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Phenolic Profile, Antioxidant, Anti-Cholinesterase, and Anti-Alpha-Amylase Activities of Algerian *Ruta graveolens*

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ABSTRACT

Ruta graveolens, belonging to the Rutaceae family, is a medicinal and aromatic plant. It is widely used in traditional Algerian medicine. As part of our contribution to promoting this medicinal plant, we conducted an in-depth study to analyze its phenolic content and evaluate its antioxidant, anti-alzheimer and antidiabetic properties. Our methodical approach consisted of studying the hydroethanolic extract of *Ruta graveolens* leaves, a plant with well-documented pharmacological properties. The extraction was carried out by maceration under agitation, using a solvent, namely 70% ethanol. The LC-ESI-MS/MS analysis was utilized to identify 14 compounds, revealing the richness of this species in polyphenols and flavonoids, particularly in hesperidin and rutin. In addition, Antioxidant activity is assessed using various recognized scientific tests, the hydroethanolic extract is distinguished by its remarkable ability to trap the DPPH radical, with an IC_{50} of 43.20±0.96 mg/mL. It also exhibits strong antioxidant activity, as evidenced by IC_{50} values of 38.01±2.96 mg/mL obtained by the ABTS test and 102.96±3.26; 312.29±6.8; 192.07±6.75 mg/mL by the FRAP, β-carotene and SNP tests respectively. The cholinesterase inhibition was also investigated in this work, specifically against cholinesterase enzymes: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Analysis of the extract revealed significant antidiabetic activity, by inhibiting α-amylase enzyme and for the first time, the Algerian *Ruta graveolens* was reported to inhibit cholinergic and α-amylase enzymes. At the end of this study, it appears that the plant studied has notable biological activities and could be an excellent natural biological product for use in the pharmaceutical field.

1. Introduction

Oxidative stress can be defined as a phenomenon when the production and accumulation of reactive oxygen species can cause damage to cells and tissues, leading to various health issues. Therefore, the balance between antioxidants and reactive oxygen species is crucial for maintaining overall health and well-being. In addition, oxidative stress has been linked to the pathogenesis of many diseases, such as cardiovascular disease, cancer

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and diabetes as well as neurodegenerative disorders, such as Parkinson's and Alzheimer's diseases (Pizzino *et al*. 2017).

Furthermore, Diabetes Mellitus is a severe, chronic condition brought on by disruption in carbohydrate, protein and fat metabolism, leading to serious health complications (Lekmine *et al*. 2023). Slowing down the rate at which glucose is released into the bloodstream after meals, ultimately leading to better blood sugar control. By reducing the amount of glucose available for absorption, this treatment can help manage blood sugar levels and prevent spikes in insulin production. This is possible through inhibiting the enzymes that catalyze the hydrolysis of polysaccharides (α-glycosidase and α-amylase), (Lekmine *et al*. 2023; Szablewski 2025). Recently, neuro degeneration has also been added to diabetes mellitus complications list (Szablewski 2025). Additionally, Inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) can enhance synaptic acetylcholine levels, thereby improving cognitive function which is relevant in the treatment of Alzheimer's disease (AD) and other neurodegenerative conditions (Orhan *et al*. 2008; Loizzo *et al*. 2008).Traditional medicine, such as plant-based treatments, has shown promise in managing oxidative stress and its associated complications. Research on the effectiveness of plants in treating hyperglycemia and neuropathy is ongoing and may provide safer and more cost-effective alternatives to synthetic drugs for disease management.

Ruta graveolens L. (Rutaceae), commonly known as rue, is a medicinal and aromatic plant traditionally used in various cultures for its therapeutic properties, including anti-inflammatory, analgesic, antispasmodic, and neuroprotective effects (Ahmed *et al*. 2010;Velmurugan *et al.* 2021). Native to the Mediterranean region, *R. graveolens* has gained considerable attention in modern phytopharmacology due to its rich reservoir of secondary metabolites and its potential in managing oxidative stress and neurodegenerative disorders (Colucci-D'Amato and Cimaglia 2020).

Phytochemical investigations have identified a wide range of bioactive compounds in *R. graveolens*, including alkaloids (e.g., graveoline, skimmianine), flavonoids (e.g., rutin, quercetin), coumarins (e.g., xanthotoxin, bergapten), and essential oils (Fakchich and Elachouri 2014) and insecticidal activities (Bouabida and Dris 2020; Bouabida and Dris 2022a). These phytoconstituents are responsible for the diverse pharmacological effects attributed to the plant. In particular, flavonoids and alkaloids have been linked to antioxidant and neuroprotective mechanisms, making *R. graveolens* a subject of interest for natural drug development (Islam *et al*. 2022).

Given the synergistic potential of its antioxidant and antidiabetic properties, *Ruta graveolens* represents a valuable candidate for the development of multifunctional agents aimed at combating oxidative deficits. The present study aims to perform a comprehensive phenolic analysis of *R. graveolens* and evaluate its *in vitro* antioxidant, cholinesterase and antidiabetic inhibitory activities.

2. Materials and Methods

2.1. Yield and Preparation of the Extract

Ruta graveolens. L leaves were collected in a good health, in the flowering stage (May 2024) from Bekkaria (35° 22′ 20″ north, 8° 14′ 32″ east), Tebessa province (located in northeastern Algeria) cleaned and rinsed in distilled water to remove dust and impurities, then dried in the shade , at ambient temperature, crushed into powder using a grinder. Next, 10 g were extracted in 100 mL of hydro ethanol (30:70) and socked for 24h. Filtered, and evaporated at 40°C; the crude extract was kept at 4°C, in dry dark glass vials until analysis.

The extraction yield was calculated using the next formula: %Yield = (Weight of dry extract/Weight of taken plant for extraction) 100 (Fenghour and Bouabida 2024)

2.2. Total Phenolic and Flavonoids Determination

The total phenolic content was determined by the Folin_Ciocalteu method. To 20 µL of Ruta extract, 100 µL of diluted ten folds of Folin_Ciocalteu reagent was added, followed by 75 µL of sodium carbonate (7.5%). The resulting mixture was left to incubate in the dark for 2 h in room temperature. The absorbance was taken at 765 nm using microplate reader. Gallic acid was used as a standard, and the result was expressed as µg of Gallic acid equivalent (GAE)/mg of extract (Müller *et al*. 2010).

The determination of the flavonoids content was based on the method of Ayoola *et al*. 2009. The mixture reaction contains an equal volume of Ruta extract and aluminum chloride (AlCl3 in 2%, 100 µL), incubated for 15 min and read at 415 nm using a microplate reader. Quercetin was used as a standard, and flavonoid content was expressed as µg of quercetin equivalent (QE)/mg of extract.

2.3. Evaluation of *Ruta graveolens* Polyphenolic Profile by LC –ESI- MS/MS

The phenolic content of R. graveolens leaves extract was determined by LC-MS/MS (Yilmaz 2020) using A Shimadzu-Nexera model ultrahigh performance liquid chromatograph (UHPLC) coupled with a tandem mass spectrometer. The reversed-phase UHPLC was performed using: an autosampler (SIL-30AC model), a column oven (CTO-10ASvp model), binary pumps (LC-30AD model), and a degasser (DGU- 20A3R model). For an optimum separation of 53 phytochemicals and overcome the suppression effects, we pretend to optimize the chromatographic conditions, for that Different coluımns such as Agilent Poroshell 120 EC-C18 model (150 mm × 2.1 mm, 2.7 µm) and RP-C18 Inertsil ODS-4 (100 mm × 2,1 mm, 2µm), different mobile phases (B) such as acetonitrile and methanol, different mobile phase additives such as ammonium formate, formic acid, ammonium acetate, and acetic acid, different column temperatures such as 25°C, 30°C, 35°C and 40°C were tried and applied until the optimum conditions were attained. We employed a reversed phase Agilent Poroshell 120 EC-C18 model (150 mm × 2.1 mm, 2.7 µm) analytical column. The column operating at a temperature of 40°C. The elution gradient was composed of eluent A (water + 5 mM ammonium formate + 0.1% formic acid) and eluent B (methanol+5 mM ammonium formate+0.1% formic acid). The following gradient elution profile was used: 20-100% B (0-25 min), 100% B (25-35 min), 20% B (35-45 min). Moreover, the solvent flow was set at 0.5mL/min, and a fixed injection volume of 4 µL was used for sample introduction.

