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Exploring the Bioactive Potential of *Cistus ladanifer* Leaves from Northern Morocco (Tangier)

Bahia Abdelfattah[1*], Amena Mrabet[1], Ayoub Simou[1], Mohamed Khaddor[1]

[1] Laboratory of Materials, Natural Substances and Environment (LAMSE), Chemistry Department, Faculty of Sciences and Techniques of Tangier, P.O. Box 416, Tangier 90000, Morocco

* Corresponding author's e-mail: bahia.abdelfattah@uae.ac.ma

ABSTRACT

The present research explored the antioxidant capacities and phytochemical profile of *Cistus ladanifer* collected from North Morocco (Tangier), to identify potential medicinal sources of antioxidants. The antioxidant qualities of the extracts were assessed using a variety of analytical methods, such as 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and oxygen radical absorbance capacity (ORAC). Fourier transform infrared (FTIR) spectroscopy, gas chromatography-mass spectrometry (GC-MS), and high-performance liquid chromatography (HPLC) were employed to examine the chemical constituents present in the plant material. Finally, ICP-AES analysis was performed to characterize the nutritional components of the extract, providing detailed insights into its valuable nutrient content. The findings revealed that the methanolic extract displayed considerable antioxidant potential, as evidenced by its powerful scavenging activities against DPPH, with an IC_{50} value of 0.115 ± 0.003 mg/ml and IC_{50} of 0.251 ± 0.006 mg/ml ABTS radicals. Moreover, it displayed a higher reducing power using the FRAP test and excellent ORAC values, with 62.74 ± 0.07 mg/g and 40.21 ± 0.7 mg/g dry weight of extract, respectively. This research also assessed the total phenolic and total flavonoid compounds, while tannins were found in high concentrations in the methanolic extract, with 69.08 ± 1.69 mg of GAE/g dW, 47.86 ± 1.76 mg of QE/g dW, and 354.98 ± 15.29 mg of TAE/g dW, respectively. The presence of phenols, benzene rings, aromatic compounds, ethers, and methylene chains was verified by FTIR analysis. Finally, HPLC-MS and GC-MS analyses revealed a rich profile of bioactive phytochemicals. This study is the first of its kind to be conducted in Tangier, Northern Morocco, shedding light on the unique chemical composition of *Cistus ladanifer* in this region.

Keywords: antioxidant capacities, chemical composition, *Cistus ladanifer*, natural sources, Nutritional components, phytochemicals, Tangier.

INTRODUCTION

Since the dawn of civilization, medicinal plants have been employed to treat a diverse array of health issues, thanks to their therapeutic properties. Despite the progress made in modern medicine, a significant number of individuals today continue to prefer traditional remedies over synthetic drugs, as they believe that these alternative treatments are less likely to cause adverse side effects compared to chemical medications. The wide range of bioactive substances, particularly the secondary metabolites that medicinal plants generate, has prompted extensive scientific studies on different plant extracts. Several studies have documented the antimicrobial, anti-inflammatory, analgesic, antioxidant, and other medicinal properties of these extracts (Dar et al., 2023; Khade et al., 2023). Morocco, with its unique bio-geographical position, is home to rich vegetation encompassing a wide array of plant species renowned for their diverse medicinal properties (Benyahya et al., 2023; Lemhadri et al., 2023; Serbouti et al., 2023). These plants are used for their vermifugal, insecticidal, antipyretic, antimicrobial, antifungal, diuretic, and

antispasmodic activities. The Tangier region in northwestern Morocco is renowned for its diverse array of medicinal plants, including species of the Cistaceae family. This family, encompassing eight genera, features Cistus as a prominent genus with 21 species (Kachmar et al., 2021; Merrouni et al., 2021).*Cistus ladanifer*, also known as labdanum, gum rockrose, or brown-eyed rockrose, is a fragrant shrub with white flowers that blooms from March to May, reaching heights of up to two meters. Its seeds emerge between July and October, showing adaptability to various climates and exceptional tolerance to cold, drought, and heat stress. This plant has a rich history of medicinal use for ailments like diarrhea and menstrual pain, with pharmacological studies highlighting its antioxidative (Barrajón-Catalán et al., 2010; Bouothmany et al., 2022; Gaweł-Bęben et al., 2020; Raimundo et al., 2018), anti-inflammatory, and pain-relieving properties (El Hamsas El Youbi et al., 2016). Extracts from *Cistus ladanifer* offer sun protection and exhibit antibacterial, antifungal, and antiproliferative effects, making them valuable for skincare and medicinal applications (Gaweł-Bęben et al., 2020). Moreover, *Cistus ladanifer* extracts have been shown to possess antibacterial, antifungal, and antiproliferative effects (Bakrim et al., 2021; Benali et al., 2020; Bouothmany et al., 2022; El Karkouri et al., 2021). In Moroccan traditional medicine, the dried leaves of *Cistus ladanifer* are specifically employed as antidiarrheal, antiacid, and antispasmodic agents (Huang et al., 2013). This article conducts a comprehensive investigation into *Cistus ladanifer* from Tangier, focusing on its secondary metabolites and medicinal effects. The study's primary objective is to evaluate the plant's antioxidant capacities using various assays, including DPPH, ABTS, ORAC, and FRAP, to determine its potential for use in therapeutic applications. Beyond its antioxidant properties, the research focuses on the bioactive molecules present in the methanolic extracts of *Cistus ladanifer*. Advanced chromatographic techniques, such as HPLC-MS and FTIR analysis, have been used to identify and characterize a wide range of compounds that contribute to the plant's medicinal potency. Additionally, GC-MS analysis following derivatization provides a more in-depth understanding of both primary and secondary metabolites found within the plant. This study combines traditional knowledge with modern scientific methodologies to reaffirm *Cistus ladanifer*'s esteemed status in Moroccan folk medicine and pave the way for its integration into contemporary healthcare practices. By elucidating the plant's secondary metabolites and antioxidant capacities, the research underscores its rich pharmacological potential, offering promising prospects for the development of natural-based therapeutic agents.

