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Elucidating the dual agro-biochemical roles of *Dittrichia viscosa* methanolic and ethanolic extracts: Biostimulant-driven germination, crop enhancement, and antioxidant synergy

Elucidação dos papéis agro-bioquímicos duais dos extratos metanólicos e etanólicos de *Dittrichia viscosa*: Germinação impulsionada por bioestimulantes, aumento da produtividade e sinergia antioxidante

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ABSTRACT

Dittrichia viscosa (L.) Greuter, a Mediterranean ruderal species, exhibits dual agro-biochemical roles as both a biostimulant and allelopathic agent. This study evaluated methanolic and ethanolic extracts for phytochemical content, antioxidant activity, and biostimulant efficacy on *Vicia faba* and *Citrus clementina*. Methanolic extracts contained significantly higher polyphenols (215.8 ± 22.7 mg GAE/g), flavonoids (112.0 ± 19.0 mg QE/g), and tannins (52.9 ± 2.0 mg/g) compared to ethanolic extracts (110.9 ± 17.6 mg GAE/g, 16.0 ± 3.1 mg QE/g, 11.2 ± 1.0 mg/g). Despite this, ethanolic extracts exhibited stronger antioxidant scavenging, with lower IC_{50} values in DPPH (0.49 ± 0.14 mg/mL) and ABTS (0.33 ± 0.22 mg/mL) assays than methanolic extracts (0.72 ± 0.05 and 0.93 ± 0.12 mg/mL, respectively). Hydroponic root assays demonstrated methanolic extracts enhanced *Vicia faba* root length significantly at 0.05 mg/mL (5.85 ± 1.32 cm) and *Citrus clementina* roots at 0.05 mg/mL (2.73 ± 0.38 cm), outperforming ethanolic extracts which showed inhibitory effects at higher doses. *In vivo* greenhouse experiments confirmed methanolic extract treatments increased *Vicia faba* seed number and weight significantly ($p < 0.001$), alongside larger seed sacs and stem sizes compared to controls and ethanol treatments. These findings validate *D. viscosa* as a promising natural bio-stimulant with antioxidant synergy for sustainable agriculture.

Index terms: *A*ntioxidant; sustainable agriculture; crop growth enhancement.

RESUMO

Dittrichia viscosa (L.) Greuter, uma espécie ruderal mediterrânica, apresenta um duplo papel agro-bioquímico como bioestimulante e agente alelopático. Este estudo avaliou o conteúdo fitoquímico, a atividade antioxidante e a eficácia bioestimulante dos extractos metanólico e etanólico em *Vicia faba* e *Citrus clementina*. Os extractos metanólicos continham polifenóis significativamente mais elevados (215,8 ± 22,7 mg GAE/g), flavonóides (112,0 ± 19,0 mg QE/g) e taninos (52,9 ± 2,0 mg/g) em comparação com os extractos etanólicos (110,9 ± 17,6 mg GAE/g, 16,0 ± 3,1 mg QE/g, 11,2 ± 1,0 mg/g). Apesar disso, os extractos etanólicos exibiram uma eliminação antioxidante mais forte, com valores IC_{50} mais baixos nos ensaios DPPH (0,49 ± 0,14 mg/mL) e ABTS (0,33 ± 0,22 mg/mL) do que os extractos metanólicos (0,72 ± 0,05 e 0,93 ± 0,12 mg/mL, respetivamente). Os ensaios de raízes hidropónicas demonstraram que os extractos metanólicos aumentaram significativamente o comprimento da raiz de *Vicia faba* a 0,05 mg/mL (5,85 ± 1,32 cm) e as raízes de *Citrus clementina* a 0,05 mg/mL (2,73 ± 0,38 cm), superando os extractos etanólicos que mostraram efeitos inibitórios em doses mais elevadas. Experimentos *in vivo* em estufa confirmaram que os tratamentos com extrato metanólico aumentaram significativamente o número e o peso das sementes de *Vicia faba* ($p < 0,001$), juntamente com sacos de sementes maiores e tamanhos de caule em comparação com os controlos e tratamentos com etanol. Estes resultados validam a *D. viscosa* como um bioestimulante natural promissor com sinergia antioxidante para a agricultura sustentável.

