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Otospermum glabrum extracts: antioxidant properties and bioactivity against fungal pathogens and Aphis fabae

Otospermum glabrum özleri: fungal patojenlere ve Aphis fabae'ye karşı antioksidan özellikler ve biyoaktivite

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ABSTRACT

The adoption of plant-based biopesticides as sustainable alternatives to synthetic chemicals in agricultural pest management has gained increasing attention due to their safety and ecological benefits. Many of these natural compounds demonstrate potent antifungal activity, mitigating foodborne fungal contamination and associated mycotoxins. This study evaluated the antioxidant, antifungal, and aphicidal potential of Otospermum glabrum (Asteraceae) extracts derived from its aerial parts. Three extracts -ethyl acetate, methanolic, and aqueous- were assessed for total polyphenolic and flavonoid contents, antioxidant activity (DPPH radical scavenging, β-carotene bleaching, and total antioxidant capacity), antifungal effects against six post-harvest and crop fungal pathogens, and aphicidal activity against the black bean aphid (Aphis fabae Scop.). The ethyl acetate extract (EaE) exhibited the highest polyphenols (57.60 \pm 0.17 μg GAE/mg) and flavonoids (49.46 \pm 0.66 μg QE/mg), while the methanolic extract (ME) demonstrated the strongest DPPH scavenging activity (IC₅₀ = $56.05 \pm 0.03 \,\mu\text{g/ml}$) and β -carotene bleaching inhibition, comparable to BHT. The aqueous and ethyl acetate extracts showed the highest total antioxidant capacity (252.60 \pm 0.20 and 249.10 \pm 0.81 μg AAE/mg, respectively). Antifungal assays revealed that ethyl acetate and methanolic extracts were the most effective, with inhibition percentages (IP) exceeding 65% against all tested fungi. Additionally, the methanolic extract at 30% concentration induced 100% mortality in A. fabae after 72 h and exhibited significant repellency (48.98 ± 8.76%). These findings highlight O. glabrum as a promising source of natural biopesticides for integrated pest management.

INTRODUCTION

Ensuring food security is essential for sustaining the growing populations of both humans and animals. Food stability can be significantly impacted by unexpected challenges in food production caused by various organisms, including bacteria, viruses, fungi, and insects (Hendel et al. 2021, Mwangi et al. 2023).

Fungal pathogens are responsible for 70-80% of microbialrelated agricultural losses, with approximately 8.000 species causing nearly 100.000 plant diseases, while recent studies report over 19.000 fungal species as phytopathogens (Deresa and Diriba 2023). Highly destructive genera such as Aspergillus, Penicillium, and Fusarium induce critical crop diseases; including corn ear rot (Fusarium spp.), cotton boll rot (Aspergillus spp.), grape blue mold (Penicillium spp.), and wheat Fusarium crown rot (Fusarium spp.), resulting in severe pre- and postharvest yield reductions (Alananbeh et al. 2024, Ghuffar et al. 2021, Zakaria 2024). At the same time, insects and pests significantly impact global food production across all stages of crop growth, harvest, and storage, leading to an estimated annual reduction of 18-20% of agricultural yield, equivalent to over US\$ 470 billion (Souto et al. 2021). The black bean aphid (Aphis fabae Scopoli), a polyphagous pest infesting over 200 plant species, is particularly destructive to fava beans, with Algerian outbreaks causing up to 50% yield loss through direct damage (sap depletion, honeydew excretion, tissue deformation/gall formation) and indirect harm as a vector for more than 30 plant viruses (Benbelkhir et al. 2024). These impacts are compounded by rising costs of chemical control measures.

Conventional management of black bean aphids and fungal diseases depends on synthetic pesticides (neonicotinoids, pyrethroids, azoles, and strobilurins) for their broadspectrum efficacy (Almogdad and Semaškienė 2021, Pandey and Rathore 2023, Pintye et al. 2024). However, their overuse endangers human health, ecosystems, and accelerates pesticide resistance (Hernández-Ceja et al. 2021, Li et al. 2024). Plant-derived biopesticides offer a sustainable alternative, combining low environmental persistence, minimal mammalian toxicity, and complex phytochemical profiles that limit resistance evolution. These attributes, alongside proven efficacy against diverse pests and pathogens, position botanical extracts as environmentally compatible tools for integrated pest management (Ahmed et al. 2020, Noureldeen et al. 2022).

Approximately 2.500 plant species from 235 botanical families show efficacy against pathogens and insects through their secondary metabolites (Ngegba et al. 2022). Notably, polyphenols serve dual protective functions, exhibiting

both potent antioxidant capacity and enhanced defense mechanisms against biotic and abiotic stressors, thereby significantly improving plant stress tolerance (Hbika et al. 2022, Šamec et al., 2021).

