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Original Article

Chemical Profiling and *in vitro* Evaluation of the Antioxidant, Anti-inflammatory, and Anti-bacterial Effects of Algerian *Solanum melongena* L.

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ABSTRACT

Aim: *Solanum melongena* L., commonly known as eggplant, is a low-calorie vegetable with high nutritional value due to its rich fiber, vitamins, minerals, and bioactive compounds, particularly polyphenols, which provide antioxidant properties. This study aimed to investigate the chemical composition and biological activities of methanolic extracts from purple *S. melongena* fruit. **Materials and Methods:** The chemical and biological properties of the methanolic extract were analyzed using both qualitative and quantitative methods. Bioactive compounds were identified through standard methods and Gas Chromatography-Mass Spectrometry (GC-MS). The antioxidant capacity was assessed using free radical scavenging (DPPH, ABTS) and ferric reducing power assays. The extract's urease inhibition and anti-inflammatory effects were also tested, and antimicrobial activity was evaluated via the agar diffusion method. **Results:** Qualitative analysis detected phenols, alkaloids, terpenoids, steroids, flavonoids, saponins, and anthraquinones. Total phenolic and flavonoid contents were found to be 81 mg GEA/kg and 75 mg QE/kg, respectively. GC-MS analysis revealed 44 chemical compounds, with quinic acid being the most abundant (>60% peak area). Most identified compounds had not been previously reported in *S. melongena L*. The methanolic extract displayed moderate antioxidant activity across DPPH, ABTS, reducing power, and phenanthroline assays and showed similar moderate effects in urease inhibition and anti-inflammatory assays. Antibacterial activity was strong against all tested species. **Discussion:** The methanolic extract of *S. melongena* L. exhibits promising antioxidant, anti-urease, anti-inflammatory, and antibacterial properties. However, further research, including metabolomic studies, is necessary to fully understand the metabolic profile and action mechanisms of the identified compounds.

Keywords: Antioxidant, phenolic, *Solanum melongena*, Gas Chromatography-Mass Spectrometry.

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INTRODUCTION

Eggplant (*Solanum melongena* L.) is an economically important vegetable plant from the Solanaceae family that originated in southern Asian countries. Around the world, several varieties of eggplant are cultivated, varying in shape, color, and size.[1] These varieties include about 1,400 species.[2] A large proportion of eggplant production is concentrated in Asia and the Mediterranean basin. In Algeria, the most commonly cultivated variety is the elongated, ovoid, dark purple eggplant.[3] It is widely grown in many parts of the country and is commonly referred to as "Bedinjan." Algeria's national production was approximately 207,548.83 tons, covering 6,626 hectares of cultivated land (Faostat Database, 2022).[4]

Solanum melongena L. contains significant quantities of macro- and micronutrients, such as fiber, protein, minerals, and vitamins, making it a fruit with high medicinal value.[5] In addition, it contains phytochemicals such as phenolic acids, flavonoids, tannins, alkaloids, and terpenoids, which have strong antioxidant activity.[6,7] This activity is attributed to their ability to neutralize oxygen radicals. Several reports have highlighted the

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biological activities of eggplant extract, including its antioxidant, antidiabetic, anti-inflammatory, antimicrobial, and anticancer properties.[8-10] For this reason, eggplant holds significant economic, pharmaceutical, and medicinal importance.

Worldwide studies have focused on the chemical and biochemical analysis of *S. melongena* L., though only a few have examined the phenolic content and antioxidant effects of Algerian eggplant. To date, there have been no reports on the chemical profile of methanolic extracts from local *S. melongena* L. fruits. This study aims to identify the phytochemicals present in these extracts using Gas Chromatography-Mass Spectrometry (GC-MS) and to evaluate their efficacy as antioxidants, anti-inflammatory, and antimicrobial agents.

MATERIALS AND METHODS

Plant material

In September 2022, we harvested *S. melongena* L. fruits (Figure 1) from the Amira Arres commune in Mila city, located in northern Algeria. The fruits were Identified by Dr. Yahia Abdewahab, Professor at the University Center of Mila, Algeria. After collection, the fresh fruits were cleaned, washed with potable water followed by distilled water, and then sliced into 1 cm-thick pieces. The slices were dried in a cool, well-ventilated area away from light. Once fully dried, the samples were ground using an electric blender. The resulting powders were stored in airtight, opaque bottles to maintain dryness, preparing them for subsequent analysis.

Extraction procedure

Thirty grams (30 g) of *S. melongena* L. fruit powder was extracted with 300 mL of methanol for 4 hr using a Soxhlet extractor. The separation of the extract from the solvent was carried out using a rotavapor at reduced temperature and pressure. All dry extracts obtained were stored in a glass bottle and kept at 4°C until use.[11]

Phytochemicals screening of the extract

We qualitatively determined the main classes of chemical compounds present in *S. melongena* L. using standard protocols, primarily based on either precipitation processes or coloring reactions. Methanolic extract of *S. melongena* (MES) was investigated for phenolic compounds and tannins (ferric chloride), flavonoids (HCl and magnesium ribbon), alkaloids (Wagner and Mayer method), triterpenes and steroids (Liebermann-Buchard method), saponins (Foam), amidon (Iode reagent), glycosides (Keller-Kilanie method), and anthraquinons (Borntrager test).[12]

Determination of total bioactive compounds

Total phenolic content

Total phenolic content (TPC) was determined with Folin–Ciocalteu's reagent (FCR) according to Aksoy *et al.* (2013).[13] Briefly, 20 µL of *S. melongena* methanolic extract was mixed with 100 µL of FCR (1:10). Then, 75 µL of Na_2CO_3 solution (7,5%) was added to the mixture and the reaction was kept in darkness at room temperature for 2 hr, the absorbance was then read at 765 nm using 96-well microplate reader (ENSPRO ®, Singapore). The TPC was expressed as micrograms of gallic acid equivalents /g of extract (µg AGE/mg) using the linear regression equation of the calibration curve plotted by gallic acid (y = 0.5206 x + 0.0029, R2 = 0.9952).

Total flavonoid content

The aluminum nitrate method was used to determine the Total Flavonoid Content (TFC), according to Topçu *et al.* (2007).[14] Methanolic extract solution (50 µL) was mixed with 130 µL of methanol, 10 µL of aluminum nitrate, and 10 µL of aqueous potassium acetate (1M). After 40 min at room temperature, the absorbance was measured spectrophotometrically (ENSPRO®, Singapore) at 415 nm. TFC was expressed as micrograms of quercetin equivalents/g of extract (y = 0.0344x + 0.0181, R2 = 0.9992).

