Total Phenolic Content, flavonoids, tannins and Antioxidant Activity of endocarps fruits of *Argania spinosa* (L.) Skeels (sapotaceae)

**H.S. Sebaa and F. Cherifi**

Département de Biotechnologie Végétale, Laboratoire des Productions, Valorisations Végétales et Microbiennes (LP2VM), Faculté des sciences de la nature et de la vie, Université des Sciences et de la Technologie d’Oran Mohamed Boudiaf, B.P. 1505, El-Mn’aour, Oran 31000, Algeria

Copyright © 2021 ISSR Journals. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**ABSTRACT**: *Argania spinosa*, Sapotaceae sole representative in Algeria and Morocco, hence its endemic in these regions. Although it is a recognised oil, forage and timber tree highly adapted to aridity. The exploitation of the argan fruits, produces considerable amounts of under or related products. These products, such as the endocarps of a fruit, recuperated after use of kernels to extract oil.

Phytochemical screening of *A. spinosa* endocarps indicated the presence of, tannins, flavonoid aglycons, proanthocyanidins, saponins and coumarins in immature endocarps. Whereas, Alkaloids and reducteurs compounds were detected only in mature endocarps. The total phenolics, total flavonoids and proanthocyanidins contents were in the ranges of 985.75 +/-0.45, 100,1 +/-0.55 and 280,47 +/-0.45 mg/g respectively in immature endocarps. In mature endocarps these compounds represent, 106,58 +/-0.45, 4,512 +/-0.55 and 104.15 +/-0.55 mg/g respectively. The 50% inhibitory concentrations (IC50) in the radical-scavenging assay against 1,1-diphenyl-2-picrylhydrazyl hydrate radical (DPPH) of immature endocarps and mature endocarps (24H, 1week) were 322; 550 and 600 μg/mL respectively. Thus, the total polyphenols (TP), total flavonoids (TF) and condensed tannins (CT) contents exhibited the highest levels in the unripe fruits. The antiradical activity trend was positively correlated to the behavior of the bioactive compounds content.

The results demonstrated that the methanol extract of the fruit endocarps of *A.spinosa* is a potential source of natural antioxidants. Thus, further work should be screened bioactivity, isolated, characterized and elucidated the bioactive compounds of the extract for medicinal value.

Antioxidant activity of extracts was expressed as percentage of DPPH radical’s inhibition and IC50 values (μg/ml).

**KEYWORDS**: Antioxidant activity; total phenolic content; DPPH assay.

1 **INTRODUCTION**

In Algeria, Arid and semi-arid plants are good sources for the production of various types of secondary metabolites which make them resistant to various environmental stress scarcity of water, salinity, pathogens etc [1]. Some endemic species are well adapted to these conditions like the argan tree. *Argania spinosa* (L.) skeels, belongs to the family Sapotaceae is endemic to the Southwestern Algeria in northern Tindouf [2]. Where it grows in over 50,670 hectares [3]. This tree is important for ecological sustainability [4]. The woodlands protect against soil erosion and desertification owing to their deep-growing roots, they shade different types of crops, and help maintain soil fertility in arid zones [5]. The argan tree also supports indigenous populations economically since almonds are used to produce argan oil [6]. Recent pharmacological studies have confirmed that *A. spinosa* have several biological effects including; antiproliferative ([7], [8], [9]), Hypolipidemic, hypocholesterolemic [10], antiatherogenic, antiradical and anti-inflammatory activities [11]. The endocarp is an inedible portion of an edible fruit, There are about 24 to 31 million tons of drupe endocarp biomass available in the world [12], which is highly underutilized.
MATERIELS AND METHODS

2.1 CHEMICALS

- Sodium carbonate is purchased from Sigma Chemical (St. Louis, MO)
- Gallic acid and Ascorbic acid were obtained from Merck
- Folin and Ciocalteu’s reagent, were obtained from POCH (Polish Chemical Reagents, Poland)
- Catechin and 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and Ascorbic acid were obtained from Sigma Chemical Co. USA.
- Aluminum chloride (AlCl₃) were from (Merck, purum)

2.2 PLANT MATERIAL

The argan fruits were collected from Oued Elma (Touaref Bouam) which is located in the city of Tindouf in Southwest Algeria. The collection site is located at an altitude of 500 m above mean sea level and at 8°00’-3°40’ latitude and 25°30’-29°40’ longitude. The voucher specimens were deposited in laboratory of production, vegetal and microbial valorization, USTO MB University.

