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Research Article

In-vivo anti-inflammatory response and bioactive compounds profile of polyphenolic-extracts from edible Argan oil (*Argania spinosa* L.), obtained by two extraction methods

Running title: In-vivo anti-inflammatory response of Argan oils

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Abstract

The present work examined and assessed the in-vivo anti-inflammatory effect of polyphenolic extracts from Moroccan edible Argan oils (*Argania spinosa* L.), extracted by two extraction processes: hand pressing (HP) and mechanical pressing (MP). Chemical properties, such as acidity, peroxide index, ultraviolet indices, total polyphenols, fatty-acid-, tocopherol-, phenolic profiling and sterol composition were measured. Then, the anti-inflammatory potential was determined by applying carrageenan, an induced paw oedema test in rats. The results revealed an anti-inflammatory effect of edible Argan oil and indicated a higher efficiency of hand-pressed oil compared to mechanical-pressed oil, supporting its traditional use in human health, related to pain and inflammations. The chemical composition of these oils was evaluated, and total polyphenols, tocopherol composition and some phenolic compounds were found highly concentrated in the hand-pressed oil.

Keywords: Polyphenolic extract; *Argania spinosa;* Edible Argan oil; Anti-inflammatory activity; Chemical composition.

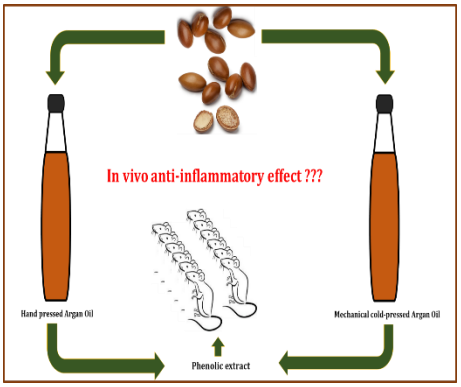
Practical application

The present study highlights and compares the in-vivo anti-inflammatory effect of polyphenolic compounds, extracted from Argan oil by two processes (hand and mechanical extraction). The study demonstrated the better quality of hand-pressed oil over mechanically pressed, supporting the traditional uses of this oil in treating several inflammations and pain-related situations. On the other hand, the edible Argan oil maybe introduced as a regular diet and food ingredient.

Abbreviations

▪ Hand pressed (HP) ▪ Mechanical cold-pressed (MP) ▪ Saturated fatty acids (SFA) ▪ Mono-unsaturated fatty acid (MUFA) ▪ Poly-unsaturated fatty acid (PUFA) ▪ Arachidic acid ($C_{20:0}$) ▪ Gadoleic acid ($C_{20:1}$) ▪ Linoleic acid ($C_{18:2}$) ▪ Linolenic acid ($C_{18:3}$) ▪ Myristic acid ($C_{14:0}$) ▪ Oleic acid ($C_{18:1}$) ▪ Palmitic acid ($C_{16:0}$) ▪ Stearic acid ($C_{18:0}$).

Graphical abstract



- In-vivo anti-inflammatory effect of polyphenolics extracted from edible Argan oils by either hand- or mechanical-pressing.

- Chemical and bioactive profiling of both hand- and mechanically pressed edible Argan oils.

- Comparative study between the two edible Argan oils.

1. Introduction

Nowadays, natural food ingredients and specific natural anti-inflammatory compounds, which have benefits on the human health receive more attention from the scientific community. Several studies were conducted on the anti-inflammatory effects of Mediterranean plants and foods (Hodge *et al* 2018, Conforti *et al.* 2008). The anti-inflammatory potential was studied in order to indicate natural, safe and effective ingredients for food diets and pharmaceutical applications. The phenolic compounds from plants exhibit a high anti-inflammatory action against chronic inflammatory diseases (Recio *et al.* 2012). No study has been reported yet on the anti-inflammatory action of Argan oils.

The Argan (*Argania spenosa* L.) forests form a locomotive for social, economic and human development in the Moroccan Southwestern regions. They are direct sources of wood, food and human existence for centuries (El Abbassi *et al.* 2014). The forests are covering a zone including the Anti-Atlas foothill mountains, the fertile Souss-valley area and the coastal region between Agadir and Essaouira (Charrouf and Guillaume 2008, Charrouf and Guillaume 2014). Argan oil was demonstrated to have significant health advantages and benefits compared to other edible vegetable oils (Charrouf and Guillaume 2008). The Argan oil shows a balanced composition, including tocopherols (Khallouki *et al.* 2003), fatty acids (Rueda *et al.* 2014), sterols (Charrouf and Guillaume 1999, Charrouf and Guillaume 2008, Charrouf *et al.* 2007), triacyl-glycerols (Charrouf and Guillaume 2014), polyphenols (Charrouf *et al.* 2007), pigments and antioxidant compounds (El Monfalouti *et al.* 2010) . Argan oil can be prepared by two extraction processes resulting in cosmetic grade from unroasted pulps, and edible grade oils from roasted pulps (Charrouf *et al.* 2002, Hilali *et al.* 2005) . Briefly, to prepare edible Argan oil traditionally (or hand pressed (HP)), the Argan fruits are broken and the kernels roasted, which then are broken using a manual millstone. The oil is extracted directly from this paste using a small quantity of warm water. On the other hand, edible Argan oil may also be prepared using a mechanic cold-pressing (MP) technique, which eliminates the step of mixing the roasted kernels paste with warm water. The oil is directly extracted using an automated procedure (Guillaume and Charrouf 2016, Matthäus *et al.* 2010). On the other hand, cosmetic oil grade is prepared directly from unroasted kernels by mechanical pressing (El Abbassi *et al.* 2014).

