAP) assays, to determine their total phenolic contents and investigate the relationship between total phenolic content and antioxidant activity.

MATERIAL AND METHODS

Samples

Two different commercial samples of extra virgin argan oil (HP and MP), produced in south-western Morocco (Taroudant), obtained by the traditional method of extraction and the half-industrialized method with cold pressing certified as ecological product, were purchased in specialist stores from Essaouira (Maroc).

Preparation of phenolic extracts

The phenolic compounds were extracted from argan oil according to the method of of Pirisi et al. [11] .Briefly, argan oil was mixed with n-hexane and methanol/water and then stirred in a vortex apparatus and centrifuged. The hydro-alcoholic solution was washed with n-hexane and then evaporated.

Antioxidant activity

DPPH free radical-scavenging activity:

The ability of argan oil (HP and MP) to scavenge the DPPH radical was determined according to the method described by Bo Huang et al. [12]. Briefly, 0.2 mM solution of DPPH in methanol was prepared and 0.5 ml of this solution was added to 2.5 mL of phenolic extract and was allowed to stand at room temperature for 30 min, and then absorbance was read at 517 nm against blank samples. A standard curve was obtained by using Trolox standard solution. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration. Using the following equation:

$$\% \text{ RAS} = [(A0 - A1) / A0] \times 100$$

Where *A0* is the absorbance of DPPH blank sample, and *A1* is the absorbance of the test solution.

Trolox equivalent antioxidant capacity (TEAC) assay:

The ABTS free radical-scavenging activity of each phenolic extract was estimated using the method described by Pukalskas et al. [13].

The ABTS radical cation (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) was produced by reacting ABTS with potassium per sulfate. The ABTS+ cation radical was produced by the reaction between 10 mL of 2 mM ABTS in H_2O and 100 µL of 70mM potassium persulphate, stored in the dark at room temperature for 24 h. The ABTS+ solution was then diluted with methanol to obtain an absorbance of 0.70 at 734 nm. Samples were prepared by diluting 100 µL of extracts (same dilution of DPPH assay) in 2 mL of the ABTS+ solution diluted with methanol and let to react for 1 min.

Absorbance was read at 734 nm. Troloxwere used as positive controls and the antioxidant activities samples are expressed as TEAC values, defined as the concentration of standard Trolox with the same antioxidant capacity of the extract under investigation.

Reducing power determination:

The ferric reducing capacity of this species was investigated by using the reducing antioxidant power (FRAP) method [15].Briefly, 0.2ml of extract, 2.5ml of phosphate buffer (0.2M, pH6.6) and 2.5ml of potassium ferricyanide K3Fe(CN)6(1%) were mixed and incubated at 50◦C for 20 min.

Then 2.5 mL of trichloroacetic acid (10% w/v) was added to the reaction mixture. Afterwards, it was centrifuged at 1000 rpm for 10 min. Finally, 2.5 ml of the upper layer was mixed with 2.5 ml of deionised water and 0.5 ml of FeCl3 (0.1%) and the absorbance was measured at 700 nm. The reducing power of the extracts was represented as ascorbic acid equivalent (mg AAE/g dw).

Determination of total phenolic content: The total phenolic contents (TPC) were measured by the Folin–Ciocalteu colorimetric method [16]. Briefly, 0.5 mL of phenolic extract was mixed with 2.5 mL of Folin-Ciocalteu reagent diluted with distilled water 1:10, followed by the addition of 4 mL of Na2CO3 (7.5 %, w/v).
The mixture is then incubated in a water bath at 45°C for 30 min and the absorbance was measured at 765nm. Gallic acid was used as a reference standard, and the results were expressed as microgram gallic acid equivalent (mg GAE)/g dry weight of plant material.