Termos para indexação: Antioxidante; agricultura sustentável; aumento do crescimento das culturas.

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Introduction

Dittrichia viscosa L. Greuter (Asteraceae), a perennial herbaceous plant from the Asteraceae family, is a ruderal species commonly found thriving along roadsides and disturbed areas of the Mediterranean region. This adaptability stems from its ability to withstand extreme environmental conditions. *D. viscosa*'s life cycle commences in March, culminating in full flowering with characteristic yellow blooms during October and November. A key feature of *D. viscosa* is the presence of numerous glandular hairs, particularly on its leaves, which secrete a sticky resin with a distinctive aroma. The tissues of *D. viscosa* are a rich reservoir of potential bioactive compounds, making it a promising resource for both agricultural and pharmaceutical applications (Sriti Eljazi et al., 2018; Qneibi et al., 2021; Paradiso, Durante, & Caretto, 2024).

Recent studies illustrate the wide range of biological activities associated with *D. viscosa*. Vuko et al. (2021) reviewed the essential oils and hydrosols extracted from this plant, detailing their antimicrobial, anti-inflammatory, and antioxidative properties, which further validate its potential applications in herbal medicine. In particular, the hydrosol and essential oils show promising potential as natural preservatives or therapeutic agents, emphasizing the plant's role beyond mere ornamental use.

D. viscosa exhibits considerable adaptability to diverse environmental stressors, attributed to its high degree of genetic plasticity. This characteristic enables it to thrive in challenging habitats, including heavily polluted mining and industrial zones, as well as arid and saline environments (De Paolis et al., 2022).

Historically, this resilient shrub has been valued in traditional medicine for its anti-inflammatory, antimicrobial, and wound-healing properties, with applications documented since antiquity (Ounoughi et al., 2020; Ben Mrid et al., 2022). Modern phytochemical analyses have attributed these therapeutic effects to its rich bioactive profile, particularly its phenolic compounds, flavonoids, and sesquiterpenes, which exhibit potent antioxidant activities (Ounoughi et al., 2020).

For instance, *D. viscosa* has emerged as a promising biostimulant in sustainable agriculture. Prisa (2020) demonstrated that foliar treatments derived from *D. viscosa* enhanced seed germination rates and biomass accumulation in *Lactuca sativa* L. (lettuce) and *Spinacia oleracea* L. (spinach) by up to 40%, while concurrently suppressing soil-borne pathogens like (*Pythium* spp). These findings align with studies highlighting the plant's sesquiterpene lactones and eudesmane derivatives, which not only promote crop resilience but also exhibit antifungal and nematicidal properties (Ben Mrid et al., 2022). Such dual functionality enhancing plant growth while providing disease protection positions (*D. viscosa*) as a viable alternative to synthetic agrochemicals, aligning with global sustainability goals (Prisa, 2020; Grauso et al., 2020).

Others claim that certain components of *D. viscosa* may serve as a significant resource for generating various bioactive compounds with antioxidant properties, warranting additional investigation to yield specific molecules applicable in agricultural or medicinal domains (Paradiso, Durante, & Caretto, 2024).

However, the plant's allelopathic potential complicates its agricultural utility. Omezzine et al. (2011) reported that aqueous extracts of *D. viscosa* inhibited seed germination and seedling growth in species like *Triticum durum* (wheat) and *Lactuca sativa*, suggesting context-dependent bioactivity. This dichotomy underscores the need for precision in application methods and dosages, as solvent choice and extraction protocols critically influence phytochemical profiles and biological effects (Ounoughi et al., 2020; Rhimi et al., 2018). For example, while methanolic extracts are rich in phenolic acids linked to antioxidant activity, ethanolic extracts may prioritize lipidic fractions with anti-inflammatory properties (Rhimi et al., 2018; Ozkan et al., 2019). Such solvent-specific variations highlight gaps in understanding the synergistic interactions between *D. viscosa*'s phytochemical networks such as polyphenol-flavonoid-tannin crosstalk and their cascading effects on plant physiology and pathogen suppression (Ben Mrid et al., 2022).