Edible Argan oil is used for medicinal and food nutritional purposes. It is frequently introduced in high-energy food compositions (El Abbassi *et al.* 2014). Moreover, several pharmacological activities were demonstrated in in-vitro investigations on edible Argan oils (Charrouf and Guillaume 2008) and they are probably due to the occurrence of various but valuable trace compounds (Khallouki *et al.* 2003). The consumption of Argan oil as a diet food is highly correlated to beneficial human-health effects (Charrouf and Guillaume 2010). Some pharmacological and chemical studies have also distinctly related the bioactive properties of the oil and its polyphenolic bioactive molecules (Owen et al. 2000a, Owen et al. 2000b).

The specific chemical profiling of edible Argan oil in relation to its various active ingredients could be the cause of a possible anti-inflammatory action. The present work aims assessing the anti-inflammatory effects by testing polyphenolic extracts resulting from two different edible Argan oils (HP and MP) on two types of edema induced in Wistar rat. The chemical composition and bioactive molecules of HP and MP were also quantified and discussed. This work presents and discusses the first study about the in vivo anti-inflammatory potential of polyphenolic extracts from edible Argan oils.

2. MATERIALS AND METHODS

2.1. Sample collection and authentication

Both edible Argan oils hand pressing (HP) according to the traditional method and mechanical pressing (MP) using cold pression, produced in southwestern Morocco (Taroudant) using roasted kernels, were studied . The Argan kernels were roasted at 110°C for 25 min.

2.2. Sample preparation and extraction

In this study the polyphenolic components were extracted from the edible Argan oils respecting the experimental protocol from Pirisi *et al.* (2000). In summary, 100 g of each sample was dissolved in pure n-hexane (500 ml) and liquid-liquid extraction with water/ methanol (50 v/v) was done. After vortexing the system was centrifuged. Finally, the

extracted solution was washed twice by n-hexane, and then methanolic fractions lyophilized over night during 12 hours (Kamal *et al.* 2016).

2.3. Physico-chemical investigation

The free acidity, peroxide index (PI), and two coefficients of absorbance (K_{232} measured at 232 nm and K_{270} measured at 270 nm) were determined according to methodology defined by the official document EEC/2568/9 from the European Commission Community (European Commission 1991). The acidity was expressed in % oleic acid. The peroxide index is given in milli-equivalents active oxygen per kilogram Argan oil (meq $O_2.kg^{-1}$).

2.4. Fatty-acid profiling

The fatty acid (FA) profiling of the edible Argan oil samples was done based on the methyl ester trans-esterification procedure using the Official Procedures of Analysis (European Commission 1991). The FA were quantified by gas chromatography (Agilent Technologies, model 6890, Wilmington, DE, USA). The system was coupled to a flame ionization detector (FID). The separation was carried out on a HP-88 capillary column (Agilent Technologies, Madrid, Spain; with the following dimensions 0.25 mm ID x 100 m x 0.20 µm film). The analytical conditions were already given in Kharbach *et al.* (2017). Briefly, helium was carrier gas, at a flow rate of 1 $mL.min^{-1}$. Detector and injector temperatures were maintained at 250 and 230°C, respectively, while 210 °C was kept for the oven temperature. Eight FA were identified and quantified by comparison to authentic standards (Sigma Aldrich, St Quentin Fallavier, France) and the results were expressed in % of total FA.

2.5. Tocopherols determination and polyphenols content

The tocopherols content was determined according to the Ce8-89/AOCS procedures (AOCS 1998). The dilution of 10 mg edible Argan oil in *n*-hexane (100 mL) was injected in a liquid chromatography system (Agilent Technologies, 1100 series, Waldbronn, Germany), in order to quantify the tocopherols. The system was coupled to a (G1321A)

fluorescence detector (Agilent). The separation was carried out using a C_{18} column (Varian, dimensions 4.6 mm× 25 cm × 5.0 µm, ChromSpher, Middelburg, The Netherlands). Isocratic mode was applied with an acetonitrile/methanol (50v/50v) mobile phase at a flow rate of 1 mL.min^{-1}, and 20 µL was injected. Both emission and excitation wavelengths were 330 and 295 nm, respectively.

The total polyphenol content in MP and HP samples was assessed following the colorimetric Folin–Ciocalteu procedure, using a gallic acid standard curve (Lister and Wilson 2001). A mass of 10 mg of polyphenolic extracts was diluted in pure methanol (10 mL) (see higher), and then 100 µL was taken and diluted with fresh prepared Folin–Ciocalteu solution (500 µL) and milli-Q water (1000 µL). A 20% m/v sodium carbonate (Na_2CO_3) (1500 µL) solution was mixed with the samples. These mixtures were incubated in the darkness for 2 hours. The absorbance was recorded at 765 nm using a UV–Visible spectrophotometer (UV-2450, Shimadzu Technologies, Tokyo, Japan) and the polyphenols content was expressed in mg gallic acid equivalents per kilogram of Argan oil (mg GEA.kg^{-1}).

2.6. Sterol composition

The sterol composition of the samples was measured following the corresponding sterols ISO procedure (ISO 12228, 2002). The capillary column DB-5 (Agilent Technologies, Spain, Madrid; with dimensions 0.25 mm ID × 30 m × 0.25-µm film) used for the sterol's separation and the same gas chromatographic instrument as for the FA quantification (see above) were applied. The temperature of the column was preserved at 280 ° C. Both detector and injector temperatures were maintained at 290 °C. The carrier gas was helium at a flow rate of 2 mL.min^{-1}. Sterol identification and quantification were done using their relative retention times and sterol standards curves. The individual sterol contents were mentioned in % of sterols, while the total sterol amount was expressed in mg.100 g^{-1} of oil.

