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Comparative Antibacterial Activity Between Embryo And Endosperm Extracts In Argania Spinosa Seeds: In Vitro And In Silico Studies

Kheira Errouane[1,*], Keltoum Dermeche[2,3], Chahrazed Aibeche[1], Riad El Houari Mezemaze[1]
[1]Laboratory of Vegetal, Microbial Productions and Valorizations (LP2VM), Department of Biotechnology, Faculty of Nature and Life Sciences, University of Science and Technology of Oran-Mohamed Boudiaf (USTO-MB), B.P. 1505, El M'Naouar, Oran 31000, Algeria.
[2]Laboratory of Environment and Sustainable Development, Faculty of Nature and Life Sciences, University of Ahmed Zabana, Bourmadia, 48000, Relizane, Algeria.
[3]Department of Living and Environment, Faculty of Nature and Life Sciences, University of Science and Technology of Oran-Mohamed Boudiaf,(USTO-MB), B.P. 1505, El M'Naouar, Oran 31000, Algeria.

*Corresponding author: Kheira Errouane, Email: kheira.errouane@univ-usto.dz.

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Abstract
The antibacterial activity of Argania spinosa endosperm and embryo extracts has been assessed seen that these seed tissues have similar masses. This activity was realized using the diffusion method. The docking results were obtained using AutoDock software. Total phenols were present approximately in double mass in the endosperm, compared with the embryo (46 vs 27.2 mg GAE/g tissue DW). Methanolic extracts exhibited an activity against Pseudomonas aeruginosa and Staphylococcus aureus with a zone of inhibition diameter ranging from 11 to 26 mm. MIC was 250 mg/ml and 125 mg/ml, respectively, in the presence of these bacteria, for the two tissues. The molecular docking highlighted the significant biological potential of catechin. Its favorable interaction with the P. aeruginosa target protein, marked by a high binding energy, for which the anchoring appears slightly more robust. We can conclude that the embryo and endosperm extracts could be effective as source of natural antibacterial agents to replace synthetic compounds that pose health and environmental problems.
Keywords: *Argania spinosa; antibacterial activity; docking; embryo; endosperm.*

INTRODUCTION

Seeds are a major source of nutrients for human and animal food. With the improvement of living conditions, high nutritional quality has become a priority objective of varietal selection **(Yang et al., 2023)**. Among dicotyledonous seeds, it is the Argania spinosa (L.) Skeels seed, (Sapotaceae). The argan species is endemic to the south-west of Algeria and Morocco **(Khechairi, 2009).** This tree in Algeria, considered as a tertiary age relic, is at its most extreme limit, forming a natural population. This ligneous is a forest tree "multi-use", each of its parts (wood, leaves, fruits, and oil) is usable, and represents a source of income and food for the user. Oil is the main product which is extracted from the almond**(Charrouf and Guillaume, 1999)**. In addition to its economic role, the argan tree plays an essential role in maintaining the ecological balance; by combating erosion, water and wind. **(Charrouf, 1995)**
Furthermore, several researchers have shown that the seed of the species studied contains a significant amount of secondary metabolites, such as phenolic compounds which are well known for their biological activities **(Khallouki et al., 2003; Berrougui et al., 2006; Charrouf and Guillaume, 2007; Charrouf and Pioch, 2009; Cabrera-Viqueet al., 2012; Hilali et al., 2020)** and since the embryo and the endosperm are of roughly similar mass; a rather rare structure in the majority of angiosperm seeds **(Camefort, 1996)**, so it appears interesting to carry out a comparative study of these two tissues. This work was devoted to the study of antimicrobial activity. This activity was chosen because of its importance to human health. Our objective was to estimate the phenolic compounds content in the argan endosperm, embryo and to compare their antibacterial potential of their extracts, using in vitro and in silico methods. We consider this contribution to be relevant for valorizing these two seed tissues in industrial sector as sources of natural components to replace synthetic components which are currently the cause of many environmental problems and diseases such as cancer.