Otospermum glabrum (Lag.) Willk. (Asteraceae) is an annual glabrous herb characterized by erect or ascending stems, 1-3 pinnatipartite leaves, and small corymbose inflorescences. The flowers feature white, tridentate ligules and blackbordered linear-obtuse bracts (Quezel and Santa 1962). As a member of the *Anthemideae* tribe, which includes bioactive generalike *Achillea*, *Artemisia*, and *Matricaria*, it likely shares their characteristic phytochemicals (phenolics, flavonoids, terpenoids, alkaloids, and coumarins) responsible for demonstrated antioxidant, antimicrobial, and insecticidal properties (El Mihyaoui et al. 2022, Kaczorová et al. 2021, Kursa et al. 2022, Silva-Beltran et al. 2023, Yang et al. 2024). This phytochemical similarity suggests that *O. glabrum* may possess comparable biological activities worthy of investigation.

To date, there is no existing documentation in the current literature regarding *O. glabrum*, aside from limited botanical and taxonomical reports. Therefore, this study aims to assess the total polyphenol and flavonoid content, explore the antioxidant and antifungal activities of *O. glabrum* extracts, and evaluate their aphicidal effects against *A. fabae*, known as the black bean aphid.

Despite the well-documented bioactivity of *Anthemideae* tribe members, *O. glabrum* remains pharmacologically unexplored, with only limited taxonomic descriptions available. This study therefore investigates the total polyphenol and flavonoid content, antioxidant and antifungal potential of its extracts, and aphicidal activity against *A. fabae*, a destructive pest of leguminous crops.

MATERIALS AND METHODS

Plant material

Aerial parts of *O. glabrum* were collected during the flowering period in April 2022 from the northern region of M'sila (35.8498° N, 4.5426° E), Algeria. The plant material was authenticated by Dr. Djamel Sarri, with a voucher specimen (OG2865QS28N) deposited in the herbarium of the Laboratory of Biology: Applications in Health and Environment at M'sila University. Following collection, the plant material was shade-dried at ambient temperature (25 \pm 2 °C) and stored in paper bags until further use.

Plant extract preparation

The aqueous extract (AE) was prepared using a standardized decoction method (Ljubuncic et al. 2005). Briefly, plant

powder (50 g) was soaked in distilled water (500 ml) and heated to 90 °C for 15 min with constant stirring. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated at 40 °C using a drying oven. The final extract was stored at 4 °C until further use.

The ethyl acetate (EaA) and methanolic (ME) extracts were prepared by maceration (Mbarga et al. 2022). Plant powder (50 g) was soaked in 450 ml either methanol (Merck 99.9%) or ethyl acetate (Merck 99.8%) with continuous stirring for 24 hours, then filtered through Whatman No. 1 filter paper. The filtrates were concentrated under reduced pressure at 40 °C and stored at 4 °C until further use.

Total phenolic content (TPC)

Total phenolic content (TPC) in *O. glabrum* extracts was determined using a modified Folin-Ciocalteu reagent (FCR) method (Elbouzidi et al. 2023). Extract aliquots (100 μl) were mixed with 500 μl of 10% FCR, followed by addition of 400 μl of 7.5% Na₂CO₃ after 4 minutes. After 2 hours of incubation at room temperature, absorbance was measured at 765 nm against a blank, using a UV-Visible Spectrophotometer (CHIMADZU UV-1280 Multipurpose). A gallic acid standard curve was used to calculate results, expressed as micrograms of gallic acid equivalents per milligram of extract (μg GAE/mg).

Total flavonoid content (TFC)

Total flavonoid content (TFC) in *O. glabrum* extracts was determined using an aluminum chloride colorimetric assay modified from Madjitoloum Betoloum et al. (2018). Each extract aliquot (1 ml) was mixed with 1 ml of 2% $AlCl_3$ solution. After 10 minutes of incubation at room temperature, absorbance was measured at 415 nm against a blank. Results were calculated from a quercetin standard curve and expressed as micrograms of quercetin equivalents per milligram of extract (μ g QE/mg).

Evaluation of the antioxidant activity

Total antioxidant capacity (TAC) assay

Total antioxidant capacity was determined using a modified phosphomolybdenum assay (Aazza et al. 2024) with ascorbic acid as standard. The reagent solution (0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate) was prepared, and reaction mixtures containing 0.1 ml extract and 0.9 ml reagent solution were incubated at 95 °C for 90 min. After cooling at room temperature, absorbance was measured at 695 nm against a blank. Results were calculated from an ascorbic acid (AA) standard curve and expressed as micrograms of AA equivalents per milligram extract (μ g AAE/mg).