Phytochemical analysis (CG/SM determination of phenolic compounds)

Bioactive molecules in our samples were identified using the technique of Gas Chromatography coupled with Mass Spectrometry (CG/MS) of type Shimadzu QP2010 operating in electronic impact mode at 70 eV, quadrupole, equipped with Columns SE30 (25 m in length, 0.25 µm in thickness of film). 1µl of carrier gas (helium) was injected in split-less mode at injection temperature 250°C and ion-source temperature 200°C. The oven temperature was programmed to move from 50°C for 0.0 min to 280°C with a rate of 5°C/min and a holding time of approximately 2 min. The parameters of the spectrometer were as follows: Pressure: 40.0 kPa, start time: 3 min, end time: 40,0 min, ACQ mode: scan, interval: 0.50 sec, scan speed: 666, start *m/z*: 40, end *m/z*: 350.[15]

Evaluation of antioxidant and enzymatic activities of the extract

We conducted all analyses on 96-well microplates and measured the corresponding absorbances using a multimode plate reader (ENSPRO®, Singapore). We calculated the 50% inhibition concentration (IC_{50}) for DPPH and ABTS using the linear regression of inhibitory activities versus extract focuses. The results of the reducing power and phenanthroline tests were shown as the concentration of the extract with an absorbance value of 0.5 (A0.5, µg/mL), as seen in the graph of absorbance versus extract concentrations.

DPPH radical scavenging activity

The antiradical activity of extracts against the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was performed following

the method described by Blois.[16] Briefly, 40 µL of the extract was mixed with 160 µL of the DPPH solution. After 30 min of incubation in the dark, the absorbance was recorded at 517 nm. A DPPH solution mixed with an equal volume of distilled water served as a control. Solutions of α-tocopherol and BHT (butylated hydroxytoluene) were used as standards. The activity is calculated following the formula:

$$\text{Antioxidant activity } (\%) = Ac - Ae/\ Ac \times 100 \ldots\ldots(1)$$

Where: **Ae** is the absorbance of the extract, and **Ac** is the absorbance of the control.

ABTS radical scavenging activity

The scavenging activity of extracts against 2,2'-azinobis-(3-ethylb enzothiazoline-6-sulfonic acid) (ABTS) free radicals was carried out following the method described by Re *et al.* (199).[17] To 40µL of extract, 160µL of ABTS solution were added (a mixture of 7 mM ABTS and 2.45 mM potassium persulfate was incubated in the dark for 14-16 hr and adjusted to 0.700 at 734 nm before use). After 10 min, the absorbance was measured at 734 nm, and BHT and BHA (butylated hydroxyanisole) were used as antioxidant standards. ABTS scavenging activity in percentage (%) was calculated using the equation (1).

The concentration range for antioxidant activities (DPPH and ABTS) is from 12.5 to 800 µg/mL (micrograms/mL).

Ferric reducing power

The ferric-reducing antioxidant power (FRAP) of *S. melongena* methanolic extract was conducted according to Benzie & Strain[18] with slight modifications. 10 µL of extract were supplemented with 40 µL of phosphate buffer (pH 6.6) and 50 µL of potassium ferricyanide (1%). After incubation of the mixture for 10 min at 50°C, 50 µL of trichloroacetic acid (10%), 30 µL of distilled water, and 10 µL of $FeCl_3$ (0.1%) were consecutively added, and the absorbance was measured at 700 nm. α-Tocophérol and ascorbic acid were used as standards.

Phenanthroline assay

The antioxidant capacity test by phenanthroline was performed following the method of Szyd Szydlowska-Czerniaka *et al.*, (2008).[19] To 10 µL of the extract, 50 µL of $FeCl_3$ (0.2%), 30 µl of Phenanthroline (0.5%) and 110 µL of mehanol were added. After 20 mn of incubation in the dark at 30°C; the absorbance was then recorded at 510 nm. BHA and BHT were used as antioxidant standards for comparaison purpose. The above equation was also used for calculate the antioxidant extent of the extract.

Urease Inhibitory Assay

Urease inhibitory activity was performed as described by Weatherburn.[20] The method is based on the measurement of ammonia production through phenol reagents. It consists of mixing 10 µL of various concentrations of extract with 25 µL of enzyme solution and 50 µL of substrate solution prepared in phosphate buffer solution (3 Mm, pH 8.2). After an incubation of 15 min at 30°C, 45 µL of phenol reagent consisting of a mixture of 1% phenol and 0.005% of sodium nitroprusside and 70 µL of alkaline reagent (2.85% NaOH and 4.7% active chloride NaCl) were added at each concentration. After 50 min, the absorbance was read at 630nm using a multimode microplate reader (ENSPRO®, Singapore). The inhibition percentage of the urease activity was calculated using the following equation:

$$\%\ \text{Inhibition} = [(\text{Abs sample} - \text{Abs control}) / \text{Abs control}] \times 100$$

Where: $\text{Abs}_{\text{control}}$ is the absorbance of the control reaction.

Figure 1: The photograph of *Solanum melongena* L. from its native habitat in Amira Arres (Mila city, Algeria).

Abs_{sample} is the absorbance of the extract.

The concentration range for anti-urease enzymatic activity is from 3.125 to 200 µg/mL (micrograms/mL).

Anti-Inflammatory activity

The *in vitro* anti-inflammatory activity of *S. melongena* methanolic extract was determined via the protein denaturation method using bovine serum albumin, as reported by Kandikattu *et al.*[21] 0.5 mL of Bovine Serum Albumin (BSA) solution (0.2% prepared in 0.05 M tris-buffered saline, pH 6.6) were mixed with 0.5 mL of plant extract solution in various concentrations (2000, 1000, 500, and 250 µg/mL). The same volume of BSA solution with 50 µL of methanol was used as the test solution control. The results were compared with diclofenac sodium as a standard. The solutions were heated in the oven at 37 °C for 15 min and then transferred to a water bath set at 70°C for 5 min. After cooling, The level of protein precipitation presented as solution turbidity was measured at 660 nm. The percentage inhibition of precipitation (protein denaturation) was determined as follows:

$$\% I = (1 - At/Ac) \times 100;$$

where: At is the absorbance of test sample.

Ac is the absorbance of control.

Antibacterial activity of the extract

The methanolic extract of *S. melongena* L. fruits was tested for its antibacterial activity using the paper disc diffusion method. The extract was assessed against four reference strains belonging to the American Type Culture Collection (ATCC): *Staphylococcus aureus* (6538 ATCC), *Bacillus subtilis* (ATCC 6633) (gram-positive bacterial strains), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) (gram-negative bacterial strains).