This study addresses these gaps by systematically evaluating the biostimulant, antioxidant of *D. viscosa*'s methanolic and ethanolic extracts. By correlating solvent-specific phytochemical profiles with functional outcomes in crop enhancement, this work aims to optimize extraction protocols for agricultural applications. Furthermore, it explores the allelopathic thresholds of *D. viscosa* extracts to reconcile its dual role as both a growth promoter and inhibitor, offering a nuanced framework for its integration into sustainable farming systems.

Material and Methods

Plant material collection and preparation

Plant samples of *D. viscosa* L. Greuter were collected from the Cheraia region, Collo area, Skikda Province, Algeria (36°59'19.9"N 6°30'19.9"E; Google Maps, 2024). The plant material underwent preparatory steps to ensure suitability for extraction: first, cleaning with distilled water to remove dust and contaminants, followed by drying at room temperature (25 ± 2 °C) in a shaded, well-ventilated environment for 10 days. Dried samples were ground to a fine powder using an electric grinder, sieved, and stored in airtight amber containers at 4 °C until analysis.

Extraction procedure

Crude extracts were prepared via maceration

Exactly 100 g of powdered plant material was submerged in 500 mL of ethanol (96%) or methanol (99.9%) in sterile glass flasks and agitated at 150 rpm for 24 h using an orbital shaker (SCILOGEX SCI-O180). The mixture was filtered through Whatman No.1 filter paper, and the filtrate was concentrated under reduced pressure (40 °C) using a rotary evaporator (DLAB RE-100-Pro, Chine). Crude extracts were stored at 4 °C until analysis.

Quantification of phytochemical constituents

Total polyphenol content

Total polyphenols were quantified using the Folin–Ciocalteu method (Waterhouse, 2011). Absorbance was measured at 765 nm, and results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g).

Total flavonoid content

Flavonoids were determined by mixing 0.5 mL extract with 1.5 mL ethanol (95%), 0.1 mL aluminum chloride (10%), and 0.1 mL sodium acetate (1 M). Absorbance was read at 415 nm against a blank (Chang et al., 2002).

Condensed tannin content

Condensed tannins were assessed using the vanillin-HCl method (Price, Van Scoyoc, & Butler, 1978). Absorbance was measured at 500 nm, with catechin as the standard.

Antioxidant activity assays

DPPH radical scavenging assay

$A_{0.1}$ mM DPPH solution was prepared by dissolving 3.94 mg DPPH in 100 mL ethanol. Extracts (0–1 mg/mL) were mixed with DPPH solution (1:1 v/v), incubated in darkness (30 min, 25 °C), and absorbance measured at 517 nm using a UV-Vis spectrophotometer (UV-1800 Shimadzu). IC_{50} values were calculated via linear regression (Blois, 1958).

ABTS radical scavenging assay

$ABTS^+$ solution was prepared by reacting 7 mM ABTS with 2.45 mM potassium persulfate (16 h, darkness). The solution was diluted with ethanol to an absorbance of 0.7 ± 0.02 at 734 nm. Extracts were incubated with $ABTS^+$ (6 min), and absorbance was measured (Re et al., 1999).

Biostimulant activity assays

Hydroponic root stimulation assay

Seeds of *C. clementina* and *V. faba* (10 seeds per group) of uniform mass (0.5 ± 0.05 g) and size were placed in glass containers with tap water (25 °C). After 48 h acclimatization, root elongation was measured in cm . Groups included:

- Group 1 (Control): Untreated water.
- Group 2:Methanolic extract (0,01 mg/mL, 0,02 mg/mL, 0,03 mg/mL, 0,0 5mg/mL and 0,1 mg/mL).
- Group 3: Ethanolic extract (0,01 mg/mL, 0,02 mg/mL, 0,03 mg/mL, 0,0 5mg/mL and 0,1 mg/mL).

Greenhouse Crop Biostimulant Assay

Seedlings of *V. faba* and *C. clementina* (n = 20 plants/group, 4 replicates) were grown in a peat-perlite substrate (3:1 v/v) under greenhouse conditions (25 °C, 16/8 h light/dark). Treatments applied weekly for 10 weeks:

Control: Water only.