MATERIAL AND METHODS

Plant material

Cistus ladanifer plants were collected for this study from the Rmilate forest in Tangier (35.7876°N,5.8653°W), situated in the northern region of Morocco. Plants were carefully selected and transported to the laboratory. The leaves of the plants were carefully isolated and left at room temperature until the leaves had dried completely.

Extraction process

Dried *Cistus ladanifer* leaves (4.5 g) were extracted for 24 h on a magnetic stirrer in 45 mL of a mixture solution of water/methanol (80 /20) and 100% water. The obtained extracts were filtered using Whatman paper and a 0.45 m syringe filter before the solvent was evaporated at a temperature of 37 °C. For analysis, the dried extracts were stored in a freezer at 4 °C.

ICP-AES for elementary characterization

We carefully weighed 0.1 g of sample (leaves powder), to which 3 ml of hydrochloric acid and 1 ml of nitric acid were added. The mixture was allowed to stand for 24 h and then heated for 2 h at 95 °C. Subsequently, the sample was diluted to 25 ml with ultrapure water. Finally, the solution was filtered through a 45 µm sieve before being analyzed using an ICP ULTIMA EXPERT apparatus.

Determination of total phenolic content

The total phenolic compound content was determined using the method described by Ennoury et al. (Ennoury et al., 2022), with minor modifications. 100 µL of the extract (1 mg/mL) was combined with 400 µL of Folin-Ciocalteu reagent (diluted in water). After adding 1 mL 7% (m/v) Na_2CO_3, the final volume was increased by distilled water to 1.6 mL. The samples were then incubated for 30 min in the dark at room temperature. Absorbance was determined at 765 nm using a spectrophotometer. The calibration curves were

prepared using a gallic acid standard, yielding the equation $y = 17.5x + 0.1113$; $R^2 = 0.9985$. The phenolic component in the dried extract weight (DW) was expressed as gallic acid equivalents (GAE) in mg/g.

Determination of total flavonoids content

The flavonoid content was evaluated using the method described by Ennoury et al. (2022). Briefly, 1800 µL of a solution containing 1 mL of acetate potassium (1M), 1 mL of aluminum chloride (10%), 10 mL of methanol (50 %), and 24 mL of distilled water were combined with 200 µL of each sample after a further 30 min in the dark at room temperature. The absorbance of the mixture was measured at a wavelength of 415 nm. Using the quercetin standard to produce the calibration curves, the following equation was obtained: $y = 5.9566x + 0.1353$; $R^2 = 0.9999$. The flavonoid content in each gram of extract was measured as mg of quercetin equivalent (QE).

Determination of total tannins content

The Folin-Ciocalteu technique was cited by Ci et al. (2016), with some modifications used to identify the tannins. 100 µL of the sample was added to 1 mL of 30% sodium carbonate solution and Folin-Ciocalteu phenol reagent (0.5 mL). After thorough shaking, the mixture was incubated at room temperature in the dark for 30 min. The absorbance was then measured at 700 nm. In the same way, calibration curves for standard tannic acid solutions were created, yielding the equation $y = 3.6129x + 0.1806$; $R^2 = 0{,}9933$. The tannin content in dried extract weight (DW) was expressed as tannic acid equivalents (TAE) in mg/g.

DPPH assay

DPPH was used to test the antiradical activity of the extracts, following a previous study (Kosani and Manojlovi, 2014). Extracts were prepared at various concentrations (1, 0.25, 0.125, 0.0625, 0.03125, and 0.015625 mg/mL). Subsequently, 750 µL of DPPH (prepared before methanol) was added to 250 µL of the extract. After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm using an ultraviolet (UV) spectrophotometer. The percentage inhibition of DPPH radical was calculated using the following Equation:

$$I\% = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100 \quad (1)$$

Sample is the absorbance of the extract, whereas control is the absorbance of the blank sample.

ABTS assay

The ABTS test was performed following the method described by Gallego et al. (Gallego et al., 2013), with minor modifications. Several concentrations were used to prepare the extracts (1, 0.25, 0.125, 0.0625, 0.03125, and 0.015625 mg/mL). A solution of ABTS radicals (7 mM), potassium persulfate (2.45 mM), and methanol was prepared and the mixture was incubated at 30 °C with an absorbance of 0.72. To conduct the test, 75 µL of each sample was added to 925 µL of ABTS radical. The mixture was then incubated for 10 min. The absorbance was measured at a wavelength of 734 nm. The percentage inhibition of ABTS radical was calculated using the following Equation:

$$I\% = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100 \quad (2)$$

Sample is the absorbance of the extract, whereas control is the absorbance of the blank sample.

FRAP assay

The method described by Arroussi et al. (2022) was used to evaluate ferric-reducing antioxidant power (FRAP). TPTZ 10nM, $FeCl_3$, and acetate Buffer 30nM were used to generate FRAP. With a ratio of 10:1:1. The diluted extract was placed in a microplate containing reactive FRAP, which had previously been incubated at 30°C. Trolox was used as a calibration curve and the findings were provided using the Trolox equivalent. Absorbance was measured at 593 nm.

ORAC assay

The ORAC test was used to assess the antioxidant activity of the extracts, following the Escribano et al. method (Escribano et al., 2017). Next, 120 µl of fluorescein 80 nM (diluted with PBS 13, 3 mM) was added to the microplate containing the sample. After reading the first fluorescence, 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH) solution was added, and the plate was immediately scanned every 2 min for 120 min. ORAC values were calculated as described by Mendes et al. (Mendes et al., 2016) using Gen5 2.0 Software (BioTek Instruments). Trolox

was used as a reference for the calibration curve. The results are expressed as mM TE/ml of the extract.