A bacterial suspension was prepared with a 0.05 Mac Farland density in sterile physiological water and then inoculated in Muller-Hinton agar by swab. After solidification, sterile discs of paper (Whatman No. 3, 6mm in diameter) impregnated with 5 µL for each concentration (50 and 100 mg/mmL) of methanolic extract tested were placed on the surface of the Petri dishes. The solvent used, DMSO (dimethyl sulfoxide), served as a negative control. The antibacterial assay plates were incubated at 37°C for 24 hr. The inhibitory zone (diameter) was measured in millimeters.[22]

Statistical analysis

All the data presented are the means of three replicates ± standard deviation. One-way analysis of variance and Student's t-test for detection of significant differences between the extracts and standard activities by the comparison of IC_{50} and A0.5 values were done using XLSTAT software (version 14.5.03, Addinsoft, France). Pearson's correlation coefficient test was used to

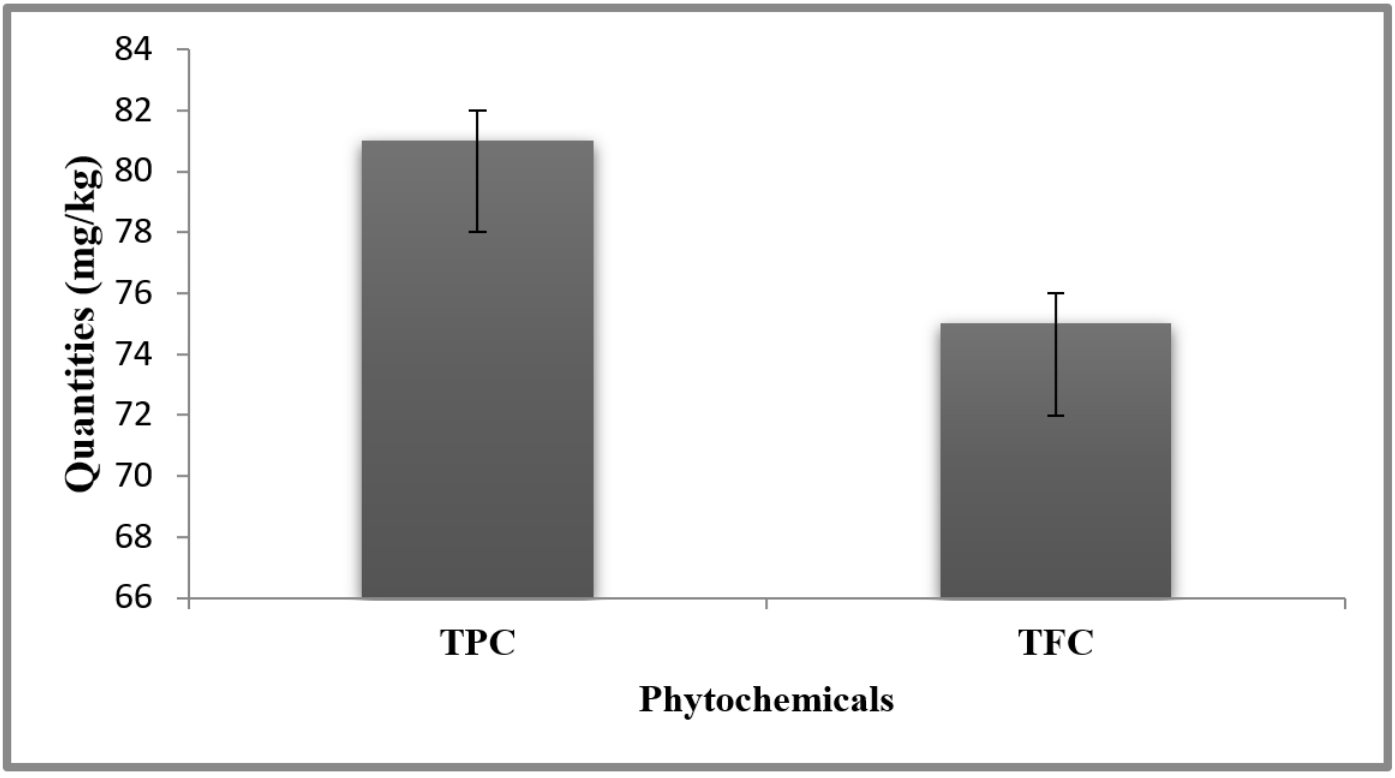

Figure 2: Total phenolic and flavonoids content in methanolic extract of *Solanum melongena* L.

Table 1: Qualitative phytochemical screening of *Solanum melongena* L.

Phytochemical family	Presence
Phenols	+++
Flavonoïdes	+++
Alcaloïdes	+++
Saponosides	++
Amidon	-
Cardiotonic glucosides	+++
Stérols and triterpenes	+++
Anthraquinones	++

+++ : abundant, ++ : moderately present, + : present, - : absen

highlight the relationship between phytochemical contents and the IC_{50} and A0.5 values of the antioxidant and urease inhibitory activities.

RESULTS

Phytochemical screening

The results of the qualitative analysis are shown in Table 1. Phenols, alkaloids, terpenoids, steroids, flavonoids, and glycosides are the most abundant classes of compounds in *S. melongena* methanolic extract. They displayed highly positive reactions with noticeable color changes. Saponins and anthraquinons were the next two classes of compounds presented in the extract.

Total phenolic and flavonoid Contents

We analyzed the methanolic extract of *S. melongena* L. for its total phenolic and flavonoid content, and Figure 2 shows the quantities of these contents. We determined the Total Phenolic Content (TPC) to be 81 mg GAE/kg, and the Total Flavonoid Content (TFC) to be 75 mg QE/kg.

Gas Chromatography-Mass Spectrometry Analysis GC-MS

To find out the different agents involved in the several activities studied herein, the GC-MS technique was used. Forty-four bioactive compounds were detected in the methanolic extract of eggplant fruit based on their elution order, mass spectrum, area, area%, and retention time, as listed in Table 2. These bioactive compounds were detected by matching their recorded mass spectra with those stored in the GC/MS Mass Spectra Library, NIST05 (National Institute of Standards and Technology). The analysis of the GC-MS chromatogram showed five major peaks (Figure 3). Three of the major peaks, constituting 52, 10; 6, 07; and 3, 26% of the peak area, were depicted by (1R, 3R, 4R, 5R). (-) Quinic acid, reflecting its substantial presence in eggplant methanolic extract.

Several phytochemical classes were also identified, namely: terpenes (Furanmethanol, tetrahydro; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl; D-Limonene and DDMP); alkaloid (3-Pyridinol and 2-Pyrrolidinone); alcohol (3-Allyl-6-methoxyphenola); propylene glycol, cyclopropyl carbinol, benzene, 1-methoxy-4-(1-propenyl) -1-Hexadecanol, 3-Allyl-6-methoxypheno); saturated and unsaturated fatty acids (n-Hexadecanoic acid; 9-Octadecenoic acid (E); 9,12-Octadecadienoic acid (Z); Octadecanoic acid; and cis,ci s,cis-7,10,13-Hexadecatrienal).

Furthermore, amide (acetamide), esters acids (butanedioic acid; monomethyl, 1,2,3-Propanetriol, monoacetate; 1,2-Benzenedicarboxylic acid, bis(2 -methylpropyl) and phenol, 2 -methoxy - 4 -(1 -propenyl) - acetate), hydrocarbons (1-Tetradecene and 1 nonadecene),coumarin (1,4 -Benzodioxin, octahydro - 2 -methylene -, trans -), lignin (2(3H) -Furanone, dihydro - 4 -hydroxyl), benzene derivates (benzene, (2,2 -dimethylbutyl - and Benzofuran, 2,3-dihydro-), amino acid methyl ester (DL-proline, 5 -oxo -,methyl ester). Researchers discovered two new compounds, 1,2,5,6-Anhydroglucitol and o-Methylisourea hydrogen sulfate, previously unreported or isolated as phytochemicals from plants.