INU1: 5% (v/v) ethanolic extract.

INU2: 5% (v/v) methanolic extract.

Shoot length, leaf area, and biomass were recorded biweekly.

Statistical analysis

All statistical analyses were conducted using GraphPad Prism 9 and Microsoft Excel 2021. One-way analysis of variance (ANOVA) was performed, followed by Dunnett's post-hoc test to compare treated samples with the control group. Data were presented as mean ± standard deviation, with statistical significance set at $p < 0.05$.

Results and Discussion

Extract yield

The yield of the crude polyphenolic extract obtained by Rotary evaporator (Rotovap) from the aerial part of the medicinal plant *D. viscosa* is characterised by a dark green color and a viscous, almost gelatinous gel form The yield of the two extracts is shown in (Table 1) below:

Table 1: Yield of extracts obtained from the plant *Dittrichia viscosa* L.

	Yield of extracts	
Sample	Ethanolic extract	Methanolic extract
Yield (%)	8.41	7.63

The yield of extracts differed significantly between the two solvents. The methanol yield is represented by the purple column and the ethanol yield by the blue column. The data shows that the ethanol yield is significantly higher than the methanol yield. In fact, the blue column exceeds the 8.6% mark, while the violet column is just above 7.4%.

The finding that ethanol yields a higher quantity of extract compared to methanol in *D. viscosa* is consistent with multiple studies examining the extraction efficacy of various solvents on plant materials. A study by Sutariya et al. (2022) observed comparable results in *Gymnosporia montana*, where ethanol extraction was substantially more effective at isolating phenolic compounds than methanol. Similarly, Altemimi et al. (2020) reported that ethanol showed superior extraction efficiency of flavonoids from *Ocimum basilicum* in contrast to methanol.

The higher yields of the ethanolic extract of *D. viscosa* may be indicative of its potential applicability in the food and pharmaceutical industries, given that higher yield often correlates with enhanced biological activity. Moreover, comparative studies, such as those by Hossen and Kato-Noguchi (2020).

These results could indicate that the ethanolic solvent is more efficient at extracting certain components from *Dittrichia viscosa*, or that the extraction conditions used were more optimal for ethanol than for methanol. This could be due to various factors, such as the polarity of the solvent, the extraction temperature, the extraction

time, and other parameters specific to the extraction process (Bouzana et al., 2023, Ounissi et al,2024 Aouzal et al., 2024).

Phytochemical composition and extraction efficiency

In the comparative Table 2 analysis of bioactive compounds, the examination of total polyphenol, flavonoid, and tannin concentrations in ethanolic and methanolic extracts of *D. viscosa* demonstrated notable disparities in their phytochemical makeup. Methanolic extracts demonstrated superior quantities of all assessed phytochemicals relative to ethanolic extracts.

Table 2: Total polyphenol, flavonoid and tannin content.

Parameter	Ethanolic extract	Methanolic extract
Polyphenol content (mg EAG/g ES)	110.96 ± 17.55	215.80 ± 22.70
Flavonoïdes content (mg EQ/g ES)	16.02 ± 3.076	112.02 ± 19.03
Tannin content (mg EAT/g ES)	11.16 ± 1.04	52.95 ± 2.02

The methanolic extract had a much greater polyphenol content, quantified at 215.8± 22.7mg EAG/g ES, in contrast to 110.96± 17.55 mg EAG/g ES in the ethanolic extract. This suggests that methanol is a more efficient solvent for extracting polyphenolic chemicals from *D. viscosa*, likely owing to its greater polarity.

The effectiveness of a solvent in extracting polyphenol is highly dependent on its polarity. Methanol is a more polar solvent than ethanol. Polyphenols, due to their hydroxyl groups and aromatic rings, exhibit varying degrees of polarity. Some polyphenols are more soluble in polar solvents like methanol, while others are more soluble in less polar solvents like ethanol. Methanol's higher polarity allows it to interact more effectively with a broader spectrum of polyphenols, leading to a higher overall yield (Dai & Mumper, 2010; Li et al., 2007). Methanol's ability to form strong hydrogen bonds is crucial for extracting polyphenols. Polyphenols often interact with plant cell wall components through hydrogen bonds. Methanol can disrupt these interactions, facilitating the release of polyphenols into the solvent (Abraham-Diego et al., 2024; Antony & Farid, 2022).