Determination of total flavonoids content: Total flavonoids content was determined spectrophotometrically by the aluminum chloride colorimetric method (Dewanto et al., 2002).
About 1 mL of dissolved sample was placed in a 10 mL volumetric flask. Distilled water was added to obtain a total volume of 5 mL and then 0.3 mL of $NaNO_2$ (5%) was added. Then 0.3 mL of $AlCl_3H_2O$ (10%) was added after 5 min and the mixture was allowed to stand for another 6 min. About 2 mL of 1 M NaOHwas added and the total volume was increased to 10 mL with distilled water.
The solution was mixed well and allowed to stand for 30 min. The absorbance was measured at 510 nm. Total flavonoid content was determined as the rutin equivalent from the calibration curve of rutin standard solutions and expressed as rutin equivalent (mg RE/g dw).

Determination of condensed tannins content: The total condensed tannins were measured according to the method of Julkunen-Titto [17]. Briefly, 50 µl of each extract was mixed with 1.5 ml of 4% vanillin (prepared with MeOH), and then 750 µl of conc. HCl were added. The mixture was then incubated at ambient temperature in the dark for 20 min. The absorbance against blank was read at 500 nm. (+)-Catechinwas used to make the standard curve and the results were expressed as mg catechin equivalents (CEQ)/ g extracts (DW).

HPLC-DAD-MS analysis condition: High-performance liquid chromatography with Diode-Array Detection and mass spectroscopy (HPLC–DAD-MS) system consisted of a binary pump (G1312A; Agilent 1100) and an autosampler (G1330B) coupled to a diode-array UV/VIS detector and a mass spectrometer equipped with an electrospray ionizer source (MS; ESI-; Micromass Quattro Micro; Waters, Milford, MA, USA).
Reversed phase HPLC separation was carried out using a zorbax C18 column Zorbax (100mm x 2.1mmx 1.7µm). The mass spectrometer was operated in negative ion mode with the following parameters: capillary voltage, 3.0 kV; cone voltage, 20 V; and extractor, 2 V. Source temperature was 100 °C, desolvation temperature was 350 °C, cone gas flow was 30 L/h, and desolvation gas flow was 350 L/h.
The mobile phase components were 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The mobile phase gradient was: 0 min, 90% A; 0–18 min, 30% A; 18–20 min, 30% A; 20–23 min, 30% A; 23–25 min, 90% A; 25–30 min, 90% A. The injection volume was 10 µL and the column temperature was 35 °C. The flow rate of the mobile phase was 0.5 mL/min. The phenolic acids, flavanols and flavonols were identified on the basis of their retention times, MS spectra and molecular-ion identification.
The liquid/liquid extraction was performed, 10 g of argan oil was weighed into a centrifuge tube and 10 mL of methanol/water (80/20, v/v) was added. The mixture was stirred for 10 min in a vortex apparatus, and the tube was centrifuged at 6000 rpm for 3 min. The methanol layer was then separated and the extraction repeated twice. The methanolic extracts were combined and filtered through a 0.2 µm PVC filter and to be used for analysis.

STATISTICAL ANALYSIS

All the assays were carried out in triplicate. The means and standard error of means (SEM) were determined using SPSS 20 statistical software. Data were expressed as the mean values ± standard deviation (SD) for each measurement.

RESULTS

Antioxidant activity of oils

Both Extracts exhibited antioxidant activity. The HP displayed higher antioxidant activity across the three testing methods (DPPH, ABTS and reducing power) compared with MP (Table 1).
As summarized in Table 1 the both extracts were able to reduce the stable, purple-coloured radical DPPH into yellow-coloured DPPH-H, the antioxidant activity of the extracts expressed as Trolox equivalents (TE) ranged from 3.46±0.03 mg TE/g dw to 4.43±0.18and HP had the strongest free radical-scavenging activity with 3.46±0.03 mg TE/g dw.
The lowest capacity to reduce DPPH was observed in MP extract from 4.43±0.18 mg TE/g dw. Similarly, HP extract exhibited the best performance in ABTS and reducing power assays with 5.14±0.18 mg TE/g dwand 2.99±0.25 mg AAE/g dw respectively.

Table 1: Antioxidant activity of HP and MP

	DPPH[a]	ABTS[b]	FRAP[c]
MP	4.43±0.18	8.39±0.34	3.38±0.09
HP	3.46±0.03	5.14±0.18	2.99±0.25

Values represent means ± standard deviations for triplicate experiments. Concentration: [b,a] mg TE/g dw,[c] mg AAE/g dw.