The mass spectrometric detection was performed using a Shimadzu LCMS-8040 tandem mass spectrometer equipped with electrospray ionization (ESI) source, operated in both negative and positive ionization modes. Collision energies (CE) were optimized to achieve efficient phytochemical fragmentation and maximum transmission of the desired product ions. The MS operating conditions were set as follows: drying gas (N_2) flow, 15 L/min; nebulizing gas (N_2) flow, 3 L/min; desolvation line (DL) temperature, 250°C; heat block temperature, 400°C; and interface temperature, 350°C. Quantification of the phytochemicals was carried out in multiple reactions monitoring (MRM) mode. The MRM method was optimized to selectively detect and quantify phytochemical compounds based on specific precursor-to-fragment ion transitions.

LC-ESI-MS/MS data were acquired and processed using Lab Solutions software (Shimadzu). All analyses were carried out in negative and positive ionization mode, and results are reported as milligrams per gram of extract.

2.4. Antioxidant Activity

To provide a comprehensive assessment of antioxidant properties of plant derived compounds, which can vary depending on the targeting pathways, multiple methods can give a faithful representation of the antioxidant capacity of natural products.

The tests were carried out in triplicate using a microplate reader, the ascorbic acid, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tocopherol were used as standard antioxidants, and the results were quantified and presented as IC_{50} and A0.50 values.

2.4.1. DPPH Assay

The free radical scavenging activity of Ruta hydroethanolic extract was determined using the DPPH (2, 2-Diphenyl-1-picrylhydrazyl) method, through the application of the technique described by Blois (1958). An aliquot (40µL) of extract was tested in different concentrations (800; 400; 200; 100; 50; 25; 12.5 µg/mL), combined with 160 µL of DPPH solution (4% in methanol). After 20 minutes of incubation in dark conditions. The sample absorbance was measured at 517 nm using a microplate reader.

2.4.2. ABTs Assay

2, 20-Azinobis (3-ethylbenzothiazoline-6 sulfonic Acid).The ABTS test was performed upon to the method of Re *et al*. (1999). A 7 mM ABTS solution was prepared in 2.45 mM aqueous potassium persulfate and incubated in the dark for 12–16 h to generate the $ABTS^+$ free radical. A sample of 40 µL of extract at various concentrations (800; 400; 200; 100; 50; 25; 12.5 µg/mL) was combined with 160 µL of ABTS solution. After 10 minute incubation period, the absorbances were quantified at 734 nm.

2.4.3. FRAP Assay

The FRAP assay of *R. graveolens* extract was evaluated using the method previously described by Oyaizu (1986). To 10 µL of diluted extract at different concentrations (200; 100; 50; 25; 12.5; 6.25; 3.125 µg/mL), with 40 µL of phosphate buffer (pH = 6.6), followed by 50 µL of 1% potassium ferricyanide[K3Fe2(CN)6]. The plate was incubated at 50°C for 20 min. After incubation, 50 µL of 10% trichloroacetic acid was added,

then 40 µL of distilled water, and finally, 10 µL of a 0.1% FeCl3 solution, consecutively. The absorbance was directly measured at 700 nm.

2.4.4. β-Carotene Assay

The β-carotene test was performed according to the method of Marco (1968). With minor modifications; a solution of β-carotene was prepared by mixing 0.5 mg of β-carotene pouder dissolved in 1 mL of chloroform with 25 µL of linoleic acid, 200 µL tween (40), and 50 mL of oxygenated water. Then the absorbance of this solution was measured and adjusted to 0.9.Next, 40 µL of the extract at different concentrations was homogenated with 160 µL of the prepared solution. The plate was placed for incubation at 45 C for 2 h; after that, the absorbance was measured at 470 nm, for 0 and 120 min.

2.4.5. Silver Nanoparticle Assay

Silver nanoparticle assay, this test was assessed upon to the method of Özyürek (2012). Briefly, 50 mL of ($AgNO_3$ (1.0 mM)) was heated for 10 min then 5 mL of Trisodium citrate (1%) was added dropwise until the color changes to pale yellow. The solution is then left at room temperature to cool. A mixture of 20 µL of diluted Ruta extract, at different concentrations, 130 µL (SNP solution) and 50 µL H_2O was prepared in a microplate well and incubated in an oven at 25°C for 30 min. Then, the absorbance was directly measured at 423 nm by a microplate reader.

2.5. Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) Inhibitory Activities

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of the extract were determined by the method of Ellman *et al*. (1961). 150 µL of sodium phosphate buffer (100 mM, pH 8.0) mixed with 10 µL of the sample solution dissolved in ethanol at different concentrations (3.125-200 µg/mL) and 20 µL AChE or BChE solution in buffer , incubated for 15 min, then 10 µL of DTNB (0.5 mM) was added. The reaction was then initiated by the addition of 10 µL of acetylthiocholine iodide (0.5 mM) or 10 µL of butyrylthiocholine chloride (0.2 mM). The absorbance of the solution was measured at 412 by the use of 96-well microplate reader for T =0 min and T=15min at 37°C. The galantamine was used as reference. The results were given as IC_{50} value (µg/mL).

2.6. Inhibition of Alpha Amylase Activity

The α-Amylase inhibitory activity was performed using iodine/potassium iodide test according to Zengin *et al*. (2014) with some modifications, In a 96 - microplate well, 50 µL of α-amylase enzyme (1U) was added to 25 µL of extract, or acarbose (as a standard) at different concentrations, and the mixture was incubated at 37°C for 10 min. Then, 50 µL of 0.1% w/v soluble starch was diluted in sodium phosphate buffer (0.1 M), added to the sample wells, and incubated again for 10 min at 37°C. After incubation, 25 µL of (1 M)HCl was added to the reaction ,After that the addition of 100 µL of iodine reagent (IKI) (5 mM I2 and 5 mMKI) was next .The absorbance was measured at 630 nm, using a spectrophotometer.

Percentage of inhibition (%) = 1 − [(Ac − Ae) − (As − Ab)/(Ac − Ae)]

Ac =Absorbance of [50 µL of sodium phosphate buffer +35 µL of methanol solvent+ 50 µL of starch solution + 25 µL of HCl+100µLofIKI]

Ae =Absorbance of [50 µL of enzyme+ 50 µL of starch +35 µL of methanolsolvent + 25 µL of HCL + 100 µL of IKI]

As =Absorbance of sample = [50 µL of enzyme +35 µL of sample + 50 µL of starch + 25 µL of HCL+ 100 µL of IKI]

Ab =Absorbance of Blank of sample = Absorbance [35 µL of sample + 125 µL of buffer + 100 µL of IKI].

3. Results

3.1. Yield and Total Phenolic and Flavonoids Content

The results showed a relatively good extraction yield of 23%. The extract was pasty with a green black color. Results of colorimetric and spectrophotometric analysis of *R. graveolens* hydroethanolic extract were showed in Table 1. In general the hydroethanolic extract was found to have a good phenolic and flavonoids contents with 95.764±2.94 µg GAE/mg, 42.5±0.4 µg QE/mg, respectively (Table 1).

3.2. Identification of *R. graveolens* Polyphenols by LC-MS/MS

The results of the quantitative content of 53 different chemical components of the *R. graveolens* sample by LC- MS/MS are given in Figure 1 and Table 2.

