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Phytochemical composition and antioxidant efficacy of *Laurus nobilis* L.: impact of sex, plant stage, and extraction solvents

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Introduction: *Laurus nobilis* L., a botanical name for bay leaf, is a widely used spice and medicinal herb. The purpose of this research is to examine the effects of solvent extraction, plant stage, and sex on the phytochemical composition and antioxidant activity of crude extracts obtained from the roots, shoots, and leaves of male and female L. nobilis shrubs that are cultivated in Northern Morocco.

Method: Primary and secondary metabolites were found by phytochemical screening, and colorimetric techniques were used to measure the total phenolic, flavonoid, and flavanol concentrations. Gas Chromatography-Mass Spectroscopy (GC-FID) was used to examine the extract's phytoconstituents. Antioxidant activity was assessed using phosphomolybdate, DPPH, and ABTS tests.

Results: According to the findings, L. nobilis is a rich source of bioactive chemicals that may be used in therapeutic and medical settings. extracts of female tree shoots and leaves had the greatest total phenolic contents. The leaves of both male and female trees had the greatest total flavonoid content. Ten phenolic chemicals, including gallic acid, quercetin, and caffeic acid, were found in variable amounts in all of the extracts according to the GC-FID analysis. According to the ABTS technique, female tree shoots and shrub leaves had the greatest levels of antioxidant activity. Furthermore, the ADME study revealed that most compounds had high intestinal absorption, fulfilling the Lipinski criterion and indicating their potential for development as oral drugs.

Discussion: The phytochemical characteristics and antioxidant activities of *L. nobilis* L. may be significantly impacted by sex, plant stage, and solvent extraction, as shown by these studies, which also highlight the plant's potential for use in the pharmaceutical, food, and cosmetics sectors.

KEYWORDS

***Laurus nobilis* L., Northern Morocco, GC-FID analysis, antioxidant activity, molecular docking, ADME analysis**

1 Introduction

Currently, there is great attention on the discovery and development of natural antioxidants to substitute a synthetic one, in foods, cosmetics, and medicinal products, which now are being limited due to their potential carcinogenicity (Parcheta et al., 2021). Medicinal and aromatic plants (MAPs) are widely studied as promising sources of natural antioxidants, owing to their bioactive compounds and potential safety (Trevizan et al., 2020). Furthermore, MAPs are endowed with important antioxidant activity. In this context, *Laurus nobilis* L. is reported to be a rich source of different bioactive substances such as phenolic compounds (acid phenolic, flavonoids, lignins, and tannins; Park and Han, 2023; Dobroslavić et al., 2021), vitamins (tocopherols; Dias et al., 2014), carotenoids (Yahyaa et al., 2015; Cvitkovic et al., 2021) and minerals (Ca, K, Mg, Fe, Mn, Cu and Zn; Guenane et al., 2016). In addition, there a few studies investigated the potential effect of sex plant on chemical composition of essential oil and crude extract of *Laurus nobilis* L. The differentiation between male and female plants is of particular interest, as it may have practical implications in optimizing the selection and cultivation of *L. nobilis* for specific uses in the food, pharmaceutical, and cosmetic industries. Understanding these chemical differences could support targeted valorization strategies and enhance the standardization of bioactive ingredients. Moreover, the choice of extraction solvent plays a critical role in the recovery, stability, and antioxidant quality of phytochemicals. Polar and non-polar solvents can extract different classes of compounds, thus affecting the antioxidant profile of the resulting extracts. Optimizing solvent selection is therefore crucial for maximizing the functional potential of natural extracts (Yahyaa et al., 2015). *L. nobilis* is a Mediterranean native tree (Khodja et al., 2021) and the most important aromatic and medicinal plant in the Rif region (North of Morocco) known for its strong healing properties (El Faqer et al., 2023). Nowadays, *L. nobilis* essential oil and its constituents are paying attention because of their relatively safe status and exploitation for potential multi-functional uses (foods and nutraceuticals; Odeh et al., 2022). Then, it can be encapsulated with zein composite nanoparticles to improve the quality and increase the shelf-life of food by estimating microbiological stability including (color, and sensory parameters; Ceylan et al., 2024). Furthermore, our present knowledge, more studies regarding the encapsulation of essential oils and/or extracts from *L. nobilis* L. and their potential utilizations in food (Tometri et al., 2020; Chaumun et al., 2020; Lima Reis et al., 2020; Dobroslavić et al., 2023), cosmetic (Shamaila et al., 2022), and medicine (Chaumun et al., 2020; Ercin et al., 2022; Sivri et al., 2023; Singh et al., 2023). Morocco with its diverse geographic areas and climates has great potential in terms of MAPs. There are a few studies focused on Morocco that investigated the total phenolic and flavonoid contents of extracts but at the present, no work detailed the chemical characterization of bioactive compounds of female and male *L. nobilis* L. grow in Northern Morocco. On the other hand, many studies were performed on *L. nobilis* L. chemical composition and their biological properties (antibacterial, antifungal, antioxidants, and anti-inflammatory) from Moroccan plant leaves and flowers (Derwich et al., 2009; Mediouni Ben Jemâa et al., 2012; Cherrat et al., 2014; Dahak et al., 2014; Taarabt et al., 2017; Nafis et al., 2020; Razzouk et al., 2022; Boussaoudi et al., 2023; Mssillou et al., 2020). More research works are needed to characterize the organic extracts chemistry of *L. nobilis* L male and female and their associated biological activities. These may offer great potential for *L. nobilis* L. valorization in foods, cosmetics, and pharmaceutical industries. The goal of the current research, therefore, was to examine the effect of the stage, plant sex, and extraction solvent on the phytochemical analysis and the antioxidant activity of different vegetative organs (roots, shoots, and leaves) from female, male, and shrubs of *L. nobilis* grow wild in Northern Morocco.

2 Materials and methods

This research is significant as it explores the complex interaction between plant sex, vegetative stage, and solvent polarity on the phytochemical composition and antioxidant efficacy of *Laurus nobilis* L. a species of high ethnobotanical and pharmacological relevance in the Mediterranean region. One limitation in previous studies is the focus solely on leaf extracts, often neglecting other organs (roots and shoots), plant sex differentiation, or extraction variables. To address this, our study employs a comparative approach using both male and female trees, analyzing multiple organs and applying two solvents of different polarities (acetone and ethanol). This allows for a more holistic understanding of compound variability and improves the relevance of the findings for pharmaceutical, nutraceutical and food applications.

2.1 Chemicals

Acetone (C3H6O), M = 58.08 g/mol, Baker, USA; Ethanol, absolute (C_2H5OH), M = 46.07 g/mol, Netherlands; Quercetin, solid ≥95% (HPLC), M = 302.24 g/mol, Sigma-Aldrich, India; Rutin hydrate, ≥94% (HPLC), powder, M = 610.52 g/mol, Sigma-Aldrich, China; Sodium carbonate, anhydrous (Na_2CO3), M = 105.99 g/mol, Biochemica, Germany; Sodium acetate, anhydrous (C_2H3O_2Na), M = 82.03 g/mol, Sigma-Aldrich, Germany; Gallic acid (3,4,5-trihydroxybenzoic acid), M = 170.11 g/mol, Sigma-Aldrich, USA.

2.2 Plant material harvest

The leaves and shoots of *L. nobilis* were harvested from male and female trees, as well as from different parts of shrubs, including leaves, shoots, and roots. The harvest was performed in November 2016 in the Tangier-Tetouan region, located in the northeast of Morocco (Figure 1). The optimal harvest point was determined based on the growth stage of the plants, which were selected at the time of peak physiological activity. Environmental conditions, such as temperature and humidity, were recorded during the harvesting period to ensure consistency across samples. Furthermore, the sampling process was replicated across multiple locations and harvests to ensure reliable data. The samples were

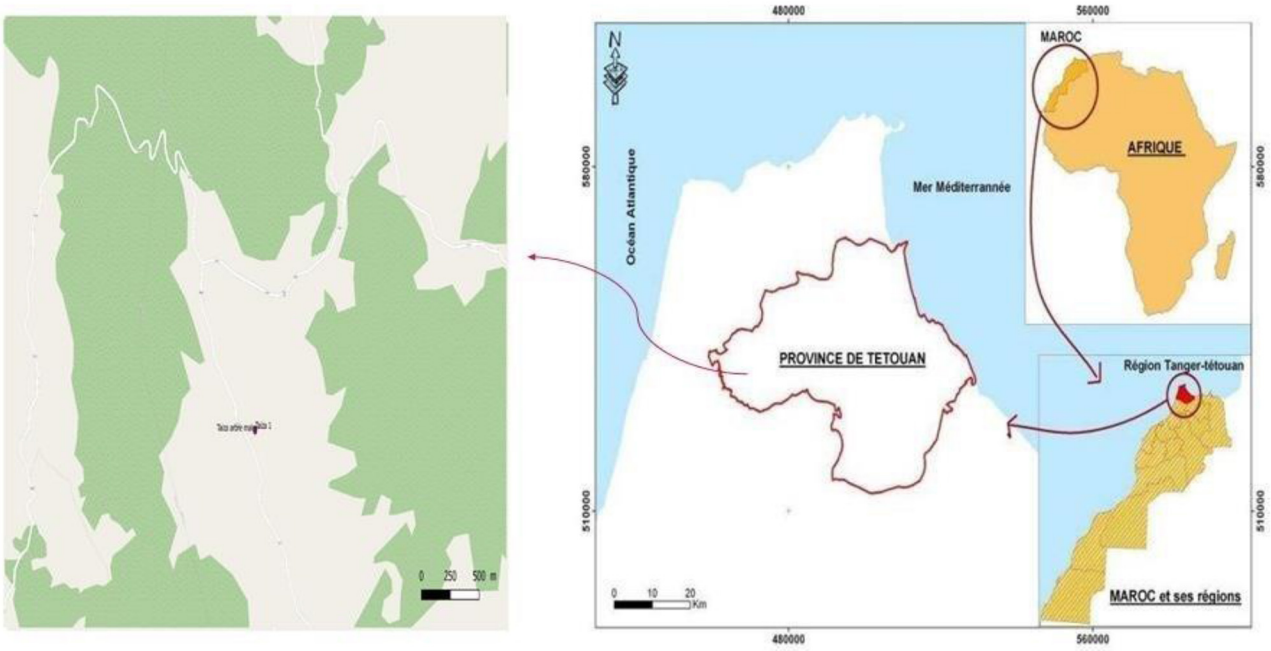


FIGURE 1
Geographic localization of the studied site in Moulay Abdeslam commune of Tetouan region, Morocco.

TABLE 1 Geographic coordinates (latitude and longitude) and altitudes of laurel trees in Tazia regions from Moulay Abdeslam commune of Tangier-Tetouan.

Locality	Latitude	Longitude	Altitude (m)	Plant material
Tazia, Tetouan, Morocco	N 35°21.652′	W005°33.739′	731 m	Female tree

carefully cleaned to remove any foreign materials. The origin, locality, latitudes, longitudes, and altitudes of the collection sites are detailed in Table 1. The plant material was authenticated and confirmed by Prof. Ahmed Lamarti, a specialist in plant physiology, at the Faculty of Sciences, Abdelmalek Essaadi University Tetouan, Morocco.

2.3 Preparation of *L. nobilis L. parts* powder

Different fresh vegetative organs of *L. nobilis* L. (leaves, shoots, and roots) have been dried for 15 days at 50 °C until stabilization of dry weight. Dried plant materials have been chopped and crushed using a knife mill MICROTRON MB 500 (KINEMATICA) at 15,000 rpm. The powder obtained has been sifted on a stainless-steel sieve Fisher Scientific LABOSI to obtain particles of average diameter <200 µm. The latter powder was used for the extraction procedure.

2.4 Reagents and plant extract preparation

2.4.1 Maceration extraction

Gram of the powder were extracted using two types of solvents with increasing polarity: acetone (C3H6O)/H_2O (70/30) and ethanol (C_2H5OH)/H_2O (70/30), using a magnetic stirrer for 1 h at room temperature at a speed of 180 rotations/min, followed by centrifugation for 15 min at 5,000 rpm. The extraction procedure was repeated twice, and the macerates were combined. The obtained extracts were then stored in a refrigerator at 4 °C until the analysis was performed.

2.4.2 Reagent preparation for phytochemical procedures

2.4.2.1 Mayer's reagent

It is a chemical reagent that is used for the detection of alkaloids. Solution (a) 1.36 g of mercuric chloride is dissolved in 60 ml of distilled water. (b) 5 g of potassium iodide is dissolved in 20 ml of distilled water. Solution (a) and (b) were mixed and the volume was adjusted to 100 ml with distilled water (Kundu et al., 2022).

2.4.2.2 Wagner's reagent

It is a universal reagent applied to alkaloid detection. 1.27 g of iodine and 2 g of potassium iodide were dissolved in 5 ml of water and the volume was adjusted to 100 ml with distilled water (John Umaru, 2018).

2.4.2.3 Starch reagent

This reactive reagent was prepared to screen starch in the samples. 1.2 g of I_2 is dissolved in 50 ml of distilled water which

TABLE 2 Phytochemical analysis procedures for *L. nobilis* L. extracts.