Free radical scavenging assay

Free radical scavenging activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay following Mokhtari et al. (2023) with some modification. Methanolic solutions of test extracts (1 ml) were mixed with 1 ml of 0.004% DPPH in methanol and incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm against a DPPH solution control, with butylated hydroxytoluene (BHT) as positive control. Radical scavenging activity was calculated as:

$$I(\%) = [(A_c - A_f)/A_c] \times 100$$

where A_c is the control absorbance and A_t is the test sample absorbance. The IC_{50} value (concentration yielding 50% inhibition) was determined from dose-response curves of inhibition percentage versus extract concentration.

β-carotene bleaching test

Antioxidant activity was evaluated using a β -carotene/linoleic acid bleaching assay modified from Tabet Zatla et al. (2023). The β -carotene stock solution was prepared by dissolving 1 mg β -carotene in 5 ml chloroform with 25 μ l linoleic acid and 200 mg Tween 40. After chloroform evaporation at 40 °C under vacuum, the residue was emulsified in 100 ml oxygen-saturated distilled water by vigorous shaking. For testing, 0.5 ml of methanolic extract solutions were mixed with 2 ml emulsion, with initial absorbance (t=0) measured immediately at 470 nm. Samples were then incubated at 50 °C for 120 min, followed by final absorbance measurement. Butylated hydroxytoluene (BHT) served as positive control. β -carotene bleaching inhibition was calculated as:

$$I\% = [(A_1 - C_1 / C_0 - C_1)] \times 100$$

where A_t is sample absorbance at 120 min, C_t is control absorbance at 120 min, and C_0 is control absorbance at t=0 min. The IC₅₀ was determined from dose-response curves of inhibition percentage versus extract concentration.

Antifungal activity test

The fungal strains used in this study; Aspergillus flavus, Aspergillus niger, Botrytis cinerea, Fusarium culmorum, Penicillium expansum, and Penicillium italicum, were obtained from the department of microbiology & biochemistry's laboratory of microbiology, M'sila University, Algeria. These molds were previously isolated and identified by Hendel et al. (2024). They were cultivated on potato dextrose agar (PDA) slants and kept at 4 °C for storage until required.

Antifungal activity was evaluated on potato dextrose agar (PDA) using a modified agar incorporation method (Fatehi et al. 2021). Extracts (AE, ME, EaA) were dissolved in DMSO and incorporated into molten PDA at 10 mg/ml. Aliquots (20 ml) were dispensed into 90 mm Petri dishes and allowed to solidify. A 6 mm mycelial disc from the growing margin of 7-day-old fungal cultures was aseptically transferred to each plate center. Control plates contained DMSO without extract. After 7 days of incubation at 25 °C, fungal growth inhibition was calculated as:

$$I\% = [(D_C - D_T) / D_C] \times 100$$

where $D_{\rm C}$ is the control colony diameter and DT is the treated colony diameter.

Evaluation of the aphicidal activity

Adult black bean aphids and Vicia faba leaves used in this study were collected in April 2024 from an infested fava bean field in the Bordj Bou Arréridj area (36.0391° N, 4.8847° E), Algeria. Aphid-infested plant sections were carefully transferred to ventilated rearing cages and transported to the laboratory under controlled conditions to preserve colony integrity. To standardize bioassays, wingless adult aphids of uniform size were selectively isolated using a fine bristle brush to minimize physical stress. The selected individuals were subsequently transferred to Petri dishes for immediate bioassay procedures. Taxonomic identification was confirmed as *A. fabae* following Martin's (1983) morphological key, with voucher specimens deposited in the laboratory archive.

Toxicity test

Aphid bioassays were conducted in 90 mm Petri dishes under controlled laboratory conditions (25 \pm 2 °C, 60 \pm 20% RH, 16:8 L:D photoperiod). Four concentrations (5%, 10%, 20%, and 30% w/v) of AE and ME were prepared by dissolving *O. glabrum* extracts in distilled water. Following Salari et al. (2010), twenty-seven Petri dishes were prepared with three replicates per concentration. Fresh *Vicia faba* leaves were immersed in extract solutions for 10 seconds, air-dried, and placed on moist filter paper in each dish. Fifteen wingless adult aphids were then introduced per dish. Control treatments received distilled water only. Mortality was assessed at 24, 48, and 72 hours post-treatment, with aphids considered dead when showing no movement of legs or antennae.

Repellency test

Repellent activity of AE and ME was evaluated using a modified choice test (Moawad and Al-Barty 2011). Twenty-

four Petri dishes (90 mm) were prepared with three replicates per concentration. Each dish contained filter paper divided into two equal sections. Pairs of surface-sterilized $V.\ faba$ leaves were prepared - one leaf immersed in extract solution (5 min) and air-dried, while the other (untreated) served as control. Fifteen wingless adult aphids were released at the center of each dish. After 24 hours at 25 \pm 2 °C, aphid distribution was recorded. Repellency percentage (RP) was calculated as:

$$RP\% = [(N_C - N_T) / (N_C + N_T)] \times 100$$

where N_C = number of aphids on control leaf and N_T = number on treated leaf.

