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Research Article

Antioxidant and anti-inflammatory activities of *Opuntia ficus-indica* L. extracts from different plant parts collected from two ecosystems in Northwestern Algeria: *in vitro* and DFT calculations studies

Abdelkader Douis[1], Ahlem Karbab[2]*, Abderrezak Djabeur[1]*, Noureddine Charef[2], Reema Ahmad Hasan Omeir[3], Hanane Sihem Sebaa[1]

[1]*Laboratoire de Productions, Valorisations Végétales et Microbiennes LP2VM, Département de Biotechnologie, Faculté des Sciences de la Nature et de La Vie, Université des Sciences et de la Technologie d'Oran Mohamed Boudiaf, USTO-MB, BP 1505, El M'naouer, Oran, 31000, Algérie*
[2]*Laboratory of Applied Biochemistry, Department of Biochemistry, Faculty of Nature and Life Sciences, Setif-1 University Ferhat Abbas 19000, Algeria*
[3]*Department of Chemistry, Applied Science University, Amman 11942, Jordan*

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*Corresponding author: Ahlem Karbab, E-mail: ahlem.karbab@univ-setif.dz; Abderrezak Djabeur, E-mail: abderrezak.djabeur@univ-usto.dz

Abstract

Opuntia ficus-indica L., a plant species that is commonly used in traditional medicine to treat diabetes, inflammation-related diseases, gastric ailments, and wounds on the skin, was studied for the first time. In this research, the phytochemistry, total phenolic compounds, *in vitro* antioxidant and anti-inflammatory capacity of the ethanolic extracts obtained from the cladodes, bark, and seeds of the plant collected from Mediterranean and Continental ecosystems are evaluated. The antioxidant and anti-inflammatory activities were further explained by DFT calculations. The phenolic acid derivatives, caffeic acid and ferulic acid, were studied to explain the structural dependency of the antioxidant activity of these compounds. In quantitative analysis, the results revealed that cladode ethanolic extract Mediterranean (CEEM), bark ethanolic extract continental (BEEC) and the seed ethanolic extract Mediterranean (SEEM) presented a high amount of total phenolic with a value of 177.33±2.01 µg gallic acid equivalent, 9.18±2.23 µg quercetin equivalent and 110.63±0.41 µg catechin equivalent/mg dry extract, respectively. Through the DPPH radical scavenging and reducing power assays, SEEM showed high *in vitro* antioxidant of the order of 0.08±0.00 and 1.03±0.03 mg/mL, respectively. Caffeic acid shows higher biological activity owing to higher HOMO energy (-6.0438 eV), higher softness 6.7536 Hartree^{-1}, while a smaller value of the energy gap (4.0292 eV). Overall, the computational results indicate that both caffeic and ferulic acids are highly reactive antioxidants while caffeic acid appears to have a slightly stronger electron-donating ability. In conclusion, the *O. ficus-indica* fruit provides effective natural antioxidants for the patient, and may prove to have potential health benefits as natural antioxidant and anti-inflammatory agents.

Keywords: *Opuntia ficus-indica* L., Polyphenols, Antioxidant activity, Anti-inflammatory activity, DFT calculations

Introduction

Oxidative stress arises from an imbalance in the oxidation and antioxidation processes within cells with health implications (Li *et al.,* 2025). Phenolic compounds are synthesized for the protection of plants. The phenolic compounds have varied biological activities, including antioxidant, anti-inflammatory, antimicrobial, anti-diabetic, neuroprotective and anticancer properties (Saad *et al.,* 2025). Their antioxidant potential is primarily associated with the presence of hydroxyl (-OH) groups on the aromatic rings, which enable the neutralization of free radicals and protection against oxidative damage (Menon, 2013). Algeria, for instance, possesses several drought and stress-tolerant species, including olive trees, carob trees, tamarix, and *O. ficus-indica* (FAO, 2022; Packirisamy *et al.,* 2023). The adaptive capacity of these species is closely associated with their ability to synthesize a wide range of natural products, particularly secondary metabolites involved in stress tolerance and defense mechanisms (Karbab *et al.,* 2026a).

Natural products, defined as chemical compounds produced by living organisms, are categorized into primary metabolites, essential for growth and development, and secondary metabolites, which serve defense, protection, or reproductive functions (Karbab *et al.,* 2026a). Among these, *O. ficus-indica* (prickly pear) is a cactus species that remains relatively underexplored and underutilized in Algeria, despite the country's extensive agricultural potential. Its local use is largely limited to seasonal fruit consumption and as a living fence. However, extensive studies conducted worldwide have demonstrated its considerable ecological, nutritional, and therapeutic value. Ecologically, *O. ficus-indica* is highly resilient to harsh environmental conditions, including drought, salinity, and poor soils. Economically, it represents a valuable source of bioactive compounds with promising pharmaceutical, nutraceutical, cosmetic, and industrial applications (Ashfaq *et al.,* 2021; Sinicropi *et al.,* 2022). These findings highlight the need for further research and valorization of this species in Algeria.