Phytoconstituents	Test procedure	Observation	References
Terpenoid	500 µL extract+ 200 µL chloroform + 300 µL con.H_2SO4	Reddish brown coloration	(Gul et al., 2017)
Saponins	2 mL extract + Olive oil (two drops)	Emulsion forms	(Gul et al., 2017)
Phlobatannins	1 mL extract + 1 mL HCl (1%) + heat	Red precipitate	(Umaru et al., 2018)
Resins	1 mL extract + 1 ml copper solution	Green precipitate	(Umaru et al., 2018)
Emodins	2 mL extract + 2 ml NH_4OH + 2 ml Benzene	Red coloration	(Rizk, 1986)
Tannins	2 mL extract + 2 ml KOH (10%)	Dirty white precipitate	(Banso, 2009; Ribéreau-Gayon, 1968
Flavonoids Alkaline reagent test	2 mL extract + 2 ml NaOH (10%)	Yellow coloration	(Gul et al., 2017)
Quinones	2 mL extract + 2 ml NaOH (10%)	Yellow, red, or purple coloration	(Harborne, n.d.)
Anthraquinones	2 mL extract + 2 ml KOH (10%)	Red coloration	(Gul et al., 2017)
Alkaloids	a) 2 ml extract + a few drops of Wagner's reagent b) 2 ml extract + a few drops of Mayer's reagent	Creamy white precipitate Brown precipitate	(Haddouchi et al., 2018)
Glycosides	2 ml extract + 2 ml $CHCl_3$ + 2 ml CH_3COOH+heat	Violet to Blue to Green coloration	(Samejo et al., 2013)
Starch	5 ml extract +10 ml NaOH +heat	Purplish-blue coloration	(Gul et al., 2017)
Proteins	2 ml extract+ NaOH (5%) + 1% Cu $(SO_4)_2$	Violet coloration	(Samejo et al., 2013)

contains 2.5 g of KI, then the solution is heated for 5 min and the volume was diluted from 250 to 500 ml (Joshi et al., 2013).

2.5 Phytochemical screening of extract

The phytochemical analysis of *L. nobilis* L. was performed for the hydro-acetonic and hydroethanolic extracts to evaluate the presence of secondary metabolites like steroids, saponins, phytosterols, phenolic compounds, tannins, flavonoids, etc. Table 2 resumes the phytochemical analysis procedures that we follow for detecting secondary compounds (metabolites) in the plant extracts with minor modifications (Joshi et al., 2013; Ribéreau-Gayon, 1986; Rizk, 1986; Harborne, 1998; Bruneton, 1999; Banso, 2009; Abdullahi, 2013; Samejo et al., 2013; John et al., 2015; Shrestha et al., 2015; Haddouchi et al., 2018; Gul et al., 2017). The qualitative data analysis is expressed as (+) for the presence and (–) for the absence of phytocompounds.

2.6 Total phenolic content quantification

The total polyphenol contents (TPC) in *L. nobilis* L. extracts were estimated by the Folin-Ciocalteu (FC) method (Singleton and Rossi, 1965; Muñiz-Márquez et al., 2018; Thu et al., 1999; John et al., 2015), with some modifications. The laurel crude extract (CE) was diluted 1:10 in distilled water and 200 µl of each extract was mixed with 1,000 µl Folin-ciocalteu reagent (FCR) 1 N diluted at 1:10 and then the solution obtained was allowed to stand at room temperature for 5 min. After adding 800 µL of Na_2CO_3 (75 g/L in distilled water), the resulting mixture was incubated in darkness for 2 h at room temperature. Finally, the absorbance was read at 765 nm by spectrophotometer UV/Vis (Lambda 2s Perkin Elmer). The analysis was reported using triplicate ($n = 3$) and the results are expressed as mg of gallic acid equivalent per gram of extract (mg GAE/g).

2.7 Total flavonoid content quantification

The total flavonoid content (TFC) of each laurel crude extract was estimated according to the aluminum chloride assay (Singleton and Rossi, 1965; Muñiz-Márquez et al., 2018; Thu et al., 1999), with slight modifications. The laurel crude extract (CE) was diluted 1:10 in distilled water and 200 µl of each sample was mixed with 1,000 µl Fol; Mbaebie et al., with slight modifications (Mbaebie et al., 2012). Briefly, each sample solution was diluted at 1:10 and 1 ml was mixed with 1 ml of aluminum chloride (2%) in a test tube. After 1 h of incubation at obscurity and room temperature, the absorbance level of each sample was measured vs. the prepared reagent blank by spectrophotometer UV/Vis at 430 nm. The TFC of each extract was expressed as milligram quercetin equivalent per gram (mg QE/g), which was calculated using the formula, $y = 0.024x - 0.119$, where, y is the absorbance at 430 nm and x is the amount of quercetin equivalent (µg/ml). All the test was achieved in triplicate ($n = 3$).

2.8 Total flavonol content quantification

The flavonol content was quantified according to the method of Awah et al. (2012) with slight modifications. Briefly, 2 mL

of properly diluted extract solution (1:3 in distilled water) was mixed with 2 mL of aluminum trichloride (AlCl3, 2%) and 3 mL of sodium acetate (C_2H3NaO_2, 50 g/L), then kept in a dark room at room temperature for 2 h and 30 min. The absorbance of the sample was measured using a UV/Vis spectrophotometer at 440 nm. The total flavonol content was expressed as mg of rutin equivalents per gram of extract (mg RE/g), based on the mean of triplicate analyses.

2.9 Gas chromatography-mass spectrometry analysis of extracts

The quantitative chemical analysis of ethanolic (C_2H5OH) and acetone (C3H6O) extracts from *L. nobilis* L. was achieved using gas chromatography coupled with a mass spectrometer from, University Center- Murcia. The column used is an agile HP-5 apolar capillary column, 30 m long, 0.320 mm in diameter, and 0.25 µm thick. Column temperature is programmed from 70 to 300 °C at 5 to 6 °C/min. The injector temperature is set at 260 °C and the detector (ionization source) at 260 °C. The carrier gas flow rate (helium) is set at 1.4 ml/min. The sample volume injected is 1 µL of crude extract. The constituents of each extract were identified by references (25 mg/ml).

2.10 *In vitro*, antioxidant activity assay

2.10.1 DPPH free radical scavenging activity assay

The antioxidant activity of plant extracts was determined using the method described by Brand-Williams et al. (1995), with slight modification. DPPH radical scavenging activity is a very cheap, suitable, and robust approach for the measurement of the antioxidant capacities of natural products (Teixeira et al., 2013). This technique is based on the capacity to scavenge synthetic free radical 2.2-diphenyl-1- picryhydrazyl (DPPH, C18H19N5O6) to react with hydrogen donors. The color changes from purple to yellow after reduction with an antioxidant (AH) or radical (R). A solution of DPPH was freshly prepared by dissolving 2 mg DPPH in 100 ml methanol (CH3OH) absolute, was vortexed thoroughly, and socked in a refrigerator until further analysis. DPPH stock solution was diluted with methanol to an absorbance of 0.90 ± 0.09 at 517 nm.

The procedure consisted of adding an aliquot of the extract (2 µl) to a cuvette containing 2 ml DPPH solution. The reaction progress and the absorbance were read at 517 nm against a blank spectrophotometer UV/Vis in a cycle for 10 min. All experiment was carried out at least in triplicate ($n = 3$) for each sample. The inhibition percentage of radical of the test samples was expressed as the ability to scavenge the DPPH radical and calculated using the following equation:

$$\text{Inhibition } (\%) = \frac{(At = 0 - At)}{At = 0} \times 100$$

Where $A_{t=0}$ = absorbance of control (t=0) and A_t = absorbance of a tested sample in 5 or 10 min.

2.10.2 ABTS scavenging activity assay

ABTS radical scavenging activity of the various crude extracts (CE) has been estimated using the method reported by Arnao et al. (1996), with some modifications. This technique is based on the neutralization of ABTS+ radical, a green chromophore by antioxidant of herbal extracts in a dose- response curve. The reaction of ABTS+ radical involves the electron-donating ability of the synthetic chromophore 2, 2–azino-bis (3 ethylbenzothiazoline-6- sulfonic acid; ABTS, $C17H19N3O6S_2$) and results in the decolorization of the radical. The ABTS+ stock solution was prepared by reacting ABTS diammonium salt ($C17H19N3O6S_2 \cdot 2NH4$; 1 mL, 50 mM) with hydrogen peroxidase (100 µl, 10 mM) and hydrogen peroxide in 25 ml of phosphate buffer solution (PBS; PH = 6.5). Then, the obtained mixture was vortexed thoroughly at room temperature until the reaction was accomplished. After that time, the ABTS. $^+$ The prepared solution was diluted until an absorbance of 1 ± 0.1 at 730 nm with a phosphate buffer solution. This fresh solution must be made at the time of use due to its low stability and rejected after usage. An aliquot of each sample (2 µl) was added to a cuvette containing 2 ml of ABTS. + solution freshly prepared. The absorbance of the reaction mixture was measured on a UV/Vis spectrophotometer at 730 nm against a blank for 6 min. The same procedure was done for the blank as a control. All solutions were used on the day of preparation, and each assay was achieved in triplicate ($n = 3$) at room temperature. The inhibition percentage of ABTS. + discoloration was calculated according to the following equation:

$$\text{Inhibition } (\%) = \frac{(At = 0At)}{At = 0} \times 100$$

2.10.3 Total antioxidant activity evaluation

The total antioxidant activity (TAA) of *L. nobilis* L. extracts was determined according to the method of Prieto et al. (1999), the procedure described by Ahmed et al., with slight modification (Ouchikh et al., 2011; Do et al., 2014; Ahmed et al., 2015; Asghari et al., 2019). This assay is based on the reduction of Mo (VI) to Mo (V) by the sample extract and the subsequent formation of a green phosphate/Mo (V) complex under acidic pH conditions, which can be evaluated spectrophotometrically at 695 nm. In brief, 0.1 ml of each plant extract was mixed with 1 ml of phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in a capped plastic tube. The test tube was incubated in a water bath at 95 °C for 90 min. Then, the mixture was cooled at room temperature, the absorbance was read at 695 nm against blank by spectrophotometer UV/Vis. Blank for each solvent was prepared using the same procedure but replacing the sample extract with an equal volume of solvent. The quantitative analysis was achieved three times for each sample. The finding of the total antioxidant activity (TAA) was presented as µg of acid ascorbic equivalents (AAE) per ml.

2.11 Molecular docking

The *in silico* molecular docking of *Laurus nobilis* L. extracts was performed using standard inhibitors Zileuton (CID: 60490), Xanthene (CID: 7107), and Celecoxib (CID: 2662), which were retrieved in 3D SDF format from the PubChem database (Available online: https://pubchem.ncbi.nlm.nih.gov/). Ligands underwent geometry optimization to ensure a stable conformation prior to docking. Using the PyMoL Molecular Graphics System (version 2.5.3), the optimized ligands were first converted to PDB format and then further processed to PDBQT format through AutoDock Tools software (ADT; version 1.5.7, The Scripps Research Institute).

The crystal structures of the target proteins Glutathione reductase (PDB: 3DK9), Lipoxygenase (PDB: 1YGE), and Cyclooxygenase-2 (PDB: 6Y3C) were retrieved from the Protein Data Bank (PDB; https://www.rcsb.org). These structures were prepared by removing crystallographic water molecules and native ligands, and by adding polar hydrogen atoms and Kollman charges using ADT. This preparation step was essential to avoid steric clashes and to ensure correct charge distributions at the binding sites. The selected target proteins are closely linked to oxidative stress and inflammation, with Glutathione reductase involved in redox balance and Lipoxygenase and Cyclooxygenase-2 in pro-inflammatory mediator synthesis, making them relevant targets for antioxidant and anti-inflammatory drug discovery (Karaman, 2020). A grid box with dimensions of 40 × 40 × 40 points and a spacing of 0.375 Å was defined and centered on the x, y, and z coordinates to encompass the active sites and potential peripheral binding regions of each protein. The prepared macromolecules were saved in PDBQT format to facilitate the subsequent molecular docking simulations. Visualization of the molecular interactions and analysis of binding affinities, especially those equal to or exceeding that of the standard inhibitors, were carried out using Discovery Studio Visualizer (Biovia et al., 2016), which also enabled the generation of interaction graphics (Farihi et al., 2023).

2.12 ADME studies

Understanding the pharmacokinetic characteristics, including absorption, distribution, metabolism, and excretion (ADME), is fundamental for comprehending how a substance operates within the body (Srivastava et al., 2020). These stages map the path of a substance from intake to elimination. Computational tools have become indispensable for predicting the ADME traits of molecules. They assist in evaluating a molecule's capacity to penetrate cellular barriers, interact with key transporters and enzymes involved in absorption and excretion, and determine its metabolic stability (Daina et al., 2017). For our molecule evaluation, we have chosen to use the SwissADME platform (Farihi et al., 2023). This platform provides an in-depth analysis of the physicochemical properties of phenolic compounds found in extracts of *L. nobilis* L., assesses their potential as therapeutic agents, and evaluates their pharmacokinetic properties, offering a comprehensive understanding of their ADME profile.