The flavonoid content exhibited a significant disparity between the two solvents. The methanolic extract exhibited a markedly elevated flavonoid concentration of 112.02±19.03 mg EQ/g ES, in contrast to 16.02±3.076 mg EQ/g ES in the ethanolic extract. This outcome underscores methanol's enhanced ability to extract flavonoids, which are essential to the plant's antioxidant characteristics.

Many flavonoids exist in plants as glycosides (bound to sugar molecules). These glycosylated forms are generally more polar than their aglycone (sugar-free) counterparts. Methanol is likely better at solubilizing these glycosylated flavonoids. (Heim, Tagliaferro, & Bobilya 2002).

The tannin content exhibited a comparable pattern, with the methanolic extract showing a higher concentration of 52.95±2.02 mg EAT/g ES, whereas the ethanolic extract had 11.16±1.04 mg EAT/g ES. This contrast highlights the effectiveness of methanol in solubilizing tannins, recognized for their antioxidant properties.

Tannins have numerous hydroxyl groups, making them capable of extensive hydrogen bonding. Methanol's ability to disrupt these hydrogen bonds, combined with its polarity, facilitates the extraction of these larger, more complex molecules (Makkar, 2003).

Effect of extraction methods on the antioxidant activity of *Dittrichia viscosa* L

The results of the antioxidant activity of ethanolic and methanolic extracts of *D. viscosa* are presented in the form of IC_{50} values, which represent the concentration required to inhibit 50% of the antioxidant activity and are reported in Table 3.

Table 3: IC_{50} antioxidant activity of *Dittrichia viscosa* L. extracts.

Sample/IC_{50}	Ethanolic extract	Methanol extract
DPPH extract (mg/ml)	0.49 ± 0.14	0.72 ± 0.059
ABTS (mg/ml)	0.33 ± 0.22	0.93 ± 0.12

In the DPPH assay, the ethanolic extract demonstrated a lower IC_{50} value (0.49519 ± 0.14 mg/ml) compared to the methanolic extract (0.722146 ± 0.59 mg/ml). This suggests that the ethanolic extract has stronger DPPH radical scavenging activity than the methanolic extract.

Even though the methanolic extract has a higher total polyphenol content, the *type* of antioxidants present in each extract is crucial for their activity. The ethanolic extract might contain specific compounds that are particularly effective at scavenging DPPH radicals. The solvent could also influence the reaction kinetics of antioxidants with DPPH. The results need to be interpreted considering the specific compounds extracted by each solvent. While methanol extracts more total polyphenols, it may be that ethanol extracts certain specific antioxidants that are more reactive with DPPH (Blois, 1958).

Similarly, the ABTS results further highlight the ethanolic extract exhibited stronger ABTS scavenging activity, with an IC50 value of (0.3356 ± 0.22 mg/ml), compared to the methanolic extract's IC_{50} value of (0.93241 ± 0.12 mg/ml). This indicates that the ethanolic extract has stronger ABTS radical scavenging activity than the methanolic extract.

These results further reinforce the idea that the ethanolic extract contains specific antioxidants that are more effective in scavenging both DPPH and ABTS radicals, despite the methanolic extract having higher overall polyphenol content.

The different extraction methods of the solvents led to different compounds with different affinities for the radicals being measured (Kedare & Singh, 2011).

Hydroponic roots stimulation

Analysis of variance of methanolic extract as a function of *Vicia faba* roots

Methanolic extract on *Vicia faba* roots

The variance analysis of the methanolic extract's effects on *V. faba* root growth over a 5-day period revealed significant differences across concentrations (Table 4). At the lowest dose (0.01), the mean root length was 4.20 ± 0.81 cm, reflecting modest growth comparable to the control (3.83 ± 0.39 cm). A pronounced enhancement was observed at 0.02, with a mean root length of 5.13 ± 1.23 cm*, indicating statistically significant stimulation compared to the control. Higher concentrations demonstrated progressively stronger effects: 0.03 yielded 5.33 ± 1.24 cm, 0.05 showed the most substantial growth (5.85 ± 1.32 cm), and the highest concentration (0.1) achieved the greatest mean root length (5.92 ± 1.29 cm*).