Seed size and colour are important characteristics for distinguishing between mature and immature fruits. The hard seeds of *Argania spinosa* are brown in color (mature endocarp) while the soft seeds are light green (immature endocarp), indicating some degree of chemical or mechanical difference between them.

2.3 METHODS

2.3.1 SAMPLE PREPARATION AND EXTRACTION

The endocarps were cleaned and dried in ETUVE at 40°C, for 48h (not exceeding 50°C) following the suggestion by [13].

2.3.2 QUALITATIVE PHYTOCHEMICAL SCREENING

The methanolic extract of was tested for the presence of sugars, reducing sugars, alkaloids, tannins, flavonoids, saponins and coumarins. The qualitative results are expressed as (+) for the presence and (−) for the absence of phytochemicals.

2.3.2.1 SCREENING FOR TANNINS

1 mL of the methanol extract (80%) was added to 2 mL of water in a test tube. 2 to 3 drops of diluted ferric chloride reagent (FeCl₃) (1%) was added and observed for green to blue-green (cathelic tannins) or a blue-black (gallic tannins) coloration [14].

2.3.2.2 SCREENING FOR ALKALOIDS

100mg of the powder was dissolved in 3mL of H₂SO₄ 1%. Then they were transferred to a water bath for five minutes. After, 1mL of the filtrated extract was treated with 5 drops of Mayer’s reagent (potassium mercuric iodide solution). Formation of whitish yellow or cream coloured precipitate indicated the presence of alkaloids [15].

2.3.2.3 SCREENING FOR FLAVONOIDS

2mL of the methanol extract (80%), few drops of concentrated HCl followed by 0,5g of magnesium turnings was added. After 3min orange or crimson red colour appeared, which indicated the presence of Flavonoids [16].

2.3.2.4 SCREENING FOR SAPONINS

2mL of distilled water was added to plant extract in a test tube and it was shaken vigorously. The foam formation higher to 1cm indicated the presence of saponins ([14], [17]).
2.3.2.5  SCREENING FOR COUMARIN

In test tube, 1g of sample was covered with filter paper moistened with 1 N NaOH. The test tube was placed for 10 minutes in a boiling water bath. After removing the filter paper, it was examined under UV light, yellow fluorescence indicated the presence of coumarins ([14], [18]).

2.3.3  QUANTITATIVE ANALYSIS

2.3.3.1  DETERMINATION OF TOTAL PHENOLIC CONTENTS

The total phenolic content of the samples was determined using the Folin-Ciocalteu’s reagent as described by[19].

Dried samples (0.2g) was used for the preparation of extract. Samples were extracted with 10ml (80%v/v) Methanol at 4°C for 24 h and one week, followed by centrifugation at 4000 tours for 10min. The extraction process was repeated thrice. The supernatants were collected and stored at -20°C for analysis. 50μL of extract was mixed with 2.5mL of water and 250μL of Folin-Ciocalteu’s reagent. After 5min, 500μL of saturated sodium carbonate solution (20% w/v in water) was added to the mixture followed by incubation at 40°C for 30min. The reaction was kept in the dark. After 1h, absorbance was measured at 760 nm with UV-Visible Spectrophotometer. Total phenols were quantified based on standard calibration curve of 0-200μg/mL gallic acid. Total phenolic content was expressed as mg gallic acid equivalents (GAE) per gram of dried sample. The result of each assay was obtained from three parallel determinations.

2.3.3.2  DETERMINATION OF FLAVONOID CONTENTS

Aluminum chloride colorimetric technique was used for total flavonoid estimation [20]. 500 μL of extracts was mixed with 1500μL of distilled water, 150μL of NaNO₂ (5%) and 150μL of aluminum chloride (AlCl₃.6H₂O, 10%). After 11min, 500μL of NaOH (1M) was added. The absorbance of the reaction mixture was measured at 510 nm with UV-Visible Spectrophotometer. The flavonoids were quantified based on standard curve of 0-150mg/L catechin. The total flavonoids were expressed as milligrams of catechin equivalents (CE) per gram of dried sample.