Antioxidant activity

To comprehensively evaluate the antioxidant capacity of S. melongena, we employed a diverse array of *in vitro* spectrophotometric techniques, including DPPH, ABTS, ferric reducing, and phenanthroline assays. Table 3 presents a summary of the extract's radical scavenging activity across these various tests. The DPPH and ABTS•+ assays, which measure the neutralization of stable colored radicals, are particularly useful for assessing antioxidant efficacy in complex biological matrices such as plant extracts. Our results demonstrated that the *S. melongena* extract possesses considerable radical-scavenging capabilities against both DPPH and ABTS, with its effectiveness exhibiting a dose-dependent relationship. Statistical analysis revealed significant differences ($p < 0.0001$) between the extract and conventional antioxidant standards (BHA and BHT), indicating a moderate level of antioxidant activity for the S. melongena extract.

We further evaluated the antioxidant capabilities of the eggplant extract using iron (III) reducing activity assays, specifically FRAP and phenanthroline methods. These tests measure the antioxidants' capacity to reduce ferric Fe (III) to its lower oxidation state. The methanolic extracts of eggplant demonstrated strong ferric reducing power, with an A 0.5 value of 111 mg/mL. However, this potency was still lower than that of the standard antioxidants used (ascorbic acid and α-tocopherol). Ascorbic acid, in particular, exhibited high reduction activity, slightly outperforming the eggplant extract. Statistical analysis revealed a significant difference between the A0.5 values of the methanolic eggplant extract and ascorbic acid (111.7 µg/mL vs. 6.77 µg/mL, $p < 0.0001$). Interestingly, when compared to α-tocopherol, the

eggplant extract's A0.5 value was relatively similar, suggesting comparable reducing power between these two antioxidants in this particular assay.

Table 3 also presents the results of the iron reduction activity assay, which measures the formation of the Fe^{2+}-phenanthroline complex. Analysis of these results indicates that the methanolic extract of eggplant demonstrated good reducing activity, with an A0.5 value of 42.61 ± 0.55 µg/mL. Nevertheless, the standard antioxidants, BHA and BHT, exhibited significantly stronger reducing activities, with A0.5 values of 0.93 ± 0.07 and 2.24 ± 0.17 µg/mL, respectively. This assay relies on the presence of reducing compounds in plant extracts, which facilitate the reduction of Fe^{3+} to Fe^{2+}. The resulting Fe^{2+} ions then form a stable complex with ortho-phenanthroline, producing a distinctive orange-red color. This color change serves as a visual indicator of the extract's reducing capacity, with the intensity correlating to the strength of the antioxidant activity.

Urease inhibitory activity

S. melongena methanolic extract exhibited moderate urease inhibitory activity (IC_{50} 800 µg/mL) compared to thiourea (11, 57 µg/mL) tested as standard. The anti-urease inhibitory activity at 200 µg/mL was 18.8% compared to 98, 90% of the control (Table 3).

Anti-inflammatory effect

The anti-denaturation activity of the tested extract at different concentrations is presented in Figure 4. At a concentration of 500 mg/mL, the positive control possesses a supreme anti-inflammatory effect with a percentage inhibition of 100%,

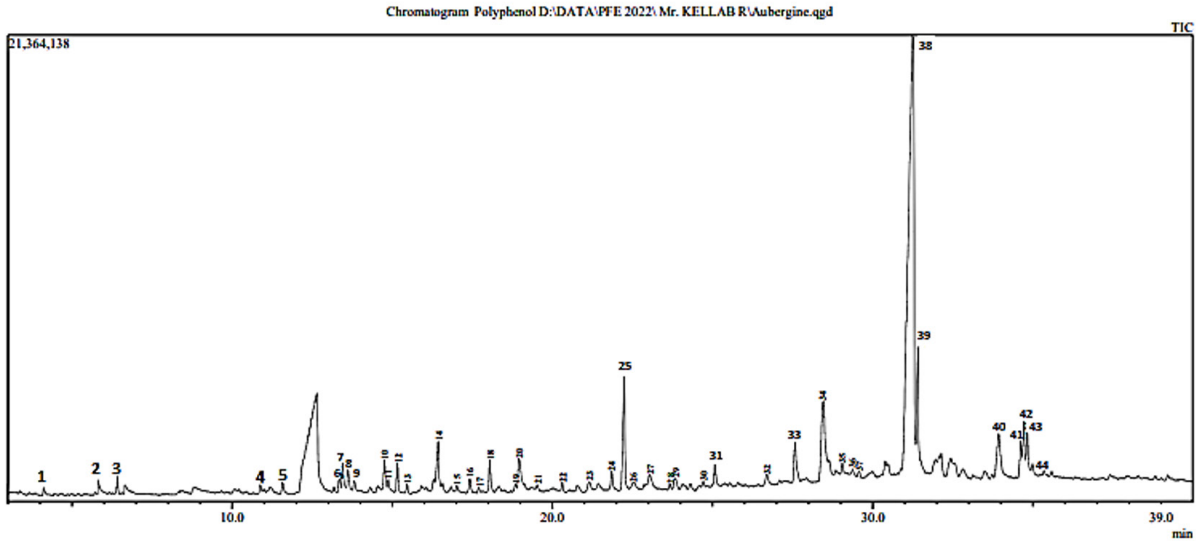

Figure 3: GC/MS chromatogram profile of the methanolic extract of *Solanum melongena* L.

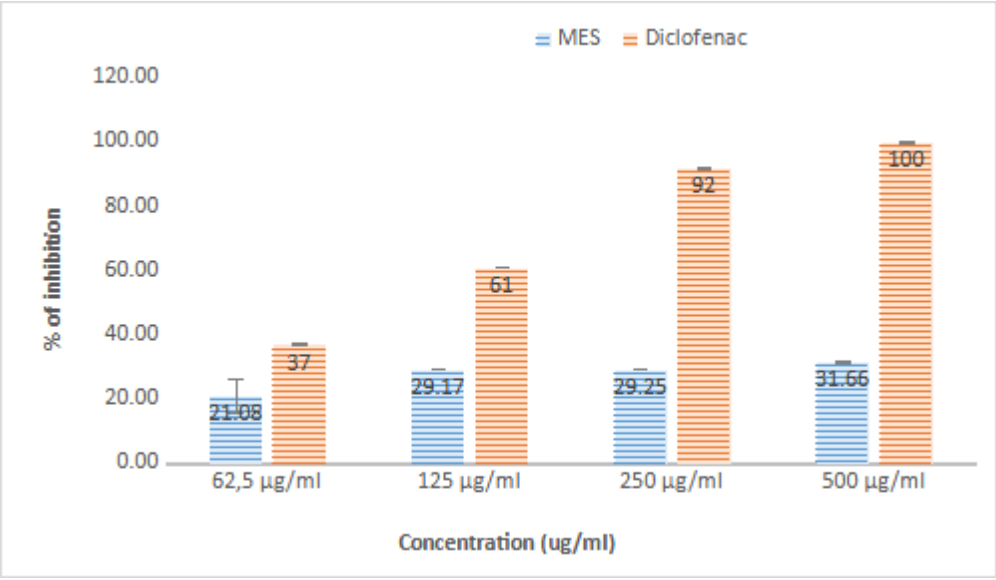

Figure 4: *In vitro* anti-Inflammatory effect of *S. melongena* and diclofenac standard.