2.7. HPLC-DAD-TOF/MS separation conditions for phenolic profiling

A liquid chromatography equipment (Agilent System Technologies, 1100, Wilmington, DE, USA) was associated to Diode-Array Detection (DAD/G1315 B), a binary pump

(Agilent/ G1312 A), and an autosampler (Agilent/ G1330B). This system was also connected to a time-of-flight detector (TOF) mass spectroscopic detector (MS) by an electrospray ionizer source (TOF-MS/ESI-; Micromass Quattro Micro; Agilent Technologies). The MS was operated in negative mode with the following operational conditions: cone and capillary voltages were 20 V and 3.0 kV; extractor, 2 V; cone gas flow 30 $L.h^{-1}$; source temperature 100 °C; desolvation temperature 350 °C; and desolvation gas flow 350 $L.h^{-1}$. The chromatographic separation was performed on a C18 column (Eclipse, XDB-C18/Zorbax, 2.1mm x 100mm, 1.7µm particle size) at a temperature 35 ºC. Chromatographic elution was carried out by applying two mobile phases, pure water with 0.1 % formic acid (A) and acetonitrile with 0.1 % formic acid (B) (v/v); applying the following gradient (v/v), 0 min, 90 % A; 0-18 min, 30 % A; 18-20 min, 0 % A; 20-23 min, 0% A; 23-25 min, 90% A; 25-30 min, 90% A, with a flow rate of 0.5 ml/min, and 10 µl injection volume. The phenolic-acids identification and quantification were done according to the MS fragmentation, retention times and calibration curves using standards from Sigma Aldrich (St Quentin Fallavier, France).

The Argan oil polyphenolic extract was obtained by the liquid/liquid extraction described higher, 10 mg of each extract was diluted in methanol (10 ml) and then three successive washes by n-hexane was done, then 1ml of sample was filtered at a syringe filter (PVDF, 0.2 µm) and was used for the HPLC-DAD-TOF/MS .

2.8. Animals

Adult male Wistar rats (180–220 g) were housed at animal care of the Laboratory of Pharmacology, Faculty of Medicine and Pharmacy of Rabat, Morocco. All Wistar rats were breeded in one room of 20 ± 1 °C with a 12 h light-12 h dark cycle and had access to standard diet and water (Sayah *et al* 2017). Wistar rats used were fasted for 18 h before oral administration of extracts. The animals were handled according to the prescribed ethical guidelines of the “Guide for the Care and Use of Laboratory Animals” made by the Moroccan Association of Animal Sciences Laboratory (MAASL), and in accordance to the guideline prescribed by the National Academy of Sciences and adapted by the National Institutes of Health.

2.9. Statistical analysis

The results of this study have been reported as mean ± standard deviation (SD). The student's t-test and the analysis of variance (one-way-ANOVA) were applied for comparing results. A p-value < 0.05 was considered significant.

3. Anti-inflammatory study

The evaluation of the polyphenolic-extract anti-inflammatory activity of edible Argan oil (*Argania spinosa* L.) on paw oedema was made by experimental trials using two stimulus : chemical stimul (Winter et al. 1963) or mechanical stimul (Riesterer and Jaques 1970)). After the stimuli, all Wistar rats fasted during 18 hours before the anti-inflammatory test and received 5 mL water with gastric gavages. The right hind paw is considered as control without treatment. The anti-inflammatory activity was expressed as percentage inhibition (%) of oedema thickness in treated animals vs the control group:

$$\% \text{ of inhibition} = (\text{mean } [V_L - V_R]_{\text{Control}} - \text{mean } [V_L - V_R]_{\text{Treated}}) / [V_L - V_R]_{\text{Control}} \times 100$$

With V_R the oedema volume of the right paw, and V_L the oedema volume of the left paw.

3.1. Induced -paw-oedema with Carrageenan

In the present study, anti-inflammatory activity was tested using the carrageenan-induced paw oedema assay, according to Winter *et al.* (1963) and Ferreira *et al.* (1971). For each polyphenolic extract (HP or MP) is a group of six male Wistar rats was applied as control and reference groups (n=6 for each group).

Injection of 0.05 mL 1% carrageenan suspension in 0.9 % NaCl into the left paw and under the sub-plantar aponeurosis was made (Nguemfo *et al* 2007), 1 hour after the oral administration of the polyphenolic compounds from the Argan oils at 300 and 500 $mg.Kg^{1}$ or of reference drug indomethacin at 20 $mg.Kg^{-1}$ (Kaushik *et al* 2012).

The volume variation was measured using a plethysmometer LE7500 (Ugo Basile, Italy) after the carrageenan injection and after at 90, 180 and 360 min.

3.2. Experimental trauma induced paw oedema in rat

An evaluation of the anti-inflammatory activity was also done according to the protocol published by Riesterer and Jaques (1970). The male wistar rats (180-220 g) were divided into different groups (n=6). Control rats received 5 $mL.Kg^{-1}$ water and the standard rats group receiving 20 $mg.Kg^{-1}$ indomethacin, while the test groups orally received different concentrations of polyphenolic compounds extract of Argan oil at 300 and 500 $mg.Kg^{-1}$. Then, 60 min after receiving the substances, a weight of 50 g was dropped to all animals (left paw) (Riesterer and Jaques 1970). A plethysmometer Digitals 7500 was used to measure the difference in volume between the left and the right hind paw at 90, 180 and 360 min.

4. Results and discussion

4.1. Chemical and bioactive profiling

Both MP and HP oils were physico-chemically investigated and 33 variables, including acidity, peroxide content, UV indices (K_{232} and K_{270}), fatty-acid-, tocopherol-, total polyphenol, sterol and phenolic composition, were determined.

The parameters composition allows classifying the analyzed MP and HP samples within the "Extra Virgin" category according to the requirements from the Moroccan official guidelines on Argan oil (Guidelines of Moroccan Normalization 2003). (Table 1)

4.1.1. Physico-chemically investigation

Acidity, peroxide content and both UV absorbances (at 232nm and at 270nm) were used to evaluate the freshness and purity of Argan oils. The acidity parameter indicates the free acidity in terms of oleic acid in Argan oils, whereas the peroxide value measures the presence of primary oxidation products. Both K_{232} and K_{270} indicate the measure of the conjugated dienes and trienes formed by the unsaturated fatty acids autoxidation (Kharbach *et al.* 2018).