2.13 Statistical analysis

All measurements were run three times ($n = 3$) and the values were averaged and given along with standard error (±SEM). Analyses were achieved with IBM SPSS Statistics 20; averages were compared by Duncan test and values beyond $p \leq 0.05$ were considered to be significant.

3 Results and discussion

3.1 Phytoconstituents classes of *L. nobilis L.*

Currently, plant extracts are increasingly marked as natural products with a beneficial effect on the human body. The present research aimed to characterize the phytochemical composition of primary and secondary compounds (metabolites) of different vegetative organs from the *L. nobilis* L. tree (leaves, shoots, and roots) and estimate their antioxidant activity. The results of the phytochemical screening of hydroacetonic and hydroethanolic extract from males and females *L. nobilis* L. (leaves and shoots) and shrubs (leaves, shoots, and roots) are shown in the following Table 3.

The phytochemical screening and qualitative analysis of hydroethanolic and hydroacetonic extracts revealed the presence of 13 known compounds were identified as terpenoids, saponins, phlobatannins, resins, proteins, tannins, quinones, anthraquinones, alkaloids, and glycosides except emodins and starch were observed in the shoots of male and female tree, well starch was found in the leaves and roots of shrubs. Both extracts showed all phytoconstituents, but comparatively, the acetone extract of *L. nobilis* was rich in phytochemicals using the percentage of presence. Generally, all the classes of phytochemicals compounds were observed with acetone in male trees, females, and Shrubs. Thus, bioactive compounds were quite abundant, possibly in the form of flavonoids and tannins which were evidenced when the sample with NaOH and KOH solutions and the formation of a yellow and dirty white precipitate were observed, respectively (Ajali et al., 2023).

In this study, the results of phytochemical screening indicate that these plants contain bioactive metabolites that may be responsible for their medicinal properties. However, the phytochemical analysis from two sexes (male and female) of *L. nobilis* L. revealed similar phytoconstituents, with large qualitative difference between all principal components. The qualitative phytochemical screening of all crud extracts from various organs of *L. nobilis* L indicated the presence of 13 phytoconstituents such as terpenoids, saponins, phlobatannins, resins, proteins, tannins, quinones, anthraquinones, alkaloids, and glycosides. Previous phytochemical investigations have declared that the *L. nobilis* L. extracts are rich source in various active metabolites communally known as active constituents which have been reported to possess various pharmacological properties (El-Saber Batiha et al., 2020; Silva et al., 2020; Kesiya and Vinoth, 2018; Assunção Barroso et al., 2018). For instance, El-Saber et al. and Abu-Dahab et al. proved that the acetonic and ethanolic extracts of *L. nobilis* L. left in various regions (Egypt and Jordan) contain terpenoids (El-Saber Batiha et al., 2020) and (Abu-Dahab et al., 2014). In addition,

TABLE 3 Phytochemical analysis of diverse parts of *L. nobilis L.*

Phytochemical tests	Extraction solvents	Male tree		Female tree		Shurbs		
		Leaves	*Shoots*	*Leaves*	*Shoots*	*Leaves*	*Shoots*	*Shurbs*
Terpenoids	70% ethanol	+++	+++	+++	+++	+++	+++	+++
	70% acetone	+++	+++	+++	+++	+++	+++	+++
Saponins	70% ethanol	+++	+++	+++	+++	+++	+++	+++
	70% acetone	+++	+++	+++	+++	+++	+++	+++
Phlobatannins	70% ethanol	+++	+++	+++	+++	+++	+++	+++
	70% acetone	+++	+++	+++	+++	+++	+++	+++
Resins	70% ethanol	+++	+++	+++	+++	+++	+++	+++
	70% acetone	+++	+++	+++	+++	+++	+++	+++
Emodins	70% ethanol	-	+	-	+	-	-	-
	70% acetone	+	++	+++	+	+++	+++	+++
Starch	70% ethanol	-	-	-	-	+	-	+++
	70% acetone	+	+	+	+	++	++	++
Proteins	70% ethanol	+	+	+++	+++	++	++	+
	70% acetone	+	+++	+++	++	+	++	+++
Tannins	70% ethanol	+++	+++	++	+++	+++	+++	+++
	70% acetone	++	++	++	++	+++	++	++
Quinones	70% ethanol	+	+	+++	+++	++	++	+
	70% acetone	+++	+++	+++	+++	+++	+++	+++
Anthraquinones	70% ethanol	+	++	++	++	++	++	++
	70% acetone	++	+++	+++	+++	+++	+++	+++
Alkaloids	70% ethanol	++*a*	+++	+++	+++	+++	+++	+++
		+++*b*	++	+++	++	+++	++	+
	70% acetone	+++	+++	+++	+++	+++	+++	+++
Flavonoids	70% ethanol	+++	+++	+++	+++	+++	+++	+++
	70% acetone	+++	+++	+++	+++	+++	+++	+++
Glycosides	70% ethanol	+++	++	+++	+++	+++	+++	+++
	70% acetone	+++	++	+++	+++	+++	+++	+++

Strongly Present (+ + +), Weakly Present (+), Moderately present (++), Absent (-).
[a]Wagner's test; [b]Mayer's test.

Silva et al. revealed the existence of quinones in distilled boiled water extract of *L. nobilis* leaves from the Brazilian region (Silva et al., 2020). Furthermore, the primary screening emphasized that all crude extracts of *L. nobilis* L. confirmed the presence of tannins, saponins, anthraquinones, resins, phlobatannins, and glycosides (Ajali et al., 2023; El-Saber Batiha et al., 2020; Silva et al., 2020; Kesiya and Vinoth, 2018; Assunção Barroso et al., 2018; Pugazhenthi and Suganthi, 2013; Qnais et al., 2012; Kesiya et al., 2015). These literature findings are in concordance with our work which scrutinized the presence of these various chemical compounds in diverse parts of this plant. However, no data was published to characterize these various phytochemical compounds and also to investigated the impact of other parameters such as the sex and plant stage in various vegetative organs of *Laurus nobilis* L.

3.2 Total polyphenol contents

For the hydroacetonic extracts, TPC ranged from 50.270 to 70.703 mg GAE/g dw. The powder from the female tree was the richest in polyphenol followed by the leaves from shrubs (60.183 mg GAE/g dw) and leaves from the female tree. For hydroethanolic extracts, TPC ranged from 40.597 to 70.443 mg GAE/g. Powder from shoots of female trees was also the richest in polyphenols followed by leaves from shrubs (60.170 GAE/g dw) and the leaves from female trees (Figure 2). The total phenol contents by acetone solvent from *L. nobilis* L., leaves found in this study were approximately similar to those described by El-Saber Batiha et al. (2020) and Kratchanova et al. (2010). However, numerous studies described in the literature that the total phenol contents by ethanol solvent from different extraction methods ranged from 27.70 to

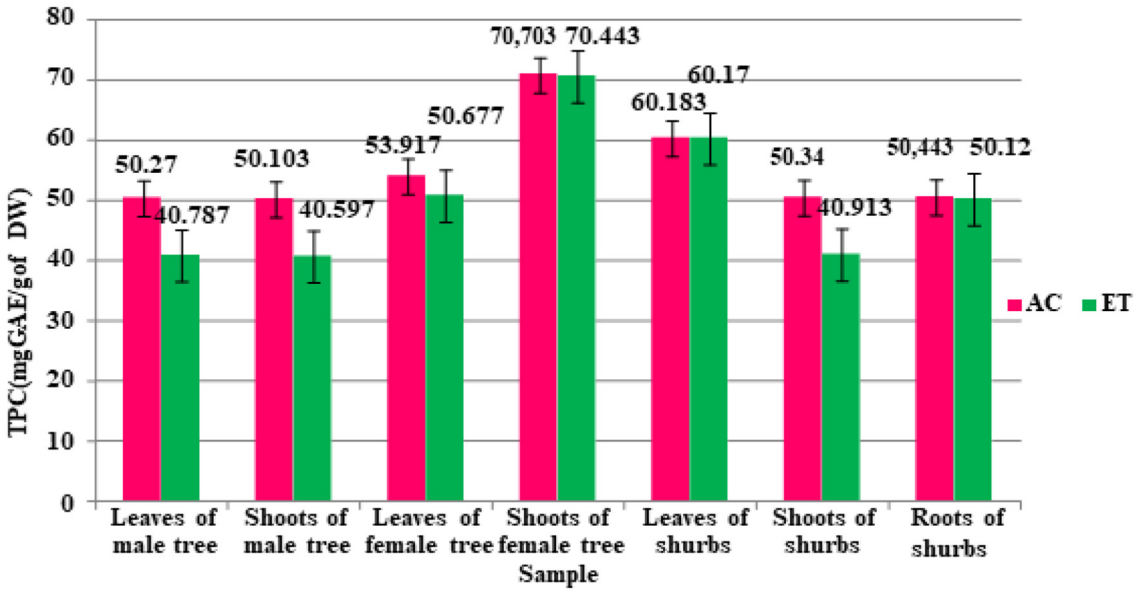


FIGURE 2
Total phenol contents of acetone and ethanolic extracts of various organs of *L. nobilis* L.

195.4 mg GAE of DW (Park and Han, 2023; Dobroslavić et al., 2021; Khodja et al., 2020; Fernández et al., 2019; Taroq et al., 2018; Siddhartha et al., 2017; Ereifej et al., 2016; Ying et al., 2015; Vinha et al., 2015; Vallverdú-Queralt et al., 2014; Ramos et al., 2012). These data were cited within the range of our study with different concentration. Indeed, our results showed that the acetone extracts with different methods of various parts of *L. nobilis* L. were rich in total phenol content (50.270 to 70.703 mg GAE/g DW; Ouchikh et al., 2011; El-Saber Batiha et al., 2020; Kratchanova et al., 2010; Ereifej et al., 2016). Notably, these findings mentioned are in agreement with our research, which estimated the quantity of phenolic compounds of *L. nobilis* L., leaves extracted with conventional and modern extraction methods such as maceration, agitation, and ultrasound. But to our knowledge, no study detailed at the moment investigated the total phenol contents from different parts and feet of *L. nobilis* L., has been published. The difference between our recent and previous studies could be attributed to variances in the solvents applied, the vegetative plant organs, but also the powder particle size and extraction methods that were analyzed. In addition, the divergences in the amount of total polyphenol contents of different parts of this aromatic species can be related to environmental conditions and geographical regions.

3.3 Total flavonoids content

Concerning hydroacetonic extracts, TFC ranged from 6.40 to 18.60 mg QE/g. Powder from the leaves of female and male trees revealed the highest TFC, followed by powder from the leaves of shrubs (12.867 mg QE/g) and shoots from female trees (10.30 mg QE/g). The lowest TFC was observed in the case of powder from shoots of male trees (Figure 3). For hydroethanolic extracts, TFC ranged from 5.767 to 19.267 mg QE/g. The powder obtained from the leaves of the female tree demonstrated the richest in TFC, followed by powder from the leaves of the male tree (17.667 mg QE/g). The poorest amount of TFC was from the roots of shrubs with a 5.767 mg QE/g (Figure 3). In previous work, the total flavonoid contents presented 11.12 mg CE/g of DW in hydroacetonic extracts of roots of *L. nobilis*, 10.46 mg CE/g of DW in hydroacetonic extract of branches, and 8.2 mg CE/g of DW in hydroacetonic extracts of leaves (Ouchikh et al., 2011), showing approximately a similar amount to that observed in this current research. Vinha et al. (2015) declared that the total flavonoid content was estimated at 20.88 mg QE/g of DW in the ethanolic extract obtained from the conventional maceration method of leaves collected in the Portugal region (Vinha et al., 2015). However, other studies have demonstrated that the hydroethanolic mg QE/g mg of extracts extracted by using conventional and advanced extraction methods from different regions contained the highest total phenol concentration varied from 153.89 mg RE/g of DW and 192.82 mg to 398.71 mg CE/g of DW (Tometri et al., 2020; Taroq et al., 2018). Furthermore, Kivrak et al. (2017), found a lower amount of total flavonoid contents (8.60 mg QE/g of DW) in hydroethanolic extracts of *L. nobilis* L., from the Turkey region. The variations in total flavonoid contents of the presented work can be probably related to intrinsic factors (such as genetic factors) and extrinsic factors including geographical factors, climatic factors, and the maturity state of the party of the plant. Furthermore, the total flavonoid contents have been impacted by other factors such as extraction method, solvent used, and methodology of research.