In the present study, LC-MS/MS analysis of *R. graveolens* ethanol water extract was performed, resulting the identification of 14 phenolic components, the highest amounts was found in hesperidin (109.61 mg/g), followed by rutin (92.237 mg/g), nicotiflorin (4.827mg/g), quinic acid (2.687 mg/g), Compounds with moderate concentrations such as Quercetin (0.333 mg/g), Chlorogenic acid (0.248 mg/g), Protocatechuic acid (0.213 mg/g), p-Coumaric acid (0.108 mg/g) and isoquercitrin (0.097mg/g) were also found, Additionally, hesperetin, caffeic acid, apigenin, naringenin and acacetin were contained in negligible amounts as shown in Table 2.

For the LC-ESI-MS/MS analysis, the phenolic substances detected can be divided into phenolic acids and flavonoids, various metabolites were identified: hesperidin, rutin, nicotiflorin, quinic acid, Quercetin, Chlorogenic acid, Protocatechuic acid, p-Coumaric acid, isoquercitrin, hesperetin, caffeic acid, apigenin, naringenin and acacetin.

Table 1. Total phenol and flavonoid content of *R. graveolens* (n=3)

Plant	Yield	TPC (µg GAE/mg)	TFC (µg GAE/mg)
R.graveolens hydroethanolic extract	23%	95.764±2.94	42.5±0.4

3.3. Antioxidant Activity

The antioxidant activity was evaluated using five methods, *in vitro*, DPPH +, ABTS, reducing power (FRAP), β-carotene, silver nanoparticle (SNP). The results of the statistical analysis of antioxidant activity

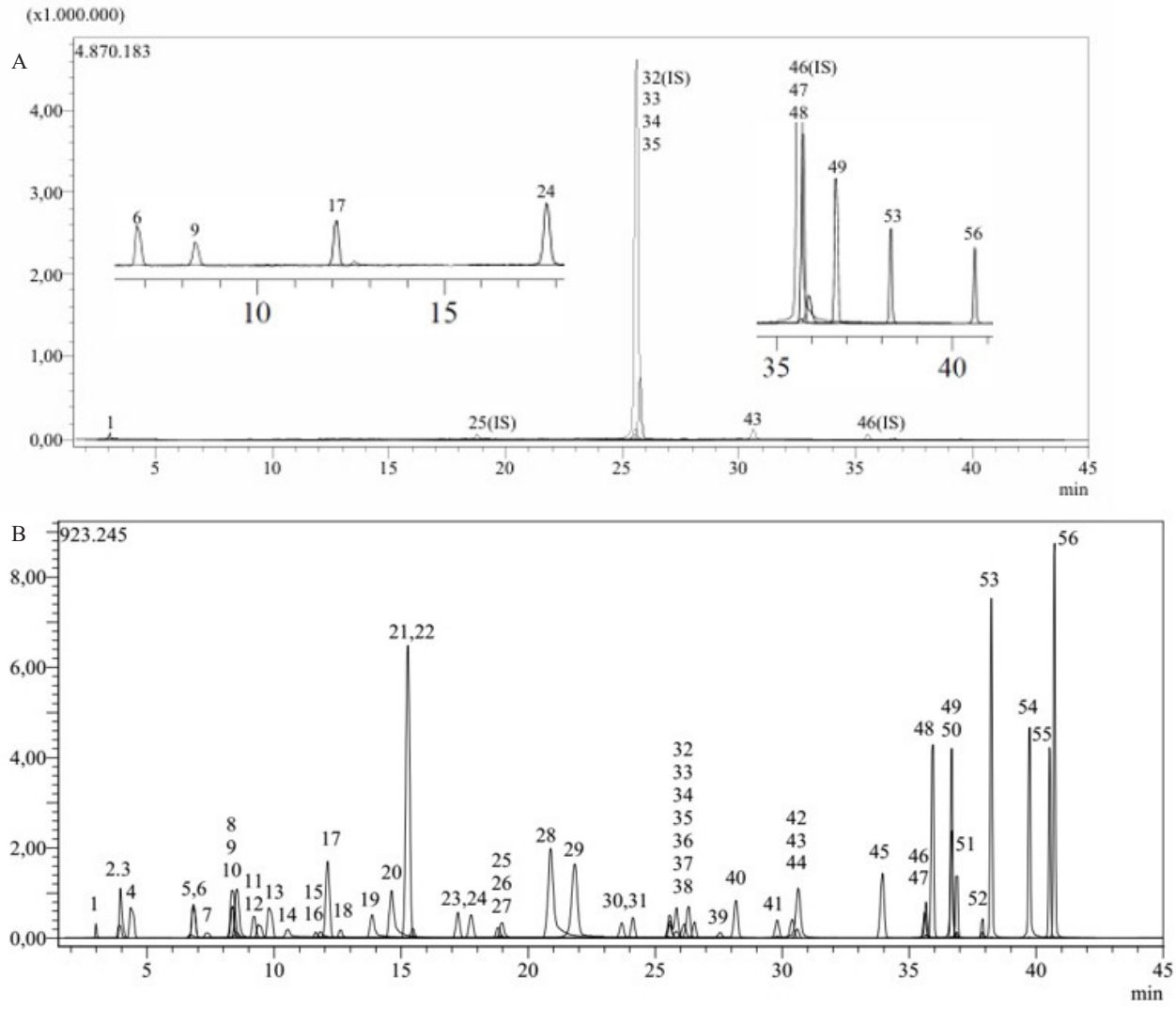

Figure 1. (A) LC-MS/MS chromatogram of standards and (B) *R. graveolens* ethanolic extract