Statistical analyses

For antioxidant and antifungal assays, all samples were analyzed in triplicate. Results are expressed as mean \pm SD (n = 3). Data were analyzed by two-way ANOVA with Tukey's multiple comparisons test using GraphPad Prism 6.01 (GraphPad Software). Statistical significance was set at p < 0.05.

Corrected mortality (CM%) data were analyzed using generalized linear models (GLMs), followed by probit analysis to determine lethal concentration (LC $_{50}$) values with their confidence intervals. A multivariate analysis of variance (MANOVA) was performed to assess aphid mortality, with extract type, concentration, and exposure time as fixed factors and mortality as the response variable. Tukey's post hoc test was used for mean comparisons at a 5% significance level. All statistical analyses were conducted using R Studio (version 1.2.5019) with R software (version 3.6.1).

RESULTS

Extraction yield

In this study, three extraction methods were employed: aqueous extraction by decoction yielded 23.91 \pm 1.43% (w/w), while methanolic and ethyl acetate extractions by maceration yielded 17.89 \pm 1.82% and 6.53 \pm 0.76% (w/w), respectively.

Polyphenolics and antioxidant activity

Table 1 presents the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity profiles of the plant extracts. Spectrophotometric analysis revealed significant differences in phytochemical composition, with ethyl acetate extract (EaE) showing the highest TPC (57.60 \pm 0.17 μg GAE/mg) and TFC (49.46 \pm 0.66 μg QE/mg), followed by methanolic extract (ME) and aqueous extract (AE) (p < 0.05).

The antioxidant properties of O. glabrum extracts, evaluated by TAC, DPPH radical scavenging, and β-carotene/linoleic acid assays, are summarized in Table 1. The AE exhibited the highest TAC value (252.60 \pm 0.20 μ g AAE/mg), followed closely by the EaE (249.10 \pm 0.81 μg AAE/mg), while the ME recorded the lowest TAC (154.92 \pm 1.06 μ g AAE/mg). DPPH radical scavenging assays revealed ME as the most potent (IC₅₀ = $56.05 \pm 0.03 \mu g/ml$), though three-fold less active than the BHT standard (21.69 \pm 0.04 $\mu g/ml$). Both AE and EaE showed minimal DPPH scavenging (IC₅₀ > 200 μ g/ml). In the β -carotene/linoleic acid system, all three extracts demonstrated significant inhibition of linoleic acid oxidation at 500 µg/ml. The ME achieved complete inhibition (100%, $IC_{50} = 7.27 \pm 0.95 \mu g/ml$), comparable to BHT, with no significant difference (p < 0.05) compared to the standard, while the AE and EaE exhibited inhibition rates of 93.42% and 89.26%, with corresponding IC50 values of $63.67 \pm 3.92 \,\mu g/ml$ and $26.66 \pm 2.70 \,\mu g/ml$, respectively.

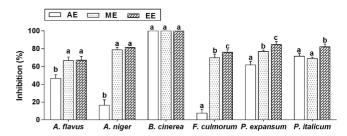


Figure 1. Inhibitory effect of the AE, ME, and EE of *Otospermum glabrum* on the tested molds. No significant differences are indicated by the same letters on each mold column (Tukey's multiple comparisons test, p<0.05; data are means $(n=3) \pm SD$)

Evaluation of the aphicidal activity

Toxicity assays against *A. fabae* revealed significant concentration- and time-dependent mortality effects for *O. glabrum* extracts (Table 2). Both extract concentration $(F(3.32) = 15.601, p = 0.000^{***})$ and exposure duration

Table 1. Polyphenolic and flavonoid contents and antioxidant activity of *Otospermum glabrum* extracts as measured by multiple assays

			Test		
Extract / Standard	Polyphenols (μg GAE/mg)	Flavonoids (µg QE/mg)	TAC (μg AAE/mg)	DPPH (IC ₅₀ , μg/ml)	β-carotene (IC ₅₀ , μg/ml)
ME	42.80 ± 0.56	17.61 ± 0.10	154.92 ± 1.06	56.05 ± 0.03	$7.27 \pm 0.95^{a^*}$
EaE	57.60 ± 0.17	49.46 ± 0.66	249.10 ± 0.81	241.23 ± 0.06	63.67 ± 3.92
AE	19.39 ± 0.16	7.23 ± 0.12	252.60 ± 0.20	267.69 ± 0.47	26.66 ± 2.70
ВНТ	-	-	-	21.69 ± 0.04	4.73 ± 0.40^{a}

^{*}Values with the same superscript letter are not statistically different according to the two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test, p<0.05. Values are means $(n = 3) \pm SD$

Evaluation of the antifungal activity

The antifungal activity of O. glabrum extracts (10 mg/ml) was evaluated by measuring fungal growth inhibition. All extracts demonstrated significant antifungal effects against the tested strains (Figure 1), with complete inhibition of B. cinerea growth observed for all treatments. Ethyl acetate extract (EaE) showed the strongest overall inhibition, followed by methanolic extract (ME), with both maintaining >65% inhibition across all fungi. Notably, EaE and ME exhibited statistically similar efficacy (P < 0.05) against A. flavus and A. niger. In contrast, aqueous extract (AE) displayed the weakest activity (7.56-71.56% inhibition), except against P. italicum, where its performance slightly exceeded ME (p < 0.05).