Opuntia ficus-indica L. (prickly pear), is a type of desert fruit that grows in arid and desert regions around the world, including the Middle East, North Africa, and South Asia. Its fruits are rich in dietary fiber, vitamins, and minerals (Salim *et al.,* 2009), and the species has long

been used in traditional medicine for the management of gastrointestinal disorders, inflammation, cardiovascular diseases, and hypercholesterolemia. In addition, *O. ficus-indica* exhibits diverse pharmacological properties, including antioxidant, anti-inflammatory, antihyperlipidemic, antidiabetic, anti-obesity, anticancer, and skin-protective activities (Sabtain *et al.,* 2021). These biological effects are attributed to the presence of numerous bioactive compounds distributed throughout the plant including the cladodes, fruits, seeds, and peel such as polyphenols, flavonoids, sterols, polyunsaturated fatty acids, carotenoids, and vitamins (Ahmed *et al.,* 2024). According to Ashraf *et al.* (2024), the synthesis of antioxydants phytochemicals by medicinal plants in high-altitude is influenced by different environmental factors (solar radiations intensity and UV exposure, temperature variation, low oxygen levels, soil composition, water scarcity and drought stress). Consequently, comparative studies across contrasting ecosystems provide valuable insights into the influence of environmental variability on the accumulation of bioactive compounds and their associated pharmacological properties (Ogwu *et al.,* 2025). To the best our knowledge, the present study aimed to evaluate the influence of two distinct ecosystems in Algeria, namely the continental ecosystem of Meknessa (west of Ouarsenis) and the Mediterranean ecosystem of Boutlelis (Oran), on the polyphenol, flavonoid, and condensed tannin contents, as well as on the *in vitro* antioxidant and anti-inflammatory activities of *O. ficus-indica* ethanolic extracts. In addition, density functional theory (DFT) calculations were performed to investigate the structural and electronic properties of selected bioactive compounds and to provide molecular-level insights into their antioxidant potential. Among the most abundance in various medical plants and extensively studied natural phenolic acids, caffeic acid and ferulic acid have been studied in this research.

Materials and methods

Plant collection

Fresh cladodes, bark, and seeds of *Opuntia ficus-indica* L. (10 kg/plant part) were collected during a single sampling campaign at the last week of July 2024 from two contrasting ecosystems in northwestern Algeria (Figure 1). The sampling sites were selected to represent distinct climatic and ecological conditions that could influence the accumulation of secondary metabolites and, consequently, the biological activities of the plant. Zone 1 corresponded to a continental ecosystem located in the Meknessa region (Ain Tarek), west of the Ouarsenis Mountains, at an altitude of 809 m, whereas Zone 2 represented a Mediterranean coastal ecosystem in the Brédéah region (Oran), at an altitude of 134 m. These sites differ in climatic and edaphic characteristics, providing a suitable basis for evaluating the effect of ecosystem variability on the phytochemical composition and biological activities of *O. ficus-indica*. The plant material was identified using the taxonomic keys of Quézel and Santa (1963). Prof. Laouer H., botanist at Laboratory of Valorization of Natural Biological Resources, University Setif 1, Algeria, recognized and authenticated the plant. A voucher specimen (050/DBEV/UFA/24) was kept in the herbarium at the Laboratory of plant and microbial production and valorization (LP2VM), Biotechnology department, University of Science and Technology of Oran Mohamed Boudiaf (USTO-MB), Oran 31000, El M'naouer, BP 1505, Algeria. After collection, the plant material was washed, air dried in shade at ambient temperature (25-30 °C) to retain their properties with a relative humidity of approximately 50-60% for three weeks in a well-ventilated area and then grounded in an electrical grinder and stored until extraction.

Extraction procedure

The ethanolic extracts of the plant were prepared form 100 g of ground cladodes, bark and seeds parts separately.

Figure 1: The different parts of *Opuntia ficus-indica* L.

First, the powder was soaked by in 700 mL of hexane and continuously shaken for 48 h, followed by filtration. Then the defatted material was extensively extracted with absolute ethanol (700 mL, three times, two days each) at room temperature. Then the solution was filtered and the solvent was recovered by evaporation in a rotavapor, at a temperature of 45 °C. The dried six ethanolic extracts (CEEC: cladode ethanolic extract continental, CEEM: cladode ethanolic extract Mediterranean, BEEC: Bark ethanolic extract continental, BEEM: Bark ethanolic extract Mediterranean, SEEC: Seed ethanolic extract continental, SEEM: Seed ethanolic extract Mediterranean) were preserved in 4 °C.

Phytochemical studies

Qualitative analysis

Phytochemical screening was carried out to determine the presence or absence of various secondary compounds in the plant extract using the following phytochemicals: alkaloids, flavonoids, polyphenols, tannins, quinones, anthraquinones, and proteins, using procedures previously outlined (Amutha & Doss, 2009). The tests depend on the appearance of characteristic colors and precipitation reactions that are useful for the detection of the respective phytochemicals.

In vitro studies

Determination of total phenolics, flavonoids and condensed tannins contents

The total phenolic contents (TPC) of the extracts were analyzed by Folin-Ciocalteu reagent following the method (Zeggar *et al.,* 2024). In brief, a mixture of 100 µL of extract and 500 µL of Folin-Ciocalteu reagent were left for reaction for 4 min, followed by addition of 400 µL of 7.5% (w/v) aqueous Na_2CO_3. Following a 90-min reaction at room temperature, absorbance was recorded at 765 nm. Total phenolic contents were calculated from a calibration curve (0-150 µg/mL) of a known concentration of gallic acid and were expressed as µg GAE/mg DE. Total flavonoid contents (TFC) of the extracts were analyzed by aluminum chloride colorimetric method (Zeggar *et al.,* 2024). In brief, a mixture of 1 mL of extract and 1 mL of $AlCl_3$ solution (w/v, 2%) were allowed to react for 10 min at room temperature. Total flavonoid contents were expressed as absorbance at 430 nm measured from a blank of methanol. Total flavonoid contents were calculated from a calibration curve (0-40 µg/mL) of a known concentration of quercetin and were expressed as µg QE/mg DE. Condensed tannins were analyzed by vanillin-HCl method as described by (Amari *et al.,* 2025). In brief, a mixture of 1 mL of extract and 1.5 mL of vanillin (in methanol, w/v, 4%) reagent were followed by addition of 750 µL of concentrated HCl. The reaction mixture was left at a temperature of 20 °C and maintained under darkness for a period of 20 min. Total flavonoid contents were expressed as absorbance at 500 nm measured from a blank. Results were calculated from a calibration curve (100-500 µg/mL) of a known concentration of catechin and expressed as µg CE/mg DE. All measurements were performed for three times (n=3).