3.4 Total flavonols content

For hydroacetonic extracts, total flavonol content ranged from 3.06 to 11.670 mg QE/g. The highest total flavonol content

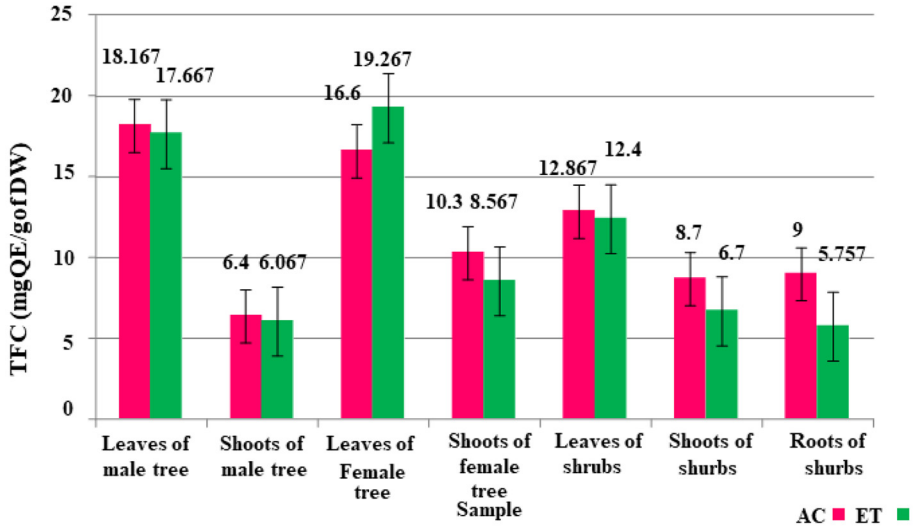


FIGURE 3
Total flavonoid content (TFC) in acetone and ethanolic extracts of different organs of *L. nobilis* L.

corresponded to the powder of leaves from the female tree (11.670), followed by the powders from the leaves of shrubs and the male tree (10.190 and 9.90). The lowest total flavonol content corresponded to the powders of shoots from shrub and male trees, respectively (3.060 and 3.190; Figure 4). For hydroethanolic extracts, total flavonol content ranged from 2.02 to 11.840 mg QE/g. The richest in total flavonol content was obtained from the leaves of the female tree with 11.840 mg QE/g followed by the leaves of the male tree (10.60 mg QE/g). The poorest amount in total flavonol contents was from roots of shrubs and shoots of male trees with 2.020 and 2.720 mg QE/g, respectively (Figure 4). Our results of total flavonol contents demonstrated that the ethanolic and acetone extracts presented similar concentrations of these bioactive compounds depending on the vegetative organs that were analyzed. However, there is little data available in the literature regarding the total flavonol content determination for *L. nobilis* L. (Guenane et al., 2016; Maleš et al., 2022). Guenane et al., analyzed ethanolic extract of Algerian *L. nobilis* leaves obtained by maceration extraction and found that TFC was 12.11 mg CE/g of DW, which was by our results for both solvents (acetone and ethanol; Guenane et al., 2016). Males et al., also indicated that the aqueous extract of *L. nobilis* L. leaves from the Croatia region contains the highest amount of TFC (1.06 g/L; Maleš et al., 2022). However, this finding cited in the literature was not sufficient, so more detailed investigations including examination of different vegetative organs of *L. nobilis* L. (roots, leaves, shoots, and fruits), extracts should be performed.

3.5 Phytochemical constituents of *L. nobilis L.* organs

GC chromatograms of hydroacetonic and hydroethanolic extracts from diverse vegetative organs of *L. nobilis* L. revealed the presence of non-volatile constituents such as phenolic compounds were isolated, purified, detected, and analyzed by GC-FID, and the findings data are demonstrated in Figures 5–13 and Tables 4, 5. A comparison between the retention times in the chromatograms of the samples and the standards allowed us to assume that the *L.nobilis* L. extracts studied contained 10 phytochemicals, Caffeic acid, hydroxybenzoic acid, rutin, synergic acid, vanillic acid, quercetin, protocatechic acid, gallic acid, and cinnamic acid. In general, the hydroacetonic extracts from various vegetative organs of *L. nobilis* L. were dominated by caffeic acid, rutin, and hydroxybenzoic acid (Table 4). Caffeic acid was observed as the main compound identified in these vegetative organs with concentrations ranging from 9.132 to 58.710%. Moreover, rutin and hydroxybenzoic acid were detected also as the main biomolecules in all organs of our plant with concentration variables (1.975–5.780%) and (1.975–5.780%) while the 7 other compounds were identified in these organs with lower concentrations. Otherwise, ferulic acid was the only molecule not detected in all hydroacetonic extracts of *L. nobilis* L. Regarding the ethanolic extracts (Table 5), 4 molecules: quercetin, syringic acid, hydroxybenzoic acid, and vanillic acid were reported in different studied organs with quercetin (2.971–60.863%) and syringic acid (1.572–10.582%) as the main compound, and whereas the root analysis showed no biomolecules examined. For other biomolecules, many chemical compounds were found including rutin, caffeic acid, protocatechuic acid, gallic acid, and cinnamic acid at lower concentrations in *L. nobilis* L., extracts. However, the GC analysis showed the absence of ferulic acid in all vegetative organs. The present study revealed that GC-FID analysis of all crude extracts obtained by hydroacetonic and hydroethanolic solvents of *L. nobilis* L. contains biomolecules such as caffeic acid, hydroxybenzoic acid, rutin, synergic acid, vanillic acid, quercetin, protocatechic acid, gallic acid, and cinnamic acid with different concentrations. Comparing these results with previous studies (Dobroslavić et al., 2021; Stefanova et al., 2020; Petkova et al., 2019; Medeiros-Fonseca et al., 2018; Bahmanzadegan et al., 2015; Shan et al., 2005) here the authors

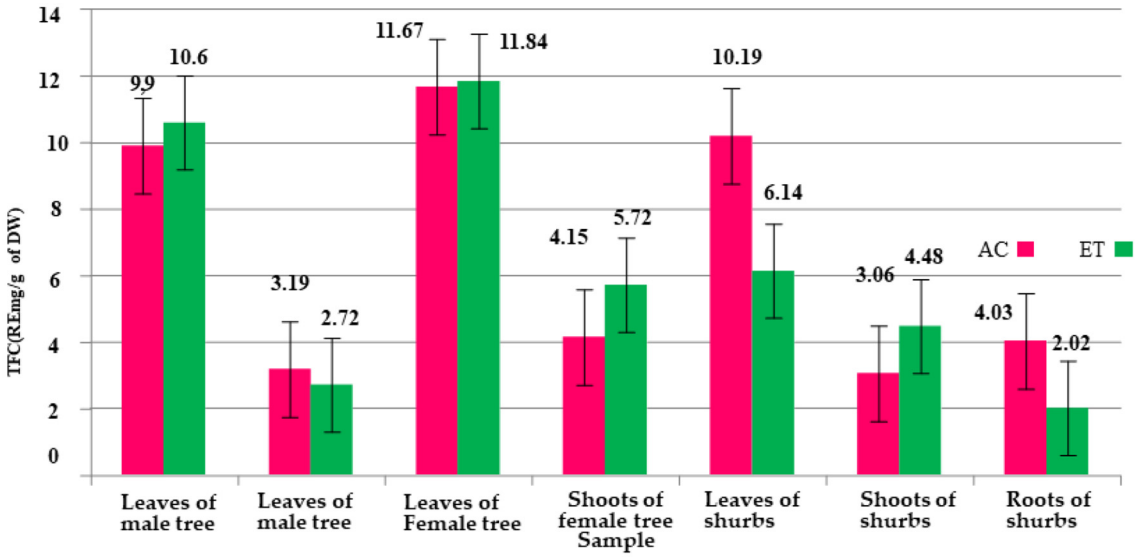


FIGURE 4
Total Flavonols content in hydroacetonic and hydroethanolic extracts of different organs of *L. nobilis*.

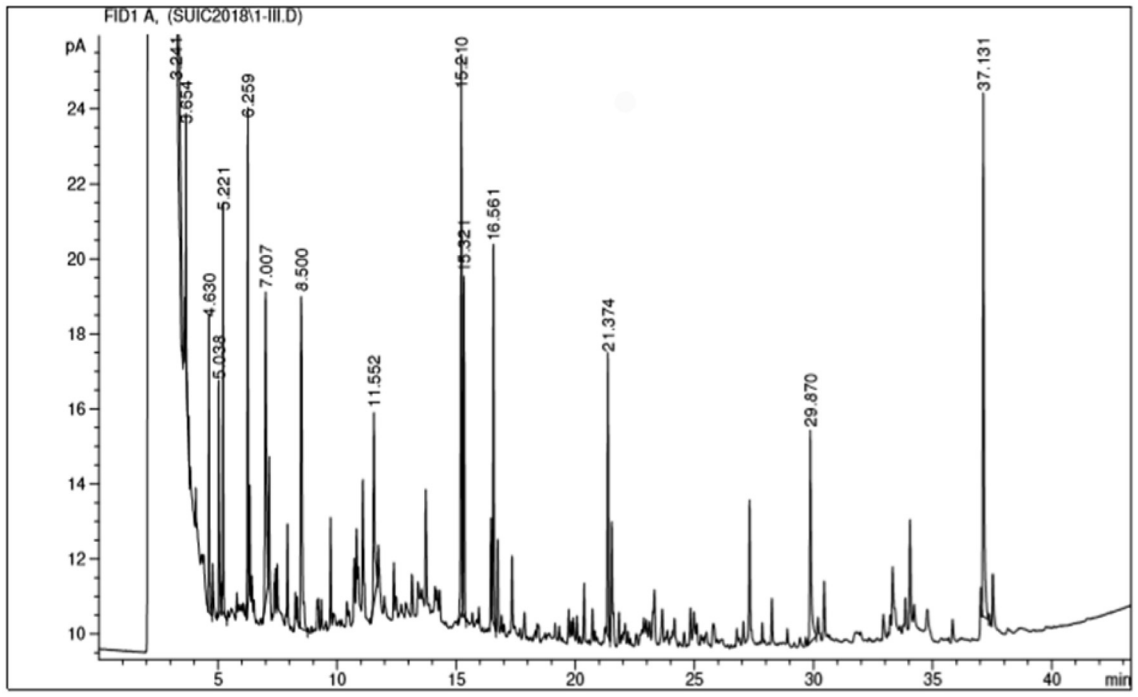


FIGURE 5
GC chromatogram of hydroacetonic extract of *L. nobilis* L., of female leaves.

examine the chemical profile of phenolic compounds by using various analytical methods (HPLC-DAD, LC-DAD, and LC-MS/MS) in others crude extracts especially from leaves and fruits, we found similar results but with the important diversity. Indeed, Dobroslavić et al. (2022) examined the chemical profile of hydroethanolic extracts of *L. noblilis* L. leaves collected from Croatia (Park and Han, 2023). The authors identified 29 compounds of which caffeic acid and protocatechuic acid were the major phenolic compounds at optimal pressurized liquid extraction (PLE) conditions. Another study on hydroethanolic extracts of the same leaves obtained by three extraction methods [conventional heat-reflux extraction, Microwave-assisted extraction (MAE), and Ultrasound- assisted extraction (UAE)] discovered the presence of 29 compounds, with caffeic acid

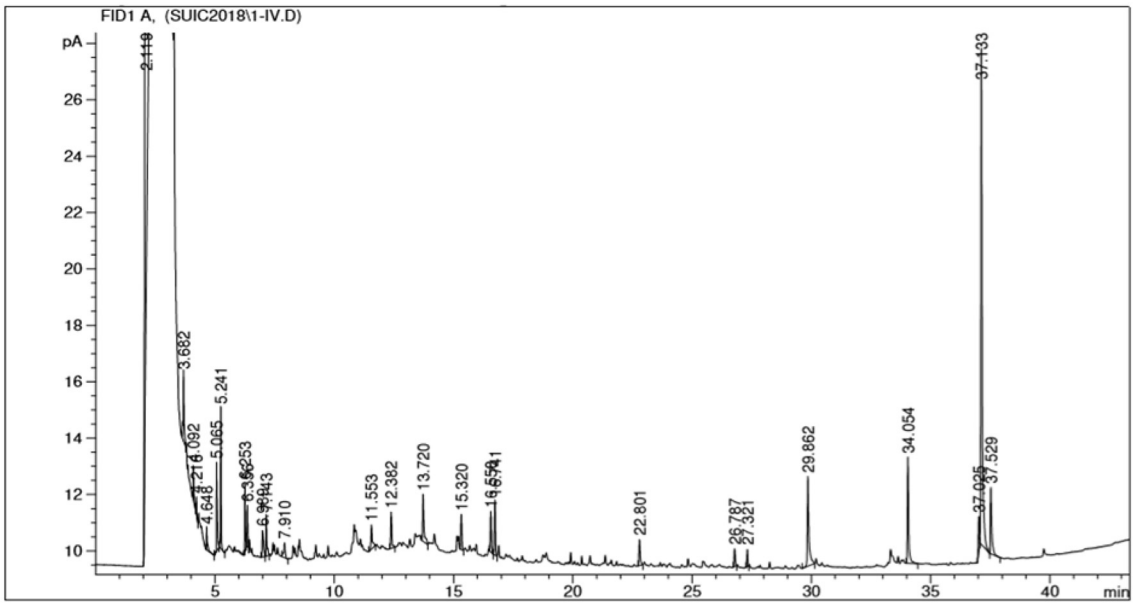


FIGURE 6
GC chromatogram of hyroacetonic extract of *L. nobilis* L., of female shoots.

