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In Vitro Antioxidant and Antimicrobial Evaluation of the Methanolic Seed Extract of Helianthus Annuus Linn. (Asteraceae) with GC-MS Profiling of Bioactive Phytoconstituents

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Abstract

Oxidative stress and the worldwide escalation of antimicrobial resistance have renewed scientific interest in plant-derived bioactive molecules as safe, sustainable sources of antioxidants and antimicrobials. Helianthus annuus Linn. (Asteraceae), the common sunflower, is a widely cultivated oilseed crop whose seeds are rich in polyunsaturated fatty acids, tocopherols, phytosterols and phenolic compounds; however, an integrated in vitro evaluation of its seed extract remains limited. The present study was designed to investigate the preliminary phytochemistry, in vitro antioxidant potential, phytoconstituent profile and antibacterial activity of the methanolic seed extract of H. annuus. Dried seed powder was Soxhlet-extracted with methanol, and the concentrated extract was screened for secondary metabolites using standard qualitative tests. Antioxidant activity was assessed by the DPPH radical-scavenging assay and total phenolic content, using ascorbic acid and gallic acid as reference standards, respectively. Volatile and semi-volatile constituents were profiled by gas chromatography-mass spectrometry. Antibacterial activity was evaluated against Staphylococcus aureus and Escherichia coli using the agar well diffusion method, and the minimum inhibitory concentration was determined and compared with that of amoxicillin. Phytochemical screening revealed the presence of tannins, flavonoids, terpenoids, phenols and proteins, whereas saponins, sterols, alkaloids, carbohydrates and glycosides were absent. The extract exhibited concentration-dependent DPPH radical-scavenging activity comparable to ascorbic acid and a total phenolic content of 32.26 mg gallic acid equivalents per gram of extract. GC-MS analysis identified eight chromatographic peaks, of which hexadecanoic (palmitic) and 9-octadecenoic (oleic) acid methyl esters, together with the pentacyclic triterpenoid lupeol acetate, were the pharmacologically notable constituents; the remaining peaks corresponded to the solvent and trace derivatisation- or column-related signals. In contrast, the extract produced no measurable zone of inhibition and showed no appreciable suppression of bacterial growth at concentrations up to 150 microgram per millilitre, whereas amoxicillin was active. These findings indicate that the methanolic seed extract of H. annuus is a promising natural antioxidant, an effect attributable to its phenolic, flavonoid and tannin constituents, although it lacks significant

antibacterial activity at the concentrations examined, warranting further bioassay-guided fractionation and dose optimisation.

Keywords: Helianthus Annuus; DPPH Radical Scavenging; Total Phenolic Content; GC-MS; Antibacterial Activity; Asteraceae

1. Introduction

Reactive oxygen species (ROS), including superoxide anion, hydroxyl radical, hydrogen peroxide and singlet oxygen, are continuously generated as by-products of normal aerobic metabolism. When their production overwhelms the endogenous defence systems, the resulting oxidative stress damages proteins, lipids and nucleic acids and contributes to the pathogenesis of ageing, cancer, cardiovascular disorders, diabetes and neurodegenerative disease [1,2]. Antioxidants limit such damage either by directly scavenging free radicals (primary antioxidants) or by preventing radical formation through metal chelation and related mechanisms (secondary antioxidants), thereby interrupting the propagation phase of oxidative chain reactions [3,4]. Although synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene are effective, concerns over their potential toxicity have driven the search for natural, plant-derived alternatives that are perceived as safer and better tolerated [5].

In parallel, the relentless rise of antimicrobial resistance has emerged as one of the foremost threats to global public health, progressively eroding the clinical utility of conventional antibiotics and intensifying the demand for novel antimicrobial scaffolds [6]. Higher plants have historically furnished a vast reservoir of structurally diverse secondary metabolites, including phenolics, flavonoids, tannins, alkaloids and terpenoids, many of which possess well-documented antioxidant and antimicrobial properties and continue to serve as templates for drug discovery [7]. Within this context, members of the family Asteraceae are of particular pharmacological interest; for example, the flower extracts of Tagetes erecta Linn. (Asteraceae) have demonstrated significant anxiolytic activity in experimental rodent models, underscoring the therapeutic potential of this family [8].