Antioxidant activity

DPPH radical scavenging assay

The DPPH scavenging potential of the extracts was investigated through the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) method, which is based on the decrease in DPPH absorption at 517 nm (Khiat *et al.,* 2026). Briefly, 50 µL of the extract/standard solutions at various concentrations were incubated with 1.25 mL of 0.004% (w/v) DPPH in methanol. The mixture was incubated for 30 min at room temperature in the dark and followed by the measurement of the absorption at 517 nm. Butylated hydroxytoluene (BHT) was employed as the positive standard. DPPH scavenging activity was calculated as percentage inhibition and calculated by the following formula: Inhibition percentage (%) = $(A_{blank} - A_{test}/A_{blank}) \times 100$.

Where A_{blank}=absorbance of the solution except the tested sample, and A_{test}=absorbance of the tested sample. IC_{50} values were calculated from the linear regression equation: y=ax+b, where y is the percentage inhibition and x is the extract concentration. The IC_{50} was obtained by setting y=50, giving: IC_{50}=(50-b/a).

Reducing power assay

The reducing abilities of *O. ficus-indica* extracts were determined based on their ferric ion (Fe^{3+})-reducing power to produce ferrous ions (Fe^{2+} ions) (Karbab *et al.,* 2026b). Briefly, the extracts (400 µL) were mixed with the phosphate buffer solution (400 µL, 0.2 M, pH 6.6) and a potassium ferricyanide solution (400 µL, w/v, 1%). The mixture was then incubated at 50°C for 20 min in a water bath. The reaction mixture was stopped by adding trichloroacetic acid (400 µL, w/v, 10%), followed by centrifugation at 3000 rpm for 10 min. Finally, a mixture of the supernatant fluid (400 µL), distilled water (400 µL), and ferric chloride (80 µL, w/v, 0.1%) was added, and then the mixture was left to stand at room temperature for 10 min. Finally, the absorbance of the mixture was determined at a wavelength of 700 nm. Increased absorbance indicated increased reducing power.

Anti-inflammatory activity

The anti-inflammatory activity of the different extracts was evaluated by the egg white protein denaturation test according to the protocol of (Akkouche *et al.,* 2012), with some modifications. This test is based on the ability of different active plant constituents to prevent protein denaturation. After washing an egg, the white and yolk were separated, and the chalazae were removed. The volume of the egg white was measured with Tris-HCl buffer (20 mM, pH 6.87) to obtain a 1:100 dilution. The mixture was stirred for 10 min, followed by filtration with strip agase. Two milliliters of the egg white suspension were mixed with two milliliters of each extract and aspirin

as a standard (at concentrations of 4.2 and 1 mg/mL) and incubated in a water bath at 70 °C for 15 min. Finally, the absorbance was calculated at 650 nm.

The percentage of protein denaturation inhibition was calculated using the following formula: % inhibition of denaturation = 100 × (AC − AS)/AC

AC=absorption of the control sample and
AS=absorption of the test sample

Computational method

Caffeic acid and ferulic acid were selected for computational study based on prior literature, as they are among the most frequently reported phenolic acids in *O. ficus-indica* due to their antioxidant activities (Riaz *et al.,* 2023). DFT analysis was performed based on literature-derived molecular structures of caffeic acid and ferulic acid. In future research, these acids will be studied experimentally to explore the relationship between their experimentally observed biological activities and the electronic properties of these acids as studied in this research. To provide further chemical insights into the electronic structures and antioxidants behavior of caffeic acid and ferulic acid, quantum chemical calculations were performed using the Gaussian 09 software package (Frisch *et al.,* 2009). Density Functional Theory (DFT) was used to fully optimize the molecular geometries of both phenolic acids without constraints. The geometry optimizations of both acids were computed using Becke's three parameter hybrid exchange functional combined with the Lee-Yang-Parr correlation functional (denoted B3LYP) (Bauernschmitt & Ahlrichs, 1996; Becke, 1993) hybrid functional, with the Pope-split valence polarized 6-311G (d,p) basis set for all atoms. This level of theory was used based on previous studies on related phenolic systems (Riaz *et al.,* 2023).

Harmonic vibrational frequency calculations were subsequently performed at the same level of theory, indicating that no imaginary frequencies were 0found and confirming that the optimized geometries correspond to the true energy minima. To give more acceptable performance for these phenolic acids with reliable and efficient calculations and estimate the results experimentally, the optimizations have been studied in aqueous media to mimic the vivo environment. Solvent effects were accounted for via the Self-Consistent Reaction (SCRF) method. Utilizing Tomasi's Polarizable Continuum model (PCM) with water as the solvent (Tomasi *et al.,* 2005). Frontier molecular orbital energies, the Highest Occupied Molecular Orbital and the Lowest Unoccupied Molecular Orbital (HOMO and LUMO, respectively (Pearson, 1986), were analyzed to compute the global reactivity descriptors including chemical hardness (η) and chemical softness (S).