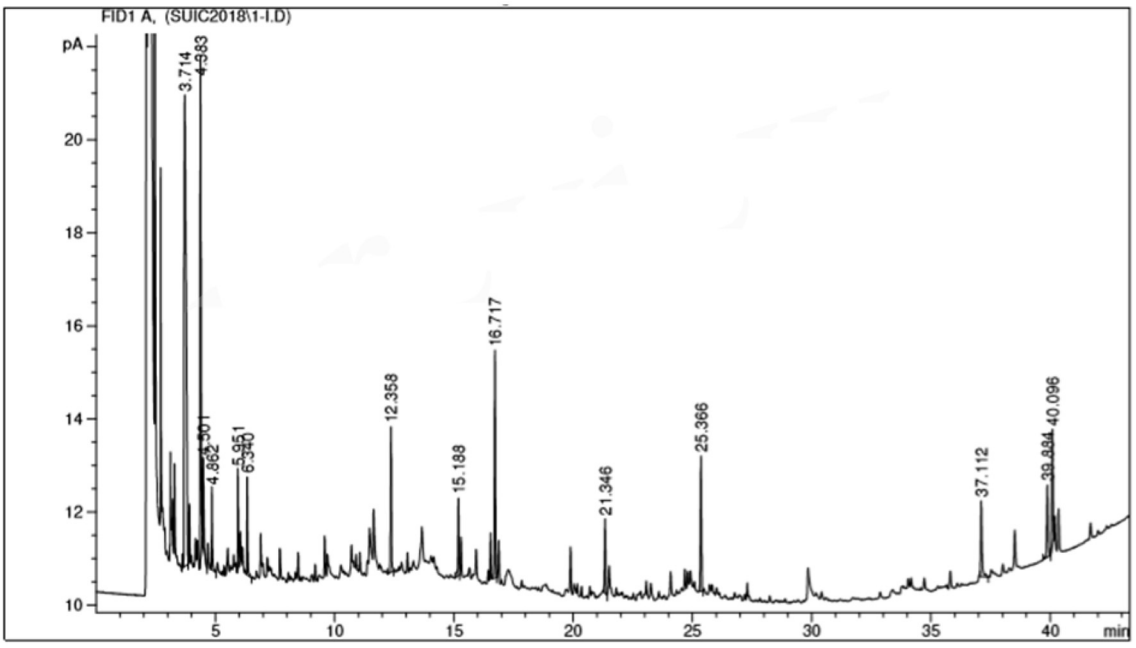


FIGURE 7
GC chromatogram of hydroacetonic extract of *L. nobilis* of male leaves.

and protocatechuic acid being the most abundant constituents with different concentration.

These present results are by our study with slight differences in concentration and identification of each compound. The results of flavonoids analysis by GC-FID showed the presence of quercetin and rutin in hydroethanolic and hydroacetonic extracts of various vegetative organs, which is in agreement with others' previous research (Stefanova et al., 2020; Medeiros-Fonseca et al., 2018; Bahmanzadegan et al., 2015; Shan et al., 2005; Lu et al., 2011; Škerget et al., 2005; Santoso et al., 2017). However, these present results are still very few so other further analyses and studies like identification and isolation of each phenolic compound (gallic acid, quercetin, kaempferol, and rutin) by other chromatographic methods (HPLC-DAD; HPLC-SM) should be carried out in the future.

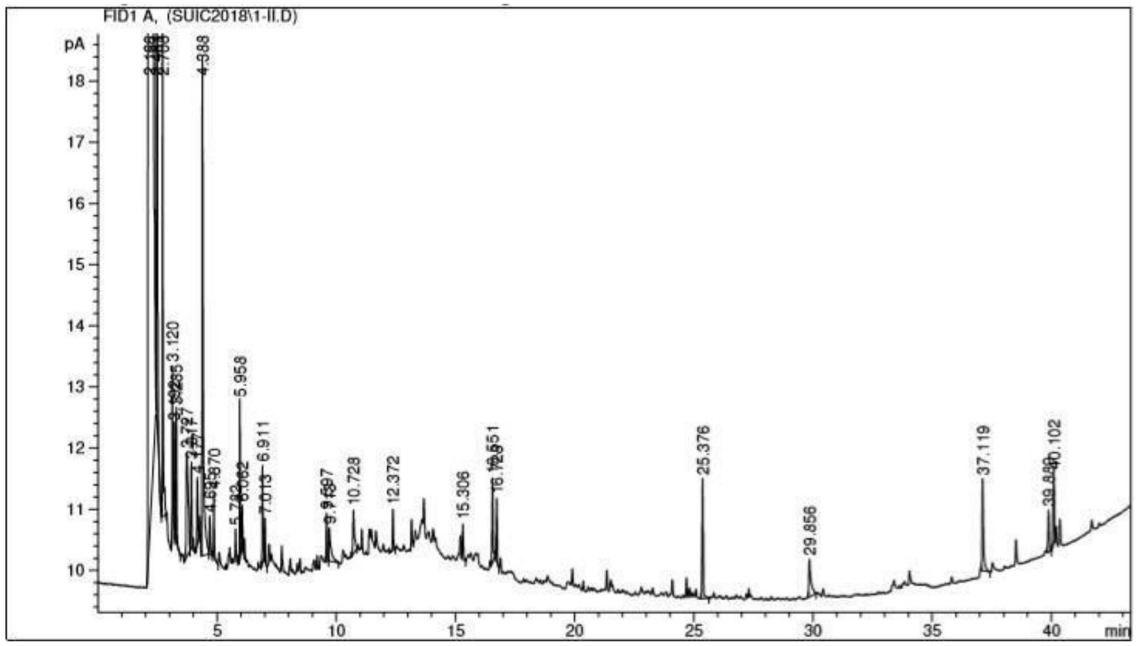


FIGURE 8
GC chromatogram of hydroacetonic extract of *L. nobilis* L., of male.

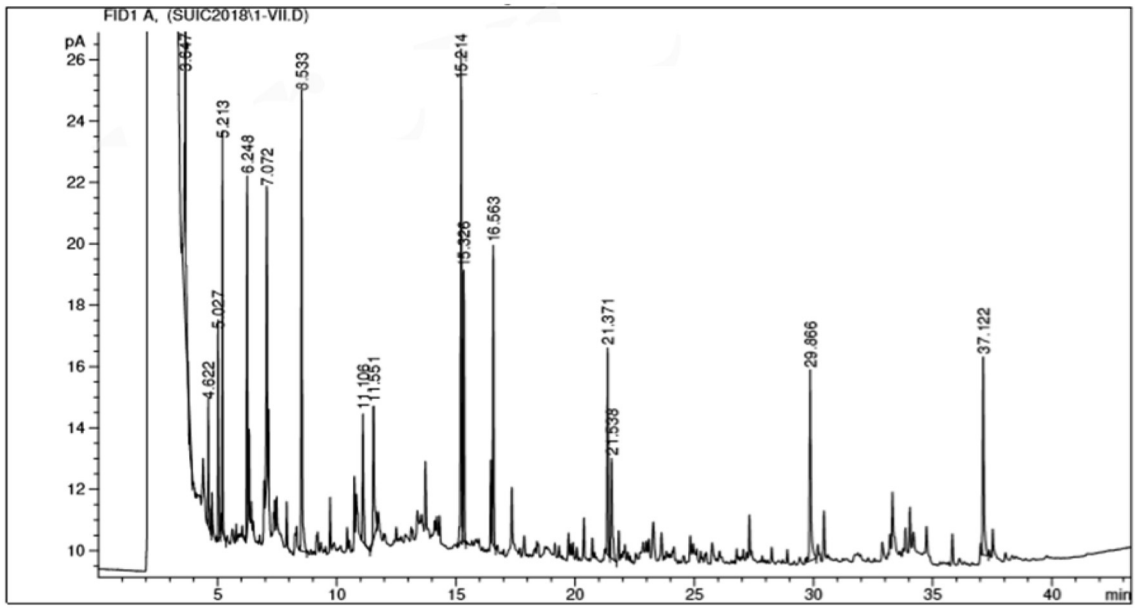


FIGURE 9
GC chromatogram of hydroethanolic extracts of *L. nobilis* L., of female leaves.

Sex affects compound concentrations through hormonal and metabolic regulation, while organs differ due to their specific physiological roles. Studies have shown that female *Laurus nobilis* may produce higher levels of some phenolic compounds, while males might have different essential oil profiles. In fact, *Laurus nobilis* is dioeucious plant, which identifying that the sex is the important niche to differentiate the bioactive compounds in male and female flowers at an early stage of development. Morphological characters of flower height, flowering time, and flower numbers were used for the sex differentiation in *L. nobilis*. Male flowers were reported to have 8–14 stamens, while female f lowers have 2–4 staminodes. On the other hand, Yahyaa et al., analyzed separately green and black fruit and revealed (E)–ocimene,-murolene, (E)–farnesene,-cadinene, and-cadinene in

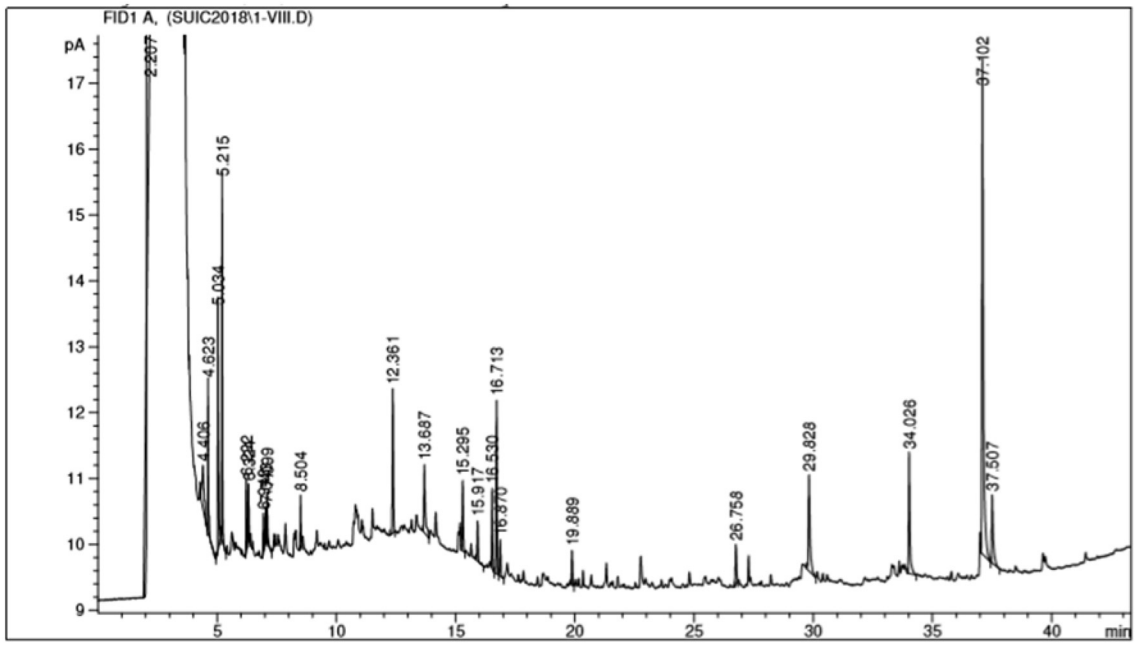


FIGURE 10
GC chromatogram of hydroethanolic extracts of *L. nobilis* L., of female shoots.