2.3.3.3  DETERMINATION OF HYDROLYSABLE TANNINS

0.2g of samples (mature and immature) was extracted with 10ml a 80% methanolic solvant for (24h) maceration for immature endocarps, 24H and one week for mature endocarps) under continuous stirring at room temperature in the dark. After filtration through watman microfibre filter paper, 3.5mL of FeCl₃ 0.01M in HCl 0.001M was added to 1mL of filtrate. The absorbance was read within 10min at 660nm. The hydrolysable tannins were quantified based standard curve of 0-150mg/L tannic acid. The concentration of hydrolysable tannins was expressed as tannic acid equivalents milligram per gram of dried samples [21].

2.3.3.4  DETERMINATION OF CATECHIC TANNINS

2mL aliquot of a freshly prepared solution of vanillin (1/100mL) in 70% sulfuric acid in added to 1mL of aqueous sample extract. The mixture is incubated in a 20°C waterbath and after exactly 15min the absorbance was measured at 500nm. The total condensed tannins in investigated samples was expressed as milligram of catechin equivalents (CE) per g of the dry sample [22].

2.3.3.5  STATISTICAL ANALYSIS

Statistical analysis Statistical analyses were carried out using Xlstat software Ver. 2017 (www.xlstat.com). Data were subjected to one-way analysis of variance (ANOVA) Values are presented as a mean ± standard deviation (SD).

2.3.3.6  DIPHENYL -1-PICRYLHYDRAZYL (DPPH) ASSAY

The stock solution f extracts were prepared in methanol to achieve the concentration of 20mg/mL. Dilutions were made to obtain concentrations of 10, 8, 6, 4, 2, 1, 0.5, 0.1 mg/mL. The reaction mixture, containing 3.9 mL of 60 μM DPPH methanolic solution and 0.1mL of the diluted test sample, was incubated for 30 min in the dark. The absorbance was measured at 515 nm. Inhibition of free radicals by DPPH in percent (I%) was calculated using this formula.
Total Phenolic Content, flavonoids, tannins and Antioxidant Activity of endocarps fruits of *Argania spinosa* (L.) Skeeels (sapotaceae)

\[
(\%) = \left( \frac{A_{\text{blanc}} - A_{\text{sample}}}{A_{\text{blanc}}} \right) \times 100
\]

Where \( A_{\text{blanc}} \) is the absorbance of the control at 30min.

\( A_{\text{sample}} \) is the absorbance of the simple at 30min.

Ascorbic acid was used as positive reference. Samples were analysed in triplicate [23].

3 RESULTS

3.1 QUALITATIVE PHYTOCHEMICAL SCREENING

The phytochemical composition of two extracts, i.e., extracts of the immature and mature endocarps of fruits of *Argania spinosa* is summarized in table 1.

<table>
<thead>
<tr>
<th>Chemical groups</th>
<th>Method</th>
<th>Mature endocarps</th>
<th>Immature endocarps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducteurs compound</td>
<td></td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Sugars</td>
<td></td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>Froth’s test</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Coumarins</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid aglycons</td>
<td>Magnesium HCl test</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Gallic tannins</td>
<td>FeCl₃ test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Catechique tannins</td>
<td></td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>HCl TEST</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Keys: Strong positive: +++; moderately positive: ++; Low positive: +; negative test: -

3.2 QUALITATIVE PHYTOCHEMICAL SCREENING

The results of phytochemical screening are summarized in table 2.