Statistical analysis

Data from *in vitro* experiments were analyzed using Student's t test for statistical significance. Data was recorded in triplicate and results were presented as mean±SD. Data analysis for this experiment was performed using GraphPad Prism-6 (GraphPad Software, San Diego, California USA, http://www.graphpad.com). Values with $p \leq 0.05$ were considered statistically significant.

Results and discussion

Phytochemical screening

Qualitative phytochemical screening was performed as a preliminary step to characterize the major classes of secondary metabolites present in the extracts. Although it was not used as a criterion for extract selection, this analysis provides essential information on the occurrence of bioactive phytochemical groups, including saponins, polyphenols, quinones, hydrolyzable tannins, proteins, anthraquinones, condensed tannins and alkaloids in ethanolic extracts of *O. ficus-indica* parts, which are known to contribute to the biological activities of medicinal plants. Furthermore, qualitative screening complements the subsequent quantitative determination of total phenolics, flavonoids, and condensed tannins, facilitating the interpretation of the observed antioxidant and anti-inflammatory activities and providing a comprehensive phytochemical characterization of *O. ficus-indica* extracts. Table 1 summarizes the results and shows the presence of polyphenols, quinones, anthraquinones, and tannins in the six extracts. Alkaloids were absent from all extracts. Saponins were found in cladodes from two regions and the bark of continental ecosystems, while proteins were detected only in cladodes from Mediterranean ecosystems.

The absence of certain phytochemical constituents may be attributed to their low intrinsic concentrations in the plant material or to limitations related to the extraction methods. According to the results of Rocchetti *et al.* (2018), cladodes contain low concentration of proteins. Likewise, Dib *et al.* (2013) reported that the alkaloids were not detected in the ethanolic extracts of cladodes or in methanolic extracts of cladodes and bark (Touré *et al.,* 2015). Whereas, El-Beltagi *et al.* (2019) reported that low alkaloid contents were reported in ethanolic extracts of pulp and bark. These observations indicate that phytochemical profiles of *O. ficus-indica* are

Table 1: Phytochemical screening of ethanolic extracts from *O. ficus-indica* parts

Compounds	CEEC	CEEM	BEEC	BEEM	SEEC	SEEM
Saponins	+	+	+	-	-	-
Polyphenols	+	+	+	+	+	+
Quinones	+	+	+	+	+	+
Proteins	-	+	-	-	-	-
Anthraquinones	+	+	+	+	+	+
Hydrolyzable tannins	+	+	+	+	+	+
Condensed tannins	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-

'-'-absence, '+'-presence, CEEC-cladode ethanolic extract continental, CEEM-cladode ethanolic extract Mediterranean, BEEC-Bark ethanolic extract continental, BEEM-Bark ethanolic extract Mediterranean, SEEC-Seed ethanolic extract continental, SEEM-Seed ethanolic extract Mediterranean

strongly influenced by plant organ specificity and extraction conditions. This current study shows that ecosystem and plant organ play a major role in determining the phytochemical content and biological effects of the ethanolic extract of *O. ficus-indica*. It has been found that the ethanolic extracts obtained from plants collected from the continental ecosystem of Meknessa and the Mediterranean ecosystem of Boutlelis have shown differences regarding phenolic content, antioxidant ability, and anti-inflammatory effect. These differences show that environmental factors and physiological role of tissues play an important part in controlling the accumulation of secondary metabolites in *O. ficus-indica*. Environmental factors like temperature, water, radiation, and soil are considered major influences on the secondary metabolism of plants (Chen *et al.,* 2022; Qaderi *et al.,* 2023). The lack of alkaloids in all types of extracts may be due either to their insufficient amount in the studied tissues or to some other factors affecting extraction specificity. Earlier researchers found that phytochemical compositions in *O. ficus-indica* differ depending on the type of organs, cultivar, geographic localization of plants, and extraction method used (Ahmed *et al.,* 2024).

In vitro studies

Total phenolic, flavonoids and condensed tannins contents

In these analyses, the quantities of total phenolic, flavonoids and condensed tannins contents were evaluated using spectrophotometric methods. The total phenolic content (TPC) was quantified using the Folin–Ciocalteu's reagent, the total flavonoids content (TFC) with aluminum chloride and the condensed tannins content (CTC) by vanillin method. The results obtained (Table 2) show significant total polyphenol contents in the six ethanolic extracts. The highest TPC were found in the cladode extracts from the two regions with a value of 177.33±2.00 and 172.08±0.41 µg GE/mg of dry extract for CEEM and CEEC respectively. For the TFC, the high content was found in the BEEC with a value of 9.18±2.23 µg QE/mg of dry extract. The high contents of condensed tannins (TC) among the six extracts was found in the SEEM with a value of 110.63±0.41 µg CE/mg of dry extract.

Table 2: Total phenolics, flavonoids and condensed tannins contents of *O. ficus-indica* extracts

Extracts	Total phenolic content (a)	Total flavonoids content (b)	Tannins content (c)
CEEC	172.08±0.41	1.44±0.33	64.57±0.82
CEEM	177.33±2.01	5.37±0.26	91.10±1.88
BEEC	146.33±0.19	9.18±2.23	81.93±0.11
BEEM	146.16±0.28	3.67±1.40	16.24±0.29
SEEC	145.83±0.33	1.03±0.18	20.16±2.53
SEEM	141.16±0.44	2.68±1.14	110.63±0.41

(a)-µg gallic acid equivalent (GE)/mg dried extract (DE), (b)-µg quercetin equivalent (QE)/mg dried extract (DE)), (c)-µg Catechin equivalent (CE)/mg dried extract (DE), CEEC-cladode ethanolic extract continental, CEEM-cladode ethanolic extract Mediterranean, BEEC-bark ethanolic extract continental, BEEM-bark ethanolic extract Mediterranean, SEEC-seed ethanolic extract continental, SEEM-seed ethanolic extract Mediterranean