Phenolic compounds

Total Phenolic compounds:

Concentration of phenolic compounds in both extracts ranged from 1.11 to 1.79 µg Gallic Acid equivalent (GAE)/mg dry extract (figure 1). A high concentration of phenolic compounds was determined in HP accounting for 1.79±0.03 µg gallic acid eq. (GAE)/mg dry extract. The lowest phenolic concentration was determined for MP (1.11 ± 0.01 µg gallic acid eq. (GAE)/mg dry extract).

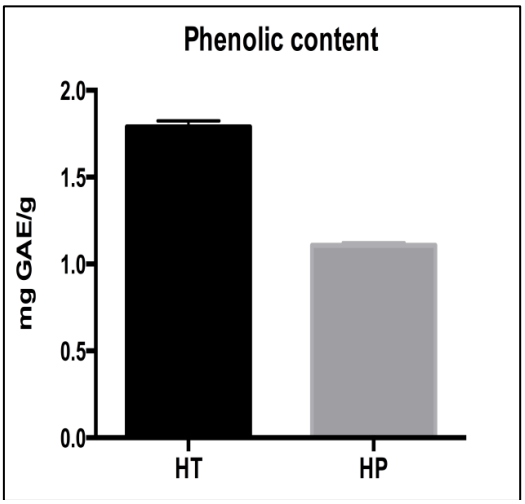

Figure 1: Total polyphenol content expressed as gallic acid equivalents (mg GAE)/ g dry extracts) in HP and MP extracts. Data are expressed as mean ± SD (*n* = 3)

Total flavonoid content

The phenolic extracts of HP and MP were characterized by the presence of considerable amount of flavonoid compounds (figure 2). The highest amount of flavonoid content was found in HP with (3.83±0.05) mg rutin equivalent RE/g dry extract, followed by MP (3.13±0.08) mg RE/g dry extract.

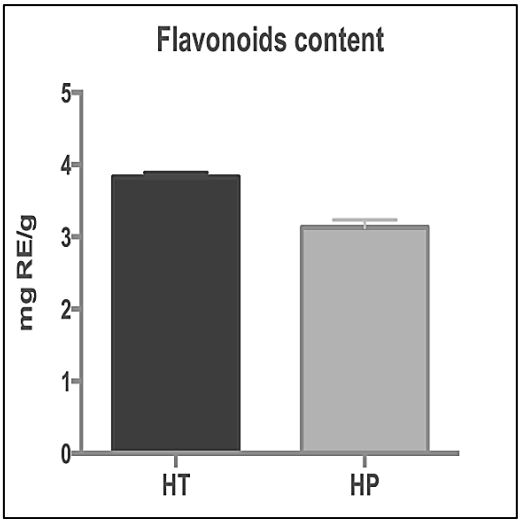

Figure 2: Total flavonoid content expressed as rutin equivalents (mg RE)/ g dry extracts in HP and MP extracts. Data are expressed as mean ± SD (*n* = 3)

Total tannin content

Figure 3 shows the total tannin content of each extract in as mg catechin equivalents (CEQ) per gram dry weight (dw). We found that HP had a significantly greater content (18.19±2.79) than MP (12.31±0.36).

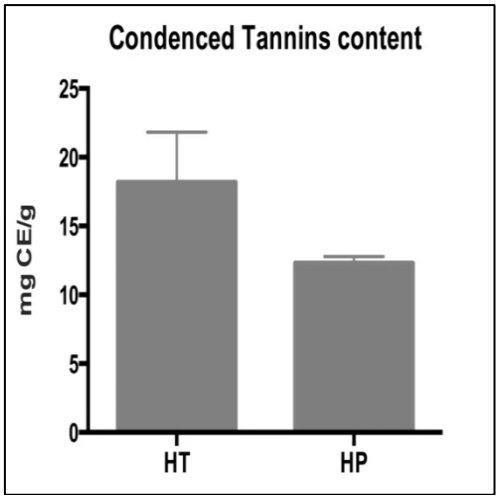

Figure 3: total tannin content of each extract in as mg catechin equivalents (CEQ) per gram dry weight (dw)

Total tannin content expressed as catechin equivalents (mg CEQ)/gdry extracts) in HP and MP extracts. Data are expressed as mean ± SD (n = 3).