Table 2. Total phenolic compounds, flavonoids and tannins in fractions expressed as gallic acid equivalents, quatechin equivalent, tannic acid equivalent respectively

<table>
<thead>
<tr>
<th>Plant materiel</th>
<th>Total phenolic content (mg GAE/g)</th>
<th>Total flavonoid content (mg QE/g)</th>
<th>Tannins Catechic hydrolysable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature endocarps (24h)</td>
<td>106.58+/-0.45</td>
<td>4.512+/-0.55</td>
<td>104.1+/-0.55 7.81+/-0.1</td>
</tr>
<tr>
<td>Mature endocarps (1week)</td>
<td>341.25+/-</td>
<td>6.82+/-0.01</td>
<td>118.77+/-5.31+/-0.1</td>
</tr>
<tr>
<td>Immature endocarps (24h)</td>
<td>985.75+/-0.45</td>
<td>100.1+/-0.55</td>
<td>280.47+/-0.45 13.5+/-0.1</td>
</tr>
</tbody>
</table>

GAE: Gallic acid equivalent; QE: Quatechin equivalent.

3.3 DIPHENYL-1-PICRYLHYDRAZYL (DPPH) ASSAY

The results of samples analysed by the antioxidant assay based on the scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals are represented in Figure 1.
The preliminary phytochemical screening of the methanol extract of endocaps of *Argania spinosa* showed the presence of various secondary metabolites of which, coumarins, saponins, flavonoid aglycons, saponin glycosides, catechique tannin and phenolic compounds in immature endocarp were higher than that of mature endocarp (Table 1). Whereas, Alkaloids and reducteurs compounds were detected only in mature endocarps.

For saponin test, endocarps fruits gave positive reactions. Reference [24] reported the presence of two new oleanene saponins isolated from the methanolic extract of the shell of *Argania spinosa*. Previous studies demonstrate that saponins have been proposed as protective agents against infective fungi [25]. According by [26], argan tree is rich in saponins, it could be considered as a source of valuable saponins.

Similarly, phytochemical screening of leaves revealed the presence of alkaloids, flavonoids, tannins. However, mainly flavonoid glycosides and aglycons were identified in the pulp of argan fruits [27]. Recently Four flavonol glycosides were identified by 1H NMR as myricitrin, quercitrin, hyperoside and myricetin 3-O-galactoside [28].

A colorimetric assay using the Folin-Ciocalteu reagent is often referenced in the literature for the determination of phenolic compounds ([29], [30], [31], [32], [33], [34], [35]) and also described in several pharmacopoeias.

The reaction between the Folin-Ciocalteu reagent and phenolic compounds results in the formation of a blue color complex that absorbs radiation and allows quantification by visible-light spectrophotometry [36].

The results of total phenol, total flavonoids and tannin contents of methanol extracts of immature and mature endocarps argan fruits are presented in Table 2. In initial observation of Table 2, the results suggest that extraction by methanol could give higher phenolic content. Reference [37] reported that methanol are said to be the most suitable solvent in the extraction of phenolic compounds due to its ability to inhibit the reaction of polyphenol oxidase that causes the oxidation of phenolics and its ease of evaporation compared to water. The results show that immature endocarps extract (985.75 mg GAE/g) had highest content of total phenol than mature endocarps (341.25 mgGAE/g for one week maceration) and (106.58 mgGAE/g for 24h maceration). Similarly the flavonoids content in immature endocarps (100.1 mg QE/g) is higher than that of mature endocarps (6.82 mg QE/g for one week maceration) and (4.512 mgOE/g for 24 h maceration). According to [38], The content of polyphenols in vegetables, like levels of other phytochemicals, can be influenced by various factors such as varieties, climatic conditions and cultural practices, maturity at harvest. In addition, the condensed tannin contents determined by a modification of the vanillin–HCl method of [39] are present in very higher abundance in endocarps of mature fruits compared with immature fruit (280.44 mg CE/g; 104.1 mg CE/g (24h) and 118.77 mg CE/g (one week) respectively.

The level of phenolic compounds in *A. spinosa* extracts was observed by [40]. In the case of leaves The levels of total polyphenols, determined by Folin–Ciocalteu’s reagent, in plant extracts varied from 447 ± 0.028 to 106.33 ± 0.062 mg/g dry weight, expressed as gallic acid equivalents (GAE) for ethyl acetate and chloroform extracts respectively. Of same in another sapotaceae (*M zapota* Leaf) the phenolic compounds is higher 194.06±1.21GAE/g.