Helianthus annuus Linn., commonly known as the sunflower, is a robust annual herb of the family Asteraceae that is cultivated worldwide primarily as an oilseed crop. The seeds are an important source of edible oil and vegetable protein and are characterised by a high oil content (40-45%) dominated by the polyunsaturated fatty acids linoleic and oleic acid, together with tocopherols and phytosterols [9,10]. Beyond their nutritional value, sunflower seeds and their extracts have been reported to exhibit a range of biological activities, including anti-inflammatory, anti-asthmatic, hypoglycaemic and antimicrobial effects, and an aqueous seed extract has been shown to alleviate asthmatic symptoms in vivo [11]. The seed contains carbohydrates, phenolics, flavonoids, tannins, saponins, phytosterols, triterpenoids and fixed oils, a phytochemical complement consistent with appreciable antioxidant capacity [12].

Despite the economic importance of the crop and several scattered reports on its constituents, an integrated investigation that simultaneously characterises the preliminary phytochemistry, quantifies the in vitro antioxidant capacity, profiles the bioactive constituents by GC-MS and evaluates the antibacterial potential of a single methanolic seed extract is comparatively scarce, and the few comparative herbal antimicrobial screenings reported in the literature have yielded variable outcomes [13]. The present study was therefore undertaken to (i) prepare and phytochemically screen a methanolic seed extract of H. annuus; (ii) evaluate its in vitro antioxidant activity by the DPPH radical-scavenging and total phenolic content methods; (iii) identify its principal phytoconstituents by GC-MS; and (iv) assess its antibacterial activity against

Staphylococcus aureus and Escherichia coli, with a view to defining the extract's pharmacological profile and its suitability as a natural source of dietary antioxidants.

2. Materials and Methods

2.1 Chemicals and Reagents

Methanol and other analytical-grade solvents were used throughout. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, gallic acid and the Folin-Ciocalteu reagent were of analytical grade and procured from standard commercial suppliers. Mueller-Hinton agar and nutrient broth were used as bacteriological media, and amoxicillin served as the reference standard in the microbiological assays.

2.2 Collection and Preparation of Seed Powder

Mature seeds of Helianthus annuus were collected, washed with fresh water and air-dried in the absence of sunlight. The dried seeds were reduced to a coarse powder using an electric grinder and stored in an air-tight container in a cool, dry, dark place until extraction.

2.3 Soxhlet Extraction

One hundred grams of the coarse seed powder was placed in a glass thimble and extracted with 250 mL of methanol at 40°C for 12 h in a Soxhlet apparatus, until the solvent in the siphon tube became colourless. The methanolic extract was concentrated on a hot plate at 35°C until the solvent had completely evaporated, and the resulting extract was used for in vitro antioxidant and antimicrobial studies.

2.4 Preliminary Phytochemical Screening

The methanolic seed extract was subjected to qualitative phytochemical screening for saponins (foam test), alkaloids (Mayer's, Dragendorff's and Wagner's tests), flavonoids (Shinoda test), tannins and phenolic compounds (ferric chloride and gelatin tests), glycosides (Keller-Killiani and Borntrager's tests), carbohydrates (Molisch and Fehling's tests), amino acids and proteins (Millon's, Ninhydrin and Biuret tests), and tests for sterols and terpenoids, following standard procedures described in practical pharmacognosy texts [14,15].

2.5 DPPH Radical-Scavenging Assay

Graded concentrations (100-500 microgram/mL) of the extract or ascorbic acid in methanol were mixed with an equal volume of 0.004% w/v DPPH solution in methanol and incubated in the dark at room temperature for 20 min. Absorbance was measured spectrophotometrically at 517 nm against a methanol blank, and the percentage radical-scavenging activity was calculated as $\%I = [(A0 - A1)/A0] \times 100$, where A0 is the absorbance of the DPPH control, and A1 is the absorbance in the presence of extract or standard [16,17].

2.6 Total Phenolic Content (Folin-Ciocalteu Method)

Total phenolic content was measured using the Folin-Ciocalteu method [18]. To 1 mL of extract or gallic acid standard solution, 5 mL of Folin-Ciocalteu reagent (diluted 10-fold) was added, followed after 3 min by 4 mL of 7.5% sodium carbonate solution. The mixture was incubated for 20 min at 25°C, and the absorbance was read at 760 nm against a reagent blank. The total phenolic content was determined from a gallic acid standard curve and expressed as milligrams of gallic acid equivalents (mg GAE) per gram of extract.