FTIR analysis

Fourier transform infrared (FTIR) spectrophotometry is widely regarded as an efficient method for detecting the chemical bonds existing in substances and their functional groups (Bunaciu et al., 2010). The methanolic extract was mixed with potassium bromide and compressed into a suitable tablet. The FTIR spectrum of the extract was recorded using an FTIR spectrophotometer in the range 400–4,000 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans were co-added (Ulpathakumbura et al., 2023).

HPLC-UV-MS analysis

The customized method, which utilized a quadrupole time-of-flight mass spectrometer with electrospray ionization and an Ultimate 3000 (Dionex) Thermo Scientific system, demonstrated effectiveness for both qualitative and quantitative analyses of the methanolic extract of *Cistus ladanifer*. The HPLC column utilized in the system for the studies was BDS Hypersil (C18, 150 × 4.6 mm × 5 µm). Solvent A (water) and solvent B (acetonitrile) were employed in the experiments, and formic acid 0.1% was added to enhance metabolite ionization. The gradient consisted of 2% solvent B in solvent A for the first minute, 25% solvent B for the subsequent minute, 45% solvent B for the next two minutes, 95% solvent B for the following minute, and 2% solvent B for the final minute. Each sample was injected in a volume of 10 µL, with a flow rate of 0.2 mL/min, and the analysis lasted for 55 min, with a 10-minute post-analysis period. The UV wavelengths ranged from 190 to 700 nm. In the mass range from m/z 40 to 1500. Fragmentation energy was maintained at 110 V, with fixed collision energies set at 10, 20, and 30 V. Gas temperatures were maintained at 350 and 400 °C, respectively, gas currents were maintained at 12 L/min each, with atomizer pressure set at 35 psi. Additionally, the voltages applied were 4000 V for the capillary and 1000 V for the nozzle.

Gas chromatography-mass spectroscopy for profiling of plant compounds

Sample derivatization

The following steps were performed to prepare the methanolic extract for GC–MS analysis. Initially, approximately 5 mg of the organic extract was derivatized by adding 200 µL pyridine and 200 µL BSTFA (comprising 1% TMCS). The resulting mixture was thoroughly mixed using a vortex mixer and subsequently subjected to 2h heating period at 70 °C within the oven, followed by 15 min cooling period at 4 °C in a refrigerator (Muazu et al., 2022). Then, after dilution with 400 µL of hexane, the sample underwent centrifugation for 5 min at 5,000 rpm (Ibragic et al., 2021). Following centrifugation, the obtained liquid was carefully dispensed into a GC vial for subsequent analyses.

GC-MS data acquisition parameters

GC-MS was performed using a GC TRACE 1300 TSQ 8000 evo with a split injection volume of 1.0 µL coupled to a mass detector. The column used was a TR – 5.30 m × 0.25 mm × 0.25 µm. The carrier gas utilized was helium, flowing at a rate of 1.5 mL/min. For injection, a programmed-temperature split/splitless injector was employed, set at 250 °C. The column oven's temperature was programmed to increase from 70 to 300 °C at a rate of 4 °C each minute, maintaining a final hold time of 30 min. Electron ionization with a mass spectrometer (70 eV) was used for analysis. Compound identification was accomplished using Nist Mass Spectral Library Version 2017 and KnowItAll software.

Statistical analysis

Every experiment was run using a minimum of three duplicates. Utilizing the SPSS 25 software for Windows, version 10.0.1, the collected data were examined. One-way ANOVA was performed to assess the statistical significance of the data obtained for the various extracts, and the Student-Newman-Keuls post hoc test was then employed to compare means that differed by $p < 0.05$. Significant differences are indicated by different letters.

RESULTS AND DISCUSSION

Elemental characterization using ICP-AES

Inductively coupled plasma atomic emission spectrometry (ICP-AES) is a technique used for the determination of elemental composition in plant samples. It is commonly used to analyze trace elements in environmental biomonitoring studies (Drava and Minganti, 2020). This method is simple, rapid, efficient, and provides

accurate and reliable results with low detection limits. Overall, ICP-AES is a valuable tool for the analysis of the elemental composition in plants, providing accurate and reliable results with wide application potential (Barros et al., 2016).

The elemental characterization results presented in Table 1 indicate that several elements, including chromium (Cr), zinc (Zn), and nickel (Ni), are undetectable in the sample. Among the detected elements, Manganese (Mn) was found at a concentration of 26.57 mg/kg, iron (Fe) at 210.5 mg/kg, magnesium (Mg) at 1916.75 mg/kg, calcium (Ca) at 8261.5 mg/kg, aluminum (Al) at 154.8 mg/kg, and potassium (K) at 6261.5 mg/kg.

The presence of essential elements, including Mg, Fe, Ca, Al, Cu, and K in the plant leaves is vital for their overall health and function. Fe is crucial for chlorophyll synthesis, photosynthesis, and the prevention of chlorosis. Mg is integral to chlorophyll molecules, aiding in sunlight absorption and sugar formation. Ca maintains cell wall integrity and promotes strong leaf and disease resistance. Cu participates in enzymatic reactions and lignin formation and provides structural support. It is worth noting that even though Cu was undetectable in plant leaves in this study, it may still be present in other parts of the plant, such as the roots. Aluminum, in small amounts, aids in enzymatic processes and may regulate stomatal conductance. K regulates stomatal function, gas exchange, and protein synthesis, which are essential for leaf function and plant growth. Together, these elements ensure optimal leaf performance and plant vitality (Kumar et al., 2019; Luyindula, 2017; Saur, 1990).

Total phenolics, total flavonoids, and tanin contents of *Cistus ladanifer* leaf extracts

The levels of total phenolic components, flavonoids, and tannins in the extracts derived from the dried aerial portions of the plant using 100% distilled water and 80% methanol-water (v/v) were compared. Table 2 presents the results. All the results were statistically significant ($p < 0.05$).