MATERIALSANDMETHODS

Plant material

The seeds of A. spinosa were obtained after decortication of the fruits. These last were collected from the area of Stidia (localized on the west coast of northern Algeria, 15 km west of Wilaya Mostaganem). The endosperm and the embryo were easily obtained after a simple separation using a razor blade **(Fig.1).**

Fig.1: Argania spinosa seed. A, Seed; B, Endosperm (left) and embryo (right) separated from the seed

Methanolic extracts preparation

The extraction was done by following the **Djeridane et al. (2006,** modified) procedure; involving the maceration of 5g of crushed samples in 100 mL of 80% methanol (from Sigma) for 24 hours (at room temperature). Subsequent filtration was carried out (this operation was repeated three times to maximize compound extraction). After evaporation of the filtrate in dry form, using a rotary evaporator (at 40°C), the resulting residue was taken up in a volume of 80% methanol and stored at 4°C until required.

Total phenolic dosage

The dosage of total phenol was performed via spectrophotometry (repeated three times), at 765 nm, following the method of **Singleton et al.** (1999). This involved utilizing 20 µL of methanolic extract and 100 µL of the Folin-Ciocalteu reagent (0.1 N, from Sigma). The mixture was allowed to stand for 3 minutes, and then 300µL of sodium carbonate solution (7.5%) was added. After adjusting to a final volume of 3 mL (by adding distilled water), the mixture was left to incubate for 90 minutes at room temperature and in the dark (to prevent phenol compounds oxidation). A calibration range of gallic acid (Sigma-Aldrich, Germany) was used. The results were expressed in milligram equivalent of gallic acid per gram of tissue DW (mg GAE/g tissue DW).

Antibacterial activity

Agar well diffusion: The antibacterial activity of the methanolic extracts against selected pathogenic bacteria **(Diao et al., 2014)**; was evaluated using standardized bacterial inocula: Staphylococcus aureusATCC25923 (Gram+), Pseudomonas aeruginosa ATCC27853 (Gram-) and Escherichia coli ATCC25922 (Gram-). This assessment was carried out through the agar diffusion technique, using bacterial growth medium «Mueller Hinton Agar, MH», 20mL/Petri dishes. Four wells of 06 mm were created on the agar for each bacterial strain, with three repetitions and a control (distilled water). The cavities formed by the wells were filled with the studied extract (100 µL per well) and the Petri dishes were then incubated at 37°C for 24-48 hours. All manipulations were conducted under sterile conditions. The inhibitory action is manifested by the formation of a halo around the wells. The results were interpreted by measuring the diameters of the inhibition zones. A substance is considered active if the diameter of the inhibition zone exceeds 8 mm **(Hammer et al., 1999)**.

Determination of minimum inhibitory concentration (MIC): The MIC is defined as the lowest concentration capable of inducing a 90% reduction in microbial growth **(Lambert et al., 2001)**. This parameter was determined using the agar dilution method **(Remmal et al., 1993)**. Physiological water was employed as a solvent to ensure optimal dispersion of the extracts within the agar. For each prepared dilution of extracts (1/2, 1/4, 1/8, 1/16), 2 mL were added to the 20 mL of MH. After the medium solidified, the strains tested were inoculated. Finally, the Petri dishes were incubated at 37 °C in an oven for 24-48 hours.

Molecular docking

The primary goal of this scientific study is to use molecular docking simulations to predict and evaluate the effectiveness of ligand in binding to the active sites of Staphylococcus aureus (PDB ID: 3WDG) and Pseudomonas aeruginosa (PDB ID: 6P8U). The structures of the target proteins were retrieved from the RCSB Protein Data Bank (PDB; http://www.rcsb.org/pdb), and ligand structures were obtained from the PubChem database. Docking calculations were carried out using AutoDockVina **(Trott and Olson, 2010)**.