Table 2: Identification of compounds in methanolic extract of Algerian *Solanum melongena* L. using GC-MS.

Peak	R.Time	Area	Area%	Name	Base *m/z*
1	4.157	1113944	0.24	Propylene Glycol	45.00
2	5.853	2156601	0.46	Acetamide	59.00
3	6.451	2125258	0.45	D-Limonene	68.05
4	10.907	1201624	0.26	Phenol acide	94.00
5	11.599	1784324	0.38	Ethanone,1-[2-(1-hydroxy-1methylethyl) cyclopropyl]- aldehyde	43.00
6	13.365	2598180	0.56	2-Pyrrolidinone alkaloids	85.00
7	13.474	5278086	1.13	2-Furanmethanol, tetrahydro-	71.00
8	13.639	4401563	0.94	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	43.00
9	13.834	2074947	0.44	Cyclopropyl carbinol	44.00
10	14.775	5375709	1.15	1,2,3-Propanetriol, monoacetate	43.00
11	14.895	1558163	0.33	Butanedioic acid, monomethyl	101.00
12	15.177	4445520	0.95	Benzene, 1-methoxy-4-(1-propenyl)-	148.10
13	15.479	1312926	0.28	1-Tetradecene	55.00
14	16.448	11784524	2.52	3-Pyridinol	95.00
15	17.030	906628	0.19	2-Furanone,3,4dihydroxytetrahydro	43.00
16	17.436	2439798	0.52	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	150.05
17	17.715	830166	0.18	Benzofuran, 2,3-dihydro-	120.00
18	18.058	5105852	1.09	3-Allyl-6-methoxyphenol	164.05
19	18.859	1127967	0.24	1,4 -Benzodioxin, octahydro - 2 -methylene -, trans -	85.00
20	18.969	9219250	1.97	1,2 –Benzenediol	110.00
21	19.534	785833	0.17	2(3H) -Furanone, dihydro - 4 -hydroxy -	44.00
22	20.316	1145669	0.24	1 -Nonadecene	83.05
23	21.152	2179715	0.47	o -Methylisourea hydrogen sulfate	44.00
24	21.857	2973118	0.64	Hydroquinone	110.00
25	22.245	22421376	4.79	DL -Proline, 5 -oxo -, methyl ester	84.00
26	22.531	2095681	0.45	Benzene, (2,2 -dimethylbutyl) -	43.00
27	23.041	4006579	0.86	D -Erythro -Pentose, 2 -deoxy -	45.00
28	23.674	1120674	0.24	Benzoic acid, 3 -hydroxy -	121.00
29	23.842	3263770	0.70	1,2:5,6 -Dianhydrogalactitol	45.00
30	24.715	799495	0.17	1 –Hexadecanol	55.00
31	25.075	3530387	0.75	Benzaldehyde, 2 -hydroxy - 4 -methyl -	136.00
32	26.690	2298392	0.49	3 -Hydroxy - 4 -methoxybenzoic acid	168.00
33	27.570	10002302	2.14	Benzoic acid, 3 -hydroxy -	138.00
34	28.450	31196632	6.67	(1R,3R,4R,5R) - (-) -Quinic acid	43.00
35	29.047	1585426	0.34	Phenol, 2 -methoxy - 4 -(1 -propenyl) -, acetate	164.05
36	29.365	957463	0.20	4 -((1E) - 3 -Hydroxy - 1 -propenyl) - 2 -methoxyphenol	137.05
37	29.571	1082620	0.23	1,2 -Benzenedicarboxylic acid, bis(2 -methylpropyl) ester	149.00
38	31.263	243649291	52.10	(1R,3R,4R,5R) - (-) -Quinic acid	60.00
39	31.398	22035980	4.71	n -Hexadecanoic acid	73.00
40	33.921	15225534	3.26	(1R,3R,4R,5R) - (-) -Quinic acid	60.00
41	34.609	7007717	1.50	9 -Octadecenoic acid, (E) –	55.00

Peak	R.Time	Area	Area%	Name	Base *m/z*
42	34.707	10283912	2.20	9,12 -Octadecadienoic acid (Z,Z) –	67.00
43	34.807	8948611	1.91	Octadecanoic acid	43.00
44	34.970	2210934	0.47	cis,cis,cis -7,10,13 –Hexadecatrienalphaty aldehydes with antibacterial activity (Nabi *et al.*, 2022)	79.00

Table 3: **Antioxidant and anti-urease potential of *Solanum melongena* L. methanolic extract.**

Extract	DPPH IC_{50} µg/mL	ABTS IC_{50} µg/mL	FRAP $A_{0.5}$ µg/mL	Phenanthroline $A_{0.5}$: µg/mL	Anti-urease IC_{50} µg/mL
MES	234.33± 2, 5	37.047±2,97	111.7 ± 1.72	42.61 ± 0,55	800
BHT	12.99± 0.41	1.29 ± 0 ,30	-	2.24 ± 0,17	-
BHA	-	1,81 ± 0,10	-	0.93 ± 0.07	-
Acide ascorbique	-	-	6.77 ± 1.15	-	-
α tocopherol	13.02± 5.17	-	34.93 ± 2.38	-	-
Thiourea	-	-	-	-	11.57± 0.68
P value	<0,0001	<0,0001	0,003	<0,0001	<0,0001

MES: Methanolic extract of *Solanum melongena* L.