2.7 GC-MS Analysis of Phytoconstituents

The methanolic seed extract was subjected to gas chromatography-mass spectrometry to identify its volatile and semi-volatile constituents. Constituents were resolved over a temperature-programmed run of approximately 34 min, and individual peaks were identified by comparison of their mass spectra and

retention behaviour with reference spectral libraries. The relative abundance of each constituent was expressed as the percentage of the total chromatographic peak area (Area%).

2.8 Antibacterial Activity by the Agar Well-Diffusion Method

Antibacterial activity was evaluated using the agar well diffusion technique against Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative) [19]. Mueller-Hinton agar plates were seeded uniformly with the standardised test cultures using sterile swabs. Wells (5 mm) were bored into the seeded agar with a sterile cork borer, and graded concentrations of the extract (25, 50, 75 and 100 micrograms) were dispensed into the wells. Plates were left for 2-3 h to allow diffusion, then incubated at 37°C for 24 h. Norfloxacin and ciprofloxacin served as the standard antibiotics against S. aureus and E. coli, respectively, and zones of inhibition were measured in millimetres.

2.9 Determination of Minimum Inhibitory and Bactericidal Concentrations

The minimum inhibitory concentration (MIC) was determined using the broth dilution method [20]. The extract was tested at 25, 50, 75, 100 and 150 microgram/mL, and amoxicillin (5-200 microgram/mL) served as the standard. Sterile nutrient broth was inoculated with the prepared bacterial suspension and treated with each extract concentration or standard before incubation at 37°C for 24 h; a tube containing only broth and inoculum served as the negative control, and growth was assessed turbidimetrically at each concentration. The minimum bactericidal concentration (MBC) was to be determined by sub-culturing tubes showing no visible growth onto fresh nutrient medium and observing for the absence of growth after 24 h.

2.10 Statistical Analysis

All antioxidant, phytochemical and microbiological assays were performed as described above, and results are reported as single-determination absorbance-derived percentages or concentrations, consistent with the original experimental protocol. The calibration curve for the total phenolic content assay was fitted by linear regression against a gallic acid standard series. Microbiological endpoints (zone of inhibition and turbidimetric absorbance for MIC) were recorded once after 24 h of incubation for each concentration tested, alongside the respective standard antibiotic run in parallel. Formal replicate statistical analysis (mean +/- SD, ANOVA or equivalent) was not performed in this study and is recommended for future validation of these findings.

3. Results

3.1 Preliminary Phytochemical Screening

Qualitative screening of the methanolic seed extract of H. annuus confirmed the presence of tannins, flavonoids, terpenoids, phenols and proteins/amino acids, whereas saponins, sterols, carbohydrates, alkaloids and glycosides were not detected (Table 1).

Table 1: Preliminary Phytochemical Screening of the Methanolic Seed Extract of H. Annuus

S. No.	Phytochemical Constituent	Result
1	Tannins	+
2	Saponins	-
3	Flavonoids	+
4	Sterols	-

5	Terpenoids	+
6	Carbohydrates	-
7	Phenols	+
8	Proteins and Amino Acids	+
9	Alkaloids	-
10	Glycosides	-

(+) Present; (-) Absent.

3.2 DPPH Radical-Scavenging Activity

The methanolic seed extract exhibited concentration-dependent DPPH radical-scavenging activity that was comparable to, and over the tested range marginally greater than, that of ascorbic acid (Table 2). Scavenging increased from 39.18% at 100 microgram/mL to 72.00% at 500 microgram/mL for the extract, against 31.40% to 65.62% for ascorbic acid over the same range.

Table 2: DPPH Radical-Scavenging Activity (% Inhibition) of the Methanolic Seed Extract of H. Annuus and Ascorbic Acid

Concentration (microgram/mL)	Ascorbic Acid (% Inhibition)	Methanolic Extract (% Inhibition)
100	31.40	39.18
200	41.60	47.60
300	52.50	56.21
400	60.06	69.60
500	65.62	72.00

Absorbance measured at 517 nm against a methanol blank.

3.3 Total Phenolic Content

The total phenolic content of the methanolic seed extract, determined against a gallic acid standard curve, was 32.26 mg GAE per gram of extract.