Table 2. Identification and quantification of *Ruta graveolens* leaves phenol

Analyte	*R. graveolens*	RT	M.I (m/z)	F.I. (m/z)	r2	LOD/LOQ (µg/L)
Quinicacid	2.687	3.0	190.8	93.0	0.996	25.7/33.3
Fumaricacid	N.D.	3.9	115.2	40.9	0.995	135.7/167.9
Aconiticacid	N.D.	4.0	172.8	129.0	0.971	16.4/31.4
Gallicacid	N.D.	4.4	168.8	79.0	0.999	13.2/17.0
Epigallocatechin	N.D.	6.7	304.8	219.0	0.998	237.5/265.9
Protocatechuicacid	0.213	6.8	152.8	108.0	0.957	21.9/38.6
Catechin	N.D.	7.4	288.8	203.1	0.999	55.0/78.0
Gentisicacid	N.D.	8.3	152.8	109.0	0.997	18.5/28.2
Chlorogenicacid	0.248	8.4	353.0	85.0	0.995	13.1/17.6
Protocatechuicaldehyde	N.D.	8.5	137.2	92.0	0.996	15.4/22.2
Tannicacid	N.D.	9.2	182.8	78.0	0.999	15.3/22.7
Epigallocatechin gallate	N.D.	9.4	457.0	305.1	0.999	61.0/86.0
Cynarin	N.D.	9.8	515.0	191.0	0.999	5.8/9.4
4-OH Benzoicacid	N.D.	10.5	137,2	65.0	0.999	68.4/88.1
Epicatechin	N.D.	11.6	289.0	203.0	0.996	139.6/161.6
Vanilicacid	N.D.	11.8	166.8	108.0	0.999	141.9/164.9
Caffeicacid	0.043	12.1	179.0	134.0	0.999	7.7/9.5
Syringicacid	N.D.	12.6	196.8	166.9	0.998	82.3/104.5
Vanillin	N.D.	13.9	153.1	125.0	0.996	24.5/30.4
Syringicaldehyde	N.D.	14.6	181.0	151.1	0.999	19.7/28.0
Daidzin	N.D.	15.2	417.1	199.0	0.996	7.0/9.5
Epicatechin gallate	N.D.	15.5	441.0	289.0	0.997	19.5/28.5
Piceid	N.D.	17.2	391.0	135/106.9	0.999	13.8/17.8
p-Coumaricacid	0.108	17.8	163.0	93.0	0.999	25.9/34.9
Ferulic acid-D3-ISh	N.A.	18.8	196.2	152.1	N.A.	N.A.
Ferulicacid	N.D.	18.8	192.8	149.0	0.999	11.8/15.6
Sinapicacid	N.D.	18.9	222.8	193.0	0.999	65.2/82.3
Coumarin	N.D.	20.9	146.9	103.1	0.999	214.2/247.3
Salicylicacid	N.D.	21.8	137.2	65.0	0.999	6.0/8.3
Cyranoside	N.D.	23.7	447.0	284.0	0.997	12.1/16.0
Miquelianin	N.D.	24.1	477.0	150.9	0.999	10.6/14.7
Rutin-D3-IS	N.A.	25.5	612.2	304.1	N.A.	N.A.
Rutin	92.237	25.6	608.9	301.0	0.999	15.7/22.7
isoquercitrin	0.097	25.6	463.0	271.0	0.998	8.7/13.5
Hesperidin	109.61	25.8	611.2	449.0	0.999	19.0/26.0
o-Coumaricacid	N.D.	26.1	162.8	93.0	0.999	31.8/40.4
Genistin	N.D.	26.3	431.0	239.0	0.991	14.9/21.7
Rosmarinicacid	N.D.	26.6	359.0	197.0	0.999	16.2/21.2
Ellagicacid	N.D.	27.6	301.0	284.0	0.999	56.9/71.0
Cosmosiin	N.D.	28.2	431.0	269.0	0.998	6.3/9.2
Quercitrin	N.D.	29.8	447.0	301.0	0.999	4.8/6.4
Astragalin	N.D.	30.4	447.0	255.0	0.999	6.6/8.2
Nicotiflorin	4.827	30.6	592.9	255.0/284.0	0.999	11.9/16.7
Fisetin	N.D.	30.6	285.0	163.0	0.999	10.1/12.7
Daidzein	N.D.	34.0	253.0	223.0	0.999	9.8/11.6
Quercetin-D3-IS	N.A.	35.6	304.0	275.9	N.A.	N.A.
Quercetin	0.333	35.7	301.0	272.9	0.999	15.5/19.0
Naringenin	0.008	35.9	270.9	119.0	0.999	2.6/3.9
Hesperetin	0.048	36.7	301.0	136.0/286.0	0.999	7.1/9.1
Luteolin	N.D.	36.7	284.8	151.0/175.0	0.999	2.6/4.1
Genistein	N.D.	36.9	269.0	135.0	0.999	3.7/5.3
Kaempferol	N.D.	37.9	285.0	239.0	0.999	10.2/15.4
Apigenin	0,018	38.2	268.8	151.0/149.0	0.998	1.3/2.0
Amentoflavone	N.D.	39.7	537.0	417.0	0.992	2.8/5.1
Chrysin	N.D.	40.5	252.8	145.0/119.0	0.999	1.5/2.8
Acacetin	0.004	40.7	283.0	239.0	0.997	1.5/2.5

Abbreviations: ND, Not detected; R.T.: Retention time, MI (m/z): Molecular ions of the standard analytes (m/z ratio), FI (m/z): Fragment ionr2: Coefficient of determination, LOD/LOQ (µg/L): Limit of detection/quantification, N.A., not applicable

of *R. graveolens* with five methods were represented in the Figure 2. Ruta extract exhibited a good antioxidant capacity with varying levels represented by the IC_{50} values, which were 43.20± 0.96; 38.01±2.96 and 312.29 ±6.8 (µg/mL) for DPPH, ABTS and β-carotene assays, respectively. On the other hand, the A0.5 values for FRAP and SNP were 102.96±3.26 and 192.07±6.75 (µg/mL). Ruta extract showed a promising inhibition with DPPH and ABTS radicals, as indicated by the IC_{50} and A0.5 values, the tested sample has lower activity than those of references BHT, BHA, α-Tocopherol and ascorbic acid.

3.4. Acetyl Cholinesterase (AChE) and Butyrylcholinesterase (BChE) Inhibitory

The results of the inhibition of enzymatic activity (AChE) and (BChE) by *R .graveolen*s extract presented in Table 4. Anti-Alzheimer activity (Table 4) shows the AChE and BChE inhibitory activities of *Ruta graveolens*

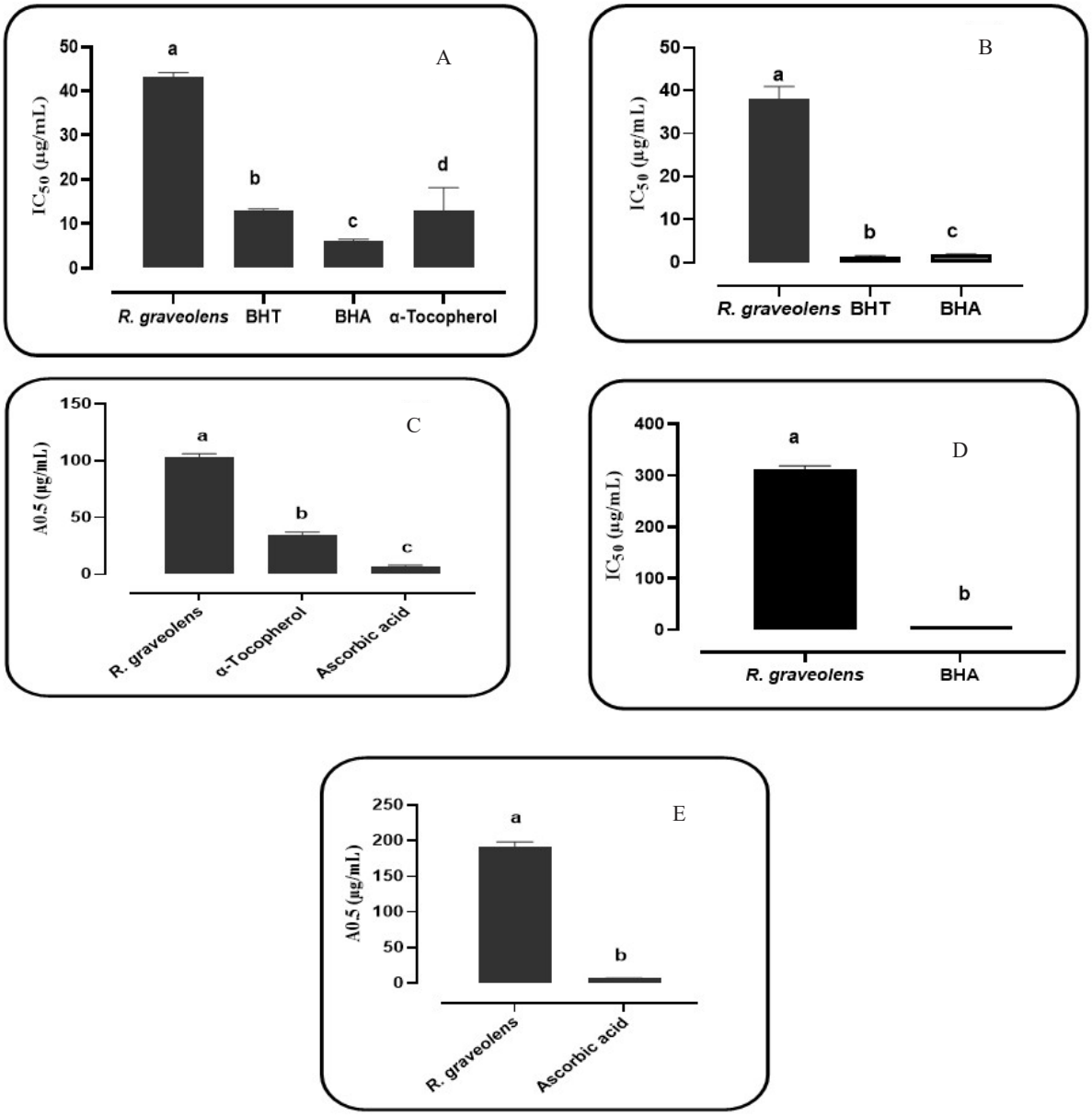

Figure 2. Antioxidant activities expressed as IC_{50} and A0.5 values of the studied extracts and standards determined by (A) DPPH, (B) ABTS, (C) FRAP, (D) β-carotene and (E) SNP assays. Results are expressed as mean ± SD. Kruskal-Wallis Test was used for statistical analysis for comparison with standards

hydroethanolic extract, compared with that of galantamine as reference. The results showed no activity against AChE, BChE enzymes at the concentration of 200 µg/mL. The galantamine was more effective than the extract with 6.27±1.15 µg/mL in AChE and 34.75±1.99 µg/mL in BChE.