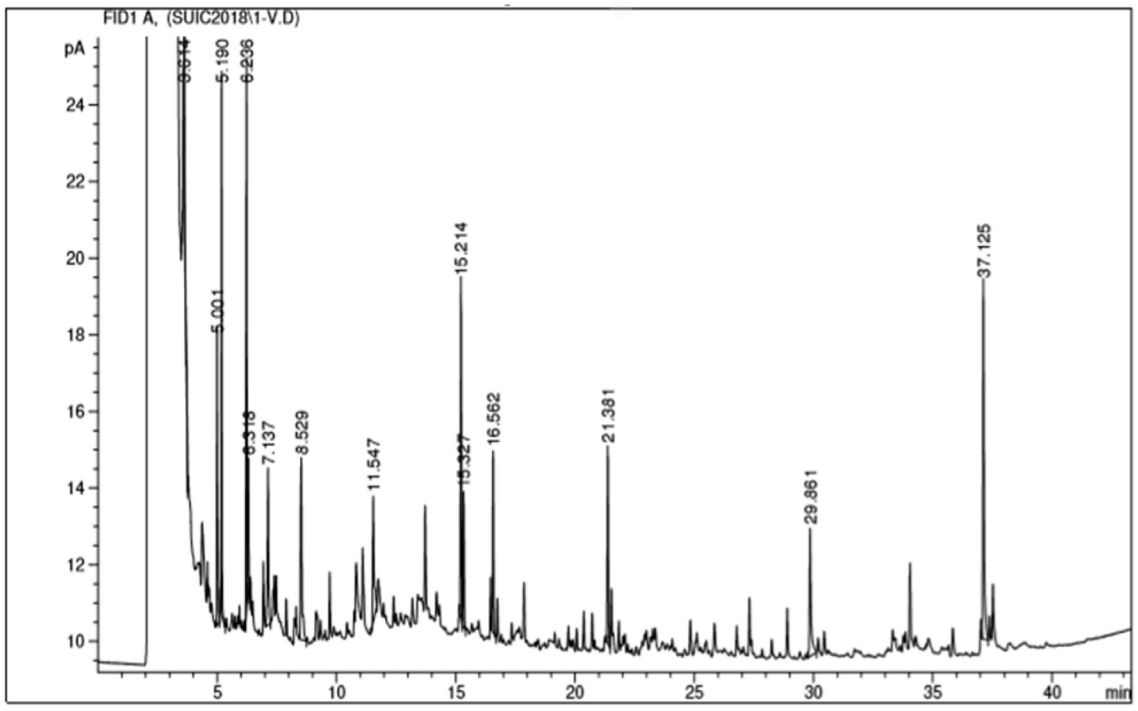


FIGURE 11
GC chromatogram of hydroethanolic extracts of *L. nobilis* L., of male leaves.

green fruits. Whereas, 1,8–cineole, (E)–ocimene, (E)–farnesene,-cardinene, and-cadinene were rich in black fruits. It was also remarkable that the fruits on the female plants contained high levels of two sesquiterpenes,-cadinene, and- cadinene. The norisoprenoid volatiles such as 6-methyl-5-hepten-2-one (MHO), pseudoionone, and- ionone were present only in the fresh pericarp of the black fruits. These norisoprenoid volatiles are distributed in numerous fruits and considered as important and potent flavor, aroma, and scent contributors in many fruits and fruit-based foods due to extremely low odor thresholds.

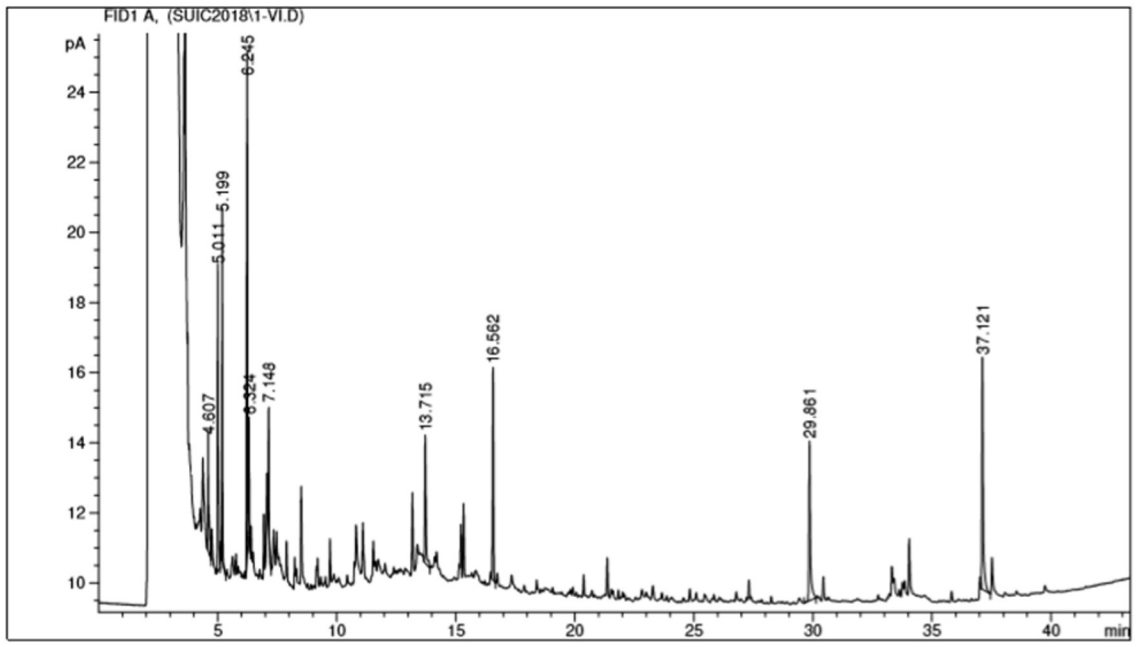


FIGURE 12
GC chromatogram of hydroethanolic extracts of *L. nobilis* of male shoots.

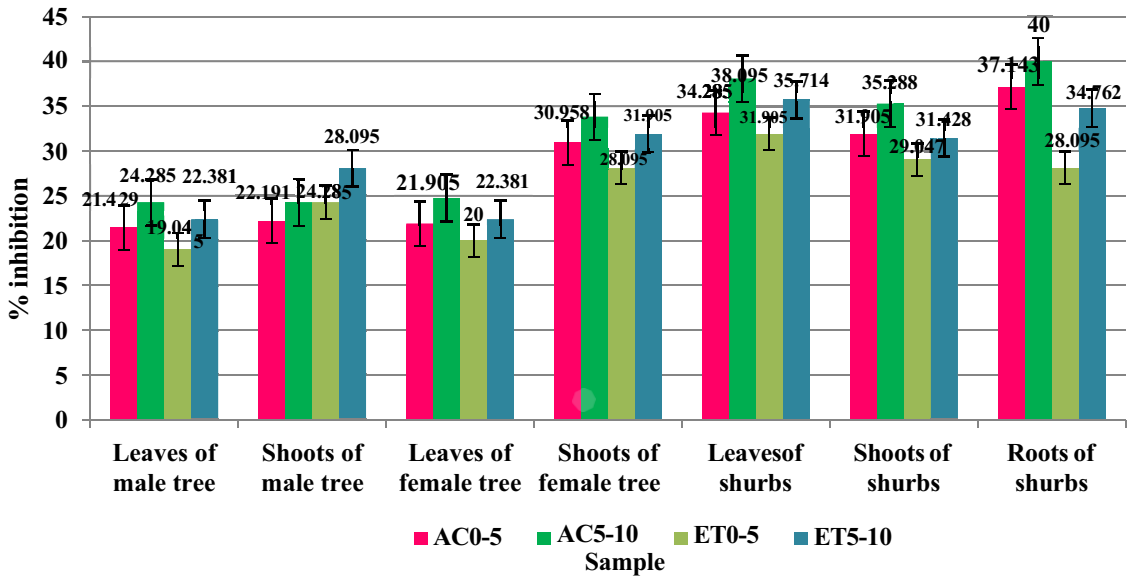


FIGURE 13
% inhibition measured by the DPPH method for 0–5 min and 5–10 min in different vegetative organs of *L. nobilis* L.

3.6 Antioxidant activity of *L. nobilis L.*

3.6.1 DPPH scavenging activity

The results of antioxidant activity (AA) in various vegetative organs of *L. nobilis* L., assessed by the DPPH method are presented in Figure 13. Both solvents of various vegetative organs demonstrated a high percentage inhibition of the free radical DPPH at different time intervals, impacting the type of plant organ and the solvent extraction. The hydroacetonic extracts of different vegetative organs of *L. nobilis* L. showed the highest capacity to scavenge the free radical DPPH compared to the hydroethanolic extracts at interval time (5–10 min). Hence, the hydroacetonic extracts of roots from shrubs of this plant possessed a strong DPPH scavenging activity compared to the other vegetative organs. However, the hydroacetonic extract of leaves of a male tree from *L. nobilis* L. exhibited higher radical

TABLE 4 Phenolic compounds of hydroacetonic extracts of *L. nobilis* L. obtained by GC-FID analysis.

Molecules	Area (%)								
	Chemical formula	Molecular weight g/mol	Leaves of male tree	Shoots of male tree	Leaves of female tree	Shoots of female tree	Leaves of shrubs	Shoots of shrubs	Roots of shrubs
Hydroxybenzoic acid	$C_7H_6O_3$	138.18	5.780	3.355	5.196	1.975	5.599	4.270	4.715
Protocatechuic acid	$C_7H_6O_4$	154.12	10.706	3.865	3.574	-	4.710	-	-
Gallic acid	$C_7H_6O_5$	170.12	6.221	8.331	-	-	2.660	-	-
Vanillic acid	$C_8H_8O_4$	168.14	-	-	7.266	1.636	4.465	-	-
Cinnamic acid	$C_9H_8O_2$	148.158	-	-	2.212	-	-	-	-
Caffeic acid	$C_9H_8O_4$	180.16	20.126	22.781	14.518	9.132	25.762	58.710	57.805
Syringic acid	$C_9H_{10}O_5$	198.1727	10.615	7.972	7.019	1.357	3.563	-	-
ferulic acid	$C_{10}H_{10}O_4$	194.18	-	-	-	-	-	-	-
Quercetin	$C_{15}H_{10}O_7$	302.236	6.368	2.452	4.877	5.299	5.454	-	-
Rutin	$C_{27}H_{30}O_{16}$	610.517	3.540	4.693	2.814	1.679	5.057	9.580	9.564
Total			63.356	30.668	47.476	21.078	57.27	72.56	72.084

(-): Not detected.

TABLE 5 Phenolic compounds of hydroethanolic extracts of *L. nobilis* L. obtained by GC-FID analysis.

Molecules	Area (%)								
	Chemical formula	Molecular weight g/mol	Leaves of male tree	Shoots of male tree	Leaves of female tree	Shoots of female tree	Leaves of shrubs	Shoots of shrubs	Roots of shrubs
Hydroxybenzoic acid	$C_7H_6O_3$	138.18	5.854	1.277	7.117	8.351	2.704	4.587	-
Protocatechuic acid	C_7H6O4	154.12	8.950	1.503	4.281	-	1.750	-	-
Gallic acid	$C_7H_6O_5$	170.12	5.791	3.087	-	-	-	-	-
Vanillic acid	$C_8H_8O_4$	168.14	5.496	1.442	10.308	8.138	2.305	-	-
Cinnamic acid	$C_9H_8O_2$	148.158	-	-	3.160	-	-	-	-
Caffeic acid	$C_9H_8O_4$	180.16	17.554	-	-	-	6.338	48.710	-
Syringic acid	$C_9H_{10}O_5$	198.1727	10.582	3.117	9.685	5.845	1.572	-	-
ferulic acid	$C_{10}H_{10}O_4$	194.18	-	-	-	-	-	-	-
Quercetin	$C_{15}H_{10}O_7$	302.236	7.979	2.971	9.062	37.682	60.863	-	-
Rutin	$C_{27}H_{30}O_{16}$	610.517	4.476	-	3.773	-	2,461	-	-
Total			66.682	13.397	47.386	60.016	77.993	53.297	-

(-): Not detected.

scavenging activity in comparison with other vegetative organs of this aromatic plant. Finally, the antioxidant capacity described by percentage inhibition of various vegetative organs of *L. nobilis* L., from acetonic and ethanolic extracts is presented follows this decreasing order:

For hydroacetonic extracts: Leaves of male tree > leaves of female tree > shoots of male tree > shoots of female tree > shoots of shrubs > leaves of shrubs > roots of shrubs.

For hydroethanolic extracts: leaves of male tree > leaves of female tree > shoots of male tree>shoots of shrubs > shoots of female tree > roots of shrubs > leaves of shrubs.

Concerning the percentages of inhibitions represented in antioxidant activity by using the DPPH method in various extracts of *L. nobilis* L. obtained with acetone and ethanol solvents in our research have been similar to other previous work (Muchuweti et al., 2007; Dudonné et al., 2009; Pacifico et al., 2014; Ishtiaque et al., 2015; Elmastaş et al., 2006; Boulila et al., 2015) but using

other solvent extraction and extraction techniques. Dudonné et *al.,* reported a DPPH analysis of Moroccan *L. nobilis* L., and the results showed a moderate percentage inhibition (18.93%) than in our crude extracts. Additionally, Ishtiaque et al. (2015) demonstrated also the modest antioxidant capacity expressed with percentage inhibition and as function dose-dependent in methanolic extract extracted by advanced method (ultrasonic extraction) of *L. nobilis* L., from the Pakistan region (Ishtiaque et al., 2015). However, our results are contradicted by other data published (Muñiz-Márquez et al., 2018, 2014; Akcan et al., 2017; Muñiz-Márquez et al., 2013). Here, we assume the highest percentage inhibition (78–94.72%) of various crude extracts obtained with numerous extraction methods (heat reflux extraction, maceration, and UAE) of *L. nobilis* L., leaves from different regions. However, at present no scientific research has been achieved on the determination of the antioxidant activity of other vegetative organs (roots, shoots, leaves, and fruits) in two feet of bay leaves (male and female). For that reason, further analysis in this field must be performed. The differences in the response of various crude extracts of *L. nobilis* L. against free radical DPPH may be due to the intervention of numerous factors including part of the plant party applied, environmental conditions, maturity stage of the plant, research methodology extraction technique, and expressed results. Also, it was concluded that these variations observed in the values can be attributed to the presence of some robust molecules in the extracts which are more capable of quenching DPPH radicals.