(F(3.32) = 71.390, p = 0.0000***) significantly influenced aphid mortality. The methanolic extract (ME) demonstrated superior efficacy, achieving 100% mortality at 30% concentration (72-h LC_{50} = 51.02%), while the aqueous extract (AE) showed 86.24% mortality (72-h LC_{50} = 46.77%). Lower concentrations (5-20%) exhibited progressive mortality increases: ME (31.11-72.49%) and AE (33.33-78.09%) across 24-72 h exposure periods.

Repellency test

Repellent activity of *O. glabrum* extracts against *A. fabae* showed concentration-dependent effects (Table 3). Both extract type (F(1.16) = 4.5, p = 0.048*) and concentration (F(3.16) = 5.19, p = 0.01071) significantly influenced repellency. The methanolic extract (ME) exhibited stronger

Table 2. Lethal concentrations of Otospermum glabrum extract against Aphis fabae aphid population following 72 h exposure

Extract	Exposure time	Concentration (%)	Corrected mortality (%)*	LC ₅₀ (%)	95% Confidence Interval	Slope ± SE	χ^2	
		5	33.33±0.00lk	49.73±0.29	46.75–55.46	1.4 ± 0.2	2.1	
	24 h	10	51.11 ± 2.22^{jihg}					
		20	$53.33l{\pm}3.84^{ihgf}$					
		30	$55.56{\pm}4.44^{\rm lihgfe}$					
		5	41.75±3.21 ^{lkj}					
AE	48 h	10	$53.49{\pm}2.06^{\rm lihgf}$	46.94±2.40	35.45–57.53	1.5 ± 0.3	2.4	
		20	$58.10{\pm}4.22^{\rm lihgfe}$					
		30	$65.08{\pm}0.79^{\rm hgfed}$					
		5	61.31±7.92 ^{lihgfe}				2.6	
	70 l	10	$69.93f \pm 3.85^{edc}$	46 77 16 02	45 77 76 02	12.02	2.6	
	72 h	20	78.09 ± 3.48^{dcb}	46.77±6.03	45.77–76.83	1.2 ± 0.3	2.6	
		30	86.24±3.13 ^b					
	24 h	5	31.11±0.00 ¹	49.74±0.25	45.53-73.24	1.3 ± 0.2	2.3	
		10	31.11±2.22 ¹					
		20	$42.22{\pm}5.87^{\rm lkj}$					
		30	$68.89{\pm}2.22^{\rm gfedc}$					
		5	44.29±2.97 ^{lkji}					
ME	40.1	10	$48.73{\pm}3.09^{kjih}$	48.37±2.06	37.67–59.77	1.4 ± 0.3	2.3	
	48 h	20	48.57 ± 5.71^{kjih}					
		30	83.65±2.55cb					
		5	59.21±5.01 ^{lihgfe}					
	72 L	10	$64.80{\pm}2.29^{\rm hgfed}$	F1 02 : 0 01	40.27. 60.02	11.02	2.1	2.5
	72 h	20	$72.49 \pm 6.27^{\text{edcb}}$	51.02±0.01	49.37–69.03	1.1 ± 0.2	2.5	
		30	100 ± 0.00^{a}					
(1.12)=0.231. P=.63	F-(2.12)=124.61 P=.0.00000	F- (3.12)=71.30 P=.0.00000						

^{*}Values are presented as mean \pm SE (n=3), significant differences were determined at P \leq 0.05; different superscript letters within a column indicate statistically significant differences at P < 0.05

Table 3. Repellent activity of Otospermum glabrum extract against Aphis fabae populations

Plant extracts	Concentration (%)	RP (%)*	Class
AE	5	12.50±00.00 ^b	I
	10	12.50 ± 00.00^{b}	I
	20	31.94 ± 10.84^{ab}	II
	30	31.94 ± 10.84^{ab}	II
ME	5	26.38±06.94 ^{ab}	II
	10	19.44 ± 06.94^{ab}	I
	20	38.88 ± 05.55^{ab}	II
	30	48.98 ± 08.76^{a}	III
value (1. 16) =4.5. P=.048	F-value (3. 16)=5.19.P=.01071		

^{*}RP - Repellency percentage (mean \pm SE, n = 3). Different superscript letters within columns indicate statistically significant differences (p < 0.05)

repellent effects (26.38-48.98%) compared to the aqueous extract (AE; 12.50-31.94%), with efficacy increasing proportionally to concentration.