3.5. Antidiabetic Activity

The antidiabetic activity expressed by α –Amylase enzyme inhibition of our extract presented in Table 5. The Ruta water ethanol extract offered an inhibition of α-amylase activity with a lower IC_{50} value (368.86±0.70 µg/mL) against (3431.01±2.72 µg/mL) for acarbose, respectively. Our extract was more effective than the reference.

4. Discussion

A hydro-ethanolic mixture was used to extract bioactive compounds from dried leaf powder of *Ruta graveolens*, and the results showed a relatively good extraction yield of 23%. The extract was pasty with a green black color. Our results of extraction yield are close to the finding of Barbouchi *et al*. (2024) who reported an extraction yield of 26.36% using ethanol as a solvent; the study was carried in Morocco. According to Bouabida and Dris (2022b), the hydro methanolic extract of the aerial part of Algerian *R. graveolens* was 19.14%. Also, the results obtained by Al qaisi *et al*. (2020) they recorded an extraction yield equal to 23.51% (w/w) of MeOH plant extract, this variation can be attributed to many factors such as : extraction methods, solvent type and concentrations, maceration time periods and plant development stage.

Precedent studies confirm the polyphenol richness of different *Ruta graveolens* extracts especially with the ethanolic extract (Pandey *et al*. 2011; Giresha *et al*. 2015; Elansary *et al*. 2020), our results of phenolic and flavonoid contents were found to be higher than that reported by Mokhtar *et al.* 2022 who reported a polyphenol content by 41.63±0.394 mg GAE/g E, the same for flavonoids with 13.97±0.33 mg EQ/gE in the phenolic extract of *R. graveolens* collected in Mostaganem, Algeria. Similarly, Yu *et al*. 2021 reported a lower phenolic 24.96±0.19 mg GAE/g DW and flavonoid contents 11.90±0.10 mg QE/g DW, Whereas, the results investigated by Barbouchi *et al.* (2024) who studied the quantitative analysis and antioxidant properties of crude extracts from different organs of three Moroccan Ruta species including *R. graveolens*, reported slightly higher phenolics at 143.79±0.72 (mg GAE/g CE) and a lower flavonoid content with 26.70±0.24(mg CAE/g CE).

The variations between these findings could be ascribed to several factors: collection time, that reflects the stage of plant development, also the geographic region, or the extraction technique (Dris and Bouabida 2023; Dris *et al*. 2024; Guenez *et al*. 2024).

The analysis revealed a rich profile of flavonoids in the *R. graveolens* leaves ethanol water extract, including hesperidin and rutin, which have high concentrations, known for their antioxidant, anti-inflammatory properties. Our findings showed a higher phenolic content compared to a previous research conducted by Mokhtar *et al.* 2022, who employed LC-PDA and identified only 9 phenolic compounds in Mostaganem (Algeria). Similarly, Caffeic acid, Chlorogenic acid, p-Coumaric acid, Protocatechuic acid, Quercetin and Rutoside were detected in the Saudi *R. graveolens* methanol leaf extract

Multiple studies have identified hesperidin, a flavanone glycoside, as the most abundant flavonoid besides rutin, in different Ruta spices (Qahtan *et al*. 2022; Szewczyk *et al.* 2022; Aouzal *et al.* 2024). The comprehensive phenolic profiles of *Ruta chalepensis* was extensively documented in the studies conducted by Loizzo *et al*. 2017 and Al-Jaberi *et al*. 2023, the methanolic and ethanolic extracts of *Ruta chalepensis* in Tunisia and Iraq respectively, confirms the presence of hesperidin.

Antioxidants can be described as compounds that inhibit food spoilage and protect the body against oxidative damage caused by free radicals, which are highly reactive molecules that can lead to oxidative stress. Antioxidants react at very low concentrations, by neutralizing free radicals through various mechanisms, by acting as reducing agents, hydrogen donors, chelators, and quenching singlet oxygen they help maintain cellular health and reduce the risk of chronic diseases (Zhong

Table 4. IC_{50} values (µg/mL) of the inhibition of enzymatic activity by *R. graveolens* extract

	(AChE) IC_{50} µg/ml	(BChE) IC_{50} µg/ml
EtOH extract	>200	>200
Galantamine	6.27±1.15	34.75±1.99

Table 5. IC_{50} values (µg/mL) of the inhibition of antidiabetic activity by *R. graveolens* extract

Inhibition	α –Amylase enzyme
Extract	379.84±6.06
Acarbose	3431.01±2.72

and Shahidi 2015; Szewczyk *et al*. 2022), in the case of DPPH and ABTS methods for free radical reduction, the antioxidant agents reduce the stable free radicals 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2-2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS),The extent of discoloration from the intense purple color of DPPH and the bluish-green color of ABTS reflects the compound's ability to scavenge free radicals or donate hydrogen atoms. For β-carotene, in an emulsion, the linoleicacidoxidation generate radicals that causes discoloration of β-carotene, which can be reduced by the antioxidants, indicating their protection against oxidative stress (Granados-Guzmán*et al*. 2017; Jianu *et al*. 2021).

The FRAP (ferric reducing antioxidant power) method measures antioxidant activity by assessing the ability of antioxidants in a sample to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), resulting in a color change that indicates the presence of antioxidants. It confirmed the presence of antioxidant activity in the leaf extract. This was indicated by the observation of a blue-purplish color formation when the extract reacted with the FRAP solution. This visual confirmation supports the extract's antioxidant properties ferrous-tripyridyl-triazine complex which has absorbtion at 593 nm (Nurinnafi'a *et al*. 2022; Then *et al*. 2003).

Previous studies have confirmed the potent antioxidant properties of *R. graveolens*, our results of the DPPH scavenging activity was consistent with the findings reported by Pavlović *et al*. 2014, were the methanolic extract of wild Rue collected at the end of flowering season showed substantial antioxidant activity in the DPPH test (IC_{50}=36.36±1.20 µg/mL). Elansary *et al*. 2020 mentioned that the methanolic leaf extract from northern Saudi Arabia showed a good activity with 21.3± 1.5 µg/ml, but contrasted with those reported by Szewczyk *et al*. 2022 and Cefali *et al*. 2019, who founds (IC_{50}=1.883 ± 0.007 mg/ml), (IC_{50} =281.02 ± 1.0 µg/ml)

Moreover, the hydro methanolic extract demonstrated a notable activity with IC_{50}= 0.10 ± 0.00 (mMTrolox g^{-1} DW) Conversely, Cefali *et al.* 2019 found that Ruta extract showed weak activity compared to our findings with an IC_{50}=587.98±0.8 µg/mL.

Regarding the β-carotene assay, our sample presented higher concentration with IC_{50}= 312, 29±6, 8 (µg/mL) compared to the BHA standard with 1.05±0.03 (µg/mL). Previous study conducted by Elansary *et al*. 2020 showed a superior antioxidant capacity compared to our result with IC_{50} =26.7±2.7 µg/mL. similarly, Mokhtar *et al*. 2022 reported a relevant activity with 0.37±0.03 (mMβ-CE/ g DW) for the phenolic extract of Algerian rue from Mostaganem.