Table 1, shows the acidity, peroxide index, ultraviolet indices (at 232nm and at 270nm) of both MP and HP samples. The values do not exceed the extra virgin specification parameters established and thus, the samples were classified in the extra virgin category (Guidelines of Moroccan Normalization 2003).

4.1.2. Fatty acid composition

The Argan oil was characterized by their specific fatty acids. Recently, a comprehensive fatty acid profiling and geographic traceability of Edible extra virgin Argan oils was done (Kharbach *et al.* 2019). The high presence of $C_{18:1}$ oleic acid, is required to range between 43 and 49% from total FA, which represents approximately 99% of the mono-unsaturated fatty acids (MUFA) composition. Further, $C_{18:2}$ linoleic acid and $C_{18:3}$ linolenic acid should account about 29-36% and ≤ 0.30% of the total fatty acids, respectively, and their sum indicates the poly-unsaturated fatty acids (PUFA) composition.

The individual and total fatty acid profiles of both MP and HP samples were reported in Table 1. The results showed a significant difference between the samples. The MP samples were highly rich in $C_{18:1}$ oleic acid and $C_{18:2}$ linoleic acid, accounting for approximately 48% and 35% compared to 44% and 30% in HP oils, respectively. Both $C_{18:0}$ stearic acid and $C_{16:0}$ palmitic acid accounted for 5.5% and 15% in MP samples compared to 11% and 4.7% in HP oils, respectively. $C_{18:3}$ linolenic acid, $C_{20:1}$ gadoleic acid, $C_{14:0}$ myristic acid, and $C_{20:0}$ arachidic acid were also quantified in the both MP and HP oils, but occur in in trace contents.

The MUFA ($C_{18:1}$ + $C_{20:1}$), PUFA ($C_{18:3}$ + $C_{18:2}$), and SFA fractions ($C_{18:0}$ + $C_{16:0}$ + $C_{14:0}$ + $C_{20:0}$), were 49%, 36% and 21% range in MP, while 44%, 30%, and 17% of the total fatty acids in HP oils. These wide intervals might relate particularly to the Argan oil extraction process (mechanical or hand pressing) (Matthäus *et al* 2010). Both MUFA/PUFA and PUFA/SFA ratios were investigated in order to assess the nutritional value and the quality of the both oils (Kharbach *et al* 2018). The results showed good similarity for the two ratios in the MP and HP oils.

4.1.3. Tocopherols composition/polyphenols content

The Argan oil is rich in antioxidants, including tocopherols (α, β, γ and δ) (Hilali *et al* 2005). The results for tocopherols from MP and HP oils are reported in Table 1. They exhibited a significant difference between the MP and HP samples. The predominant compound is γ-tocopherol occurring at 715 $mg.kg^{-1}$ in MP samples and at 801 $mg.kg^{-1}$ in HP samples ; followed by δ-tocopherol with 56 (HP) and 103 $mg.kg^{-1}$ (MP); α-tocopherol with 24 and 42 $mg.Kg^{-1}$ in HP and MP samples, respectively; while the β-

tocopherol showed concentrations of 1 $mg.kg^{-1}$ (HP) and 3 $mg.kg^{-1}$ (MP). The total tocopherol contents in MP and HP samples were 860 to 884 $mg.kg^{-1}$, respectively. However, the roasting process of Argan kernels before oil extraction resulted in a decreased of α-tocopherol amount (Hilali *et al* 2005). The tocopherols composition variability might relate to several factors, such as extraction process, storage, harvesting time and geographical origin (Gharby *et al.* 2011, Hilali, Charrouf, Aziz Soulhi, Hachimi and Guillaume 2005, Kharbach *et al* 2017, Kharbach *et al* 2018).

The polyphenol content was much higher in HP samples, with 108 mg $GAE.kg^{-1}$ oil, than in the MP samples with 88 mg $GAE.kg^{-1}$. The results are summarized in Table 1. The polyphenol content thus varied significantly depending to the Argan oil type (mechanical or traditional pressing). In addition, Argan oil shows a high polyphenol amount in comparison to several other oils (Marfil *et al.* 2011).

4.1.4. Sterol composition

Phytosterols form an essential non-saponifiable fraction from Argan oil with highly nutritional and pharmacological properties (El Abbassi *et al* 2014, Hilali, Charrouf *et al* 2005).

Schottenol, followed by spinasterol, stigma-8–22-dien-3β-ol, and Δ−7-avenasterol are the predominant sterols, while campesterol and cholesterol were found in trace amounts in the MP and HP samples. Schottenol occurred at 47% (HP) and 49% (MP) of the total sterols, while spinasterol varied between 41% (HP) and 44% (MP). Schottenol and spinasterol exhibit a beneficial effects on cholesterol metabolism and biological activities towards the nervous system (El Kharrassi *et al* 2014). Δ-7-avenasterol (5.5–6.0%) and the stigma-8–22-dien-3β-ol (5.1–5.6%) also quantified in the HP and MP samples, respectively (Table 1). Fraud in Argan oils maybe handled by evaluating the level of campesterol (Hilali *et al* 2007).

The MP samples showed a higher total sterols content (211 $mg.100^{-1}$ g) than the HP samples (195 $mg.100^{-1}$ g) in. A significant difference between the samples was revealed. The results were in accordance with the sterol composition of extra virgin Argan oil determined in Kharbach *et al.* (2018). Numerous studies reported on the sterols composition of Argan oils and demonstrated that several factors might influence their

concentration including the extraction process (mechanical or hand-press preparation) (Matthäus *et al* 2010).