DISCUSSION

Extraction yields followed the polarity-dependent trend: ethyl acetate extract (EaE) < methanolic extract (ME) < aqueous extract (AE), consistent with the higher efficiency of polar solvents for phytochemical extraction. This pattern reflects the differential solubility of plant metabolites, where solvent polarity critically influences extraction efficiency (Molole et al. 2022). The superior yield of AE likely results from both the high polarity of water and the elevated temperature during decoction, which enhances solubility of polar compounds including flavonoids, alkaloids, and polysaccharides (Kumar et al. 2023). Similar polarity-yield relationships have been documented in related Anthemideae genera; *Achillea* (Toplan et al. 2022) and *Artemisia* (Trifan et al. 2022), confirming our observations.

Total phenolic (TPC) and flavonoid (TFC) contents exhibited a consistent solvent polarity-dependent trend: aqueous < methanolic < ethyl acetate extracts. This pattern reflects the preferential solubility of polyphenolic compounds - including phenolic acids, terpenes, and methoxylated flavonoid aglycones - in less polar solvents (Palaiogiannis et al. 2023). The aqueous extract's lower yields may result from both the limited solubility of non-polar phenolics in water and potential thermal degradation during decoction (Lezoul et al. 2020). These findings align with previous reports on Algerian Matricaria chamomilla (Khennouf et al. 2013) and Achillea species (Kaczorová et al. 2021), where chloroform and ethyl acetate consistently outperformed polar solvents in extracting polyphenols, confirming that medium-polarity solvents optimally recover these bioactive compounds. Comparative analysis reveals superior polyphenolic content in O. glabrum relative to related species. Our TPC and TFC values significantly exceeded those reported for Achillea species (TPC: 16.34-27.48 μg GAE/mg; TFC: 11.31-27.13 μg QE/mg) (Mehmood et al. 2022) and approached the highest levels documented in Artemisia species (methanol extracts: 106.34 µg GAE/g in A. vulgaris; 47.74 µg RE/mg in A. annua) (Trifan et al. 2022). Notably, our extracts consistently outperformed the lowest-yielding chloroform extracts of A. absinthium (TPC: 5.78 µg GAE/g; TFC: 0.37 µg RE/mg). These findings position O. glabrum as a particularly rich source of bioactive polyphenols, with significant potential for biologically protective applications.

The antioxidant results revealed an inverse relationship between *O. glabrum*'s total phenolic/flavonoid content (TPC/TFC) and total antioxidant capacity (TAC), with the

aqueous extract (AE) showing the highest TAC despite lower TPC/TFC levels. This apparent discordance results from three key factors: the presence of non-phenolic antioxidants (e.g., carotenoids, tocopherols) not detected by TPC assays, structural efficacy of specific compounds that disproportionately contribute to antioxidant activity, and synergistic interactions between phytochemicals that enhance overall activity. These findings align with reports in Artemisia species (Guenane et al. 2024, Trifan et al. 2022), confirming that antioxidant potential cannot be predicted by polyphenolic content alone. Notably, O. glabrum's TAC (up to 252.60 µg AAE/mg) exceeded values reported for related species like Achillea coarctata (226.82 µg AAE/ mg) and Matricaria chamomilla (100-124 µg AAE/mg), highlighting its exceptional antioxidant profile (Albayrak and Silahtarlıoğlu, 2019).

The DPPH radical scavenging results revealed that the EaE of O. glabrum, despite containing the highest levels of TPC and TFC, exhibited significantly lower antioxidant activity compared to the ME. This apparent contradiction stems from the structural specificity of DPPH radicals, which preferentially react with flavonoids containing hydroxyl groups on the β-ring and polyhydroxy aromatic acids (Mammeri et al. 2022), rather than responding to overall phenolic concentration. The superior activity of the ME can be attributed to its greater polarity, which enhances extraction of hydroxyl-rich phenolic compounds that are particularly effective in radical neutralization (Kaczorová et al. 2021). These findings are consistent with studies on Achillea species (Kaczorová et al. 2021, Şabanoğlu et al. 2019) and Artemisia species (IC50: 107.2-227 µg/ml) (Ranjbar et al. 2020), confirming that antioxidant efficacy depends more on molecular structure than total polyphenol content. Notably, O. glabrum's ME (IC₅₀ = 56.05 µg/ml) showed stronger radical scavenging activity than most reported values for related Anthemideae species, including various populations of Matricaria chamomilla (IC₅₀: 19.23-73.35 μg/ ml) (Hassanpour et al. 2020), highlighting its exceptional antioxidant potential despite having only moderate TPC levels. This pattern underscores the importance of considering both chemical composition and specific antioxidant mechanisms when evaluating plant extracts for their radical scavenging capacity.