In the reducing abilities, both FRAP and SNP assays demonstrated less effective capacity with A0.5:102, 96± 3, 26 and 192, 07± 6, 75 µg/mL respectively. Compared to the Ascorbic acid standard, which had an A0.5 value of 6.77±1.15 and 7.14 ±0.05 µg/mL. Our findings are contrasted with those reported by Elansary *et al*. 2020 and Yu *et al.* 2021. The silver nanoparticle assay results are obtained for the first time with *Ruta graveolens* leaves; No previous research has been conducted.

Overall, the diverse range of bioactive compounds present in plant extract highlights their potential as promotes agents against oxidative stress. Flavonoids are a group of non-enzymatic compounds with large health benefits, including, antioxidant, anti-inflammatory and cardioprotective effects, These involve rutin, hesperidin, quercetin, which were confirmed in different recent studies (Choi *et al*. 2021; Rahmani *et al*. 2023; Lee *et al*. 2024; Hu *et al*. 2025).These compounds were identified and detected by means of LC-ESI- MS/MS analysis, as represented in Table 2 They are capable of scavenging free superoxide radicals, reducing α-tocopherol radicals, hydrogen donors and metal chelators, inhibiting enzymes such as oxidases, this ability can be attributed to the phenolic hydroxyl groups attached to ring structures. Also the presences of Phenolic acids, specially, hydroxycinnamic acids, mainly, caffeic acid, coumarin and Chlorogenic acids which were identified in our plant extract, known to be a powerful antioxidant molecules (Carocho & Ferreira 2012). Besides, the observed variations in antioxidant efficacy may also be significantly influenced by the synergistic effect of these bioactive molecules. The obtained results clearly prove that the ethanolic extract of Ruta owns enhanced antioxidant properties that may be attributed to their phenolic and flavonoid composition.

A study conducted by Talic *et al*. 2014, the authors demonstrated that the methanolic and aqueous extracts of *R. graveolens* shows an inhibition percentage at a rate of 29.0±2.8; 32.2±1.5 respectively with butyrylcholinesterase (BChE) and 73.8±5.7; 80.0±0.8 with acetyl cholinesterase (AChE) both at the concentration of 400µg/mL. The same results were recorded by Wszelaki *et al.* 2010, where the hexane extract shows a good activity at the concentration of 400 µg/mL. It has been documented that a variety of natural substances from various chemical classes isolated from *R .graveolens* have been tested for their inhibitory

effects on (AChE) and (BChE) enzymes, In brief 16 chemical constituents of *Ruta graveolens* were tested in silico for their ability to inhibit AChE enzyme, only four constituents showed activity : high activity was recorded with Arborinine (IC_{50} 34.7±7.1 µM) a medium activity with Isoplatydesmine and norgraveoline (IC_{50} 205.6±16.3 µM 197.3±18.0 µM) in order, a weak activity with 6,7,8-Trimethoxycoumarin (IC_{50} 395.8 ±68.5 µM) , the other compounds showed no activity (IC_{50} > 500 µ M) (Rollinger *et al*. 2008). Further research was needed to fully understand the potential benefits of these treatments and determine their effectiveness for treating Alzheimer's both *in vitro* and *in vivo*.

α -Amylase is a digestive enzyme that catalyses the hydrolysis of carbohydrates 'starch/glycogen' into digestible forms, facilitating the absorption of nutrients in the body, produced by salivary glands and pancreas book, This enzyme plays a crucial role in carbohydrates metabolism, Understanding the mechanisms of α-amylase inhibition may lead to the development of new therapeutic strategies for metabolic disorders (Lekmine *et al.* 2023).

In our study, the *R. graveolens* extract demonstrated strong inhibition against-amylase enzyme compared to the reference (acarbose), a study carried out by Pandey *et al*. 2011 confirms the potential activity of Ruta ethanolic extract with an inhibition rate of 72.53% at 200 µg/mL.

According to the literature, many studies have reported the in vivo antidiabetic effect of Ruta extracts and rutin, highlighting their potential effect that leads to a notable amelioration of hyperglycemia, hyperlipidemia, enzyme regulation, and insulin production (Ahmed *et al*. 2010; Velmurugan *et al*. 2022). The secondary metabolites present in *Ruta graveolens* extract may be effective against diabetes. Previous studies proved that phenols can be functional against α-amylase and α-glycosidase enzymes through various mechanisms by interacting in both hydrophobic and hydrogen bonds within polyphenols and the key amino acids constituting the active site enzymes. These interactions can lead to the inhibition of carbohydrate digestion and absorption, eventually helping to control blood glucose levels. Additionally, the antioxidant properties of phenols in Ruta extracts may also contribute to their potential antidiabetic effects by reducing oxidative stress and inflammation associated with diabetes (Asgar 2012, Rasouli *et al*. 2017; Velmurugan *et al*. 2022).

In Conclusion, The current research focused on the phenolic composition of Ruta leaves extract, the antioxidant, cholinesterase and α-amylase inhibition activities were evaluated. After the analysis with LC-ESI-MS/MS, fourteen compounds were identified, hesperidin and rutin was the most abundant. This study reported for the first time the anti-alzheimer and antidiabetic activities of the Algerian *Ruta graveolens*. Additionally, the antioxidant activity was assessed using four methods: DPPH, ABTS, β -carotene, FRAP and SNP, the water ethanol extract of Ruta leaves confirms a very good activity especially with DPPH and ABTS assays. The obtained results highlighted the potential uses of *Ruta graveolens* as a natural source for antioxidants and as a general healthcare agent, with applications in metabolic dysfunction diseases such as diabetes.

Conflict of Interest

The authors have no conflict of interest to declare.

References

Ahmed, O.M., Moneim, A.A., Yazid, I.A., Mahmoud, A.M., 2010. Antihyperglycemic, antihyperlipidemic and antioxidant effects and probable mechanisms of action of *Ruta graveolens* infusion and rutin in nicotinamide-streptozotocin-induced diabetic rats. *Diabetologia Croatica*. 39, 15.

Al-Jaberi, A.M.Z., Al-Fadal, S.A.M., Abdul-Jalil, T.Z., Al-Wafi, H., 2023. HPLC isolation of rutin, hesperidin and quercetin from *Ruta chalepensis* extract growing in Iraq. P*harmacognosy Journal*. 15, 606-611. https://doi.org/10.5530/pj.2023.15.127

Al-Qaisi, Y., Khleifet, K.M., Atallah, S., 2020. *In vitro* quantitative assessment of viability of *Echinococcus granulosus* protoscoleces after treatment with *Ruta graveolens* L. methanolic extract. *Journal of Pharmaceutical Research*. 13. https://doi.org/10.31838/ijpr/2021.13.01.487

Aouzal, B., Slimani, S., Kamah, F., Ounissi, I., Bouacha, A., Elmokli, S., 2024. LC–MS/MS phytochemical analysis of *Ruta montana* and the hepatic preventive effects in male rats exposed to tebuconazole. *Global NEST Journal*. 26. https://doi.org/10.30955/gnj.06877

Asgar, M.A., 2012. Anti-diabetic potential of phenolic compounds: a review. *International Journal of Food Properties*. 16, 91-103. https://doi.org/10.1080/10942912.2011.595864

Ayoola, G., Ipav, S., Sofidiya, M., Adepoju-Bello, A., Coker, H., Odugbemi, T., 2009. Phytochemical screening and free radical scavenging activities of the fruits and leaves of *Allanblackia floribunda* Oliv. (Guttiferae). *International Journal of Health Research*. 1, 85-93. https://doi.org/10.4314/ijhr.v1i2.47920