Quantification of phenolic compounds (HPLC-DAD-MS)

The analysis of phenolic compounds in argan oils was performed by HPLC-DAD-MS method. The phenolic profiles vary according to the processes of the preparation of the argan oil. As shown in Figure 1 and Table 2, ten phenolic compounds were quantified and significant differences between HP and MP were observed, such as gallic acid, p-hydroxybenzoic acid, vannilic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, epicatechine and quercetin. The changes in the contents of the quantified phenolics in HP showed that ferulic acid presented the highest concentration (4.02 mg/ Kg). The second major compound was syringic acid with a concentration at (3.72 mg/Kg). Other minor phenolic compounds were detected in traces. However, The MP argan oil was rich in the ferulic acid (4.45 mg/Kg), syringic acid (3.60 mg/Kg), p-hydroxybenzoic acid (1.98 mg/Kg), vannilic acid (1.59 mg/Kg) and caffeic acid (1.28 mg/Kg); respectively. This result is similar to the data previously reported by Rueda et al., 2016 [18].

Table 2: Phenolic compounds content in Argan oils (milligrams per kilogram (mg/Kg))

		HP	MP
Phenolic acids (Mean ± SD)	Gallic acid	0.24 ± 0.13	0.22 ± 0.15
	Pyrogallol	ND	ND
	Chlorogenic acid	ND	ND
	p-Hydroxybenzoic acid	1.43 ± 0.58	1.98 ± 0.71
	Vannilic acid	1.63 ± 0.81	1.59 ± 0.64
	Caffeic acid	1.10 ± 0.19	1.28 ± 0.67
	Syringic acid	3.72 ± 0.62	3.60 ± 0.66
	p-Coumaric acid	0.47 ± 0.32	0.52 ± 0.41
	Ferulic acid	4.02 ± 2.09	4.45 ± 2.67
	Sinapic acid	0.25 ± 0.03	0.23 ± 0.09
	Salycilyc acid	ND	ND
	Rosmarinic acid	ND	ND
	Resveratrol	ND	ND
	Pyrocatechol	ND	ND
	Epicatechine	0.12 ± 0.15	0.14 ± 0.11
	Quercetin	0.19 ± 0.14	0.12 ± 0.13
Flavonoids			
	Catechin	ND	ND
	Rutin	ND	ND
	Tannic acid	ND	ND
ND = Not detected SD= Standard deviation			