The resulting binding poses were analyzed using Discovery Studio Visualizer to characterize ligand–protein interactions within the binding pocket. The inhibition constant (Ki) was estimated using the equation Ki=exp (ΔG/RT), where ΔG, R, and T are the docking energy, gas constant (1.9872036×10-3 kcal/mol) and ambient temperature (298.15 K), respectively **(Yung-Chi and Prusoff, 1973)**.

The validation for the docking methodology was performed by re-docking, with RMSD, given a good measure of the match between their atomic positions on an atom by atom basis. A docking algorithm reproduces a crystallographic conformation if its calculated RMSD is below 2.0 Å.

Statistical analysis

The statistical tests for this study were performed using ANOVA. The experiments were conducted in triplicate to assess measurement variability and the data were presented as the mean value ± standard deviation.

RESULTS AND DISCUSSION

Total phenolics determination

The total phenolic (aromatic substances) content was observed almost twice in the endosperm than in the embryo, registering 46±0.21 and 27.2±0.4 mg GAE/g tissue DW, respectively, at maturation (**Fig.2**).

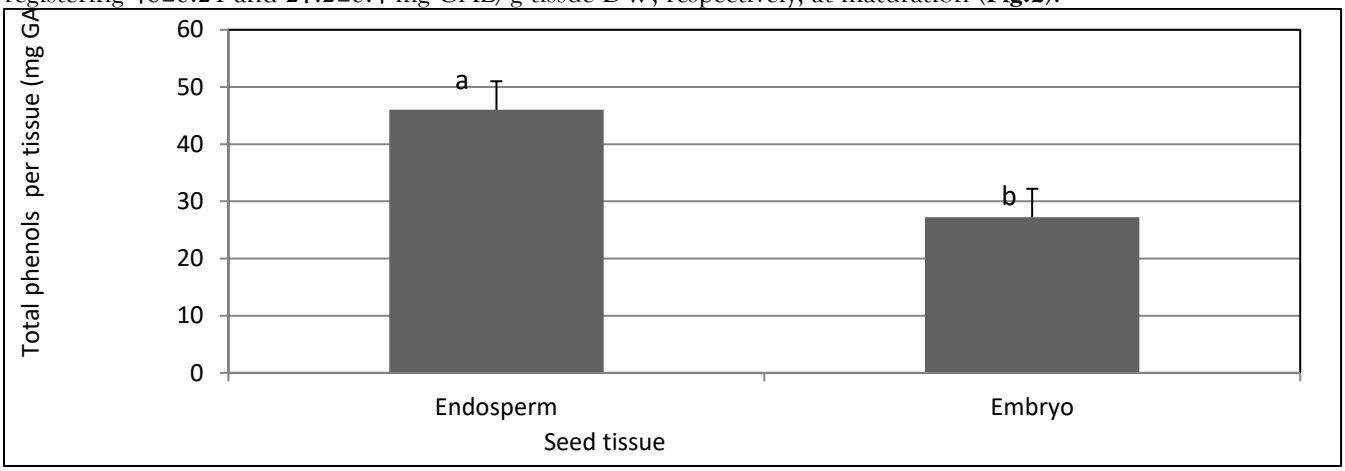

Fig. 2 Total phenols per tissue in argan seed.

These results correspond to those mentioned for the tocopherols (antioxidants) in the same tissues by study of **Errouane et al. (2015)**, which presented higher tocopherol content in the endosperm; with γ-tocopherol (the predominant isomer comprising 85–92% of total tocopherols) nearly twice as abundant as in the embryo (514 vs. 261 mg/kg oil). This amount of tocopherols in the endosperm could be related to its higher linoleic acid percentage **(Kamal-Eldin and Andersson, 1997)**. So we can suggest that the estimated content of total phenolic compounds in the argan seed might be related to the significant presence of tocopherols, which play an important role in the protection of the lipids and cell membranes against oxidation. On the other hand, the bioactive compounds studied are also characterised by their antimicrobial properties **(Muanda, 2010).**

Khallouki et al. (2003) demonstrated that the Moroccan argan oil, extracted from whole kernels, contains extremely low quantities of phenolic compounds (<5.0 mg/kg). Using the technique of gas chromatography-mass spectrometry (GC/MS), these authors identified the following major phenolic; vanillic, syringic, and ferulic acids, in addition to tyrosol. Among these, ferulic acid was exclusively detected in the acid hydrolysates of the methanolic extracts, suggesting its conjugation to a sugar.

