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Original Research Article

Phytochemical profiling, antioxidant potential and genoprotective activity of *Inula graveolens*

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Abstract

Purpose: *To investigate the phytochemical content, antioxidant properties, and DNA-protective effect of methanolic extracts of Inula graveolens leaves from Southern Iraq.*

Methods: *Leaves of Inula graveolens were pulverized and extracted using Soxhlet extraction with petroleum ether (40 – 60 °C) and 70 % (aqueous) methanol by maceration. The petroleum ether extract was analyzed using gas chromatography – mass spectrometry (GC-MS) and the methanolic extract was profiled by high-performance liquid chromatography (HPLC) analysis. Spectrophotometric analysis was performed to measure total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC). human genomic DNA assay was used to determine genoprotective activity.*

Results: *Extraction with methanol yielded 19.8 ± 1.2 % (w/w), significantly higher than petroleum ether (2.1 ± 0.3 %). Linoleic acid (3.568 %), oleic acid (1.842 %), stigmasterol (1.458 %), squalene (1.354 %) and palmitic acid (1.711 %) were the major constituents in the petroleum ether extract. The most abundant compounds identified in the methanolic extract were gallic acid (90.8 ppm), apigenin (71.5 ppm), cinnamic acid (66.9 ppm), luteolin (62.5 ppm), caffeic acid (61.5 ppm) and quercetin (60.3 ppm). Total phenolic content (TPC) was 141 ± 3.2 mg GAE/g, TFC was 153.8 ± 4.1 mg QE/g, and TAC was 0.87 ± 0.04 mg AAE/g. The methanolic extract showed a strong concentration-dependent DNA-protective effect (90.47, 78.01 and 64.43 % at 500, 250 and 100 µg/mL, respectively) against UV/H_2O_2-induced oxidative damage.*

Conclusion: *The methanolic extract of I. graveolens contains abundant phenolic and flavonoid compounds with significant antioxidant and genoprotective effects.*

Keywords: *GC-MS, HPLC, Inula graveolens, Phenolic compounds, Phytochemical profiling*

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INTRODUCTION

Inula graveolens (L.) Desf., commonly known as sweet inula or sticky fleabane, is an annual aromatic herb of the Asteraceae family; the genus *Inula* has about 100 species native to temperate areas of Europe, Asia and North Africa [1,2]. In more traditional local applications, *I. graveolens* has been used in different folk medicinal systems as an anti-inflammatory, antimicrobial, antispasmodic, and respiratory remedy for chronic bronchitis and spasmodic coughs, a cardiac tonic, and a natural anticancer remedy in traditional medicine [3,4]. These uses have stimulated many phytochemical studies in an effort to identify and clarify bioactive constituents with beneficial effects.

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In a recent study, isobornyl acetate 50.8 %, borneol 18.3 %, τ-cadinol 6.2 % were detected in an Algerian plant oil [5]. In contrast, bornyl acetate (68.5 %), borneol (7.7 %), camphene (4.6 %), epi-α-cadinol (4.0 %), and eicosane (3.2 %) were identified as the main components by GC-MS analysis of the essential oil from Turkish *I. graveolens* [6]. Also, bornyl acetate (33.4 – 39.6 %), borneol (19.3 – 21.4 %) and τ-cadinol (9.2 – 11.3 %) have been identified in a Tunisian population [7]. The methanol extract significantly inhibited oxidation in the β-carotene/linoleic acid system and showed DPPH radical scavenging activity, indicating a strong correlation between antioxidant activity and phenolic acid content [6,7]. This study therefore investigated the phytochemical composition and genoprotective activity of *I. graveolens* from the Southern Iraqi region (Al-Siba) against UV/H_2O_2-induced DNA damage.

EXPERIMENTAL

Plant collection and authentication

The leaves of *Inula graveolens* (L.) Desf. were collected at full flowering (April, 2024) from Al-Siba, nearly 45 km south of Basra Governorate, Iraq (30°23′ N, 47°45′ E). The plant material was taxonomically authenticated by Ula Al-Mousawi (Department of Pharmacognosy, College of Pharmacy, University of Basrah), and a voucher specimen (reference No. IG/SIBA-04/2024) was deposited at the herbarium for future reference.

Sample preparation

The leaves of *Inula graveolens* were thoroughly cleaned and shade-dried at room temperature (25 – 30 °C) for 14 days until a stable weight was achieved. Dried plant material was mechanically pulverized using a laboratory mill (Retsch SM100, Germany) to obtain a finely powdered homogeneous sample. This powder was kept at 4 °C in light-protective and humidity-free amber-glass containers until further use.