3.4 GC-MS Profile of Phytoconstituents

GC-MS analysis of the methanolic seed extract resolved eight chromatographic peaks (Table 3). After the dominant solvent peak (dimethyl sulfoxide, Area% 99.11), the chemically meaningful constituents were hexadecanoic acid methyl ester (palmitic acid methyl ester, Area% 0.21) and 9-octadecenoic acid methyl ester (oleic acid methyl ester, Area% 0.35), together with an octamethyl-substituted triterpenoid (Area% 0.10) and the pentacyclic triterpenoid lupeol acetate (Area% 0.11). The remaining peaks (methane isocyano-, an N-trifluoroacetyl-tris(trimethylsilyl) derivative and dodecamethylcyclohexasiloxane) were present at trace or negative-baseline area fractions consistent with solvent, derivatisation or column-bleed artefacts rather than genuine plant constituents.

Table 3: Complete GC-MS Peak Report for the Methanolic Seed Extract of H. Annuus

Peak	Rt (min)	Area	Area %	Height	A/H	Base m/z	Tentative Identity
1	1.176	3044942	0.12	4930901	0.62	41.00	Methane, isocyano- (trace)
2	5.169	2485323375	99.11	58228312	42.68	62.85	Dimethyl sulfoxide (solvent)
3	9.911	-93434	-0.00	37365	-2.49	73.05	N-(Trifluoroacetyl)-N,O,O'-tris(trimethylsilyl)- derivative (trace)
4	12.398	70869	0.00	45484	1.56	73.10	Cyclohexasiloxane, dodecamethyl- (trace/column)
5	20.182	5329539	0.21	1374910	3.88	74.05	Hexadecanoic acid, methyl ester (palmitic acid methyl ester)
6	21.925	8748440	0.35	1455443	6.01	55.05	9-Octadecenoic acid, methyl ester (oleic acid methyl ester)
7	30.753	2455457	0.10	420910	5.83	218.15	4,4,6a,6b,8a,11,11,14b-Octamethyl-triterpenoid derivative
8	31.737	2714463	0.11	309671	8.77	43.05	Lup-20(29)-en-3-ol, acetate (lupeol acetate)
Total	-	2507593651	100.00	66802996	-	-	-

Rt = retention time; A/H = area-to-height ratio; Base m/z = base peak mass-to-charge ratio.

3.5 Antibacterial Activity

In the agar well-diffusion assay, the standard antibiotics produced clear, concentration-dependent zones of inhibition against both test organisms: norfloxacin against S. aureus (9-16 mm) and ciprofloxacin against E. coli (10-16 mm) at concentrations of 10-100 micrograms (Table 4). The methanolic seed extract produced no measurable zone of inhibition (0 mm) against either organism at any of the concentrations tested (25-100 micrograms).

Table 4: Zone of Inhibition (mm) of Standard Antibiotics and the Methanolic Seed Extract Against S. Aureus and E. Coli

Test / Strain	10 microgram	25 microgram	50 microgram	75 microgram	100 microgram
Norfloxacin - S. aureus	9	10	11	14	16
Ciprofloxacin - E. coli	10	11	13	15	16
Extract - S. aureus	-	0	0	0	0
Extract - E. coli	-	0	0	0	0

0 = No Measurable Zone of Inhibition; (-) = Concentration Not Tested for that Agent.

3.6 Minimum Inhibitory Concentration

Amoxicillin progressively reduced turbidity with increasing concentration and was active (absorbance approaching zero) at 100 microgram/mL against E. coli and at 25 microgram/mL against S. aureus (Table

5). In contrast, the extract failed to reduce turbidity at any concentration up to 150 microgram/mL against either organism, and therefore, a definable MIC could not be established within the tested range; consequently, the minimum bactericidal concentration could not be determined.

Table 5: Minimum Inhibitory Concentration Assay (Turbidimetric Absorbance) of Amoxicillin (Standard) and the Methanolic Seed Extract

Agent / Strain	5	10	25	50	75	100	150	200
Amoxicillin - E. coli	0.18	0.13	0.11	0.07	-	0.01	-	0
Amoxicillin - S. aureus	0.02	0.14	0.02	0.02	-	0	-	0
Extract - E. coli	-	-	0.28	0.63	0.56	0.53	0.55	-
Extract - S. aureus	-	-	0.26	0.30	0.23	0.24	0.22	-

Concentrations in microgram/mL; Values are Optical Density (Absorbance). A Fall in Absorbance Towards Zero Indicates Growth Inhibition. (-) = Concentration Not Tested for that Agent.