The quantified amounts were extremely low. Trace amounts of p-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid and vanillin were also identified. The main polyphénols were resorcinol (-)-epicatechin and (+)-catechin **(Charroufand Guillaume, 2007)**.

According to **Guillaume and Charrouf (2005); Hilali et al. (2020),** the proportion of phenols in argan oil is low but their impact on its biological properties is very important (caffeic acids, 4-hydroxybenzoic, vanillic, syringic, ferrulic 4-o-glycosylated, oleuropein, 3-hydroxypyridine (3-Pyridinol), 6-methyl-3-hydroxypyridine and catechol, resorcinol, vanillyl alcohol, tyrosol, 4 hydroxy-3-methoxyphenethyl, epicatechin and catechin.

Antibacterial Activity of endosperm and embryo

After comparing the diameter of the inhibition zones with the sensitivity scale **(Ponce et al., 2003)** and evaluating the results, we assume that Staphylococcus aureus (Gram +) was extremely sensitive with an average of 26 ± 0.5 mm and 25±0.6 mm **(Fig. 3)**,also Pseudomonas aeruginosa (Gram-)exhibits sensitivity with 11 ± 0. 1 mm and 11.3 ± 0.7 mm**,** respectively, for the embryo and endosperm's extract. These results demonstrate the efficacy of these extracts against both Gram-positive and Gram-negative bacteria, which may be due to the effect of phenolic compounds **(Cowan 1999; Muanda, 2010).**

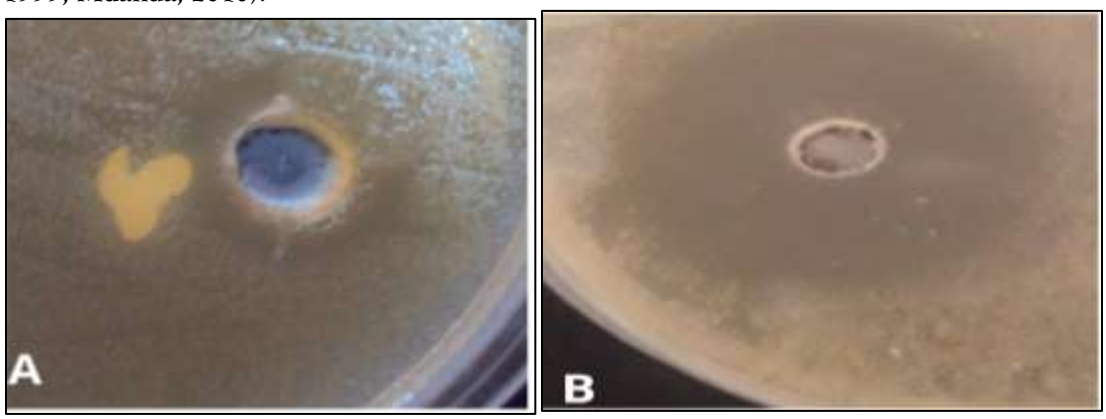

Fig. 3. Inhibitory effect of methanolic extract from argan seed tissues, on Staphylococcus aureus and Pseudomonas aeruginosa. A, Inhibition zone diameter observed for P. aeruginosa in the presence of methanolic extract; B, Methanolic extract on S. aureus.

The MICs were measured using only one method on four strains. The values obtained were 125 ± 0.8 mg/mL for S. aureus and 250 ± 0.1 mg/mL for P. aeruginosa.