Extraction procedures

Soxhlet extraction for non-polar constituents

The dried powdered plant material (50 g) was extracted continuously with 500 mL petroleum ether (boiling range 40 – 60 °C, analytical grade, Sigma-Aldrich) by Soxhlet extraction (Buchi B-811, Switzerland) for 8 h at a cycle rate of 5 – 6 per h. The obtained extract was concentrated at 40 °C under reduced pressure with a rotary evaporator (Heidolph, Germany). The dried lipophilic extract was stored at 4 °C prior to GC-MS analysis, and the extraction yield was determined gravimetrically.

Maceration extraction for polar constituents

Polar phenolics were extracted from 50 g of powdered plant material by maceration with 500 mL of 70 % (v/v, HPLC-grade) aqueous methanol in a stoppered Erlenmeyer flask at room temperature (22 ± 2 °C). The extraction was performed for three days under orbital shaking at 120 rpm. The resulting mixture was strained through Whatman No. 1 filter paper. The residue was re-extracted two additional times, under the same conditions using fresh solvent (2 × 250 mL). The filtrates were consolidated and concentrated in a rotary evaporator (at 40 °C), lyophilized using Free Zone 4.5 equipment (Labconco, USA), and afterwards kept at 4 °C.

Gas chromatography – mass spectrometry (GC-MS)

The dried petroleum ether extract (10 mg) was dissolved in 1 mL n-hexane (GC grade) and filtered using a 0.22 µm PTFE syringe filter. Gas chromatography–mass spectrometry (GC-MS) analysis was carried out on an Agilent 7890B GC system (Agilent Technologies Corp, USA) coupled to a mass analyzer, control module, and interface of the same brand and model. Separation was performed on an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) under standard conditions (flow rate of carrier gas (helium) was 1.0 mL/min, oven temperature program was 60 °C (hold 2 min), ramp at 10 °C/min to 300 °C in 10 min, temperatures were 250 °C and 230 °C for the injector and ion source). Acquisition of mass spectra was performed in electron ionization (EI) mode at 70 eV over a range of scan (m/z 35 to 550), followed by compound identification by comparing the obtained mass spectra with those in the National Institute of Standards and Technology (NIST) Mass Spectral Library (Version 14) and published retention index information.

HPLC analysis (methanolic extract)

Quantitative analysis of phenolic compounds in the methanol extract was carried out using a Shimadzu LC-20AD HPLC system (Shimadzu, Japan) with a C18 reverse-phase column (Phenomenex Luna; 250 mm × 4.6 mm, 5 µm). The mobile phase was (A) water containing 0.1 % formic acid (v/v) and (B) methanol. The gradient elution was programmed as follows: 10 % solvent B was maintained from 0 to 5 min, followed by a linear increase from 10 to 60 % B

between 5 and 25 min. From 25 to 35 min, the proportion of solvent B was further increased from 60 to 90 %, after which 90 % B was maintained for 35–40 min before re-equilibrating the column with solvent A. The flow rate was 1.0 mL/min, injection volume was 20 µL, column temperature was maintained at 30 °C, and detection performed at wavelengths of 280 nm (phenolic acids) or 320 nm (flavonoids). Individual compounds were identified and quantified by comparison with authentic external standards (Sigma-Aldrich, purity > 98 %). Results were reported as ppm of dry extract.

Total phenolic content (TPC)

Total polyphenolic content (TPC) was quantified using a colorimetric assay [8]. Diluted methanol extract (0.5 mL of methanol extract (1 mg/mL)) was combined with 2.5 mL 0.2 N Folin-Ciocalteu reagent (Sigma) and set aside for 5 min. Thereafter, 2.0 mL 7.5 % (w/v) sodium carbonate was added and incubated at room temperature in the dark for 60 min. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). Total polyphenolic content (TPC) was reported as milligrams of gallic acid equivalents per gram of extract (mg GAE/g of extract).

Total flavonoid content (TFC)

Total flavonoid content (TFC) was measured by the aluminum chloride colorimetric method [9]. Briefly, 1.0 mL of extract (1 mg/mL) was diluted by mixing with 3 mL 95 % ethanol, 0.2 mL of aluminum chloride solution (10 %, w/v), 0.2 mL of potassium acetate (1 M), and 5.6 mL of distilled water [9]. Absorbance was measured at 415 nm after a 30 min incubation at 25 °C. Flavonoid content was expressed as mg of quercetin equivalents per gram of dry extract (mg QE/g extract).