The noted decline in root growth with elevated extract concentration signifies phytotoxicity, indicating that the extract harbours chemicals detrimental to plants at heightened levels. This is a prevalent occurrence noted with numerous plant extracts (Duke, 1985). Specific chemicals in the extract may disrupt mitosis or cell elongation in the root meristem, thereby inhibiting root growth (Holmsen & Hess, 1984).

Plant hormones, such as auxins and gibberellins, are essential for root formation. Compounds in the extract may interfere with the synthesis, transport, or signalling of these hormones, resulting in growth suppression (Grossmann, 2003). Certain phytotoxic chemicals provoke oxidative stress in plant cells by elevating the generation of reactive oxygen species (ROS). Oxidative stress can impair cellular components and impede growth (Mittler, 2002).

Bashir and Al-Habib (2020), for example, explored how cytokinins and auxins, among other plant hormones, can induce root development a process that would help to explain the increased root length seen in *V. faba* in this work. This implies that the active chemicals in *D. viscosa* might operate similarly to these known growth regulators, therefore supporting the knowledge that plant extracts can efficiently control growth parameters. Treatments using chemicals related to auxins may similarly encourage lateral root development, according to Kulkarni et al. (2006), therefore demonstrating a consistent mechanism by which some plant extracts improve root development. Besides, studies by Hossen and Kato-Noguchi (2020) emphasizes root sensitivity to allelochemicals, so validating the results of the present work showing increasing doses of *D. viscosa* extract linked with improved root development, so indicating the presence of stimulatory bioactive components in the extract. Maksimović, Markovic and Hasanagić (2021) underlined, meanwhile, how particular plant extracts might block germination or initial growth phases, so highlighting a complexity in the effects of various plant extracts depending on their composition and the target plant species.

Like the improvements seen in *V. faba,* Jantasorn, Pongsupap and Oiuphisittraiwat (2021) noted improved root and shoot development in rice seedlings by use of *Caesalpinia sappan* extractions. This emphasizes the need for *D. viscosa* as a possible growth regulator since it implies that different plant extracts might boost development in different ways depending on their biochemical makeup and the recipient plant species.

Ethanolic extract on *Vicia faba* roots

The ethanolic extract had a diminished impact on *V. faba* roots growth relative to the methanolic extract. At the minimal dose of 0.01, the average root size was 4.37 ± 0.708, indicating modest growth during the 5-day research duration. As the concentration rose to 0.02 and 0.03, the average root sizes decreased to 3.73 ± 0.548 cm and 3.52 ± 0.553 cm, respectively, signifying a dose-dependent diminishment in growth enhancement. At a dose of 0.05, the mean root size was 3.48 ± 0.423 cm, which further diminished to 3.35 ± 0.449 cm at the maximum concentration of 0.1 Table 5.

Table 4: Analysis of variance (Dunnett's comparison test) of the effects of methanolic extract as a function of *Vicia faba* root size over the 5-day period(N=4).

Treatment	Day 00	Day 01	Day 02	Day 03	Day 04	Day 05	Mean ± SD (cm)
Control	3.80	3.20	4.00	3.70	4.20	4.10	3.83 ± 0.39
0.01	4.20	3.70	4.20	3.50	5.70	3.90	4.20 ± 0.81
0.02	5.20	5.00	4.6	3.50	7.00	4.50	5.13 ± 1.23*
0.03	5.70	5.00	6.10	4.00	7.2	4.00	5.33 ± 1.24*
0.05	5.90	5.20	5.30	4.50	7.70	3.90	5.85 ± 1.32*
0.1	6.00	5.50	6.50	5.50	8.00	4.00	5.92 ± 1.29

Note: The asterisk (*) indicates a statistically significant difference ($p < 0.05$). N= number of experimental repetitions per treatment.

Table 5: Analysis of variance (Dunnett's comparison test) of the effects of ethanolic extract as a function of *Vicia faba* root size over the 5-day period (N=4).