Barbouchi, M., Benzidia, B., Elamrani, K., Sabiri, M., Idrissi, M.E., Choukrad, M., 2024. Phytochemical screening, quantitative analysis and antioxidant properties of crude extracts from stems, leaves, and flowers of three Ruta species. *Kuwait Journal of Science*. 51, 100287. https://doi.org/10.1016/j.kjs.2024.100287

Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. *Nature*. 181, 1199-1200. https://doi.org/10.1038/1811199a0

Bouabida, H., Dris, D., 2020. Effect of rue (*Ruta graveolens*) essential oil on mortality, development, biochemical and biomarkers of *Culiseta longiareolata*. *South African Journal of Botany*. 133, 139-143. https://doi.org/10.1016/j.sajb.2020.07.005

Bouabida, H., Dris, D., 2022a. Biological toxicity of *Ruta graveolens* essential oil against three species of Diptera *Drosophila melanogaster*, *Culex pipiens* and *Culiseta longiareolata*. *Journal of Vector Borne Diseases*. 59, 320-326. https://doi.org/10.4103/0972-9062.353272

Bouabida, H., Dris, D., 2022b. Phytochemical constituents and larvicidal activity of *Ruta graveolens*, *Ruta montana* and *Artemisia absinthium* hydro-methanolic extract against mosquito vectors of avian plasmodium (*Culiseta longiareolata*). *South African Journal of Botany*. 151, 504-511. https://doi.org/10.1016/j.sajb.2022.05.017

Carocho, M., Ferreira, I.C., 2012. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*. 51, 15-25. https://doi.org/10.1016/j.fct.2012.09.021

Cefali, L.C., Ataide, J.A., Fernandes, A.R., Sanchez-Lopez, E., De Oliveira Sousa, I.M., Figueiredo, M.C., Ruiz, A.L.T.G., Foglio, M.A., Mazzola, P.G., Souto, E.B., 2019. Evaluation of *in vitro* solar protection factor (SPF), antioxidant activity, and cell viability of mixed vegetable extracts from *Dirmophandra mollis* Benth, *Ginkgo biloba* L., *Ruta graveolens* L., and *Vitis vinifera* L. *Plants*. 8, 453. https://doi.org/10.3390/plants8110453

Choi, S., Park, H., Lee, K., 2021. A comparative study of rutin and rutin glycoside: antioxidant activity, anti-inflammatory effect, effect on platelet aggregation and blood coagulation. *Antioxidants*. 10, 1696. https://doi.org/10.3390/antiox10111696

Colucci-D'Amato, L., Cimaglia, G., 2020. *Ruta graveolens* as a potential source of neuroactive compounds to promote and restore neural functions. *Journal of Traditional and Complementary Medicine*. 10, 309-314. https://doi.org/10.1016/j.jtcme.2020.05.002

Dris, D., Bouabida, H., 2023. Assessment of larvicidal and pupicidal activities of *Mentha piperita* essential oil and effects in biomarkers and morphometric aspects against two mosquito species (*Culiseta longiareolata* and *Culex pipiens*). *International Journal of Tropical Insect Science*. 43, 1897-1909. https://doi.org/10.1007/s42690-023-01067-7

Dris, D., Bouabida, H., Seghier, H., Gheraibia, S., 2024. Larvicidal activity of methanolic and aqueous extracts of *Lavandula dentata* and *Nerium oleander* flowers against the vector of avian malaria *Culiseta longiareolata*. *Tropical Biomedicine*. 41, 441-449. https://doi.org/10.47665/tb.41.4.004

Elansary, H.O., Szopa, A., Kubica, P., Ekiert, H., El-Ansary, D.O., Al Mana, F.A., Mahmoud, E.A., 2020. Polyphenol content and biological activities of *Ruta graveolens* L. and *Artemisia abrotanum* L. in northern Saudi Arabia. *Processes*. 8, 531. https://doi.org/10.3390/pr8050531

Ellman, G.L., Courtney, K.D., Andres, V., Featherston, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*. 7, 88-95. https://doi.org/10.1016/0006-2952(61)90145-9

Fakchich, J., Elachouri, M., 2014. Ethnobotanical survey of medicinal plants used by people in Oriental Morocco to manage various ailments. *Journal of Ethnopharmacology*. 154, 76-87.

Fenghour, H., Bouabida, H., 2024. GC/MS analysis, antimicrobial, fungicidal activities and toxicity of *Rosmarinus officinalis* essential oil plant from Algeria. *Polish Journal of Environmental Studies*. 34, 2665-2672 https://doi.org/10.15244/pjoes/188054

Giresha, A.S., Anitha, M.G., Dharmappa, K.K., 2015. Phytochemical composition, antioxidant and *in vitro* anti-inflammatory activity of ethanol extract of *Ruta graveolens* L. leaves. *International Journal of Pharmacy and Pharmaceutical Sciences*. 7, 272-276.

Guenez, R., Dris, D., Grabsi, S., Bouabida, H., 2024. Larvicidal activity of an *Origanum majorana* plant in two mosquito species: *Culex pipiens* and *Culiseta longiareolata* (Diptera: Culicidae). *Journal of Entomological Research*. 48, 162-166. https://doi.org/10.5958/0974-4576.2024.00033.2

Granados-Guzman, G., Salazar-Aranda, R., Garza-Tapia, M., Castro-Rios, R., De Torres, N.W., 2017. Optimization and validation of two high-throughput methods indicating antiradical activity. *Current Analytical Chemistry*. 13. https://doi.org/10.2174/1573411013666170118111516

Hu, Y., Liang, X., Li, X., Han, Y., Lang, G., 2025. *Rutin ameliorates* imiquimod-induced psoriasis-like skin lesions by inhibiting oxidative stress injury and the inflammatory response in mice via the Keap1/Nrf2 signaling pathway. *Scientific Reports*. 15, 20712 https://doi.org/10.1038/s41598-025-08451-y

Islam, F., Khadija, J.F., Harun-or-Rashid, M., Rahaman, M.S., Nafady, M.H., Islam, M.R., Akter, A., Emran, T.B., Wilairatana, P., Mubarak, M.S., 2022. Bioactive compounds and their derivatives: an insight into prospective phytotherapeutic approach against Alzheimer's disease. *Oxidative Medicine and Cellular Longevity*. 2022, 1-22. https://doi.org/10.1155/2022/5100904

Jianu, C., Goleț, I., Stoin, D., Cocan, I., Bujancă, G., Mișcă, C., Mioc, M., Mioc, A., Șoica, C., Lukinich-Gruia, A.T., Rusu, L., Muntean, D., Horhat, D.I., 2021. Chemical profile of *Ruta graveolens*, evaluation of the antioxidant and antibacterial potential of its essential oil, and molecular docking simulations. *Applied Sciences*. 11, 11753. https://doi.org/10.3390/app112411753

Lee, G.B., Kim, Y., Lee, K.E., Vinayagam, R., Singh, M., Kang, S.G., 2024. Anti-inflammatory effects of quercetin, rutin, and troxerutin result from the inhibition of NO production and the reduction of COX-2 levels in RAW 264.7 cells treated with LPS. *Appl Biochem Biotechnol.* 196.8431-8452. https://doi.org/10.21203/rs.3.rs-3914566/v1

Lekmine, S., Benslama, O., Kadi, K., Martín-García, A.I., Yilmaz, M.A., Akkal, S., Boumegoura, A., Alhomida, A.S., Ola, M.S., Ali, A., 2023. LC/MS-MS analysis of phenolic compounds in *Hyoscyamus albus* L. extract: *in vitro* antidiabetic activity, *in silico* molecular docking, and *in vivo* investigation against STZ-induced diabetic mice. *Pharmaceuticals*. 16, 1015. https://doi.org/10.3390/ph16071015

Loizzo, M.R., Saab, A.M., Tundis, R., Menichini, F., Bonesi, M., Piccolo, V., Statti, G.A., Cindio, B.d., Houghton, P.J., Menichini, F., 2008. *In vitro* inhibitory activities of plants used in Lebanon traditional medicine against angiotensin converting enzyme (ACE) and digestive enzymes related to diabetes. *Journal of Ethnopharmacology*. 119, 109-116. https://doi.org/10.1016/j.jep.2008.06.003

Loizzo, M.R., Falco, T., Bonesi, M., Sicari, V., Tundis, R., Bruno, M., 2017. *Ruta chalepensis* L. (Rutaceae) leaf extract: chemical composition, antioxidant and hypoglycaemic activities. *Natural Product Research.* 32, 521-528. https://doi.org/10.1080/14786419.2017.1326491

Marco, G.J., 1968. A rapid method for evaluation of antioxidants. *Journal of the American Oil Chemists' Society*. 45, 594-598.