Total antioxidant capacity (phosphomolybdenum method)

The phosphomolybdenum reduction method was used to assess total antioxidant capacity [10]. Aliquots of 0.3 mL from each extract (1 mg/mL) were mixed with 3.0 mL reagent solution (0.6 M sulfuric acid, sodium phosphate (28 mM) and ammonium molybdate (4 mM)). The mixture was incubated at 95 °C in a water bath for 90 min. Absorbance was measured against a blank (methanol) at 695 nm after cooling to room temperature. Results were expressed as mg of ascorbic acid equivalents per gram of dry extract (mg AAE/g extract), using 5-0.05mg/ml of ascorbic acid.

Genoprotective activity

Assessment of DNA damage was conducted using genomic DNA obtained from human white blood cells (WBCs). The DNA extraction process was conducted with the FAVORGEN DNA extraction kit, adhering to the manufacturer's guidelines. The genomic DNA was subjected to photolysis using UV light while being exposed to hydrogen peroxide (H_2O_2). Subsequent damage inflicted on DNA was evaluated using agarose gel electrophoresis. Three polyethylene microcentrifuge tubes were each supplemented with 5 µL aliquots of human genomic DNA, at 11.3 ng/µL. The tubes were then supplemented with three distinct concentrations of *I. graveolens* methanolic extract (500, 250, and 100 µg/mL). The fourth tube was used as a control for irradiation (CR) without *I. graveolens* extract. All tubes were compared with an intact DNA sample to evaluate the protection assay. Each tube was supplemented with 5 µL of 3 % hydrogen peroxide (H_2O_2) solution.

Subsequently, the tubes were positioned onto the surface of a UV transilluminator emitting light at 300 nm and left undisturbed for 10 min at ambient temperature. Thereafter, all the samples were subjected to electrophoresis on a 1 % agarose gel and ImageJ software was used to analyze the results by applying the following formula: "DNA damage% = Conc. (ng) of the untreated DNA * Area under curve of the treated DNA in presence of plant sample / Area under curve of the untreated DNA". Area under the curve obtained from the ImageJ software.

Statistical analysis

Statistical analysis was done using the Statistical Package for the Social Sciences (SPSS, version 26.0, IBM Corp., USA). Experiments were performed in triplicate, and data were reported as mean ± standard deviation (SD), and compared using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. $P < 0.05$ was considered statistically significant.

RESULTS

Extraction yields

The petroleum ether extract was an oily greenish residue (2.1 ± 0.3 % w/w). The methanolic extract was a brownish hygroscopic powder (19.8 ± 1.2 % w/w), suggesting an approximately nine-fold greater extraction efficiency compared to the non-polar solvent.

Gas chromatography – mass spectroscopy (GC-MS)

The GC-MS analysis of the petroleum ether extract revealed the presence of eight major compounds. The identified compounds were fatty acids, one triterpene precursor, and phytosterols. In addition to a low percentage of 0.407% 2-Octadecanone (ketone) and 0.113% 2,4-Di-tert-butylphenol (phenol). Among these compounds, linoleic acid was the highest (3.568 %), while 2,4-di-tert-butylphenol was the least (Table 1).

High-performance liquid chromatography (HPLC) of methanolic extract

Analysis of the 70 % aqueous methanol extract using HPLC showed a complex phenolic profile made up of six significant compounds (Table 2, Figure 1).

Total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC)

The TFC was higher than the TPC, which indirectly suggests that flavonoids form a large part of the phenolic pool (Table 3).

Genoprotective activity

The extract showed 90.47 %, 78.01 %, and 64.43 % protection at 500, 250, and 100 µg/mL, respectively, in a dose-dependent manner ($p < 0.05$, Figure 2).

Table 1: Gas chromatography – mass spectrometry of petroleum ether extract of *Inula graveolens*

RT (min)	Compound Name	TIC % Area	Chemical formula
17.931	2,4-Di-tert-butylphenol	0.113	$C_{14}H_{22}O$
23.328	Hexadecanoic acid (Palmitic acid)	1.711	$C_{16}H_{32}O_2$
24.524	2-Octadecanone	0.407	$C_{18}H_{36}O$
26.175	Linoleic acid	3.568	$C_{18}H_{32}O_2$
26.262	Oleic acid	1.842	$C_{18}H_{34}O_2$
26.581	Stearic acid	0.871	$C_{18}H_{36}O_2$
31.328	Squalene	1.354	$C_{30}H_{50}$
33.638	Stigmasterol	1.458	$C_{29}H_{48}O$

Total ion chromatogram (TIC)