The total phenolic (TPC), flavonoid (TFC), and tannin contents of *Cistus ladanifer* leaf extracts were calculated as gallic acid equivalents (GAE) using a standard curve ($R^2 = 0.9985$). Using the quercetin calibration curve ($R^2 = 0{,}9999$), the TFC was expressed as quercetin equivalent (QE). Tanin was quantified using the tannic acid standard curve ($R^2 = 0{,}9933$).

The average concentrations of phenols in aqueous and methanolic extracts varied from 20.93 to 69.23 mg GAE/g dW, flavonoids from 23.77 to 47.55 mg QE/g dW, and tannin from 290.57 to 359.72 TAE/g dW. The highest concentrations of tannin (359.72 TAE/g dW), flavonoids (47.55 mg QE/g dW), and total phenols (69.23 mg GAE/g dW) were found in the methanolic extract.

The methanolic extract had higher total phenolic, flavonoid, and tannin contents. These substances are secondary metabolites in plants that contribute to growth and development, protect against parasites and pathogens, and help generate free radicals (Sayah et al., 2017). Phenolic compounds are a significant class of molecules that support the antioxidant capacity of plant extracts and have been shown to exert various biological effects (Sayah et al., 2017).

Alcoholic solvents have frequently been used for the extraction of phenolic compounds from natural sources, yielding a substantial total extract. The use of alcohol-water mixtures has proven to be more effective for extracting phenolic constituents than single-component solvent systems (Poonguzhali et al., 2023). Incorporating a small quantity of water into an organic solvent commonly generates a more polar environment, thereby enhancing polyphenol extraction (Spigno et al., 2007). Therefore, the phenolic compounds abundance observed in the 80% (v/v) methanol-water extract can be explained by the polarity of the solvent, which enhances extraction efficiency.

Determination of antioxidant capacity

Within the human body, there is a continuous generation of free radicals and various reactive oxygen species. These have been associated with numerous ailments such as diabetes, cancer, neurological disorders, and the aging process (Valko et al., 2007). Although synthetic antioxidants have demonstrated their effectiveness, their usage is linked to significant adverse health consequences

Table 1. Elemental composition analysis of *Cistus ladanifer* leaves

Elements	Mn	Fe	Mg	Ca	Cu	Al	K	Cr	Zn	Ni
mg/kg	26.57	210.5	1916.75	8261.5	2.775	154.8	6261.5	–	–	–

Table 2. The total phenolic, flavonoids, and tannin contents present in aqueous and methanolic extracts of *Cistus ladanifer*

Parameters	Aqueous extract	Methanolic extract
Total phenolic content (mg of GAE/g dW)	21.18 ± 2.1 [a]	69.08 ± 1.69 [b]
Total flavonoids content (mg QE/g dW)	23.93 ± 0.1 [a]	47.86 ± 1.76 [b]
Tannin content (mg of TAE/g dW)	288.03 ± 12.69 [a]	354.98 ± 15.29 [b]

Note: GAE: gallic acid equivalents; QE: quercetin equivalents; TAE: tannic acid equivalents; DW: dry weight. Values are expressed as mean ± SE of four replicates. The different superscript (a, b) values significantly differ (p<0.05) from those of the other extracts in the same row.

(Shahidi and Zhong, 2010). Consequently, the exploitation of medicinal plant extracts as a viable resource of antioxidants, which may offer minimal to no negative side effects, presents an important alternative approach to support overall well-being. This research involved assessing the antioxidant power of *Cistus ladanifer* leaf extracts to bind the free radical DPPH, ABTS cation, and ORAC, as well as their antioxidative ferric reductase activity (FRAP). Table 3 presents the results. TE: Trolox equivalents: the different superscripted (a,b) values differ significantly (p<0.05) from the other extracts in the same row. IC 50%: concentration of the sample needed to neutralize 50% of DPPH and ABTS free radicals. DPPH is an organic nitrogen compound that is known for its stability and widespread availability in commercial applications. It exhibited a pronounced absorption peak at 517 nm (Blois, 1958). The free radical transitions from purple to yellow as it accepts either lone-pair electrons or hydrogen radicals. These findings indicated that the methanolic extract had an excellent scavenging effect, with an IC50 value of 0.115 ± 0.003 mg/ml. In the ABTS assay, the antioxidants facilitated the reduction of the ABTS + cation to its non-oxidized form, ABTS, resulting in a noticeable decolorization effect. The methanolic extract had a strong capacity to quench ABTS + at 0.251 ± 0.006 mg/ml. Furthermore, using the FRAP method, the yellow coloration of the test solution shifts to various shades of green and blue, and these changes are contingent on the reducing capacity exhibited by each sample. When reducers are present, they assist in the reduction of the Fe3+/ferricyanide complex, employed in this method, to its ferrous state. (Gülçın et al., 2003). In this assay, the methanolic extract exhibited the highest antioxidant content (62.74 ± 0.7 mg TE/g dW).

The ORAC method, originally devised by Cao, Alessio, and Cutler in 1993, involves assessing the reduction in a protein's fluorescence due to the alteration of its structure resulting from oxidative harm caused by peroxyl radicals (ROO•). This approach quantifies the capacity of antioxidants within a sample to shield proteins from oxidative harm(Zulueta et al., 2009). In this test, the methanolic extract displayed an antioxidant capacity of 40.21±0.7 mg TE/g dW.