The β -carotene bleaching assay revealed that *O. glabrum*'s EaE extract, despite having the highest TPC/TFC levels, showed the weakest antioxidant activity, underscoring that polyphenols play only a limited role in preventing lipid peroxidation (Faraone et al. 2018). This paradox reflects the system's dependence on non-phenolic antioxidants (carotenoids, tocopherols) that more effectively quench lipid-derived radicals (Othman et al. 2014), as previously

observed in *Achillea* species (Agar et al. 2015, Gharibi et al. 2013). Notably, *O. glabrum* extracts demonstrated superior inhibition of linoleic acid oxidation compared to related Anthemideae species - exceeding the 30-90% range reported for *Achillea* (Gharibi et al. 2015) and matching or surpassing *Artemisia* species (*A. campestris*: 88.03%; *A. herba-alba*: 67.56%; *A. absinthium*: 48.7%) (Bouguerra et al. 2020, Mourad et al. 2018). These results position *O. glabrum* among the most effective antioxidant species in the tribe, while confirming that lipid peroxidation inhibition depends on specific antioxidant profiles rather than total phenolic content.

The antifungal activity of O. glabrum extracts showed a strong positive correlation with their total phenolic and flavonoid content, consistent with the established antimicrobial properties of polyphenolic compounds (Lagnika et al. 2016). This relationship explains the observed variability in efficacy across extracts, where ethyl acetate extracts with higher TPC/TFC demonstrated superior antifungal activity compared to aqueous extracts. The differential sensitivity of fungal species to specific secondary metabolites (Salem et al. 2019) and the polarity-dependent extraction efficiency of bioactive compounds further account for these variations. Our findings align with current literature demonstrating that organic extracts typically outperform aqueous extracts in antimicrobial activity (Rahim et al. 2023), and moderately polar solvents optimally extract antimicrobial phytochemicals (Lagnika et al. 2016).

The aqueous extract (AE) showed unexpectedly high activity against *Penicillium italicum*, potentially due to the fungus' sensitivity to water-soluble compounds, and synergistic effects of non-phenolic phytochemicals (alkaloids, glycosides, terpenoids) with demonstrated antifungal properties (Chioma et al. 2021, Onanuga and Oloyede 2022). This species-specific response likely reflects differences in fungal cell wall composition and metabolic pathways that influence susceptibility to particular phytochemical structures (Silva-Beltran et al. 2023).

Otospermum glabrum extracts exhibited superior antifungal activity compared to related Anthemideae species. While Matricaria aurea showed moderate inhibition (31.48-53.44%) against Aspergillus spp. (Rizwana et al. 2016), and Artemisia campestris methanol extract achieved 51.96-56.47% inhibition against Botrytis cinerea and P. expansum (Hendel et al. 2021), O. glabrum demonstrated consistently stronger efficacy. The variable performance of Achillea and Artemisia species (18-63.82% inhibition) (Andreu et al. 2018, Kursa et al. 2022, Salem et al. 2019) further highlights

O. *glabrum*'s potential as a promising candidate for the development of new biofungicides, particularly given the economic importance of the target pathogens.

The aphicidal activity of *O. glabrum* extracts arises from its rich range of secondary metabolites, including phenolics, flavonoids, terpenoids, and alkaloids (Li et al. 2024, Noureldeen et al. 2022). These bioactive compounds disrupt key physiological processes in aphids; deterring feeding, impairing development, and reducing reproductive capacity (Lebbal et al. 2023). Our findings align with documented efficacy of plant extracts against multiple aphid species (*Aphis fabae*, *Macrosiphum rosae*, *Brevicoryne brassicae*, *Aphis craccivora*) (Abdel-Rahman et al. 2019, Ahmed et al. 2020, Thakshila et al. 2022).

Otospermum glabrum demonstrated particularly strong insecticidal effects against A. fabae, with methanolic extract (ME) achieving 100% mortality at 72 h (highest concentration) versus 80% for aqueous extract (AE). ME also showed superior repellency (48.98% maximum) compared to AE (31.94% maximum). This differential bioactivity reflects variations in extract composition, where ME's lower polarity likely enhanced extraction of non-polar bioactive compounds with greater aphid toxicity.