4.1.5. Phenolic profiling analysis

The pharmacological potential of Argan oil is generally related to the antioxidants, specifically the polyphenolic compounds (El Monfalouti *et al.* 2010). The level of polyphenols in Argan oil can be very low (Charrouf and Guillaume 2007). In the literature, the polyphenol content of virgin Argan oil is reported to be higher than that of other edible vegetable oils but lower than that of virgin olive oil (Cabrera-Vique *et al.* 2012). Earlier Khallouki *et al.* (2003) studied the phenolic compounds in Argan oil using GC-MS and only four phenolics were quantified (i.e. ferulic acid, vanillic acid, syringic acid and tyrosol).

The actual study, the phenolic composition was assessed in both HP and MP oils by HPLC-DAD-TOF/MS. The phenolic profile changes significantly according to the preparation process of Argan oil (HP or MP). The recorded results (Table 1) showed the presence of ten phenolic components, including ferulic-, syringic-, p-hydroxybenzoic-, vanillic-, caffeic, p-coumaric-, gallic-, sinapic acids epicatechin and quercetin. The HP phenolic extract exhibited a high ferulic acid content (4.56 $mg.kg^{-1}$), following by syringic acid, 3.61 $mg.kg^{-1}$; p-hydroxybenzoic acid, 2.05 $mg.kg^{-1}$; vanillic acid, 1.64 $mg.kg^{-1}$; caffeic acid, 1.34 $mg.kg^{-1}$; and p-coumaric acid, 0.63 $mg.kg^{-1}$. The MP phenolic extract revealed a high concentration in ferulic acid, 4.10 $mg.kg^{-1}$; followed by the syringic acid concentration 3.91 $mg.kg^{-1}$; vanillic acid, 1.73 $mg.kg^{-1}$; p-hydroxybenzoic acid, 1.53 $mg.kg^{-1}$; caffeic acid, 1.22 $mg.kg^{-1}$; and p-coumaric acid, 0.58 $mg.kg^{-1}$. Other phenolic components were quantified in trace concentrations in both oils (below 0.50 $mg.kg^{-1}$).

The effect of the preparation processes (resulting in cosmetic, edible, and traditional oils) on the phenolic composition of Argan oils and their related press cakes was demonstrated by using GC-MS (Rojas *et al* 2005). The results showed that the phenolic composition was directly influenced by the preparation process. For instance, 6 phenolic compounds were quantified in MP edible Argan oil (i.e. 3-hydroxypyridine, 6-methyl-3-hydroxypyridine, catechol, resorcinol, 4-hydroxy benzyl alcohol, and tyrosol) (Rojas *et al.* 2005). The phenolic composition of the Argan fruit parts (roasted and unroasted kernels, pulp, shell, and press cake) was also studied and revealed large differences, whereas eleven

compounds were identified by HPLC-ESI-MS (i.e. catechin-hydrate, epicatechin, epigallocatechin gallate, hyperoside, isoquercitrin, myricetin, phloridzin, procyanidin-B1, procyanidin-B2, quercitrin, and rutin) (El Monfalouti *et al.* 2012). Recently, a highly informative study of the polyphenol composition of Argan fruit (flesh or pulp) were presented in Khallouki *et al.* (2015), and 32 polyphenolic compounds were identified by HPLC-ESI-MS/MS. The results exhibited the presence of catechins (39%), followed by flavonoids (28%), procyanidins (26%), free phenolic acids (6%) and phenolic acid glycosides (1%) of a total of 15.4 g/kg of Argan fruits. Therefore, the polyphenolic composition from Argan oil need more investigation and several factor that can influence it occurrence should be tested.

4.1.6. Anti-inflammatory activity

Many plant-derived products are rich in bioactive polyphenols, of which some present powerful anti-inflammatory, antioxidant properties or health-promoting properties (Gorzynik-Debicka *et al* 2018). Various studies have suggested that the dietary intake of edible oils, e.g. olive oil, containing phenolic compounds, could reduce chronic inflammatory disease development (Cicerale *et al.* 2012). Carrageenan- and experimental trauma-induced paw edema in Wistar rats is an appropriate animal model to examine the antiedematous effect of different bioactive components like from plant extracts (Abe *et al.* 2003, Hajhashemi *et al.* 2003, Santos and Rao 2000, Sforcin *et al.* 2009). Though this study helps to screen the anti-inflammatory effect of phenolic compounds, only few information is obtained about their mechanism.

Induction of paw edema by carrageenan is a method extensively used to verify the anti-inflammatory activity of many substances. The edema, which is a result of carrageenan injection in the left foot, is a biphasic event (Vinegar *et al.* 1969). The first phase, which occurs between 0 and 150 min after the injection of carrageenan, has been attributed to serotonin, bradykinin and histamine on vascular permeability (Panthong *et al* 2007, Vinegar *et al* 1969, Yonathan *et al* 2006). Authors have declared that serotonin and histamine are released during 1h30min, whereas bradykinin is released during 2h30min after carrageenan injection (Mequanint *et al* 2011, Panthong *et al* 2007). The second phase, which occurs between 2h30min and 6 h post-carrageenan injection, contains the secretion of prostaglandins (Gomes *et al* 2008, Mequanint *et al* 2011). Carrageenan is

sensitive to cyclo-oxygenase and not to lipoxygenase inhibitors and is used to test the effect of nonsteroidal anti-inflammatory drugs which first inhibit the cyclooxygenase implicated in prostaglandins synthesis. It has been reported that the inhibition of carrageenan-induced inflammation stops after 360 min (Bounihi *et al* 2013).