Because of the ability of specific polyphenols to bind to reactive oxygen species (ROS), a high concentration of polyphenol substances in extracts is generally linked to robust antioxidant activity. (Glevitzky et al., 2019). The reason methanolic extract exhibits potential antioxidant activity lies in the high quantities of polyphenolic compounds present within it. Polyphenols are well-known antioxidants, capable of scavenging free radicals, and reducing oxidative stress in the body (Liu et al., 2023; Bobrysheva, 2023). Methanol, as a solvent, is particularly effective at extracting these compounds from plant material, resulting in a concentrated extracts with high antioxidant capacity (Altemimi et al., 2017). Therefore, the combination of methanol-water extraction and the presence of polyphenolic compounds leads to the observed potent antioxidant activity in the methanolic extract compared to the aqueous extract with lower concentrations of phenolic compounds. Our findings confirm the results of previous

Table 3. Antioxidant activity of aqueous and methanolic extracts from *Cistus ladanifer* leaves measured using DPPH radical scavenging capacity, ferric reducing power (FRAP), Trolox equivalent antioxidant capacity (ABTS), and oxygen radical absorbance capacity (ORAC) Assays: mean values of four replicates ± standard deviation.

Solvent extraction	DPPH mg/ml IC 50%	Abts mg/ml IC 50%	ORAC mg TE/g dW	FRAP mg TE/g dW
Methanolic extract	0.115±0.003 [a]	0.251±0.006 [a]	40.21 ±0.7 [a]	62.74±0.07 [a]
Aqueous extract	0.149±0.004 [b]	0.517±0.04 [b]	12.62±0.8 [b]	44.66±0.08 [b]

research. According to (Amensour et al., 2010), the methanolic extract had a greater level of antioxidant activity, inhibiting DPPH with an efficiency of 87.72%, additionally, according to research published by Gaweł-Bęben et al. (Gaweł-Bęben et al., 2020), *Cistus ladanifer* extracts from Poland showed considerable antioxidant activity, with an IC50 range of 4.08 to 10.20 g/mL. Furthermore, based on a previous study by Bouothmany et al. (Bouothmany et al., 2022), the ethanolic extract showed a higher scavenging activity than the other extracts, with an IC50 of 266.6 0.828 g/ml. The aqueous extract also showed a relatively strong antioxidative activity, as previously reported by Bakrim et al. (2021). The differences in the antioxidant activity of *Cistus ladanifer* extracts might be due to changes in the extraction solvents used, the locations where samples were collected, and the plant parts used. Accordingly, the extracts from *Cistus ladanifer*, gathered from various geographic locations, have been identified as potent candidates with strong antioxidant properties (Amensour et al., 2010; Barrajón-Catalán et al., 2011; Blois, 1958; Gaweł-Bęben et al., 2020; Glevitzky et al., 2019; Gülçın et al., 2003; Shahidi and Zhong, 2010; Valko et al., 2007; Zulueta et al., 2009). This attribute has been linked to their substantial abundance of phenolic acids and flavonoids (Amensour et al., 2010; Barrajón-Catalán et al., 2011; Guimarães et al., 2010).

Identification of methanolic extract by FTIR analysis

FTIR analysis identified the bonds and functional groups within diverse chemical compounds. In addition, FTIR spectroscopy offers a precise depiction of the complete array of chemical bonds within the specimens by employing straightforward and rapid sample-handling techniques (Yunitasari et al., 2022). Within the absorption spectrum ranging from 4000–400 cm^{-1}, organic compounds are crucial to phytochemical research, as they exhibit unique fingerprint bands enabling their identification. The spectrum of the methanolic extract sample is illustrated in Figure 1 and presented in Table 4. The peak at 3310.10 cm^{-1} signifies the presence of alcohols and phenols, attributed to the stretching vibrations of the OH groups (Sutariya et al., 2023). The aromatic ring was identified by a peak at 1605.51 cm^{-1}, indicative of C=C stretching vibrations (Nsofor et al., 2023). Furthermore, the existence of a peak at 1708.83 cm^{-1} is associated with the carboxylic group (C=O vibrations). Alkaline compounds were suggested by the C-H stretching vibrations at 2928.35 cm^{-1}. At 1514.35 $cm^{-1,}$ the peak corresponds to the stretching vibrations (C=C) indicating the presence of aromatic compounds. The compound exhibits alkynes, as evidenced by a strong peak at 629.08 cm^{-1}, within the reference range of 610–700, corresponding to the C=C-H–H and C-H bending functional groups. The peak at 1439.40 cm^{-1} indicates the presence of methylene groups, while 1044.86 cm^{-1} suggests the existence of ethers. The aromatic group was confirmed by a C-H "loop" (Ramya et al., 2022) at 878.25 cm^{-1} within the reference range of 675-900. Additionally, medium peaks at 1346.21 cm^{-1} (C-N stretching), 1180.09 cm^{-1} (C-O stretching), and 750 cm^{-1} (C-H bending) indicate the presence of aromatic amines, ethers, alcohols, esters and aromatic groups respectively.

Table 4. Identified compounds in the methanolic extract by FTIR analysis

Wavenumber (cm^{-1})	Functional groups	Mode of vibration	Identified phytocompounds
3310.10	O-H	stretch	alcohols, phenols
2928.35	C-H	stretch	alkaline
1708.83	C=O	stretch	carboxylic acid
1605.51	C=C	ring stretch	aromatics (type benzenic)
1514.35	N-O	stretch	nitro compound
1439.40	O-H	bend	carboxylic acid
1346.21	O-H	bend	phenols
1180.09	C-N	stretch	alkyl ether
1044.86	S=O	stretch	sulfonic acid
878.25	C-H "loop"	stretch	aromatic group
750.63	$-(CH_2)n-$, CH_2	rocking	CH_2 rocking in methylene chains in hydrocarbons
629.08	-C=C-H; C-H	bend	alkynes