3.6.2 ABTS scavenging activity

In the present experiment, all tested extracts of *L. nobilis* L., possess the highest antioxidant capacity on the ABTS test (Figure 14). The results revealed that the extraction solvent and plant part studied influenced the capacity scavenging ability of ABTS free radical. As can be observed, the hydroacetonic extracts achieved the strongest capacity, followed by the hydroethanolic extracts of different vegetative organs at different intervals. The capacity to scavenge the ABTS free radical by various extracts of both solvents follows this increasing order:

For hydroacetonic extract at an interval time (5–10 min): leaves of shrubs > shoots of female tree > roots of shrubs > leaves of male tree > shoots of shrubs > shoots of male tree > leaves of the female tree.

For hydroethanolic extract at an interval time (5–10 min): leaves of shrubs > leaves of male tree > shoots of female tree> shoots of shrubs > shoots of male tree > roots of shrubs > leaves of the female tree.

The result of the present study revealed also that the scavenging of the ABTS radical by all crude extracts (CE) was found to be consistent with anterior research (Dudonné et al., 2009; Muñiz-Márquez et al., 2014). These authors have reported a positive agreement in percentage inhibition (18.61 and 47.71%, respectively) with our research. Moreover, many studies have reported the highest antioxidant activity against ABTS free radical with IC_{50} ranged from 0.086 to 1.391 mg/ml in all crude extracts of *L. nobilis* L. leaves obtained by using several extraction methods and solvents extractions (Kim and Kim, 2021). However, some authors have preferred to express antioxidant activity as a function of trolox equivalent value (TAEC; 0.21–1.001 mmol TE/g of DW) in different crude extracts (specially ethanolic and methanolic extracts) obtained by numerous extraction techniques such as infusion, maceration, and Soxhlet (Vallverdú-Queralt et al., 2014; Lu et al., 2011; Speroni et al., 2011; Dall'Acqua et al., 2009; Unver et al., 2009). Therefore, comparing these literature results with our findings is more difficult because they are the great variability of the parameters calculated so more research for standardization and optimization of these parameters and other conditions (pH, temperature, extraction methods, and processing time) in the estimation of antioxidant activity must be performed (Cano et al., 2003).

3.6.3 Total antioxidant activity

The assessment of total antioxidant activity (TAA) using phosphomolybdate assay of different plant organs in both extraction solvents (acetone and ethanol) from *L. nobilis* L., was achieved in Figure 15. The data obtained are expressed in mg/g of ascorbic acid equivalent (AAE). Our finding displayed that all extracts tested in this study indicated an interesting antioxidant capacity in both extraction solvents. The leaves of the female tree exhibited the highest total antioxidant activity (TAA) in ethanol (3.133 ± 0.158 mg AAE/g of DW) and acetone (3.025 ± 0.144 mg AAE/g of DW), respectively than other vegetative organs. The orders of antioxidant activities in both solvents (acetone and ethanol) were: Leaves of female tree > leaves of shrubs > shoots of shrubs > leaves of male tree > shoots of female tree > roots of shrubs > shoots of the male tree. In previous studies described in the literature reviews, the antioxidant activity of *L. nobilis* L. leaves and other organs (branches and roots) determined by phosphomolybdate assay has varied significantly. However, it's extremely difficult to compare the results of this work with different studies because researchers can set different parameters to determine the total antioxidant activity. Ouchikh et al., found that different vegetative organs of *L. nobilis* L. from Tunisia demonstrated a great total antioxidant activity (TAA) expressed as ascorbic acid equivalents (AE) and gallic acid equivalents (GE) in the acetonic extract obtained by magnetic stirring for 30 min (Ouchikh et al., 2011). Albayrak et al., studied three different extracts of *L. nobilis* L. leaves collected from Turkey and they showed that all extracts had potential antioxidant activity. The higher value was found in methanolic extract (196.98 ± 2.1 mg AAE/g extract), followed by boiling water (69.50 ± 0.0 mg AAE/g extract) and decoction (35.65 ± 0.1 mg AAE/g extract; Albayrak et al., 2012). In Morocco, Taroq et al., investigated the solvent effect on the total antioxidant activity of *L. nobilis L.* and aqueous extract exhibited a higher reducing molybdate 550 mg E vitamin C/g DW than that of the ethanolic (160 mg E vitamin C/g DW) and methanolic extract (150 mg E vitamin C/g DW), whereas the crude extract by acetate solvent demonstrated lower total antioxidant activity with value 28.6 ± 2.07 mg EvitC/g DW.

Furthermore, the total antioxidant activity was significantly higher in all crude extracts and the authors reported that the strong antioxidant activity of water extract was probably due to the presence of the highest content of phenols in its extracts (Taroq et al., 2018). However, Guenane et al., reported a lower value (0.211 ± 0.016 in the ethanolic extract of *L. nobilis* L. leaves harvested in Algeria (Guenane et al., 2016).

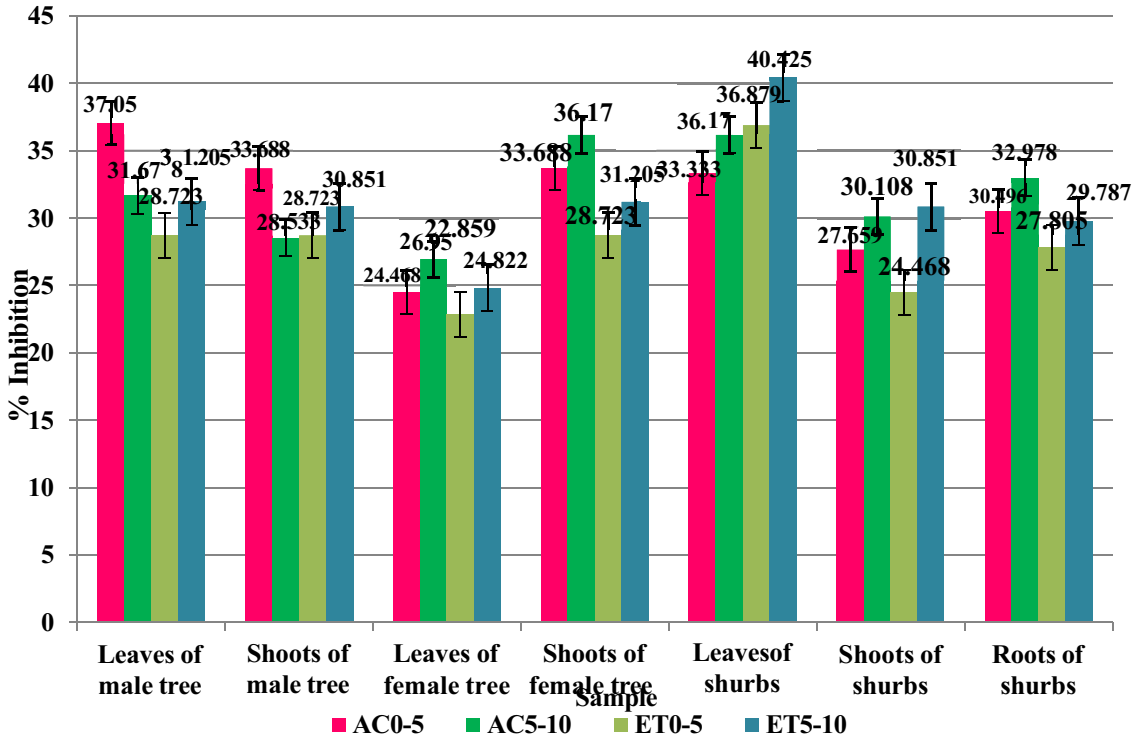


FIGURE 14
% inhibition measured by the ABTS method for 0–5 min and 5–10 min in different vegetative organs of *L. nobilis* L.

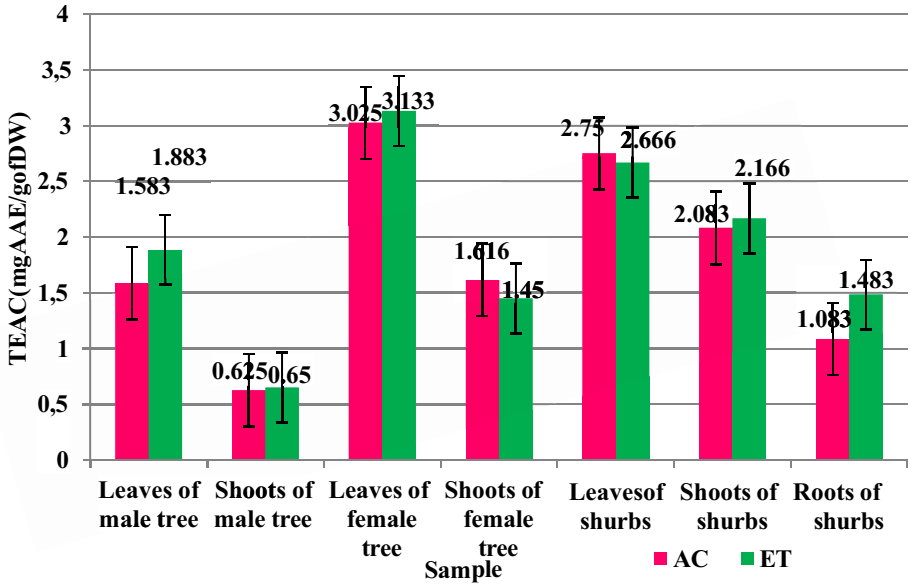


FIGURE 15
Total antioxidant capacity measured by phosphomolybdate assay (TAC) in different vegetative organs of *L. nobilis* L., in Northern Morocco.

3.7 Molecular docking results

To understand the impact of *L. nobilis L.* extracts on antioxidant activities, we performed molecular docking of the bioactive components present in these extracts with the corresponding molecular receptors. These compounds were tested against four proteins: glutathione reductase (PDB: 3DK9), lipoxygenase (PDB: 1YGE), and cyclooxygenase (PDB: 6Y3C). The results of these tests provide insights into the potential therapeutic effects of *L. nobilis L.* extracts in managing oxidative

stress and inflammatory conditions. Glutathione reductase (GR) is an essential enzyme that helps cells fight against oxidative stress, a risk factor for the development of many diseases (Karaman, 2020). Inhibiting glutathione (GSH), a crucial antioxidant, could offer therapeutic benefits given its connection to excessive oxidative stress, which is associated with numerous diseases. When carefully implemented, this strategy can have synergistic effects with other therapeutic modalities (Güller, 2021). The results revealed that all molecules exhibit a lower affinity for glutathione reductase compared to xanthine a standard inhibitor (−8.2 kcal/mol; Table 6), except rutin, which showed good affinity toward glutathione reductase (−8.9 kcal/mol). Rutin stood out for its good affinity, interacting efficiently with several amino acid residues of the enzyme. More specifically, it demonstrated hydrogen bond interactions with Ser 225 and Glu 386 (Figure 16a). These results underscore the promising capability of Rutin as a candidate deserving further exploration as an antioxidant agent. Lipoxygenases (LOX) are enzymes that play a key role in the metabolism of polyunsaturated fatty acids. The oxidative metabolism of fatty acids by LOX can lead to the production of reactive oxygen species (ROS), which contribute to oxidative stress, a condition associated with various chronic diseases. Therefore, finding molecules with high affinity for the enzyme lipoxygenase is crucial not only for developing therapeutic strategies to treat inflammation-related diseases but also for mitigating oxidative stress, thereby offering potential antioxidant benefits (Filipsky et al., 2015). The results revealed that all molecules exhibit a lower affinity for lipoxygenase compared to Zileuton a standard inhibitor (−7.2 kcal/mol; Table 6), except rutin and quercetin, which showed good affinity toward lipoxygenase. Rutin stood out for its good

TABLE 6 Molecular binding affinities (Kcal/mol) between phytochemicals identified in extracts of *L. nobilis* L with glutathione reductase (PDB: 3DK9), Lipoxygenase (PDB: 1YGE) and Cyclooxygenase (PDB: 6Y3C).