As noticed in Table 2 and 3, both the injection of carrageenan into the sub-plantar tissue and experimental trauma of the left foot induced edema development in the control groups, which peaked (0,57 ± 0,014 and 0,71 ± 0,014 mL, respectively) in paw volume, 180 min after the induction. This confirms that carrageenan injection and experimental trauma provokes an acute inflammatory reaction into the left hind paw. Polyphenolic extracts from Argan oils (HP and MP) at 300 and 500 $mg.kg^{-1}$, p.o., reduced the edema in the first and second phases of carrageenan-inflammation (significance $p<0.05$). Both HP and MP effects on carrageenan-edema (Table 4 and Figure 1) were dose- and time-dependent; 180 min after carrageenan administration, HP and MP extracts at 500 $mg.kg^{1}$ showed a good inhibition activity with a peak effect of 72% for HP and 47% for MP. This effect from HP was not statistically different ($p < 0.05$) from that of the reference drug indomethacin at a dose of 10 mg/kg with an inhibition value of 72,63% (Table 4).

In the trauma-induced edema, the polyphenolic extracts of Argan oil (HP or MP) decreased edema in the different phases of the inflammatory response. The effect of HP and MP on experimental trauma was also dose dependent, as shown in Table 5 and Figure .2 with a significant and higher inhibition (56% for HP and 44% for MP after 180 min) produced at 500 mg/kg. The effect is in accordance to that caused by indomethacin (20 $mg.kg^{-1}$, 84.08 % inhibition). The inhibition of paw volume during the inflammation by polyphenolic extracts from the two Argan oil (HP and MP), was also reported by Masresha *et al* (2012). The polyphenolic extracts from edible Argan oil HP and MP, thus have an anti-inflammatory activity by inhibiting the secretion of inflammatory mediators; histamine and serotonin furthermore suppressing cytokine and prostaglandin (Bounihi *et al* 2013). However, the real mechanism of this anti-inflammatory action of the polyphenolic extracts from the edible Argan oils used in the present study is yet not clear and more investigation should be established.

The anti-inflammatory potential of the traditional edible Argan oil was found better than that of the oil obtained by mechanical pressing. This result directly indicates the effect of the preparation process on the therapeutic activity of the edible Argan oils.

Further investigation should be done in order to characterize and isolate the bioactive molecules responsible for the anti-inflammatory potential.

5. Conclusion

It can be concluded that the edible Argan oils have a good anti-inflammatory potential, in accordance with their traditional use in treating several inflammations and pain diseases. The in vivo anti-inflammatory effect of two types edible Argan oil, extracted by hand pressing and mechanical pressing was demonstrated. The results revealed that the hand-pressed oil has a higher anti-inflammatory effect than the mechanically pressed. Furthermore, polyphenols and tocopherols were more concentrated in the manually pressed oil.

Conflict of interest

The authors declare there is no conflict of interest.

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Table captions

Table 1. Chemical composition presented as mean value ± standard deviation (SD), N=5 in each oil. MP, mechanical pressing; HP, hand pressing.

Chemical parameter	EVAO regulation	MP			HP		
		Mean ± SD	Min	Max	Mean ± SD	Min	Max
Quality indices							
Acidity (%)	≤ 0.80	**0.35^{b} ± 0.02**	0.25	0.34	0.21^{a} ± 0.01	0,2	0,23
Peroxide index (meq O_2.kg^{-1})	≤ 15	**2.0^{b} ± 0.17**	2.0	2.5	1.9^{a} ± 0.20	1,7	2,1
K_{232}	≤ 2.52	**1.30^{b} ± 0.02**	1.23	1.32	1.25^{a} ± 0.03	1,2	1,3
K_{270}	≤ 0.35	0.20^{a} ± 0.02	0.20	0.24	**0.22^{b} ± 0.01**	0,2	0,24
Fatty Acids (% of total fatty acid)							
$C_{14:0}$	≤ 0.20%	0.15^{a} ± 0.02	0.14	0.17	0.15^{a} ± 0.02	0.13	0.18
$C_{16:0}$	11.50 -15.00%	**14.64^{b} ± 0.25**	14.20	15.19	11.80^{a} ± 0.32	11.12	12.96
$C_{18:0}$	4.30-7.20%	**5.52^{b} ± 0.22**	4.94	6.09	4.70^{a} ± 0.20	4.60	4.88
$C_{20:0}$	≤ 0.50%	**0.36^{a} ± 0.02**	0.33	0.37	0.35^{a} ± 0.05	0.28	0.44
$C_{18:1}$	43.10-49.00%	**47.88^{b} ± 0.42**	45.28	48.63	43.87^{a} ± 0.52	43.12	44.68
$C_{20:1}$	≤ 0.50%	0.42^{a} ± 0.03	0.43	0.49	**0.46^{b} ± 0.02**	0.42	0.50
$C_{18:2}$	29.30-36.00%	**35.19^{b} ± 0.51**	34.19	36.98	30.22^{a} ± 0.70	28.34	32.19
$C_{18:3}$	≤ 0.30%	**0.26^{b} ± 0.02**	0.21	0.29	0.12^{a} ± 0.04	0.10	0.15
SFA	-	**20.50^{b} ± 0.40**	18.50	22.67	17.18^{a} ± 0.46	17.15	19.90
MUFA	-	**48.50^{b} ± 0.61**	47.22	49.15	44.36^{a} ± 0.51	43.36	45.12
PUFA	-	**35.80^{b} ± 0.52**	34.50	36.54	30.39^{a} ± 0.79	33.40	35.42
MUFA/PUFA ratio	-	1.37^{a} ± 0.03	1.35	1.40	**1.44^{b} ± 0.04**	1.39	1.48
PUFA/SFA ratio	-	1.71^{a} ± 0.03	1.65	2.10	**1.78^{a} ± 0.04**	1.70	1.89
Tocopherols (mg.kg^{-1} of oil)							
α-tocopherol	18-75	**42.23^{b}** ± 2.52	38.26	46.16	24.46^{a} ± 2.51	22.22	27.82
β-tocopherol	01-05	**3.07^{b} ± 0.40**	2.90	3.20	1.22^{a} ± 0.14	1.12	1.90
ɣ-tocopherol	640-810	715.42^{a} ± 7.80	654.44	760.50	**800.77^{b} ± 6.52**	797.30	807.41
δ-tocopherol	54-110	**103.22^{b} ± 4.19**	96.88	110.55	57.70^{a} ± 3.10	55.28	60.81
Total tocopherol	600-900	860.15^{a} ± 9.26	800.70	910.03	**884.11^{b} ± 7.80**	752.59	807.51
Total polyphenols (mg GAE.kg^{-1})	-	87.7^{a} ± 0.80	70.80	95.04	**108.1^{b} ± 4.12**	83.30	120.05
Phenolic compounds (mg.kg^{-1})							
Gallic acid	-	**0.29^{b} ± 0.11**	0.18	0.41	**0.24^{a} ± 0.12**	0.12	0.36
p-Hydroxybenzoic acid	-	1.53^{a} ± 0.46	1.07	1.99	**2.05^{b} ± 0.50**	1.55	2.55