Treatment	Day 00	Day 01	Day 02	Day 03	Day 04	Day 05	Mean ± SD (cm)
Control	1.80	2.90	4.40	5.00	5.70	6.4	4.37 ± 0.708
0.01	2.10	2.10	3.70	4.70	4.80	5.00	3.73 ± 0.548
0.02	2.00	2.30	2.60	4.70	4.50	5.00	3.52 ± 0.553
0.03	2.20	2.20	4.20	4.30	4.50	4.50	3.65 ± 0.461
0.05	2.20	2.10	4.00	4.20	4.20	4.20	3.48 ± 0.423
0.1	1.70	2.20	4.00	4.00	4.00	4.20	3.35 ± 0.449

Note: The asterisk (*) indicates a statistically significant difference ($p < 0.05$). N= number of experimental repetitions per treatment.

A comparative study of the two extracts reveals that the methanolic extract is superior in enhancing onion root growth, especially at the 0.05 concentration, which yielded the largest mean root size (6.63 ± 0.585 cm) among all tested circumstances. The ethanolic extract, conversely, exhibited a continuously diminished effect, with its optimal concentration (0.01) producing a mean root size of just 4.37 ± 0.708 cm.

According to Rice (1984), the presence of phytotoxic substances, also known as allelochemicals, within the extract is suggested by the fact that the ethanolic extract causes a significant inhibition of the growth of the roots of the *V. faba* plant. Allelochemicals have the potential to disrupt a wide variety of physiological processes that are necessary for the growth of plants. These processes include cell division (Holmsen & Hess, 1984), hormone control (Grossmann, 2003), nutrient uptake (Marschner, 2012), and photosynthesis (Duke & Powles, 2009).

Concentration appears to play a critical role in determining the efficacy of plant extracts. The methanolic extract's optimal concentration is consistent with findings in literature that dictate specific thresholds for growth stimulation, where excessive concentrations can lead to phytotoxicity (Jantasorn, Pongsupap & Oiuphisittraiwat, 2021). Conversely, the ethanolic extract's lowest recorded mean root size of 4.37 cm at the 0.01 concentration implies a lack of responsiveness that may relate to a lower concentration of bioactive constituents or the influence of growth inhibitors present in the extract at higher concentrations (Sladonja et al., 2021).

In terms of integrated pest management (IPM), the use of *D. viscosa* extracts may provide a natural, sustainable approach to controlling pest populations and enhancing the growth of *V. faba*. This aligns with recent studies emphasizing the importance of incorporating plant extracts into IPM strategies to promote sustainable agriculture (Perdomo et al., 2020). Furthermore, the genetic diversity and resilience of *V.* facilitated through the application of *D. viscosa* extracts can assist in developing crop varieties that are more tolerant to both biotic and abiotic stresses (Khazaei et al., 2021).

Methanolic extract effects on *Citrus clementina* roots

The effects of methanolic extract concentrations on *C. clementina* seed root growth were analyzed over a 5-day period using Dunnett's comparison test. The results demonstrated a clear concentration-dependent impact on root elongation. At the lowest concentration (0.01), the mean root size was 1.83 ± 0.0664 cm, showing limited stimulation of root growth compared to the control (Day 0, 1.125 cm). As the concentration increased to 0.02, the mean root size improved slightly to 1.98 ± 0.111 cm, indicating moderate enhancement of root development.

At a concentration of 0.03, the root growth increased further, with a mean size of 2.04 ± 0.167 cm, suggesting an optimal extraction of bioactive compounds contributing to enhanced root elongation. The most significant growth was observed at the 0.05 concentration, where the mean root size reached 2.73 ± 0.377 cm. This concentration produced a statistically significant effect, as indicated by the asterisk (*), highlighting its efficacy in promoting root development.