Comparative analysis reveals differential aphicidal efficacy among Anthemideae species. While Artemisia judaica ethanolic extract achieved 100% Aphis fabae mortality within 2 hours at 12.5 mg/ml (Acheuk et al. 2017), O. glabrum required higher concentrations and longer exposure (72 h). Conversely, Matricaria chamomilla aqueous extracts showed only 35% mortality after 120 h (Binias and Gospodarek 2017), demonstrating O. glabrum's intermediate potency. Against other aphid species, Achillea millefolium essential oil exhibited superior contact toxicity (LC₅₀ 0.34%) and repellency (58.1%) to Myzus persicae (Czerniewicz et al. 2018), while Artemisia spp. ethyl acetate extracts showed complete Macrosiphoniella sanborni control within 7 days (Yang et al. 2024). O. glabrum displayed faster toxicity but weaker repellency than these counterparts, suggesting distinct mode-of-action profiles. Beyond aphids, Artemisia absinthium methanol extract caused 100% Sitophilus oryzae mortality within 24 h (Dane et al. 2016), whereas O. glabrum required 72 h for complete A. fabae control. However, O. glabrum achieved comparable efficacy at lower concentrations than A. absinthium against Tribolium castaneum (93.3% mortality at 95 mg/ml; Naimi et al. 2025). These variations likely reflect species-specific susceptibility and differential phytochemical bioavailability.

While this study provides the first evidence of *O. glabrum*'s bioactivity, its laboratory-scale design using crude extracts

limits field relevance and mechanistic understanding. Future work should employ bioassay-guided fractionation (HPLC/LC-MS) to isolate active compounds, validate efficacy in field trials, and expand testing to diverse pests/pathogens. Investigating synergies with existing biocontrol agents would further support its integration into sustainable pest management systems.

This study provides the first comprehensive evaluation of Otospermum glabrum as a source of bioprotective agents, demonstrating significant antioxidant, antifungal, and aphicidal activities. The ethyl acetate extract showed superior phenolic and flavonoid contents, correlating with enhanced bioactivity, while the methanolic extract exhibited the strongest aphid toxicity. These findings highlight the influence of extraction solvents on phytochemical recovery and biological efficacy. As a novel botanical resource, O. glabrum shows exceptional potential for developing multitarget, eco-friendly biopesticides. Future research should focus on isolating active compounds through bioassayguided fractionation and validating field efficacy to facilitate its integration into sustainable crop protection strategies that address both pest management and food security challenges.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The author declared no conflict of interest.

ÖZET

Tarımsal zararlı yönetiminde sentetik kimyasallar yerine bitki bazlı biyopestisitlerin benimsenmesi, güvenlikleri ve ekolojik faydaları nedeniyle giderek artan bir ilgi çekmektedir. Bu doğal bileşiklerin birçoğu güçlü antifungal aktivite göstererek gıda kaynaklı mantar kontaminasyonunu ve ilişkili mikotoksinleri azaltmaktadır. Bu çalışmada, glabrum'un (Asteraceae) toprak Otospermum kısımlarından elde edilen ekstraktların antioksidan, antifungal ve afisidal potansiyeli değerlendirilmiştir. Üç ekstrakt -etil asetat, metanolik ve sulu- toplam polifenolik ve flavonoid içerikleri, antioksidan aktiviteleri (DPPH radikal süpürme, β-karoten ağartma ve toplam antioksidan kapasitesi), altı hasat sonrası ve bitki fungal patojenine karşı antifungal etkileri ve bakla yaprakbitine (Aphis fabae Scop.) karşı afisidal aktiviteleri açısından değerlendirilmiştir. Etil asetat özütü (EaE), test edilen özütler arasında en yüksek

polifenol (57.60 \pm 0.17 µg GAE/mg) ve flavinoid (49.46 \pm 0.66 µg QE/mg) seviyesine sahipken; metanolik özüt (ME), en vüksek DPPH temizleme aktivitesi (IC50= 56.05±0.03 ug/ml) ve β-karoten ağartmasını önlemede en yüksek etkinliğini göstererek BHT'ninkine esdeğer bir inhibitör aktivitesine ulasmıstır. Sulu özüt ve etil asetat özütü, en yüksek toplam antioksidan kapasitesini göstermistir (sırasıyla 252.60±0.20 µg AAE/mg ve 249.10±0.81 AAE/ mg). Antifungal testlerine göre etil asetat özütü ve metanolik özütü tüm test edilen funguslara karsı %65'i geçen inhibisyon yüzdeleri (IP) ile en belirgin etkiyi ortaya koymustur. Ek olarak, %30 konsantrasyonundaki metanolik ekstrakt, 72 saat sonra A. fabae'de %100 ölüme neden olmus ve önemli bir kovucu etki ($\%48.98 \pm 8.76$) göstermistir. Bu bulgular, O. glabrum'un entegre zararlı yönetimi için umut verici doğal bir biyopestisit kaynağı olduğunu göstermektedir.

Anahtar kelimeler: *Otospermum glabrum*, polifenoller, antioksidan aktivite, antifungal aktivite, afisidal aktivite

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