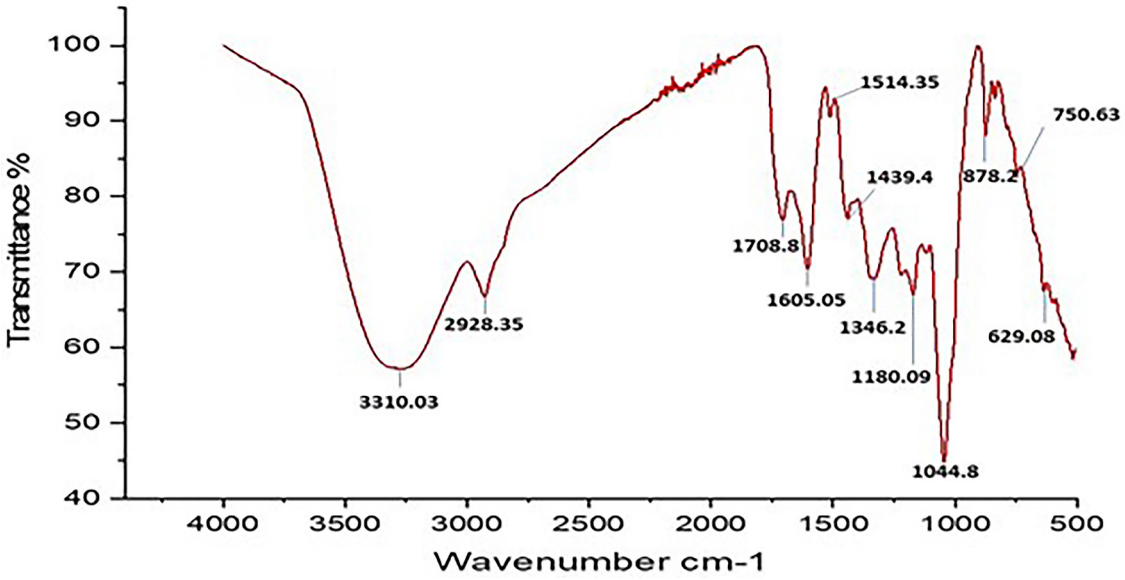

Figure 1. IR Spectra of the methanolic extract

Qualitative analysis of *Cistus ladanifer* leaf extracts by HPLC-UV-MS

Two ionization techniques, positive and negative, were employed to perform HPLC-ESI-MS analyses to further elucidate of *Cistus ladanifer* methanolic extract. The negative ion mode yielded the most favorable results and will be the subject of the discussion. The chromatographic profile of the methanolic extract of Dittrichia viscosa is depicted in Figure 2, while Table 5 provides putative identifications based on retention time, maximum absorption wavelength and precursor ions. The identification of these compounds in the methanolic extract of *Cistus ladanifer* leaves sheds light on the plant's phytochemical composition and it's potential medicinal properties. Gallic acid, quinic acid, and shikimic acid are known for their antioxidant and anti-inflammatory effects, suggesting that *Cistus ladanifer* may possess therapeutic benefits for conditions related to oxidative stress and inflammation (Bai et al., 2022). The presence of epigallocatechin and gallocatechin, both catechins commonly found plants, hints at potential cardioprotective and anticancer properties of the extract (Sebastiani et al., 2021; Wen et al., 2022). Ellagic acid and its xyloside derivative, along with quercetin glucoside, exhibit strong antioxidant and anticancer activities, further supporting the potential health benefits of *Cistus ladanifer* (Ceci et al., 2018; Saadullah et al., 2020). The flavonoids kaempferol diglycoside, apigenin, and myricitrin contribute to the extract's antioxidant and anti-inflammatory properties (Olszowy-Tomczyk

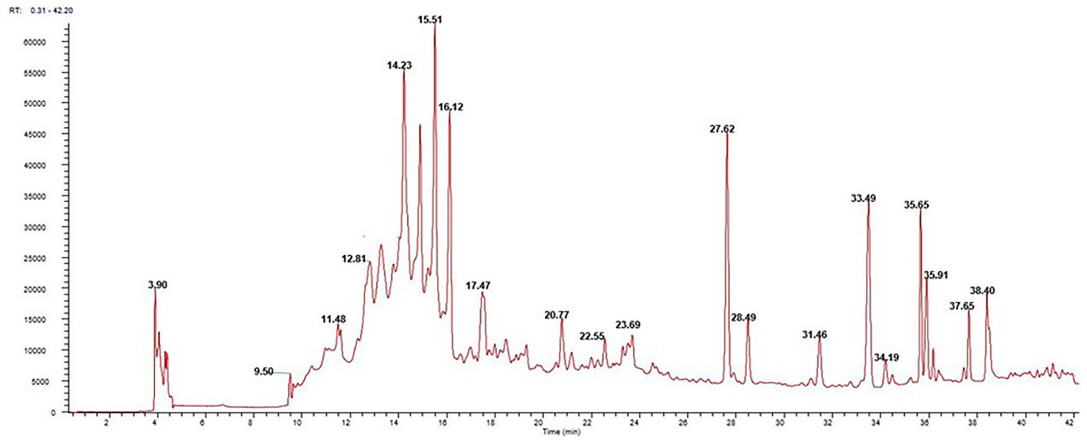

Figure 2. Chromatographic profile of *Cistus ladanifer* leaves methanolic extract by HPLC

Table 5. Identified compounds in *Cistus ladanifer* leaves methanolic extract by HPLC-UV-MS