Vanillic acid	-	**1.73^{b} ± 0.52**	1.21	2.25	1.64^{a} ± 0.32	1.32	1.96
Caffeic acid	-	1.22^{a} ± 0.21	1.01	1.43	**1.34^{b} ± 0.27**	1.07	1.61
Syringic acid	-	**3.91^{b} ± 0.64**	3.27	4.55	3.61^{a} ± 0.63	2.98	4.23
p-Coumaric acid	-	0.58^{a} ± 0.31	0.27	0.89	**0.63^{b} ± 0.27**	0.36	0.90
Ferulic acid	-	4.10^{a} ± 1.02	3.08	5.12	**4.56^{b} ± 1.63**	2.93	6.19
Sinapic acid	-	**0.25^{a} ± 0.03**	0.22	0.28	0.23^{b} ± 0.05	0.18	0.28
Epicatechin	-	**0.22^{b} ± 0.09**	0.13	0.31	0.17^{a} ± 0.05	0.12	0.22
Quercetin	-	**0.26^{b} ± 0.10**	0.16	0.36	0.14^{a} ± 0.03	0.11	0.17
Sterols (% of total seterols)							
Cholesterol	≤ 0.40	**0.36^{b} ± 0.02**	0.35	0.40	0.24^{a} ± 0.02	0.23	0.29
Campesterol	≤ 0.40	**0.38^{b} ± 0.02**	0.33	0.40	0.17^{a} ± 0,02	0.15	0.22
Δ^{7}-Avenasterol	4.00 - 7.00	**6.01^{b} ± 0.20**	5.80	7.00	5.53^{a} ± 0.14	5.31	5.77
Stigma-8-22-dien-3β-ol	3.20 - 5.70	**5.60^{b} ± 0.11**	5.45	5.86	5.10^{a} ± 0.13	4.90	5.40
Schottenol	44.00 - 49.00	**48.70^{a} ± 0.47**	47.90	49.26	47.30^{a} ± 0.26	47.08	48.05
Spinasterol	34.00 - 44.00	**43.76^{b} ± 0.97**	42.19	44.27	41.02^{a} ± 0.41	31.15	42.11
Total sterols (mg.100g^{-1} of oil)	≤ 220 mg/100g	**211.12^{b} ± 13.02**	209.32	219.10	196.15^{a}± 15.53	190.15	212.72

The lowercase letters indicated significant differences in the same line (t-test); EVAO, extra virgin Argan oil; SFA, Saturated Fatty Acids; MUFA, Monounsaturated Fatty Acids and PUFA, Polyunsaturated Fatty Acids; The bold numbers represent the highest mean in each category.

Table 2: Effect of the polyphenolic extract from Argan oil (*Argania spinosa* L.) on carrageenan-induced rat paw edema.

Treatment group	Dose (mg.kg^{-1})	Mean edema volume (left -right paw) (mL)		
		1 h 30 min	3h	6h
Control 1	-	0.386 ± 0.016	0.570 ± 0.014	0.481 ± 0.014
Indomethacin	10	0.128 ± 0.014*	0.156 ± 0.016*	0.181 ± 0.014*
MP	300	0.298 ± 0.011*	0.350 ± 0.015*	0.32 ± 0.019*
MP	500	0.261 ± 0.017*	0.300 ± 0.018*	0.288 ± 0.02*
HP	300	0.200 ± 0.015*	0.250 ± 0.013*	0.230 ± 0.011*
HP	500	0.133 ± 0.014*	0.157 ± 0.019*	0.141 ± 0.014*

Values are expressed as mean ± SD; SD, standard deviation; (n=6 of each group); n, number of rats; HP, hand pressed; MP, mechanical cold-pressed; $p < 0.05$ statistically significant compared to the control. Reference drug is Indomethacin.

Table 3: Effect of the polyphenolic extract from Argan oil (*Argania spinosa* L.) on trauma -induced rat paw edema.

		Mean edema volume (left-right paw) (mL)		
Treatment groups	**Dose (mg.kg^{-1})**	**1 h 30 min**	**3h**	**6h**
Control 2	**-**	**0.458 ± 0.017**	**0.710 ± 0.014**	**0.550 ± 0.014**
Indomethacin	**20**	**0.060 ± 0.014***	**0.113 ± 0.016***	**0.135 ±0.010***
MP	**300**	**0.420 ± 0.015**	**0.511 ± 0.015***	**0.475 ± 0.014***
MP	**500**	**0.350 ± 0.019***	**0.398 ± 0.013***	**0.368 ± 0.015***
HP	**300**	**0.311 ± 0.015***	**0.372 ± 0.011***	**0.343 ± 0.019***
HP	**500**	**0.236 ± 0.014***	**0.310 ± 0.019***	**0.277 ± 0.017***

Values are expressed as mean ± SD; SD, standard deviation; (n=6 of each group); n, number of rats; HP, hand pressed; MP, mechanical cold-pressed; p < 0.05 statistically significant compared to the control. Reference drug is Indomethacin.