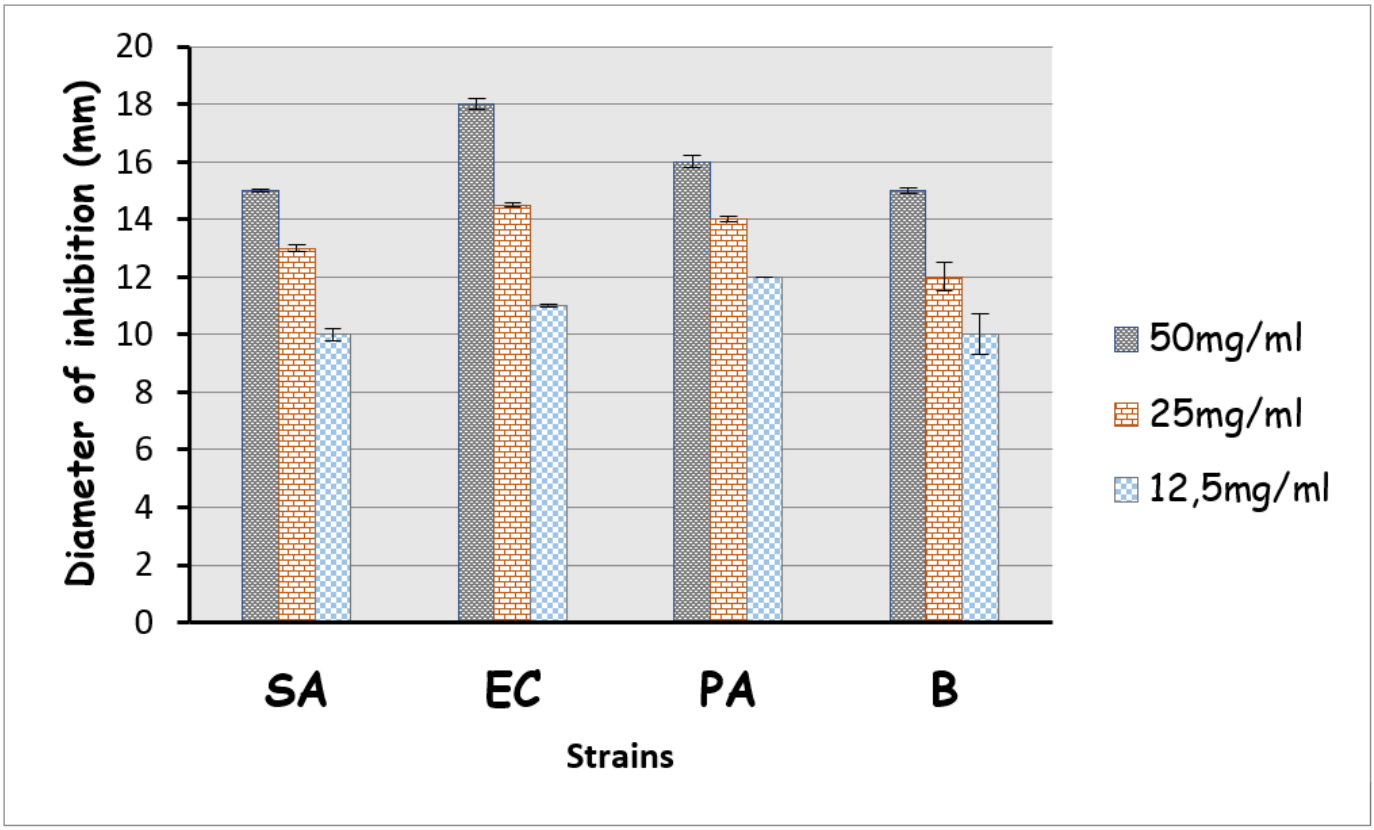

Figure 5: Antimicrobial inhibition zone diameters obtained with *Solanum melongena* methanolic extract against EC: *Escherichia coli*; SA: *Staphylococcus aureus; PA: Pseudomonas aerugenosa;* B: *Bacillus subtilis.*

Table 4: Pearson's correlation coefficients between TPC, TFC and antioxidant, anti- urease and anti-inflammatory activities of *Solanum melongena* L. methanolic extracts.

	DPPH	ABTS	FRAP	Phenanthroline	Anti-Urease
TPC	0,994^{ns}	0,486^{ns}	-0,449 ^{ns}	-0,875^{ns}	-1,00***
TFC	0,994^{ns}	0,486^{ns}	-0,449 ^{ns}	-0,875^{ns}	-1,00***

TPC: Total phenolic content**; TFC;** Total flavonoid content**; ns:** Non significant (p ˃ 0, 05); *** Correlation significant at $p < 0.0001$.

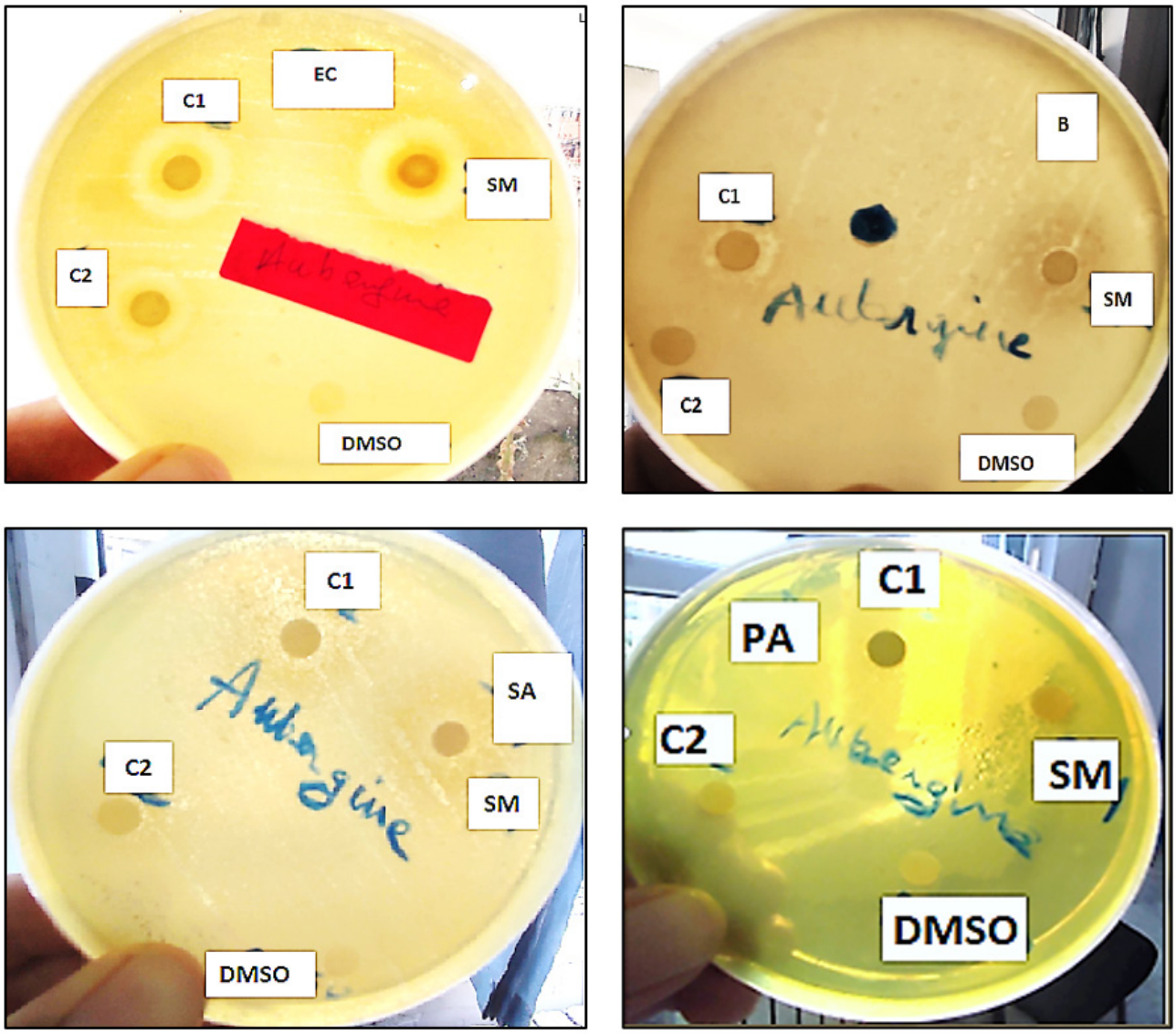

Figure 6: Antibiogram results of *S.melongena* methanolic extract against the tested strains and control. EC: *Escherichia coli,* B: *Bacillus subtilis,* PA: *Pseudomonas aerugenosa*, SA: *Staphylococcus aureus,* SM: Stock solution (50 mg/mL), C1: 100 mg/mL, C2: 50 mg/mL, DMSO: dimethyl sulfoxide (negative control).

while the extract presents a moderate anti-inflammatory effect (31.66%) at the same concentration. Therefore, the MES had an undeniable aptitude to maintain the three-dimensional structure of functional proteins.