RT (min)	UV λ (nm)	$[M-H]^-$	MS/MS	Molecular formula	Proposed compound
3.90	270,220	169.013	125, 169	$C_7H_6O_5$	Gallic acid
9.50	191, 239	191.055	173, 129, 111	$C_7H_{12}O_6$	Quinic acid
11.48	210	173.024	129, 125	$C_7H_{10}O_5$	Shikimic acid
12.81	276, 235	305.059	289, 137	$C_{15}H_{14}O_7$	Epigallocatechin
14.23	278, 235	305.059	137	$C_{15}H_{14}O_7$	Gallocatechin
15.51	255, 355	595.1294	301	$C_{26}H_{27}O_{16}$	Ellagic acid-7-xyloside
16.12	242, 278	301.029	257, 229	$C_{14}H_6O_8$	Ellagic acid
17.47	260, 360	463.088	301, 151	$C_{21}H_{19}O_{12}$	Quercetin glucoside
20.77	265, 346	593.130	447, 285	$C_{30}H_{25}O_{13}$	Kaempferol diglycoside
22.55	266, 346	593.128	447, 285	$C_{30}H_{25}O_{13}$	Kaempferol diglycoside
23.69	270, 336	269.045	137	$C_{15}H_{10}O_5$	Apigenin
27.62	269, 345	287.060	153	$C_{16}H_{12}O_6$	Kaempferol methylether
28.49	269, 345	287.060	153	$C_{16}H_{12}O_6$	Kaempferol methylether
31.46	283	339.124	179	$C_{16}H_{22}O_8$	Caffeoyl-glucose
33.49	269, 350	624.141	463, 301	$C_{27}H_{29}O_{17}$	Quercetin diglycoside
34.19	254, 365	301.029	151, 178	$C_{15}H_{10}O_7$	Quercetin
35.65	254, 367	479.089	317, 151	$C_{21}H_{20}O_{12}$	Myricitrin
35.91	272, 336	283.061	231, 225	$C_{16}H_{11}O_5$	Apigenin methylether
37.65	275	615.118	593, 453, 285	$C_{39}H_{32}O_{15}$	Kaempferol dicoumaroyl glucose
38.40	266, 350	313.071	255, 235,	$C_{17}H_{13}O_6$	Kaempferol dimethylether

and Wianowska, 2023; Yoon et al., 2023), while caffeoyl-glucose and quercetin diglycoside offer additional bioactive components with potential health-promoting effects. The presence of kaempferol derivatives, including methylether, dicoumaroyl glucose, and dimethylether, underscores the diversity of bioactive compounds present in *Cistus ladanifer* leaves, suggesting a rich source of natural products for pharmaceutical and nutraceutical applications. Overall, the identification of these compounds highlights the pharmacological relevance of *Cistus ladanifer* and warrants further investigation into its potential medicinal uses.

Profiling plant compounds by gas chromatography-mass spectroscopy analysis

The chemical composition of the *Cistus ladanifer* plant was analyzed using GC-MS, which identified 36 compounds that accounted for a total of 98.28% (Figure 3, Table 6). This rich variety

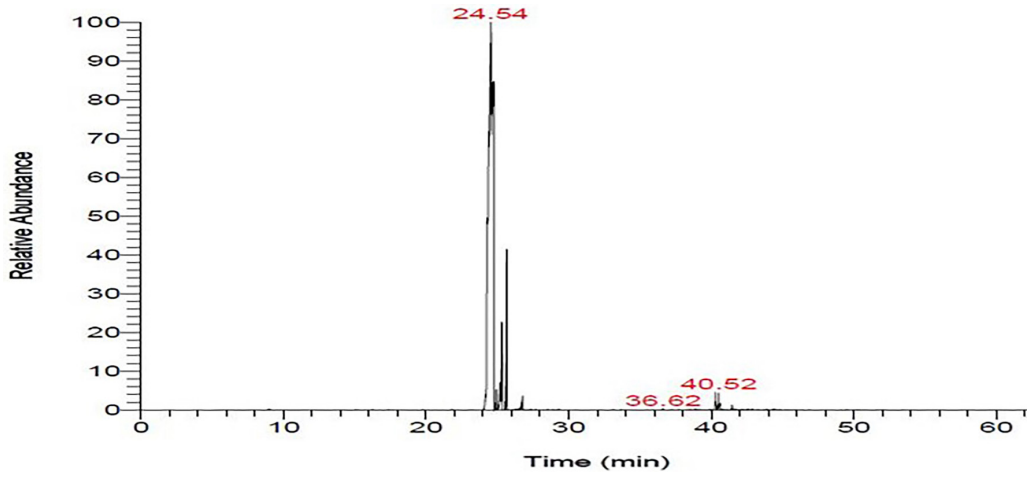

Figure 3. Chromatogram profile of GC-MS analysis of methanolic extract

Table 6. Identified compounds in methanolic extract via GC-MS analysis

No	t_{ret}, min	Identified compounds	% Peak area
1	24.11	Methyl α-D-glucofuranoside, 4TMS	3.37
2	24.12	α-D-Ribopyranoside, 3TMS	0.02
3	24.17	D-Erythro-Pentofuranose, 2-deoxy-1,3,5-tris-O-(trimethylsilyl)-	0.03
4	24.24	1-Methoxy-12-isopropyltricyclo [5.3.2.0(2,6)] dodeca-8,11-dien-3,10-dione isomer	1.25
5	24.25	4-isopropyl-3-phenylcyclohexanol	0.35
6	24.28	β-Galactofuranose-5TMS	0.7
7	24.3	Puqienine B	3.15
8	24.32	2-Acetyl-5,8-dimethoxy-1,4-naphthoquinone	1.82
9	24.4	Galactinol	0.12
10	24.43	Cinnamic acid	3.46
11	24.46	Pyruvaldehyde (propanal, 2-oxo-)	0.09
12	24.53	Shikimic Acid, Methyl Ester, 3TMS	16.9
13	24.62	1,2,3,4,5,6-Hexa-o-trimethelsilyl-myo-inositol	0.43
14	24.67	2,3-Bis(4-tert-butoxystyryl) furan	0.09
15	24.68	methyl octacosanoate	4.19
16	24.69	Methyl α-D-glucofuranoside, 4TMS derivative	0.85
17	24.7	2-(Acetamido) thia-5-benzylidene-3-butyl-4H-imidazolidin-4-one	0.22
18	24.74	5,6,7,8-tetrahydronaphthalene-2,3-dicarboxylic acid dimethyl ester,	5.83
19	24.75	Benzo[b]carbazole-7,10-dione	0.1
20	24.76	1,2,3,4-Tetrakis-O-(trimethylsilyl) pentopyranose, Per(trimethylsilyl)-D-fructose, Trimethylsilyl 1,2,3,5-tetrakis-O-(trimethylsilyl) hexofuranuronate	0.68
21	24.76	2-Phenylnaphthalene	4.48
22	25.18	Myo-inositol, 5-deoxy-1,2,3,4,6-pentakis-o-(trimethylsilyl)	2.94
23	25.28	Glucoheptonic acid, 7TMS	7.37
24	25.29	((4,5-Bis[(trimethylsilyl)oxy]tetrahydro-3-furanyl) oxy)(trimethyl)silane	3.9
25	25.3	1,4-dioxane, 2,5-dihydroxy-3,6-bis(hydroxymethyl)tetrakis-o-(trimethylsilyl)-	3.79
26	25.63	Quinic acid-pentaTMS	16.47
27	25.65	Cyclopentane-1,2,4-trione, 3,3,5,5-tetrakis(trim ethylsilyloxy)-	6.9
28	26.3	Glucopyranose-5TMS	0.21
29	26.7	D-mannonic acid	0.49
30	28.55	2,3,4,5,6-Pentakis-O-(trimethylsilyl)hexose	0.13
31	29.33	Hexadecanoic acid	2.68
32	36.62	2-(3-Methyl-but-1-ynyl)-cyclohexene-1-carboxaldehyde	1.84
33	40.52	1-kestose, 11TMS	2.18
34	40.6	Pregnan-11-one, 17-hydroxy-3,20-bis[(trimethylsilyl)oxy]-, (3α,5β,20S)-	0.27
35	40.61	13,17-Seco-5α-pregn-13(18)-en-20-one	0.45
36	41.45	6,7-Dihydroxycoumarin-β-D-glucopyranoside, penta-TMS	0.93