Table 2: Phenolic compounds quantified by HPLC in *Inula graveolens* methanolic extract

Compound Name	Concentration (ppm)
Apigenin	71.5
Caffeic acid	61.5
Cinnamic acid	66.9
Gallic acid	90.8
Luteolin	62.5
Quercetin	60.3

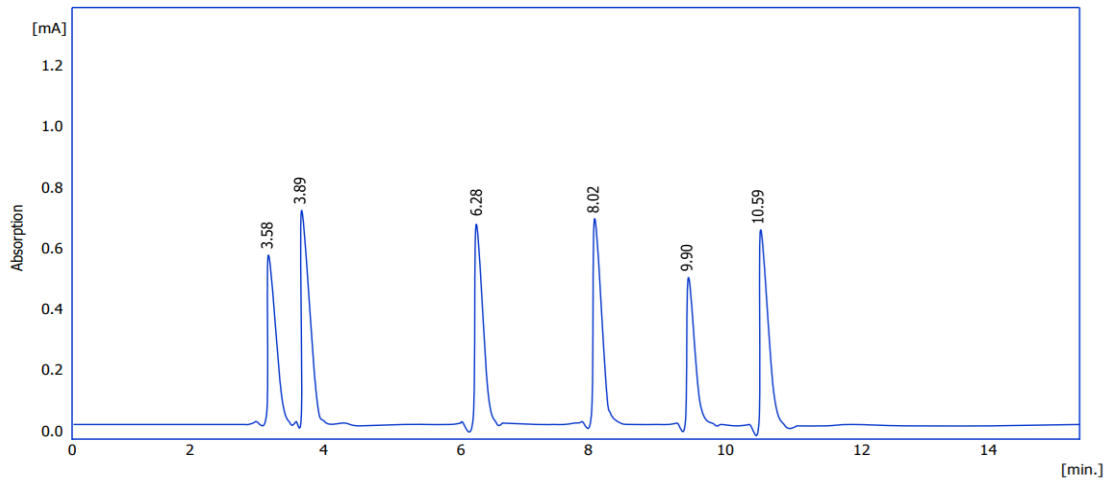

Figure 1: High-performance liquid chromatography (HPLC) of methanolic extract (phenolic acids – 280 nm; flavonoids – 320 nm)

Table 3: The TPC, TFC, and TAC of *Inula graveolens* methanolic extract

Parameter	Value
Total Phenolic Content (TPC)	141 ± 3.2 mg GAE/g DE
Total Flavonoid Content (TFC)	153.8 ± 4.1 mg QE/g DE
Total Antioxidant Capacity (TAC)	0.87 ± 0.04 mg AAE/g DE

Values are expressed as mean ± SD (n = 3). Dry extract (DE), gallic acid equivalents (GAE), quercetin equivalents (QE), ascorbic acid equivalents (AAE)

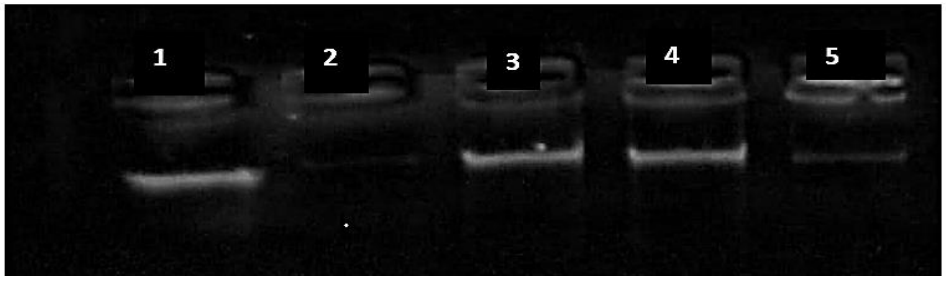

Figure 2: DNA damage protection assay of *Inula graveolens* methanolic extract. Ability of Achillia methanolic extract to protect DNA from damage caused by UV-light and (3% v/v) H2 O2 Lane 1: Control (untreated DNA), Lane 2: DNA UV-irradiated and treated with (3% v/v) H2 O2, Lanes 3–5: Achillia methanolic extract at concentrations of 500, 250 and 100, µg/ml, respectively, with DNA exposure to UV-light and 3% v/v H2 O2

DISCUSSION

The findings revealed that 70 % aqueous methanol achieved a higher yield compared to petroleum ether, which might be due to basic differences in solvent composition, consistent with previous studies [11]. The composition examined by GC-MS was dominated by fatty acids, a triterpene precursor, and phytosterols. This composition is significantly distinct from the essential oil profiles of *I. graveolens* widely reported in the literature, with predominant volatile oxygenated monoterpenes such as bornyl acetate and borneol [12]. This may be due to different protocols used in the extraction procedure. Dried plant powder was extracted with petroleum ether using Soxhlet extraction; however, prolonged heating may result in the evaporation and subsequent loss of highly volatile monoterpenes, thereby reducing their recovery [13]. This process, rather than extracting polyphenols, mostly extracts higher molecular weight and less volatile lipids and triterpenes.