Our results consolidate those listed in the literature: **Mokadem et al. (2023),** reported that ethanolic extracts of whole argan almonds (from Algeria) have good inhibitory activity against several microorganisms (E. coli, Pseudomonas, Salmonella, Aspergillus flavus, A. niger, Fusarium, S. aureus and Streptococcus thermophilus), of which S. aureus and S. thermophilus showing high sensitivity to the extract. **Cherifi and KaidHarche (2019)** observed a substantial antibacterial potential in the methanolic extract of Algerian argan leaves against bacteria E.coli, B. subtilis, E. faecalis and P. aeruginosa (17-23.5 mm/MIC: 31.25 to 250 mg/mL).

As outlined by **Cowan (1999)**, phenols function as mechanisms of plant defense against predation by microorganisms, insects, and herbivores. Some, such as quinones and tannins, are responsible for plant pigmentation. According to the same authors, major classes of antimicrobial phenolic compounds are as follows: Simple phenols, phenolic acids, quinones, flavonoids, flavones, flavonols, tannins and coumarins. Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group of phenylpropane derived compounds existing in a highly oxidized state. Both tarragon and thyme, medicinal familiar herbs, contain caffeic acid, which is effective against bacteria, fungi and viruses. Catechol and pyrogallol, hydroxylated phenols, shown to be toxic to microorganisms. Catechol has two-OH groups, while pyrogallol has three. The site and number of hydroxyl groups on the phenol group are believed to influence their relative toxicity to microorganisms, with evidence suggesting that increased hydroxylation leads to heightened toxicity. The high activity of the phenolic components may be further explained in terms of the alkyl substitution into the phenol nucleus, which is known to enhance the antimicrobial properties of phenols **(Dorman and Deans, 2000)**. S. aureus, an opportunistic pathogen, has the potential to induce various illnesses in humans, ranging from self-resolving conditions to life-threatening conditions. This bacterium is one of the main causes of food poisoning, resulting from the consumption of food contaminated with enterotoxins. Staphylococcal food poisoning is characterized by a sudden onset of nausea, vomiting, abdominal pain, cramps, and diarrhea. P. aeruginosa is responsible of pneumonia cases and hospital-acquired urinary tract infections. However, it is less common in the general population, and rarely causes severe infections **(Le Loir et al., 2003; Murray et al., 2003)**.

Molecular docking

The observed antibacterial activity was promising, as evidenced by the negative binding affinity values obtained for the interactions between catechin and the target bacterial proteins. In particular, docking results revealed binding energies of approximately −7.3 kcal/mol for P. aeruginosa (PDB: 6P8U) and −7.2 kcal/mol for S. aureus (PDB: 3WDG), reflecting favorable and largely comparable ligand–protein interactions in both cases. Consistent with these findings, the inhibition constants (Ki) of 4.45 μM for P. aeruginosa and 5.27 μM for S. aureus (**Table 1**) indicate that catechin acts as an effective inhibitor of the bacterial enzymes investigated, with a slightly higher predicted affinity toward the P. aeruginosa target. Although this inhibitory activity appears marginally more pronounced against the last bacterium. The small difference between the two Ki values suggests that catechin exhibits broadly comparable efficacy against both bacterial species. These findings revealed the presence of several hydrogen bonds between catechin and the target proteins, with hydrogen bonding recognized as a major contributor to the stability of the ligand–protein complexes.

Figure 4 summarizes the binding mode of (+)-catechin within the active sites of the two bacterial targets, as illustrated by the 3D (A, C) and 2D (B, D) interaction maps. In the S. aureus complex (PDB: 3WDG), catechin is primarily stabilized by conventional hydrogen bonds involving Gln A:261 and acidic residues such as Asp A:231 and Asp B:80, supporting directional polar anchoring and a well-defined ligand orientation within the pocket. In addition, a π–σ contact with Val B:82 reinforces the docking pose by contributing complementary aromatic/hydrophobic packing that can further stabilize the bound conformation. In the P. aeruginosa complex (PDB: 6P8U), conventional hydrogen bonds, notably with Leu A:184 and Ile B:35, are complemented by weaker carbon–hydrogen contacts that can cumulatively strengthen ligand retention within the binding site.