of compounds is essential in providing valuable insights into the plant's biological effects and its overall chemical composition. Among the identified compounds, methyl α-d-glucofuranoside stood out as a representative of the carbohydrate class, indicative of the plant metabolic pathways involving sugars. Puqienine B, characterized as an alkaloid compound, suggests the presence of secondary metabolites with potential pharmacological significance (Jiang et al., 2005). In addition to primary and secondary metabolites, phenolic compounds are abundant, exemplified by cinnamic acid, which is known for its antioxidant and antimicrobial properties (Geetika Vaisnav et al., 2023). The presence of shikimic acid, methyl ester further underscores the plant biosynthetic pathways, potentially contributing to the synthesis of aromatic amino acids and secondary metabolites. Shikimic acid is applied in the pharmaceutical sector and serves as a key ingredient in the production of antiviral medication against the H1N1 influenza virus (Priyanka Singh et al., 2020). Interestingly, the derivatization treatment introduced silyl groups to certain compounds, enhancing their detectability and stability in GC-MS analysis. Compounds such as glucoheptonic acid, 7TMS, and ((4,5-Bis((trimethylsilyl)oxy) tetrahydro-3-furanyl) oxy)(trimethyl)silane are indicative of this derivatization process. These silylated derivatives provide valuable insights into the chemical transformations facilitated by derivatization, offering a deeper understanding of the chemical profile of the plant. Quinic acid was detected at a significant concentration and its presence was further confirmed by HPLC-UV-MS analysis. Quinic acid (QA) is a natural organic compound found in various plants and fruits. It has been studied for its diverse biological activities, including antioxidant, antimicrobial, antidiabetic, anticancer, and wound-healing effects (Ercan and Doğru, 2022; Huang et al., 2023). Moreover, lipid-related compounds, such as methyl octacosanoate, indicate the presence of structural components vital for cellular membranes and signaling pathways. Aromatic compounds, such as 2-Phenylnaphthalene and 5,6,7,8-tetrahydronaphthalene-2,3-dicarboxylic acid dimethyl ester, contribute to the plant's aromatic profile and may play roles in defence mechanisms or interspecies interactions. The identification of cyclic compounds, including 1,4-dioxane, 2,5-dihydroxy-3,6-bis(hydroxymethyl)tetrakis-o-(trimethylsilyl)- and 3,3,5,5-Tetrakis((trimethylsilyl)oxy)-1,2,4-cyclopentanetrione, adds further complexity to the chemical composition of *Cistus ladanifer*. These cyclic structures may serve as precursors for bioactive compounds or participate in metabolic processes essential for plant growth and adaptation.

The present analysis of the methanolic extract of *C. ladanifer* offers valuable insights into its chemical composition, showcasing a wide variety of metabolites. The derivatization process enhances our understanding of a plant's chemical profile, exploring possibilities for further discovery of its ecological significance and potential applications in pharmacology and natural product research.

CONCLUSIONS

Our research presents novel insights into the phytochemical composition, antioxidant capabilities, and nutritional value of *Cistus ladanifer* leaves sourced from Tangier, Morocco, a region previously underexplored in this context. While the medicinal properties of *Cistus ladanifer* are recognized, our findings contribute new data on its rich bioactive constituents, specifically identifying a substantial presence of total phenolic compounds, flavonoids, and tannins. Crucially, we have discovered that both phenolic and non-phenolic compounds significantly contribute to the antioxidant properties, which are pivotal in combating oxidative stress-related diseases. This aspect has not been thoroughly quantified in previous studies, especially within this geographical variant of the plant.

Moreover, our detailed analysis of the micro and macronutrient profiles of these leaves provides fresh evidence of their nutritional importance, which could lead to practical applications in health supplements and functional foods. This study is the first to apply a comprehensive set of analytical techniques including infrared and various chromatographic methods to assess both the medicinal and nutritional potential of *Cistus ladanifer* leaves from this specific locale.

For future research, we propose employing solvent fractionation and comprehensive analysis of each extract to dissect further the individual components responsible for the medicinal and nutritional properties observed. This approach could enhance our understanding of the synergistic effects of these compounds and solidify the plant's role as a valuable natural resource for both pharmacological use and nutritional supplementation.

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