Fig. 1.  *IC*₅₀ values of endocarp fruits of *Argania spinosa* plant for the DPPH test
The decrease of phenolic compounds biosynthesis in endocarps during fruit ripening might be evolution phenomena. According to sebba [41], the ultrastructural studies of the immature endocarps of A. spinosa have shown a high concentration of phenolic compounds. During fruit ripening, endocarps lignification (pit hardening) occurs during intermediate stage, the phenolic compounds considerably decrease This phenomenon might be related to the formation of the large lignified parietal masses, as phenols are lignin precursors ([42], [43]). Like lignin, tannins are also synthesized via secondary metabolism pathways, which are thought to be competitive with lignin since both draw on the same precursors of the phenylpropanoid pathway. Such a hypothesis is supported by our observations, since the phenolic compounds disappear during endocarp lignification. Similarly, the apparent reduction in tannin content that is commonly seen during the ripening of many types of fruit [44]. Thus, the highest levels were reached in the unripe stage and then decreased to attain the lowest levels at the end of the maturity.

Tannins are considered to be polyphenolic metabolites of plants with a molecular weight larger than 500 and with the ability to precipitate gelatin and other proteins from solution [45] (Mehansho et al., 1887). Based on their structure, tannins can conveniently be divided into two groups, hydrolyzable and condensed tannins. Condensed tannins, also known as proanthocyanidins, are polymeric flavonoids [46]. Catechins and some low-molecular weight PA have received considerable attention owing to their various biological activities, in particular their effects on arteriosclerosis [47] (Masquellier, 1988) and their oxygen free radical scavenger ability (Ricardo-da-Sila, 1991). On the other hand, a considerable research revealed the anticarcinogenic, antimutagenic, antimicrobial (antibacterial, antiviral) and antioxidant effects of tannins ([48], [49], [50], [51]).

Fig; 1 shows the IC50 (μg/mL) values of methanolic extracts of mature and immature endocarp fruits for free radical scavenging activity by DPPH. It was observed that the extract of immature endocarps exhibited the highest radical-scavenging activity (lowest IC50 value= 322 μg/mL) than mature endocarps (600 μg/mL. 550 μg/mL; 24H and 1week respectively). The antioxidant capacity followed the same change pattern shown by the bioactive compounds content which confirms the high correlation between the amounts of bioactive compounds and antioxidant capacity assessed by DPPH test ([52], [53]). The scavenging effect of vitamin C (312 mg/mL) was nearly equal to that of immature endocarps.

The total antioxidant activity of some Sapotaceae species was assessed based on scavenging activity of DPPH free radicals, among them the fruit extract of Chrysophyllum cainito L (known commonly as star apple or caimito) showed the highest antioxidant activity (IC50=40 uM) in the DPPH assay [54]. Seeds extract of Manilkara zapota showed high antioxidant activity [55]. Argania spinosa showed antioxidant properties via protection against free radical-induced erythrocyte haemolysis and its ability to potentiate the antioxidant effect of Vitamin E [56]. Fruit extract of Argania spinosa showed potent antioxidant activity [57], Pouteria sapota fruit extract showed antioxidant activity [58]. The extract of three parts; leaves, fruits and stem of Pouteria campechiana reported as antioxidant ([59], [60]). The antioxidant activity of phenolic compounds isolated from Manilkara zapota fruits was studied [61]. Leaves extract of Manilkara hexandra showed antioxidant activity [62].

4 CONCLUSION

The results demonstrated that the methanol extract of the fruit endocarps of Argania spinosa is a potential source of natural antioxidants. Thus, further work should be screened bioactivity, isolated, characterized and elucidated the bioactive compounds of the extract for medicinal value. However, the ripening was accompanied by a re-gression in phenolic compounds correlated with down trend of antioxidant activity. Therefore, the endocarps of fruits immature were considered as more interesting source of natural antioxidant for pharmaceutical uses.

ACKNOWLEDGEMENT

The authors would like to thank to the Laboratory of productions, vegetal and microbial valorizations of Oran Science and Technology University for the support during the study. Thanks to Houaria Achouri and Riad X, Technician, for his assistance during experimental work in the laboratory.
REFERENCES


Total Phenolic Content, flavonoids, tannins and Antioxidant Activity of endocarps fruits of *Argania spinosa* (L.) Skeels (Sapotaceae)