Antibacterial activity

The extract was tested against four reference strains from the American Type Culture Collection (ATCC): *S. aureus* and *B. subtilis* (gram-positive bacterial strains), as well as *E. coli* and *P. aeruginosa* (gram-negative bacterial strains). The methanolic extract of eggplant inhibited the growth of nearly all the tested strains, showing a notable antibacterial effect in a concentration-dependent manner (Figures 5 and 6). Higher extract concentrations resulted in greater inhibitory activity against the tested strains. The eggplant methanolic

extract exhibited slightly stronger inhibitory activity against gram-negative bacteria compared to gram-positive bacteria.

DISCUSSION

The most common chemicals found in the methanolic extract of *S. melongena* (eggplant) were phenols, alkaloids, terpenoids, steroids, flavonoids, and glycosides. These findings align with previous studies on different eggplant varieties, which also reported the same chemical classes.[23-25] There were also small amounts of saponins and anthraquinones, but no starch. This shows that the glycosides found are not starchy carbohydrates. Additionally, other research has identified compounds like anthocyanins, carotenoids, and vitamins in eggplants, which contribute to their significant pharmacological and medicinal properties.[1,5] This comprehensive range of bioactive compounds underscores the health benefits and medicinal potential of eggplants.

The methanolic extract of *S. melongena* presented a TPC of 81 mg GEA/kg and a TFC of about 75 mg QE/kg. Our results are lower than TPC of different Turkish eggplant cultivars (615 to 1389 mg SE/kg)[26] and of Italian Black Beauty and Violetta Lunga eggplant variants (75, 4 to 64,8 mg CAE/100g fresh samples).[27] Indeed, this result was expected since our sample was taken during the hot season. Hydroxycinnamic Acids (HCA) and their derivatives were reported to be the most prevalent class of phenolic acid conjugates in *S. melongena*, with chlorogenic acid being the single most abundant phenolic compound.[1,28]

Piao *et al.*[29] studied TFC in fruits and leaves of *S. melongena* from 15 countries; they concluded that the flavonoid content differed according to the region. The same authors suggest that the flavonoids are quantitatively greater and qualitatively more diverse in leaves than in fruit. The difference in the polyphenol and flavonoid content between samples results from the difference in extraction method (polarity of the solvents, time, temperature, sample ratio, etc.) as well as the chemical characteristics of the samples and the environmental conditions (soil, type of microclimate, stage of harvesting, and season).[30,31]

The results showed that Quinic acid is present in large quantities in the methanolic extract of eggplant. All the reports up today are in agreement that chlorogenic acids are the most widely phenolic acids distributed in eggplant fruit.[1, 28] An increase in temperature above 23°C induces hydrolysis, isomerization, and degradation of polyphenols.[32] In our study, the harvesting period (hot season) could cause the hydrolysis of chlorogenic acid to quinic acid. Other phenolic acids were also identified as hydroquinone, phenol, 1,2-benzenediol, benzoic acid, 3-hydroxy-, and 2-furanone, 3,4-dihydroxytetrahydro. The main phenolic acids present in eggplant fruit were beneficial and well known for their biological activities, offering them a potential use in the treatment of metabolic and cardiovascular diseases.[33]

The present study isolated the remaining phenolic compounds *from S. melongena* for the first time, with the exception of phenolic acids, three terpenes (furanmethanol, tetrahydro, DDMP, and D-imonene), and acetamide. Researchers have found that plants that contain some of these phytochemicals can do a lot of different biological things, like fight cancer, inflammation, free radicals, and microbes.[34-37] Some of the isolated phytochemicals possess potential benefits in industry as flavor and fragrance agents.[38,39]

The study demonstrates antioxidants' ability to scavenge radicals in complex biological matrix, such as plant extracts. The extract showed good radical-scavenging activities, with moderate activity compared to BHA and BHT standards. Chokthaweepanich *et al.*[40] have studied the antioxidant activity of three species of eggplant, including *S. torvum, S. violaceum*, and ten varieties of the commercial *S. melongena*. The Thailand Makhuea yao muang variety exhibited the highest DPPH scavenging activity compared to the other varieties and species. Another study by Boubekri *et al.*[41] reported IC_{50} values with greater inhibitory activity of the radical ABTS (between 433.4 and 1149.2 µg/mL) for five cultivars of Algerian *S. melongena* L. using a cyclic voltammetry assay. In the same context, several parts of the eggplant were studied by Contreras-Angulo *et al.*[42] who indicated that the leaf had the highest antioxidant activity (DPPH and ABTS) compared to the root, stem, and fruit. All these results differ from our study; this could be attributed to the extraction process, part-analyzed variety, climate, and time of harvesting.

The relationship between phytochemical content and antioxidant activities was well established and proved worldwide,[43-45] and a higher number of hydroxyl groups in the structure was correlated with good antiradical activity.[46] In our study, Pearson's coefficients revealed weak correlations (Table 4) between the antiradical scavenging activities (DPPH and ABTS) and TPC and TFC contents.

The antioxidant potential of eggplant extract was also determined by its iron reducing activity, with methanolic extracts showing potent ferric reducing power. BHA and BHT had strongest reducing activities. The obtained results could be related to its polyphenol content, especially flavonoids and phenolic acids containing hydroxyl groups, which serve as electron donors and are known for their important capacity to reduce activity. Apak *et al.*[47] suggested that the potent antioxidant activity of a flavonoid is significantly correlated to the presence of some specific structures in their aromatic and heterocyclic rings (double bonds, 3- and 5-hydroxyl groups, etc.,).

A relevant study looked at the antioxidative activity of ten different types of eggplant using the FRAP assay. The EC_{50} value was between 15,32 and 29,93 ug/mL.[5] The aqueous and ethanolic extracts of the stalk and fruit of *S. melongena* showed the same trend.[48] We got results for the phenanthroline reduction assay that are similar to those found for the butanolic fraction of an

Algerian *Sonchus oleraceus* L. (42, 89 µg/mL).[49] They were also somewhat similar to those found for the dichloromethane fraction of an Algerian *Artemisia campestris* L.[50] According to Pearson's coefficients (Table 4), the present study found no significant impact of the phenolic compounds (phenols and flavonoids) on reducing power activity (FRAP and phenanthroline).

We can attribute the weak correlation between antioxidant activities (scavenging and reducing activities) and the content of polyphenols and flavonoids to either the interactions between active and non-active compounds in the extracts, which reduce the inhibitory efficiency of the active ones,[45] the ineffective extraction of antioxidant compounds using methanol as solvents, or the absence of these compounds in sufficient quantities to exert antioxidant activities.