The variability observed in TPC among *O. ficus-indica* extracts can be attributed to the differences in extraction solvent, plant organ, and geographical origin. The TPC observed in *O. ficus-indica* cladode extracts in the present study was lower than that reported for methanolic extracts by El-Hawary *et al.* (2020) with a value of 236.5 µg GE/mg dry extract but higher than values reported for ethanolic extracts by (Allai *et al.,* 2017) with a value of 111.2±0.58 µg GE/mg dry extract, highlighting the strong influence of solvent polarity on phenolic extraction efficiency. In contrast, the TPC values obtained for bark extracts were comparable to those reported by El Mannoubi (2023) for methanolic extracts of green bark (141.71±0.13 µg GE/mg dry extract), as well as to butanolic fruit extracts (148.91±0.95 µg GAE/mg dry extract) reported by Mohammed *et al.* (2025), suggesting that bark part represent a significant amount of phenolic compounds in *O. ficus-indica*. Furthermore, the TFC recorded in this study exceeded the values previously reported by Ali *et al.* (2022) for ethanolic bark extracts from Egypt (1.30±0.23 mg/g dry extract; and from Portugal (3.01±0.14 mg/g dry extract), (Pereira *et al.,* 2019). These variations may be attributed to differences in extraction solvents, plant organ specificity, geographical origin, and environmental conditions, all of which are known to influence secondary metabolite biosynthesis and accumulation in O. ficus-indica. This enhancement may reflect differences in environmental conditions, plant maturity, or stress exposure, all of which are known to modulate flavonoid biosynthesis. Overall, the findings confirm that both extraction parameters and ecological factors play a crucial role in determining the phenolic and flavonoid composition of *O. ficus-indica* extracts. As observed from the quantitative analysis, the extracts of cladode possessed the highest amount of total phenolic content, especially CEEM and CEEC, while the highest levels of condensed tannin were found in seed extracts, especially SEEM. This difference can be explained through the different physiological functions of the plant parts. Cladode is a photosynthetic organ which is constantly under environmental pressure and therefore needs antioxidants to combat the oxidative damage within the cell. However, seeds possess phenolic compounds and tannins as protective structures for protection against diseases, predators, and also maintenance of seed viability (Isah, 2019; Qaderi *et al.,* 2023).

Antioxidant activity

DPPH radical- scavenging activity

The presence of phytochemical molecules such as polyphenols and flavonoids in our food makes the antioxidant capacity more important in the body. The antioxidant activity of different ethanolic extracts of *O. ficus-indica* was measured according to the DPPH test, where the absorbance was estimated at 517 nm. The present results showed that SEEM present a high antioxidant activity with an IC_{50} of 0.08±0.00 mg/mL followed by SEEC and CEEC for CEEM with an IC_{50} of 0.09±0.00 and 0.09±0.01 mg/mL, respectively. The DPPH radical scavenging activity of the cladode extracts obtained in the present study was

higher than that reported by Touré *et al*. (2015), who recorded an IC_{50} value of 1.20 mg/mL. For bark extracts, the antioxidant activity observed was comparable to that reported by Ali *et al.* (2022) for ethanolic bark extracts of *O. ficus-indica* with an IC_{50} of 0.55±0.01 mg/mL, and was also consistent with the an IC_{50} value of 0.61±0.01 mg/mL reported by Ferreira *et al.* (2023). Regarding seed extracts, Touré *et al.* (2015) reported a remarked antioxidant activity, with an IC_{50} value of 0.18 mg/mL, indicating a higher free radical scavenging potential of seed derived extracts compared with cladode and bark extracts. These differences in antioxidant capacity may be attributed to variations in phenolic and flavonoid composition, extraction solvent, and plant organ specificity.

Reducing power activity

Table 1 below shows the antioxidant activity curves of the reducing powers of the ethanolic extracts of *O. ficus-indica*. In this assay, all of the extracts demonstrated some ability to donate electrons to reduce Fe^{3+} to Fe^{2+} ions. The highest effective concentration was presented by SEEM with an IC_{50} of 1.03±0.03 mg/mL, followed by SEEC with a value of 1.82±0.00 mg/mL. These values are interesting compared to the results of Berrebah (2019), where the average IC_{50} of reducing power of seeds extracts from several regions was 7.68±0.59 mg/mL. The variation in reducing power among *O. ficusindica* extracts likely reflects differences in the nature and concentration of phenolic antioxidants present in distinct plant organs and ecosystems. For instance, significant variability in total phenolic and antioxidant profiles has been observed among *O. ficusindica* seed extracts from different cultivars, which was attributed to geographical origin and extraction conditions (Bouaouich *et al.,* 2023). Similarly, comprehensive profiling of *O. ficusindica* root extracts demonstrated a consistent correlation between phenolic content and multiple antioxidant assays, including iron reduction, reinforcing the role of phenolic constituents in reducing power (Benramdane *et al.,* 2025). The stronger reducing power of seed extracts in the present study, particularly from the Mediterranean ecosystem, may therefore be due to higher concentrations or a more active composition of reducing phenolics compared with bark and cladode extracts. In contrast, the relatively weak reducing power of bark extracts may reflect a higher proportion of structural components (Karbab *et al.,* 2020, 2025), where phenolic composition correlates with antioxidant performance across assays. The described distinctions of the ecosystems demonstrate the effect of the environment on the metabolic reactions of *O. ficus-indica*. The Mediterranean and continental ecosystems studied in the current paper have various climatic features that may impact the response of the plant under certain stressful conditions and the accumulation of different metabolites. A number of abiotic stresses, such as drought, high temperatures, and enhanced solar radiation, trigger the activation of the phenylpropanoid pathways for the production of phenols and flavonoids. This environmental-induced reaction serves as an adaptive strategy helping plants survive in the stressful environment (Chen *et al.,* 2022; Qaderi *et al.,* 2023). The antioxidant