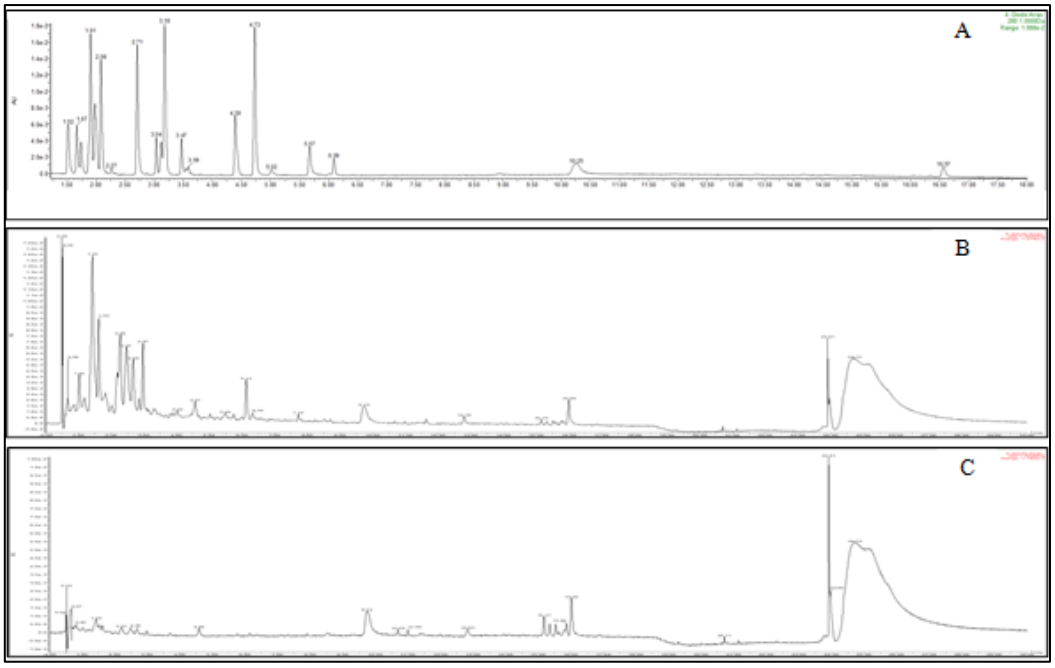

Figure 4: A, B and C, HPLC-DAD chromatograms at 280 nm of phenolic standards, HP and MP argan oils, respectively

DISCUSSION

The argan oil has been attributed with a range of pharmacological characteristics, including anti-inflammatory, analgesic, phagocytic, Antithrombotic and hypoglycemic properties [9,19-20]. Thus the present study was undertaken with the aim to compare the antioxidant potentials of traditional and industrial argan oil, known to be widely used in Moroccan food and folk Moroccan medicine, using three widely known methods (DPPH, ABTS and FRAP) and to find any correlation between the antioxidant activity and total phenolic, total flavonoid contents of the argan oil.The three tests clearly proved that both extracts possess considerable antiradical and antioxidant properties. Therefore, the argan oil derived from hand pressed extraction had the highest antioxidant activity owing to its high content of phenolic compounds flavonoids and tanins.

Similar results were found by Cadi et al. (2013), which revealed that argan oil derived from hand pressed extraction, has a protective activity against H_2O_2 (oxidant agent) toxicity in *Tetrahymena Pyriformis*. Also, a study of El babili et al., [20] has reported that Argania spinosa had promising antioxidant activity. Additionally, our results are in agreement with previous work of Amzal et al. [21] .who demonstrated that the saponins compounds extracted from A. spinosa had promising antioxidant properties.

Therefore, the argan oil derived from hand pressed extraction had the highest antioxidant activity owing to its high content of phenolic compounds flavonoids and tanins. Several studies have reported variations in the biological activities and phenolic content of extracts prepared using different extraction techniques [22-24]. In traditionnal extraction, the highest activity observed can be explained by the presence of residue from the extraction meal, rich in saponins, (Cotton 1888, Battino 1929 Charrouf 1991) known for its strong antioxidant [25,26].

Several chemical analysis of the argan oil showed the presence of sterols (295 mg/100 g oil), tocopherols (637 mg/kg oil), polyphenols (3263 µg/kg oil), and carotenes (545 mg/100 g oil), which are strong scavengers and high reducing effect [6,28,29,30]. Since, our results suggest that the antioxidant effect of the *A. spinosa* oil could be related to its high content of phenolic compounds.

The results of our study also showed a positively high correlation between total phenolic content in various extracts of two vegetable oil extract of *A. spinosa* derived from two different methods of extraction hand pressed and mechanical cold-pressed and antioxidant capacities using two methods DPPH, ABTS and FRAP assays. These results were in accordance with other researches, many studies revealed that phenolic compounds are major antioxidant constituents in medicinal plants, vegetables, fruits, and spices [31] and there are direct relationships between their antioxidant activity and total phenolic content [32].

CONCLUSION

The results obtained from this study suggest that argan oil is a strong radical scavenger and can be seen as potential source of natural antioxidants for medicinal and commercial uses. However the hand pressed extraction technique, exhibited better antioxidant activities and higher phenolic contents.

COMPETING INTERESTS

All contributing authors declare no competing interests.

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