4. Discussion

The co-occurrence of phenolic compounds, flavonoids and tannins in the phytochemical screen is mechanistically relevant, because these classes of secondary metabolites are recognised as powerful, chain-breaking antioxidants whose hydroxyl groups donate hydrogen atoms or electrons to quench free radicals [4,21]. The qualitative profile obtained here is broadly consistent with earlier reports on the phytochemistry of sunflower seed [12].

The progressive reduction of the violet DPPH radical to its pale-yellow form reflects the hydrogen-donating capacity of the phenolic and flavonoid constituents identified in the extract, whose radical-scavenging efficiency is governed by the number and arrangement of their hydroxyl groups [21,22]. The substantial total phenolic content (32.26 mg GAE/g) provides a mechanistic basis for this antioxidant activity, since a positive correlation between total phenolic content and radical-scavenging capacity is well established across plant extracts [5,23]. This antioxidant capacity is consistent with the broader recognition of plant phenolics as natural radical scavengers of nutritional and therapeutic value [2,3].

The GC-MS profile was dominated, after the solvent peak, by fatty acid methyl esters (palmitic and oleic acid methyl esters) and the pentacyclic triterpenoid lupeol acetate. The predominance of unsaturated fatty acid esters is consistent with the known lipid profile of H. annuus seed, which is characteristically rich in linoleic and oleic acid [9,10]. The detection of lupeol acetate is noteworthy, since lupeol and its esters have been reported separately to exhibit antioxidant, anti-inflammatory, and other pharmacological activities and may contribute to the overall bioactivity of the extract [24]. The remaining minor peaks, present at trace or near-zero area fractions, most plausibly represent solvent-, derivatisation- or column-related signals rather than genuine plant secondary metabolites, and were not considered further.

In contrast to its antioxidant activity, the extract did not display measurable antibacterial activity against S. aureus or E. coli at the concentrations examined, unlike the standard antibiotics norfloxacin, ciprofloxacin, and amoxicillin. Although flavonoids and other phenolics are recognised to possess intrinsic antibacterial properties through membrane disruption and enzyme inhibition [25], the lack of measurable activity here most plausibly reflects insufficient concentrations of the relevant constituents in the crude extract at the doses examined, the predominance of non-polar lipidic constituents revealed by GC-MS, and the limited diffusion of such constituents through the aqueous agar matrix. This is consistent with the

variability reported in comparative herbal antimicrobial screenings, in which crude polar extracts of otherwise antioxidant-rich plants frequently show weak or absent activity against standard Gram-positive and Gram-negative panels unless tested at considerably higher concentrations or after solvent-partition enrichment [7,13]. The escalating clinical relevance of antimicrobial resistance means that such negative antibacterial findings remain informative, since they help delineate which botanicals merit further bioassay-guided fractionation and which do not [6].

The present findings should be interpreted in light of certain limitations. Only a single crude methanolic extract was examined, over a comparatively narrow antibacterial concentration range (up to 150 microgram/mL) and against two bacterial species, and antioxidant/phenolic estimations were performed as single determinations rather than as replicate means with standard deviation, precluding formal statistical comparison. Future work should extend testing to a wider concentration range and additional microbial strains, incorporate bioassay-guided fractionation of the non-polar and polar sub-fractions, and quantify individual phenolic and flavonoid constituents by high-performance liquid chromatography to more precisely define the constituents responsible for the observed antioxidant activity.

5. Conclusion

The present investigation demonstrates that the methanolic seed extract of Helianthus annuus Linn. is a rich source of phenolic compounds, flavonoids, tannins and terpenoids and possesses notable in vitro antioxidant activity, reflected by its concentration-dependent DPPH radical-scavenging capacity comparable to ascorbic acid and a total phenolic content of 32.26 mg GAE/g of extract. GC-MS profiling confirmed the presence of fatty acid methyl esters and the triterpenoid lupeol acetate as the principal identifiable constituents. Owing to these antioxidant properties, the seed may be regarded as a potential natural source of dietary antioxidants. However, under the conditions of this study, the extract did not exhibit significant antibacterial activity against Staphylococcus aureus or Escherichia coli at concentrations up to 150 microgram/mL, in contrast to the reference antibiotics. Further bioassay-guided fractionation, evaluation at higher concentrations, testing against additional microbial strains, and isolation of the active constituents are recommended to fully define the pharmacological potential of H. annuus seed.

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