Table 3: Antioxidant activity of ethanolic extracts of *O. ficus-indica* and standards

Extracts/ standards	Inhibition concentration (IC_{50}) mg/mL	
	DPPH radical scavenging	Reducing power
CEEC	0.09±0.01^{ns}	2.37±0.10***
CEEM	0.15±0.05***	5.13±0.84***
BEEC	0.24±0.01***	14.26±0.41***
BEEM	0.52±0.05***	7.97±1.47***
SEEC	0.09±0.00^{ns}	1.82±0.00***
SEEM	0.08±0.00^{ns}	1.03±0.03***
BHT	0.08±0.00	ND
Vitamin C	ND	0.03±0.01

CEEC-cladode ethanolic extract continental, CEEM-cladode ethanolic extract Mediterranean, BEEC-bark ethanolic extract continental, BEEM-bark ethanolic extract Mediterranean, SEEC-seed ethanolic extract continental, SEEM-seed ethanolic extract Mediterranean. ND-not determined. ns-no significant difference, '***'-p<0.001 as compared with the control

tests established that the differences noted in phytochemical content among extracts had biological significance. Seed extracts, more so SEEM extracts, exhibited the highest activities in scavenging for DPPH radicals and in reducing power due to their high level of condensed tannins and phytochemicals. Nevertheless, it cannot be assumed that the antioxidant activity is dependent solely on the phenolic compounds since antioxidant activity is based on structural chemistry, hydroxylation pattern, molecule interactions, and synergism of the various metabolites (Panche *et al.,* 2016).

Anti-inflammatory activity

Anti-inflammatory drugs prevent protein denaturation and maintain stable protein structures. *In vitro* anti-inflammatory activity results using the protein denaturation inhibition assay show greater activity in the ethanolic extract of *O. ficus-indica* bark BEEM (4 mg/ml) with a percentage of 65.66±3.85%, followed by the extract of cladodes CEEM (55.76±2.57%) (Table 4).

The comparative study between the two regions shows a significant disparity in anti-inflammatory potential between the extracts. Superior inhibitory activity was found in the Mediterranean ecosystem. The results obtained show that the ethanolic extracts of the cladodes and bark of *O. ficus-indica* possess significant anti-inflammatory activities according to the egg white protein denaturation inhibition test in comparison with seed extract. The results of Chafaa *et al.* (2024) show that *O. ficus-indica* by-products have a good anti-protein denaturation effect. According to this test, it was found that the Mediterranean bark extract (BEEM) was the most effective inhibitor of protein denaturation, while the Mediterranean cladode extract (CEEM) was the second most effective. Such activity may be associated with the protective function of bark tissues, because they are characterized by the accumulation of secondary metabolites for protection from various stresses and pathogens. It can be assumed that phenolic substances, flavonoids, and tannins contained in these extracts may provide such an anti-inflammatory effect due to their antioxidant properties (Albuquerque *et al.,* 2021; Tatipamula & Kukavica, 2021; Saad *et al.,* 2025).

Table 4: *In vitro* anti-inflammatory activity of ethanolic extracts of *O. ficus-indica* and standard

Extracts/ standards	Inhibition percentage (%)/ concentration (mg/mL)		
	1	2	4
CEEC	11.85±0.21***	20.85±1.73***	24.90±1.00***
CEEM	12.57±1.61***	29.5±2.12***	55.76±2.57***
BEEC	8.71±0.20***	9.71±0.85***	15.78±2.72***
BEEM	13.57±4.24***	24.85±4.64***	65.66±3.85***
SEEC	15.64±2.72***	18.28±0.20***	34.76±1.31***
SEEM	18.21±6.47***	19.57±5.25***	28.71±7.07***
Aspirin	80.42±0.51	76.00±1.40	96.85±1.11

CEEC-cladode ethanolic extract continental, CEEM-cladode ethanolic extract Mediterranean, BEEC-bark ethanolic extract continental, BEEM-bark ethanolic extract Mediterranean, SEEC-seed ethanolic extract continental, SEEM-seed ethanolic extract Mediterranean, ND-not determined. '***'-p<0.001 as compared with the control

DFT calculations study

Among the most common phenolic acids (Riaz *et al.,* 2023), have been extracted from *O. ficus-indica* plant through column-chromatography, caffeic and ferulic acids were chosen to be studied computationally. DFT calculations were utilized to perform a comparative frontier molecular orbital (FMO) analysis and investigate the electronic properties underlying the biological antioxidant capacities of these acids. To shed more light on the isomers of ferulic acid, the more stabilized one, (E)-3-(4-hydroxy-3-methoxyphenyl) acrylic acid, were studied. The optimized geometries of caffeic and ferulic acids are shown in Figures 2 and 3, respectively. The results of calculated chemical parameters to optimize the phenolic acids as shown in Table 5. The energy of the highest occupied molecular orbital E_{HOMO} is a key parameter reflects a molecule's ability to donate electrons. As it increases, the tendency to release electrons increases. That contributes to the stabilization of free radicals (Muthu & Prabhakaran, 2014). On the other hand, lowest unoccupied molecular Orbital E_{LUMO} is another key factor that reflects a molecule's ability to accept electrons. Where lower E_{LUMO} values facilitate electron acceptance. As summarized in Table 5, caffeic acid exhibits slightly higher HOMO energy **(**-6.0438 eV**)** compared to ferulic acid **(**-6.1808 eV**)**, indicating that caffeic exhibits a slightly stronger electron-donating tendency, which is a key step in radical quenching. This aligns with our experimental data where caffeic acid showed better performance than ferulic acid.