	GR (PDB: 3DK9)	LOX (PDB: 1YGE)	COX (PDB: 6Y3C)
Compounds	Affinity (Kcal/mol)	Affinity (Kcal/mol)	Affinity (Kcal/mol)
Inhibitor standard	−8.2	−7.0	−8.1
Hydroxybenzoic acid	−6.5	−5.5	−6.2
Protocatechuic acid	−6.3	−6.5	−6.5
Gallic acid	−6.3	−6.0	−6.6
Vanillic acid	−5.1	−6.5	−6.2
Cinnamic acid	−4.8	−6.7	−6.1
Caffeic acid	−4.8	−7.1	−6.6
Syringic acid	−4.9	−5.6	−6.0
Ferulic acid	−4.9	−6.0	−6.3
Quercetin	−6.4	−8.7	−8.5
Rutin	**−8.9**	**−9.1**	**−9.3**

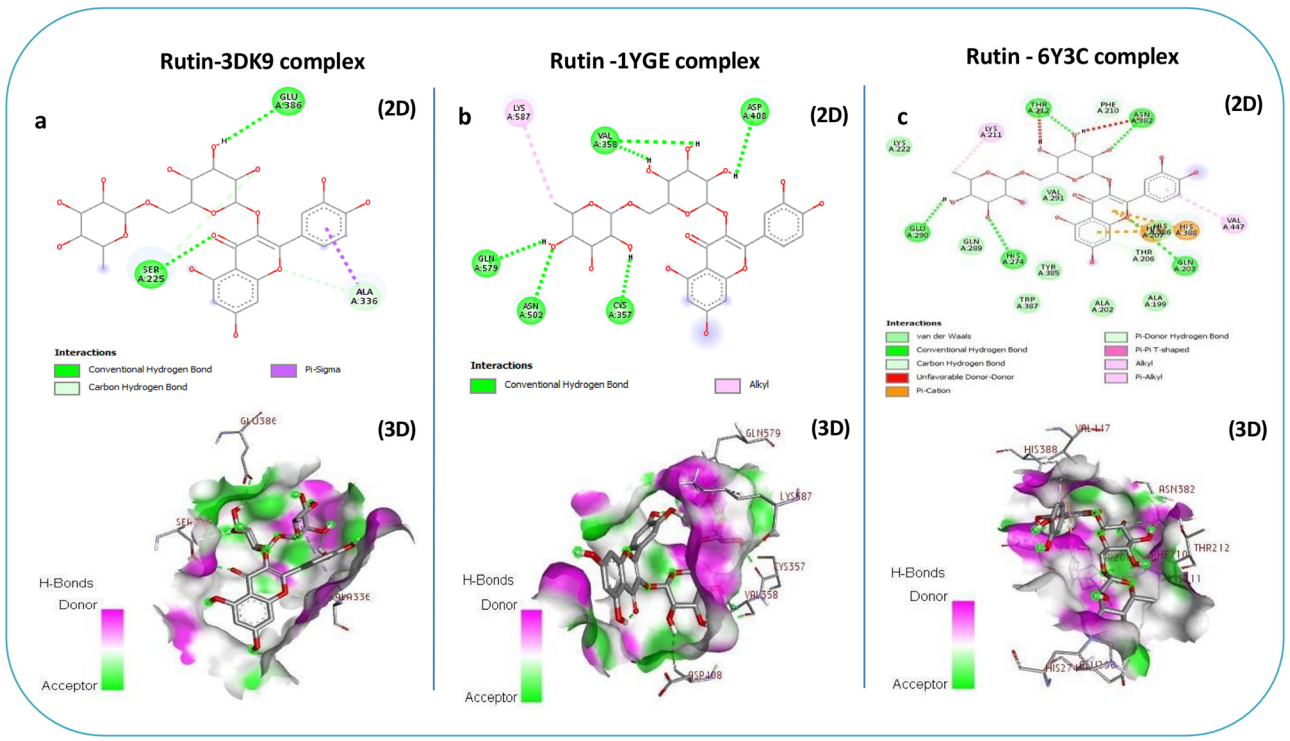


FIGURE 16
2D and 3D Binding Interactions of the phytochemicals identified in extracts of *L. nobilis* L with glutathione reductase (PDB: 3DK9) **(a)**, Lipoxygenase (PDB: 1YGE) **(b)** and Cyclooxygenase (PDB: 6Y3C) **(c)**.

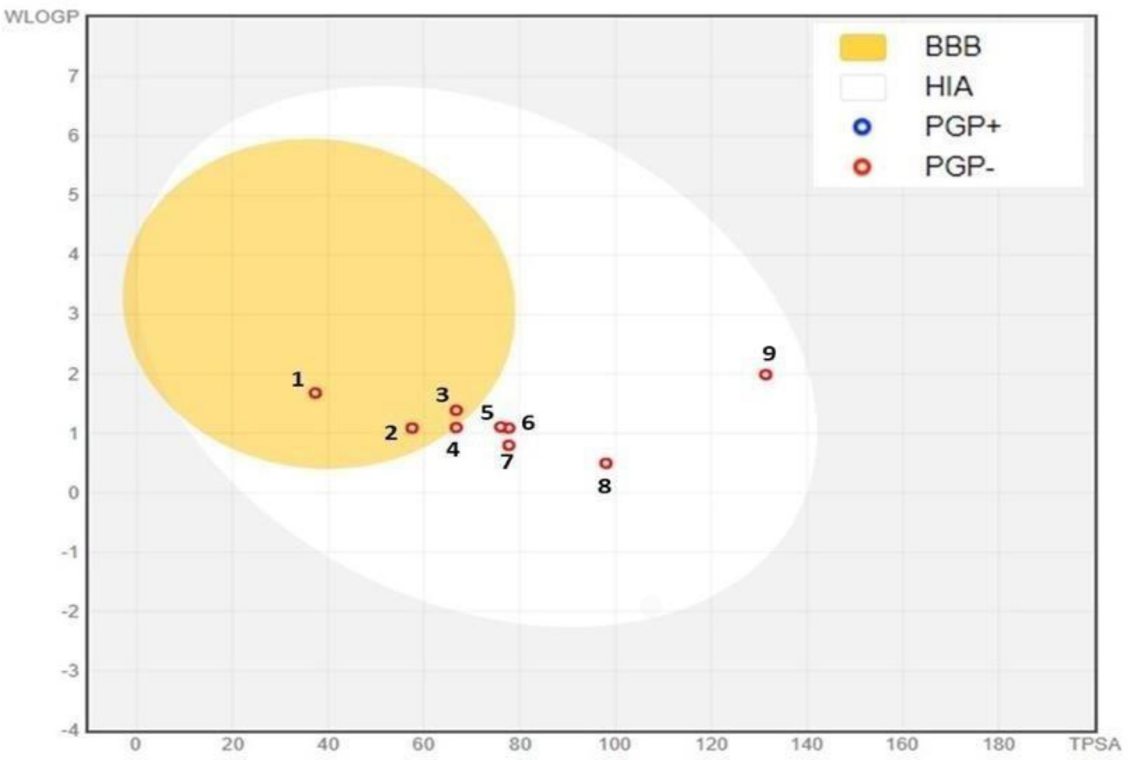


FIGURE 17
BOILED-Egg Model of the GI absorption and BBB permeability of phytochemicals identified in extracts of *L. nobilis* L. (1) Cinnamic acid, (2) Hydroxybenzoic acid, (3) Ferulic acid, (4) Vanillic acid, (5) Syringic acid, (6) Caffeic acid, (7) Protocatechuic acid, (8) Gallic acid (9) Quercetin. PGP-: non-substrate of P-glycoprotein, PGP+: P- glycoprotein substrate.

affinity (−9.1 kcal/mol) interacting efficiently with several amino acid residues of the enzyme. More specifically, it demonstrated hydrogen bond interactions with Cys 357, Val 358, Asp 408, Asn 502 and Gln 579 (Figure 16b). These results underscore the promising capability of rutin as a candidate deserving further exploration for its anti-inflammatory properties and potential antioxidant benefits.

Cyclooxygenase (COX) plays a key role in the biosynthesis of prostaglandins involved in inflammation. By inhibiting COX, it is possible to reduce the production of ROS, thereby reducing oxidative stress and associated cellular damage (Cicalau et al., 2021). The results revealed that all molecules exhibit a lower affinity for cyclooxygenase compared to Celecoxib a standard inhibitor (−8.1 kcal/mol; Table 6), except rutin and quercetin, which showed good affinity toward cyclooxygenase. Rutin stood out for its good affinity (−9.3 kcal/mol) interacting efficiently with several amino acid residues of the enzyme. More specifically, it demonstrated hydrogen bond interactions with Gln 203, Thr 212, His 274, Glu 290, and Asn 382 (Figure 16c). These results underscore the promising capability of rutin as a candidate deserving further exploration for its anti-inflammatory properties and potential antioxidant benefits.

3.8 ADME analysis

Early pharmacokinetic assessment using computer models enables the swift identification of potential drug candidates and optimizes the development process. These evaluations help mitigate the risk of adverse effects, lower the chances of drug development failures, and improve clinical success rates (van de Waterbeemd and Gifford, 2003). According to Lipinski's rule of five and Veber's rule, compounds intended for oral drug development should generally adhere to the following criteria, with no more than one exception (1) no more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms), (2) an octanol-water partition coefficient log P (MLogP) below 5, (3) a molecular weight under 500 daltons, and (4) no more than 5 hydrogen bond donors (Lipinski et al., 2012). In our study, we observed that all the phytochemicals identified in the ethanolic extract of *L. nobilis* L. examined met Lipinski's criteria, indicating their potential suitability for oral drug development, except Rutin with MW>500 g/mol. Our investigation revealed that Cinnamic acid (TPSA = 37.3Å², WLogP = 1.6), Hydroxybenzoic acid (TPSA = 57.5 Å², WLogP = 1.0) and Ferulic acid (TPSA = 66.7 Å², WLogP = 1.3) are capable of crossing the BBB (blood–brain barrier). This is illustrated by the yellow region in Figure 17. Additionally, all of the

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TABLE 7 Pharmacokinetic properties (ADME) of phytochemicals identified in extracts of *L. nobilis* L (*in silico*).

Compounds	Physicochemical properties					Lipophilicity		Drug-likeness		Pharmacokinetic		
	MW g/mol	HBA	HBD	TPSA Å^2	Rotatable Bonds	MlogP	WlogP	Lipinski's	Verbier's	GI absorption	BBB Permeation	CYP1A2 inhibitor
Hydroxybenzoic acid	138.1	3	2	57.5	1	0.9	1.0	0	0	High	Yes	No
Protocatechuic acid	154.1	4	3	77.6	1	0.4	0.8	0	0	High	No	No
Gallic acid	170.1	5	4	97.9	1	−0.1	0.5	0	0	High	No	No
Vanillic acid	168.1	4	2	66.7	2	0.7	1.1	0	0	High	No	No
Cinnamic acid	148.1	2	1	37.3	2	1.9	1.6	0	0	High	Yes	No
Caffeic acid	180.1	4	3	77.7	2	0.7	1.0	0	0	High	No	No
Syringic acid	198.1	5	2	75.9	3	0.4	1.1	0	0	High	No	No
Ferulic acid	194.1	4	2	66.7	3	1.0	1.3	0	0	High	Yes	No
Quercetin	302.2	7	5	131.3	1	−0.5	1.9	0	0	High	No	Yes
Rutin	610.5	16	10	296.4	6	−3.8	-1.6	3	1	Low	No	No

compounds analyzed were determined to be P- glycoprotein non-substrates (PGP-) (Figure 17), indicating that they are less likely to be effluxed by cells, which can enhance their bioavailability and therapeutic effectiveness. This characteristic is crucial for ensuring that the compounds maintain sufficient intracellular concentrations to exert their desired pharmacological effects (van de Waterbeemd and Gifford, 2003). A molecule that is strongly absorbed by the intestine offers significant advantages in terms of bioavailability, efficacy, convenience, and tolerance, making it a promising candidate for the development of oral medications (Stillhart et al., 2020). Notably, all the phytochemicals examined in our study demonstrate high intestinal absorption, except Rutin (Table 7). Our analysis revealed that the majority of compounds are neither inhibitors nor substrates of CYP450 enzymes, particularly CYP1A2, except Quercetin, which was identified as a CYP1A2 inhibitor (Table 7). This finding suggests a reduced risk of interference with drug metabolism, thereby enhancing the safety profile of the phytochemicals. Additionally, the lack of interaction with CYP450 enzymes minimizes the potential for adverse drug-drug interactions, making these compounds more suitable for use in combination therapies (Taşçioglu et al., 2021). This characteristic further underscores their potential as safe and effective therapeutic agents.

4 Conclusion

This study, conducted for the first time on both male and female trees of *Laurus nobilis* L. from the Tangier-Tetouan region in northern Morocco, revealed notable differences in phytochemical profiles. Both acetonic and ethanolic extracts exhibited high levels of phenolic, flavonoid, and flavonol compounds, particularly in female shoots and shrub leaves. Phytochemical analysis confirmed the presence of major bioactive molecules such as caffeic acid, hydroxybenzoic acid, rutin, syringic acid, vanillic acid, quercetin, protocatechuic acid, gallic acid, and cinnamic acid. The strong antioxidant activity observed across all extracts is likely attributed to this rich composition. Among these, rutin and quercetin stand out for their potential health benefits and warrant further investigation as candidates for oral therapeutic applications. Overall, Within, this scientific research showed that L. nobilis L. is a naturally rich source of bioactive molecules including rutin and quercetin which can be made it for prevention of various diseases and also suggest that can be used in the food, cosmetics, and pharmaceutical industries. However, more specific research in the future should be performed to attract the beneficial effect of this aromatic species in various fields including food, cosmetics, and phytotherapy.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Author contributions

IB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. AF: Data curation, Formal analysis, Methodology, Resources, Validation, Writing – original draft, Writing – review & editing. BO: Formal analysis, Resources, Writing – original draft. AB: Formal analysis, Resources, Writing – original draft. MB: Data curation, Methodology, Project administration, Writing – review & editing. AS: Methodology, Project administration, Supervision, Writing – original draft. RH: Data curation, Investigation, Resources, Writing – original draft. ES: Data curation, Resources, Software, Writing – review & editing. AL: Data curation, Investigation, Methodology, Supervision, Writing – review & editing. NE: Methodology, Visualization, Writing – review & editing.

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