Mokhtar, M., Youcefi, F., Keddari, S., Saimi, Y., Elhaou, S.O., Cacciola, F., 2022. Phenolic content and in vitro antioxidant, anti-inflammatory and antimicrobial evaluation of Algerian *Ruta graveolens* L. *Chemistry & Biodiversity.* 19, e202200545. https://doi.org/10.1002/cbdv.202200545

Müller, L., Gnoyke, S., Popken, A.M., Böhm, V., 2010. Antioxidant capacity and related parameters of different fruit formulations. *LWT - Food Science and Technology.* 43, 992-999. https://doi.org/10.1016/j.lwt.2010.02.004

Nurinnafi'a, A.M.U., Artini, K.S., Permatasari, D.A.I., 2022. Total flavonoid content of lemongrass leaf (*Cymbopogon citratus* (DC.) Stapf) extract and antioxidant activity with FRAP. *Journal of Fundamental and Applied Pharmaceutical Science.* 30-36, progress. https://doi.org/10.18196/jfaps.v3i1.15556

Orhan, I., Aslan, S., Kartal, M., Şener, B., Başer, K.H.C., 2008. Inhibitory effect of Turkish *Rosmarinus officinalis* L. on acetylcholinesterase and butyrylcholinesterase enzymes. *Food chemistry*. 108, 663-668. https://doi.org/10.1016/j.foodchem.2007.11.023

Oyaizu, M., 1986. Studies on products of browning reactions: antioxidative activities of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition.* 44, 307-315. https://doi.org/10.5264/eiyogakuzashi.44.307

Özyürek, M., Güngör, N., Baki, S., Güçlü, K., Apak, R., 2012. Development of a silver nanoparticle-based method for the antioxidant capacity measurement of polyphenols. *Analytical Chemistry.* 84, 8052-8059. https://doi.org/10.1021/ac301925b

Pandey, P., Mehta, A., Hajra, S., John, J., Mehta, P., 2011. Antioxidant property, total phenolic content and inhibition of α-amylase activity of *Ruta graveolens* L. leaves extract. *Journal of Pharmacy Research.* 4, 1735-1737.

Pavlović, D.R., Vukelić, M., Najman, S., Kostić, M., Zlatković, B., Mihajilov-Krstev, T., Kitić, D., 2014. Assessment of polyphenol content, *in vitro* antioxidant, antimicrobial and toxic potentials of wild growing and cultured rue. *Journal of Applied Botany and Food Quality*. 87, 175-181. https://doi.org/10.5073/JABFQ.2014.087.025

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., Bitto, A., 2017. Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity.* 2017, 8416763. https://doi.org/10.1155/2017/8416763

Qahtan, A.A., Faisal, M., Alatar, A.A., Abdel-Salam, E.M., 2022. Callus-mediated high-frequency plant regeneration, phytochemical profiling, antioxidant activity and genetic stability in *Ruta chalepensis* L. *Plants.* 11, 1614. https://doi.org/10.3390/plants11121614

Rahmani, S., Naraki, K., Roohbakhsh, A., Hayes, A.W., Karimi, G., 2023. The protective effects of rutin on the liver, kidneys, and heart by counteracting organ toxicity caused by synthetic and natural compounds. *Food Science & Nutrition.* 11, 39-56. https://doi.org/10.1002/fsn3.3041

Rasouli, H., Hosseini-Ghazvini, S.M., Adibi, H., Khodarahmi, R., 2017. Differential α-amylase/α-glucosidase inhibitory activities of plant-derived phenolic compounds: a virtual screening perspective for the treatment of obesity and diabetes. *Food & Function.* 8, 1942-1954. https://doi.org/10.1039/c7fo00220c

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine.* 26, 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Rollinger, J., Schuster, D., Danzl, B., Schwaiger, S., Markt, P., Schmidtke, M., Gertsch, J., Raduner, S., Wolber, G., Langer, T., Stuppner, H., 2008. *In silico* target fishing for rationalized ligand discovery exemplified on constituents of *Ruta graveolens*. *Planta Medica.* 75, 195-204. https://doi.org/10.1055/s-0028-1088397

Szablewski, L., 2025. Associations between diabetes mellitus and neurodegenerative diseases. *International Journal of Molecular Sciences.* 26, 542. https://doi.org/10.3390/ijms26020542

Szewczyk, A., Marino, A., Molinari, J., Ekiert, H., Miceli, N., 2022. Phytochemical characterization and antioxidant and antimicrobial properties of agitated cultures of three rue species: *Ruta chalepensis*, *Ruta corsica*, and *Ruta graveolens*. *Antioxidants.* 11, 592. https://doi.org/10.3390/antiox11030592

Talić, S., Dragičević, I., Ćorajević, L., Martinović Bevanda, A., 2014. Acetylcholinesterase and butyrylcholinesterase inhibitory activity of extracts from medicinal plants. *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina.* 43, 11-14.

Then, M., Szentmihályi, K., Sárközi, Á., Szöllosi Varga, I., 2003. Examination on antioxidant activity in the greater celandine (*Chelidonium majus* L.) extracts by FRAP method. *Acta Biologica Szegediensis.* 47, 115-117. http://abs.bibl.uszeged.hu/index.php/abs/article/download/2357/2349

Velmurugan, C., Subramaniyan, V., Ilanthalir, S., Fuloria, S., Sekar, M., Fuloria, N.K., 2022. Evaluation of anti-diabetic and wound healing potential of Ethiopian plant *Ruta graveolens* in diabetic induced rat. *Natural Volatiles & Essential Oils Journal.* 2022, 5629-5640.

Wszelaki, N., Kuciun, A., Kiss, A., 2010. Screening of traditional European herbal medicines for acetylcholinesterase and butyrylcholinesterase inhibitory activity. *Acta Pharmaceutica.* 60, 1-10. https://doi.org/10.2478/v10007-010-0006-y

Yilmaz, M.A., 2020. Simultaneous quantitative screening of 53 phytochemicals in 33 species of medicinal and aromatic plants: a detailed, robust and comprehensive LC–MS/MS method validation. *Industrial Crops and Products.* 149, 112347. https://doi.org/10.1016/j.indcrop.2020.112347

Yu, M., Gouvinhas, I., Rocha, J., Barros, A.I.R.N.A., 2021. Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. *Scientific Reports.* 11, 89437. https://doi.org/10.1038/s41598-021-89437-4

Zengin, G., Sarikurkcu, C., Aktumsek, A., Ceylan, R., Ceylan, O., 2014. A comprehensive study on phytochemical characterization of *Haplophyllum myrtifolium* Boiss. endemic to Turkey and its inhibitory potential against key enzymes involved in Alzheimer, skin diseases and type II diabetes. *Industrial Crops and Products.* 53, 244-251.

Zhong, Y., Shahidi, F., 2015. Methods for the assessment of antioxidant activity in foods. *Journal of Functional Foods.* 18, 287-333. https://doi.org/10.1016/b978-1-78242-089-7.00012-9