The frontier energy gap E_{GAP}, defined as the difference between HOMO and LUMO energies, reflects the kinetic stability and reactivity of the molecules. The molecules become less stable and more biologically active with smaller E_{GAP}. Caffeic acid exhibited a slightly smaller energy gap (4.0292 eV) and lower chemical hardness (2.0146 eV) compared to ferulic acid (ΔE=4.1519 eV); η=2.0760 eV). In addition, caffeic acid showed slightly higher chemical softness (S=6.7536 Hartree^{-1}). The electronic descriptors show a smaller energy gap and higher global softness of caffeic acid, indicating that it is more chemically reactive and polarizable. These enhance the molecule's reactivity toward reactive oxygen species and inflammatory mediators

Table 5: Quantum chemical parameters including the energy of highest occupied molecular orbital (E_{HOMO}), energy of the lowest unoccupied molecular Orbital (E_{LUMO}), the energy gap between LUMO and HOMO (E_{GAP}), the chemical hardness (η) and softness (S), for both caffeic and ferulic acids.

Quantum chemical parameters	Caffeic acid	Ferulic acid
Highest Occupied Molecular Orbital (E_{HOMO}), Hartree	-0.22207	-0.22714
Highest Occupied Molecular Orbital (E_{HOMO}), eV	-6.0438	-6.1808
Lowest Unoccupied Molecular Orbital (E_{LUMO}), Hartree	-0.07400	-0.07456
Lowest Unoccupied Molecular Orbital (E_{LUMO}), eV	-2.0136	-2.0289
Energy Gap E_{GAP} ($\Delta E=E_{LUMO}-E_{HOMO}$), Hartree	0.14807	0.15258
Energy Gap E_{GAP} ($\Delta E=E_{LUMO}-E_{HOMO}$), eV	4.0292	4.1519
Chemical Hardness (η=ΔE/2), eV	2.0146	2.0760
Global Chemical Softness (S=1/2η), Hartree^{-1}	6.7536	6.5539

eV-the electron volt unit of energies calculated. All calculations performed at B3LYP/6–311G (d,p) (SCRF=water) levels of theory and the formulas used to calculate these descriptors were adopted according to (Riaz *et al.*, 2023)

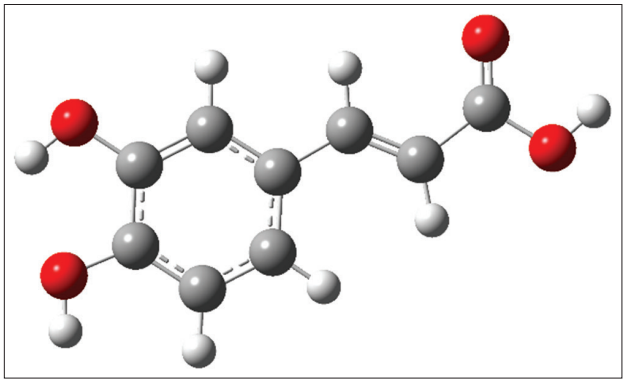

Figure 2: The optimized structure of caffeic acid

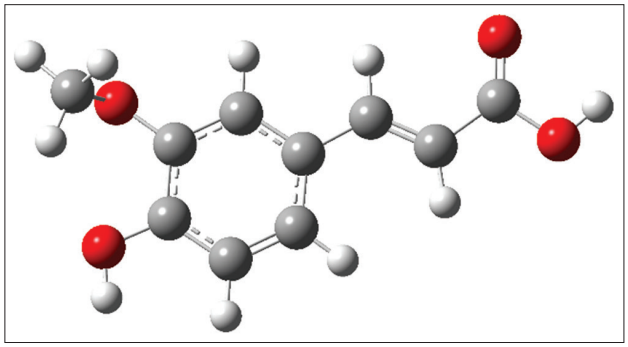

Figure 3: The optimized structure of ferulic acid

and contribute to its antioxidant and anti-inflammatory potential. Moreover, the observed differences in electronic properties can be explained by structural variation between the two phenolic acids. In caffeic acid, the presence of the ortho-dihydroxyl group on the aromatic ring (catechol moiety) facilitates donor electrons and enhances stability of phenoxy radical. Where, the substitution of a hydroxyl (-OH) group in caffeic acid with a methoxy (-OCH) group

in ferulic acid is decreasing donating ability and increasing rigidity of the molecular structure.

The DFT analysis gives a theoretical explanation of the experimental antioxidant activity observed in this research. Antioxidants work by donating electrons to unstable molecules called free radicals, helping prevent cellular damage. Furthermore, the presence of conjugated aromatic rings and hydroxyl groups can help donate electrons and stabilize reactive oxygen species, showing a radical scavenging effect. This may contribute to oxidative imbalance, which is closely associated with inflammatory activities. The important values examined were the higher HOMO energy and lower HOMO-LUMO energy gap of caffeic acid, suggesting a greater tendency to donate electrons, which may contribute to the radical scavenging activity of phenolic acids that exist in the extract. Thus, the computational findings corroborate the experimental results by providing the molecular mechanism of the antioxidant behavior.