Table 4: Percentage inflammation inhibition by polyphenolic extract from edible Argan oils (*Argania spinosa* L.) on carrageenan-induced rat paw edema. p.o., oral route; * $p < 0.05$ statistically significant compared to the reference drug (Indomethacin).

		Percentage inhibition of edema (%)		
Treatment group	**Dose ($mg.kg^{-1}$) p.o.**	**1 h 30 min**	**3h**	**6h**
Indomethacin	**10**	**66.83**	**72.63**	**62.37**
MP	**300**	**22 .79***	**38.59***	**33.47***
MP	**500**	**32.38***	**47.36***	**40.12***
HP	**300**	**48.18***	**56.14***	**52.18***
HP	**500**	**65.54**	**72.45**	**70.68***

Table 5: Percentage inflammation inhibition by polyphenolic extract from edible Argan oils (*Argania spinosa* L.) on experimental trauma-induced rat paw edema. p.o., oral route; * p < 0.05 statistically significant compared to the reference drug (Indomethacin).

		Percentage inhibition of edema (%)		
	Dose ($mg.kg^{-1}$)			
Treatment group	**p.o**	**1 h 30 min**	**3h**	**6h**
Indomethacin	**20**	**86.89**	**84.08**	**75.45**
MP	**300**	**8.29***	**28.02***	**15.78***
MP	**500**	**23.58***	**43.94***	**33.09***
HP	**300**	**32.09***	**47.60***	**37.63***
HP	**500**	**48.47***	**56.33***	**49.63***

Figure legend list

Figure .1. Effects of Indomethacin and polyphenolic extract of edible Argan oil (*Argania spinosa* L.) on carrageenan –induced rat paw oedema.

Figure .2. Effects of Indomethacin of polyphenolic extract from edible Argan oil (*Argania spinosa* L.) on experimental trauma–induced rat paw oedema.

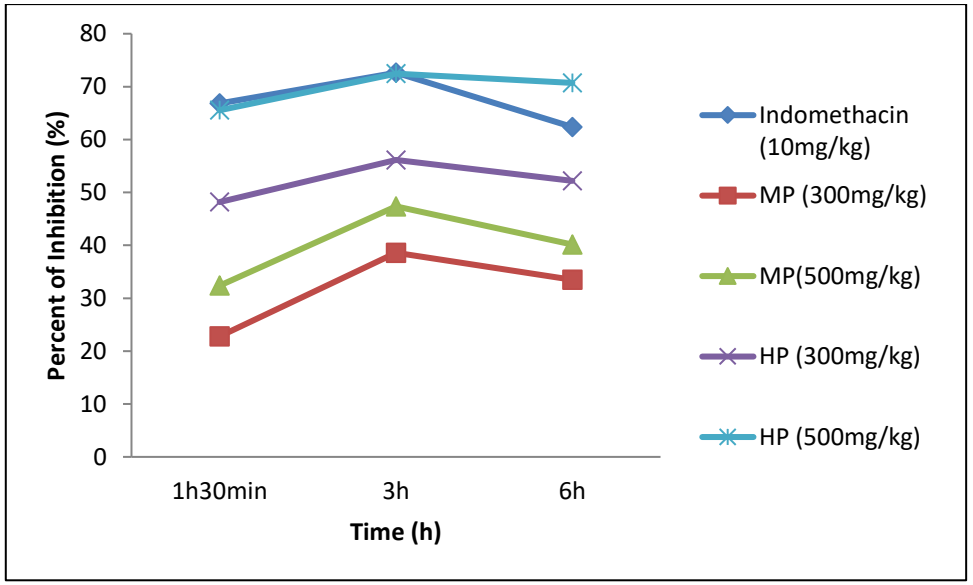

Figure .1. Effects of Indomethacin and polyphenolic extract of edible Argan oil (*Argania spinosa* L.) on carrageenan –induced rat paw oedema.

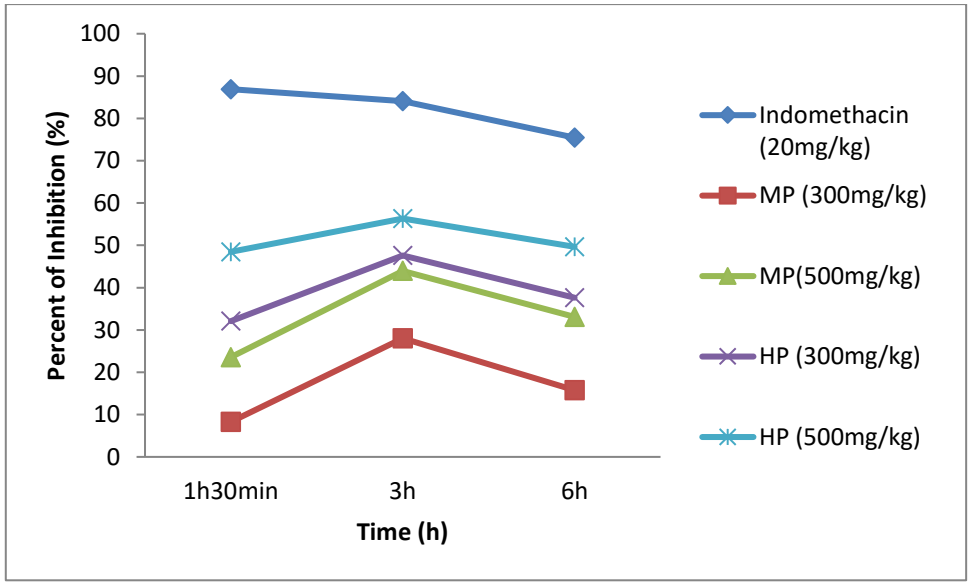

Figure .2. Effects of Indomethacin of polyphenolic extract from edible Argan oil (*Argania spinosa* L.) on experimental trauma–induced rat paw oedema.