Moreover, the aromatic scaffold of catechin engages in multiple π-type interactions, including a π–cation contact with Arg A:188, a π–anion interaction with Asp B:88, a π–sulfur contact with Met B:89, and π–alkyl contacts with Pro B:37, collectively suggesting a multipoint anchoring pattern within the pocket.

Table 1. List of the names, codes, Binding energies (ΔG, kcal/mol) and inhibition constants (Ki, μM) of the two biological targets.

Targeted proteins	BDP ID	Ligand	Binding scores (kcal/mol)	Ki (μM)
S. aureus	3WDG **(Wang et al., 2014)**	(+)-catechin CID:9064	-7.2	5.27
P. aeruginosa	6P8U **(Horchaniet al., 2022)**		-7.3	4.45

Overall, the cooperative interplay between directional hydrogen bonding and π-mediated contacts (hydrophobic and electrostatic reinforcement) provides a physicochemical basis for catechin retention in both binding sites and supports the formation of potentially stable catechin–protein complexes. According to **Dermeche et al.** (2025), the molecular docking simulations revealed that flavonoids (type of phenolic compounds) had a high affinity for important enzymes, implying considerable biological properties.

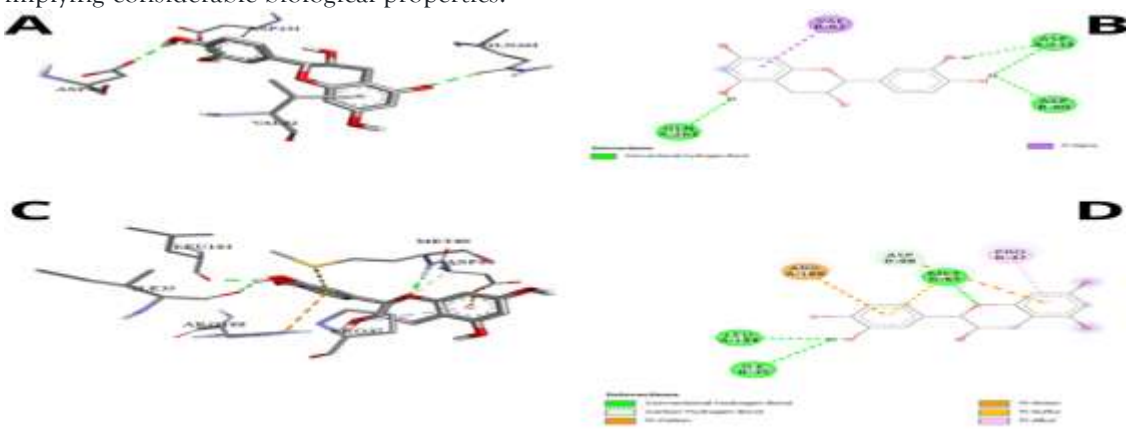

Fig. 4. 3D and 2D interactions of catechin with bacterial strains during molecular docking. A, 3D; B, 2D interactions

of catechin with S. aureus binding site (PDB: 3WDG)**;** C, 3D; D**,** 2D interactions of with P. aeruginosa binding site (PDB: 6P8U).

CONCLUSION

Significant differences in phenolic compounds content were recorded between the tissues of argan seed; of which these substances were more important in the endosperm. The antibacterial activity of the methanolic extract revealed that S. aureus was extremely sensitive for both seed tissues. Molecular docking analyses demonstrated that catechin forms energetically stable complexes with the investigated bacterial target proteins. The results obtained could be considered very encouraging for the use of extracts from endosperm end embryo as natural antimicrobial agents and justify the continuation of the study of other biological activities on this seed by comparing these two tissues (such as anti-inflammatory, antioxidant, anti-diabetic, anti-cancer activities, ….). Also, further analyses of other bioactive components would be necessary to carry them out.

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Conflict of Interest The authors have no conflict of interest

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