To confirm the validity of our computational study, the calculated energies were compared with previously reported literature values. The energy gap obtained for caffeic acid (4.0292 eV) closely matches the values reported by Riaz *et al.* (2023) (4.054 eV). Moreover, the ferulic acid's HOMO energy (-6.1808 eV) show good agreement with the DFT studies on hydroxycinnamic acid derivatives (Bakalbassis *et al.,* 2001). The smaller E_{GAP} and higher global softness of caffeic acid improves its reactivity in chemical transformations in aqueous biological system, as well as in the gas phase that was previously studied. This agreement with literature (Riaz *et al.,* 2023) confirms that the computational method used in this study is reliable and appropriate for describing the electronic behavior of these acids and consistent with experimental antioxidant assays (DPPH and ABTS).

This study used Density Functional Theory (DFT) calculations to better understand the biological activities of caffeic acid and ferulic acid that act as antioxidants. Antioxidants work by donating electrons to unstable molecules called free radicals, helping prevent cellular damage. One important value examined was the Highest Occupied Molecular Orbital (HOMO) energy, indicates how easily a molecule can donate electrons. In general, molecules with higher HOMO energies can donate electrons more readily and may show stronger antioxidant activity. The results showed that caffeic acid has a slightly higher HOMO energy (-6.0438 eV) than ferulic acid (-6.1808 eV). Although the difference is small, it suggests that caffeic acid may have a slightly greater ability to donate electrons and neutralize free radicals. The study also evaluated the energy gap (ΔE_{gab}), which represents the difference between the HOMO and LUMO energy levels. A smaller energy gap generally indicates that a molecule is more chemically reactive. The results revealed that caffeic acid has a slightly smaller energy gap (4.0292 eV) compared to ferulic acid (4.1519 eV). Caffeic acid also showed lower chemical hardness (2.0146 eV) and slightly higher chemical softness (6.7536 $Hartree^{-1}$), suggesting that it may respond more readily during chemical reactions. These differences can be explained by their molecular structures. Caffeic acid contains a hydroxyl (-OH) group that is replaced by a methoxy ($-OCH_3$) group in ferulic acid. This structural change affects how electrons are distributed throughout the molecule, leading to the small differences observed in their electronic properties. The biological findings of experiments have been confirmed by the DFT calculations. The calculated molecular descriptors such as frontier molecular orbitals energy, HOMO-LUMO gap, electronegativity, and chemical hardness give the data on the ability of molecules to transfer electrons and chemical reactivity. Molecules possessing desirable electron properties can help stabilize radicals and be involved in the mechanism of antioxidant activity. Hence, *in vitro* tests together with DFT calculations have given complementary proofs that show how molecular features of selected compounds cause biological effects (Muthu & Prabhakaran, 2014).

This research has generated important information regarding the chemical profile, antioxidant properties, and anti-inflammatory potential of *O. ficus-indica* harvested in two different ecosystems in northwestern Algeria. There are a number of drawbacks that need to be highlighted, though. Firstly, the biological potential was assessed only through in vitro experiments, which may not account for the real situation of physiological processes in the organism. Secondly, while qualitative phytochemical analysis and the estimation of total phenolic compounds, flavonoids, and condensed tannins was conducted, the particular active components behind the biological potential were neither identified nor quantified using more advanced analytical approaches like (HPLC-MS/MS or LC-MS/MS phytochemical analyses). Furthermore, the samples were harvested during one campaign and in two ecosystems, which does not allow assessing the seasonal and geographical variability. Finally, DFT calculations were done for some selected compounds and give theoretical insight that needs to be proven experimentally.

Further research should aim at conducting a complete metabolic profile of *O. ficus-indica* collected from various environments in Algeria using chromatography and spectrometry methods. Moreover, there is a need for conducting pharmacological experiments, toxicity tests, and mechanistic studies to validate the biological activities and toxicity of the extracts. An increase in sampling at various seasons will help to better comprehend the role of ecological factors in influencing the phytochemical constituents of the plants. Moreover, the combination of computational methods such as molecular docking and molecular dynamics simulations with experimental validation will help to better understand the molecular mechanisms involved in the antioxidant and anti-inflammatory activities of *O. ficus-indica*.

Conclusion

The results of this study demonstrate that ethanolic extracts of *O. ficus-indica* contain significant levels of polyphenols, flavonoids, and condensed tannins, which

likely contribute to the strong antioxidant activity observed across the different samples. These findings confirm that *O. ficus-indica* is a rich source of bioactive compounds, particularly phenolic constituents, which have well-documented beneficial effects on human health by mitigating oxidative stress and reducing the risk of related diseases. Given its adaptability to extreme environmental conditions and its potential applications in food and health sectors, *O. ficus-indica* represents an important species for both nutritional and pharmaceutical purposes. Overall, the computational results indicate that both caffeic acid and ferulic acid are highly reactive antioxidant compounds while caffeic acid appears to have a slightly stronger electron-donating ability. These findings support the antioxidant activities observed in the experimental assays, including the DPPH and ABTS radical scavenging tests. Based on these results, a recommend promoting large-scale cultivation of this species and exploring the industrial exploitation of its bioactive compounds in functional foods, nutraceuticals, and other health-related applications.

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Author contributions

Abdelkader Douis and Ahlem Karbab: Writing–original draft, Software, Methodology, Conceptualization. Abderrezak Djabeur, Noureddine Charef, Hanane Sihem Sebaa and Reema Ahmad Hasan Omeir: Validation, Software, Data curation. Ahlem Karbab, Noureddine Charef and Abderrezak Djabeur: Writing–review & editing, Validation, Supervision, Software, Conceptualization. All authors approved the final manuscript.

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