Linoleic acid (C18:2, ω-6) is an essential polyunsaturated fatty acid with anti-inflammatory effects [14]. The high concentrations of oleic acid and palmitic acid indicated the lipid-rich nature of the non-polar fraction. Detection of stigmasterol and squalene suggested the presence of notable non-volatile isoprenoid compounds. The presence of 2,4-di-tert-butylphenol (a phenolic antioxidant) possessing antimicrobial and anti-inflammatory effects [15] correlated with other Inula species.

High-performance liquid chromatography (HPLC) confirmed that 70 % aqueous methanol is highly effective in extracting a wide variety of hydrophilic phenolic compounds from *I. graveolens*. This observed profile is similar to those previously reported for the species [16,17]. Gallic acid, the most abundant compound (90.8 ppm), is an effective, naturally occurring antioxidant with verified metal-chelating and free radical scavenging properties [2]. Quercetin, luteolin and apigenin are examples of flavonoids with various pharmacological properties including antioxidant, anti-inflammatory and anticancer effects [18]. Caffeic and cinnamic acids are phenolic acids with antioxidant and antimicrobial properties [19]. A previous study has reported the presence of phenolic compounds such as chlorogenic acid, quinic acid, hyperoside, protocatechuic acid and quercetin in ethanol and water extracts of *I. graveolens* using UHPLC-MS/MS analysis [16]. This study revealed the presence of quercetin, which is consistent with previous reports [16]. On the other hand, chlorogenic acid was not identified in this study, which may be due to differences in plant material (geographical origin, harvest season), extraction solvent system (70 % methanol vs absolute methanol or ethanol) and/or HPLC detection conditions.

One study on the total phenolic concentration of three Turkish Inula species (flowers, fruits and whole plant samples) reported values from 21.1 ± 0.8 to 190.9 ± 6.1 mg GAE/g extract [18]. Thus, the results were within expected ranges for the analyzed sample. The TPC (141 ± 3.2 mg GAE/g) and TFC (153.8 ± 4.1 mg QE/g) values presented in this study were among the higher

value reported to date. An earlier study recorded a TPC of 5.36 ± 0.32 mg GAE/g, and a TFC of 3.49 ± 0.05 mg QE/g, which are approximately 26-fold and 44-fold differences compared with this study [17]. This large discrepancy is likely due to a combination of factors, which include geographic and chemotypic variation [20], extraction solvent composition, and the nature of the biological material and phenological stage [21]. The relatively high TFC revealed that flavonoids constituted a large part of the phenolic pool, and the HPLC profile of the plant identified apigenin, luteolin and quercetin as dominant constituents. The TAC was lower than the TPC and TFC. This difference is not surprising because the phosphomolybdenum assay quantifies total reducing capacity involving all electroactive molecules, while the Folin-Ciocalteu and aluminum chloride methods detect phenolic hydroxyl groups and flavonoid structures, respectively. The methanolic extract showed effective concentration-dependent protection from UV/H_2O_2-induced DNA damage, with 90.47 % protection at 500 µg/mL. This activity may be associated with higher TPC and TFC, further corroborating findings from a previous study [6]. The human DNA scission assay is an established model system for measuring the protective effect of molecules against oxidative stress [22].

The high genoprotection at 500 µg/mL is highly comparable with DPPH radical scavenging activity reported in a previous study [6], substantiating a close association between phenolic content and free radical-scavenging potential. Also, the observed genoprotection at concentrations as low as 100 µg/mL might be relevant to clinical settings, especially since oxidative damage to DNA plays a direct role in mutagenesis, carcinogenesis, and aging pathways. This activity may be due to the high concentration of phenolics and flavonoids. Future studies on the *in vivo* safety and activity of the extract, purification, characterization, and molecular elucidation of the bioactive compounds should be pursued.

CONCLUSIONS

The methanolic extract of *I. graveolens* contains abundant phenolic and flavonoid compounds with significant antioxidant and dose-dependent DNA-protective effects.

DECLARATIONS

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None.

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare no conflict of interest.

Contributions of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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