Urease is a key enzyme catalyzing the degradation of urea to ammonia, which confers a suitable acidic environment for the proliferation and persistence of urease-positive pathogens. Plant and food extracts with urease inhibition capacity could be used as natural drugs in the treatment of some gastrointestinal diseases caused by urease-producing bacteria such as *H. pylori*. Our study is the first to study the anti-urease capacity of *S. melongena* fruit. Our result is close to that reported by Mahernia *et al.*,[51] of two consumed herbal belonging to Solanaceae family, *Datura inoxia* Miller and *Datura stramonium* L., with IC_{50}s of 935,10 and 719.30, respectively. A strong relationship between anti-urease and TFC was registered (Table 4). Several urease inhibitors are reported in the literature, including polyphenols, especially flavonoids, alkaloids, and terpenoids; they act as competitive substrates with the urea on the active site or as chelating factors of the binuclear co-factor nickel.[52,53]

Inflammation involves various processes, such as increased blood flow, heightened blood vessel permeability, and protein denaturation. Protein denaturation includes alterations in electrostatic hydrogen, hydrophobic, and disulfide bonding. Thus, agents that prevent protein denaturation are considered potent anti-arthritic and anti-inflammatory drugs.[54] Various in vitro methods are used to evaluate anti-inflammatory activity, including hemolysis inhibition, proteinase inhibition, protein denaturation inhibition, and lipoxygenase inhibition.[55] In our study, the extract demonstrated a moderate anti-inflammatory effect at a concentration of 500 mg/mL, suggesting its ability to maintain the three-dimensional structure of functional proteins. This may be attributed to the main bioactive compounds isolated previously from this species, such as polyphenols, alkaloids, and flavonoids.[56] Bailey-Shaw *et al.*[57] found that the weak anti-inflammatory effect of some plant extracts could be due to the antagonistic interaction of multiple compounds in the crude extract, which may interfere with or mask each other's activity. They recommended the separation or concentration of the crude extract before testing. Another study demonstrated that the *in vivo* anti-inflammatory activity of the aqueous extract of *S. melongena* leaves, using the carrageenan-induced paw edema method, showed significant activity in a dose-dependent manner. The extract exhibited a percentage inhibition of 42.62%, compared to the standard drug aspirin, which showed 64.5%.

The eggplant methanolic extract showed significant antibacterial activity against four ATCC strains: *S. aureus, B. subtilis, E. coli*, and *P. aeruginosa*. Higher concentrations resulted in greater inhibitory activity, with slightly stronger activity against gram-negative bacteria. this could be attributed to the fact that Gram+ bacteria are endowed with a complex cell wall structure compared to Gram-, which may impede the entry of antimicrobial compounds contained in the extract. The antibacterial effect of eggplant was widely investigated.[10,58-60] It may be attributed to the presence of alkaloids and phenolic compounds, especially quinic acid (a derivative of chlorogenic acid), which is considered the predominant phenolic compound detected in our study.

In general, polyphenols stop bacteria from growing by stopping the production of cell walls, breaking down proteins, and stopping DNA replication.[61] The presence of saponins could also cause Brinjal's antibacterial effect; they interfere with the cell wall's surface tension.[62] What we found was similar to what Al-Janabi & Al-Rubeey.[63] They found that *S. melongena* could kill *E. coli, S. aureus, B. subtilis*, and *P. vulgaris* by forming an inhibition zone with a diameter of 12, 16, 20, and 17 mm, respectively, at 50 mg/mL using the well diffusion agar method.

CONCLUSION

This study contributes new insights into the chemical composition and biological properties of the methanolic extract of *Solanum melongena* L. from Algeria. The identification of 44 bioactive compounds, including quinic acid as a major component, presents novel findings, particularly as most of these compounds have not been previously reported in eggplant. The methanolic extract exhibited significant antibacterial, antioxidant, anti-inflammatory, and urease inhibitory activities, highlighting its therapeutic potential.

However, several gaps remain in this investigation. Notably, the mechanisms of action underlying the interaction of these bioactive compounds remain unclear. Future studies should aim to elucidate these mechanisms through metabolomics and molecular-level analysis. Additionally, the extract's moderate activity compared to standard antioxidants suggests that optimizing extraction methods and exploring other solvent systems could enhance its efficacy.

In conclusion, the methanolic extract of *S. melongena* L. has promising pharmaceutical applications. It offers potential in the development of antioxidant, antibacterial, and anti-inflammatory treatments. To close the existing gaps, we recommend further research focusing on bioavailability, *in vivo* studies, and formulation optimization for potential therapeutic use.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval for this study titled "Chemical Profiling and *in vitro* Evaluation of the Antioxidant, Anti-Inflammatory, and Anti-Bacterial Effects of Algerian *Solanum melongena* L." was obtained from the Ethics Committee of the Abdelhafid Boussouf University Center, Algeria.

ABBREVIATIONS

ABTS: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); **ATCC:** American Type Culture Collection; **BHA:** Butylated hydroxyanisole; **BHT:** Butylated hydroxytoluene; **BSA:** Bovine Serum Albumin; **DPPH:** 1,1-diphenyl-2-picrylhydrazyl; **DMSO:** Dimethyl sulfoxide; **FRAP:** Ferric-reducing antioxidant power; **GC-MS:** Gas Chromatography-Mass Spectrometry; **IC_{50}:** Half-maximal inhibitory concentration; **MES:** Methanolic extract of Solanum melongena; **TFC:** Total Flavonoid Content; **TPC:** Total Phenolic Content.

SUMMARY

This study focused on exploring the chemical and biological properties of methanolic extracts from purple *Solanum melongena* L. fruit. The qualitative and quantitative analyses revealed a rich composition of bioactive compounds, including phenols, alkaloids, terpenoids, steroids, flavonoids, saponins, and anthraquinones. The total phenolic content was measured at 81 mg GEA/kg, while flavonoids were approximately 75 mg QE/kg. GC-MS analysis identified 44 compounds, with quinic acid predominant at over 60% of peak area, many of which were previously unreported for *S. melongena* L.

The methanolic extracts exhibited moderate to strong antioxidant activity in assays such as DPPH, ABTS, and ferric reducing power, as well as significant anti-urease and anti-inflammatory effects. The extracts also demonstrated potent antibacterial activity against all tested bacterial strains using the agar diffusion method.

While these findings highlight the therapeutic potential of S. *melongena* L. as a source of antioxidants and antimicrobials, further research, particularly in metabolomics, is necessary to comprehensively understand the metabolic profile and interactions of the identified compounds.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Rabah Kellab, Fouzi Boulkenafet, and Simonetta Lambiase designed the study, conducted data analyses, and wrote the manuscript. Serine Amokrane and Zoubida Benmakhlouf conducted experiments and analyzed antimicrobial and antioxidant activities. Abdelouahab YAHIA and Azeddine Bounamous helped in writing the manuscript and conducted data analysis. Fahd Al-Meklafi and Mohammed Wadaan helped in writing the manuscript.

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