However, at the highest concentration of 0.1, root growth declined, with a mean size of 1.77 ± 0.113 cm, indicating a potential inhibitory effect caused by excessive concentrations of the methanolic extract. These findings suggest that while the methanolic extract exhibits strong growth-promoting properties, excessive concentrations may lead to phytotoxicity, reducing its effectiveness. The decline in root growth at the highest concentration (0.1) indicates a potential phytotoxic effect. High concentrations of phenolic compounds or other secondary metabolites may disrupt cellular processes, such as enzyme activity, and cause membrane damage, leading to oxidative stress and growth inhibition. For instance, high concentrations of caffeic acid, a common phenolic acid, have been shown to inhibit seed germination and seedling growth in various plant species (Leather & Einhellig, 1985). An excess of solutes in the growing medium can affect the osmotic potential which impacts water uptake and growth and contributes to phytotoxicity (Corwin, Rhoades, & Šimůnek, 2007).

Analysis of variance of methanolic extract as a function of *Citrus clementina* roots

Table 06 presents a Dunnett's comparison test of methanolic extract effects on *C. clementina* seed root sizes over 5 days, revealing a significant increase in root size at the 0.05 concentration (Mean ± deviation: 2.73±0.377* cm).

Methanolic and ethanolic extract effects on *Citrus clementina* roots

The ethanolic extract also showed concentration-dependent effects on root growth over the same 5-day period, although its overall efficacy was lower compared to the methanolic extract. At the 0.01 concentration, the mean root size was 2.03 ± 0.157, indicating a moderate stimulation of root elongation. Increasing the concentration to 0.02 resulted in a mean root size of 2.03 ± 0.143, which was comparable to the previous concentration, suggesting a plateau in growth enhancement.

At the 0.03 concentration, the mean root size was 1.97 ± 0.243 cm, showing a slight reduction in root growth, indicating diminishing returns with higher concentrations. Further increases in concentration to 0.05 and 0.1 resulted in mean root sizes of 1.68 ± 0.252 cm and 1.72 ± 0.140, respectively, reflecting a consistent inhibitory effect at these levels.

The limited growth-promoting efficacy of *D. viscosa* ethanolic extract, particularly at elevated concentrations, aligns with documented phytotoxic responses to ethanol-soluble allelochemicals. This plateau or dose-dependent decline in bioactivity reflect either (1) reduced extraction efficiency of growth-promoting compounds (e.g., auxin-like molecules) at higher solvent ratios or (2) the dominance of inhibitory secondary metabolites, such as phenolics or terpenoids, which accumulate preferentially in ethanol.

For instance, ethanolic extracts of *Phytolacca americana* (1,000–4,000 µg/mL) and *Begonia* 'Dragon Wing' (6% v/v ethanol) suppressed seed germination and seedling development in *Brassica pekinensis* and *Digitaria sanguinalis* (Xiaohong et al., 2012; Sillmann & Mattiuz, 2024). These inhibitory effects are mechanistically linked to ethanol's capacity to solubilize allelochemicals that disrupt membrane integrity (e.g., lipid peroxidation) and amplify reactive oxygen species (ROS) generation, leading to oxidative stress. Such findings corroborate the trade-off between extraction efficiency and phytotoxicity in ethanol-based protocols.

In contrast, low-dose applications of *Melilotus officinalis* ethanolic extract enhanced root elongation and seedling vigor by 15–20% relative to controls (Xiaohong et al., 2012). This biphasic response stimulation at low concentrations vs. inhibition at high doses mirrors *hormesis*, a phenomenon wherein mild biochemical stress (e.g., from flavonoids or low-dose phenolics) transiently activates antioxidant defenses (e.g., SOD, CAT enzymes) or nutrient assimilation pathways.

A direct comparison between the methanolic and ethanolic extracts reveals significant differences in their efficacy. The methanolic extract outperformed the ethanolic extract across all concentrations, with the 0.05 concentration of the methanolic extract achieving the highest mean root size (2.73 ± 0.377 cm) (Table 6). In contrast, the ethanolic extract exhibited its best performance at the 0.01 and 0.02 concentrations, both achieving mean root sizes of 2.03 ± 0.157 cm and 2.03 ± 0.143 cm (Table 7), respectively, which were notably lower than the optimal performance of the methanolic extract. Both extracts displayed concentration-dependent effects, with optimal growth observed at intermediate concentrations and inhibitory effects at the highest concentrations (0.1).

Biostimulation assay on crops

Biostimulation assay on *Vicia faba*

Figure 1 illustrates the effects of methanol and ethanol treatments on the growth and characteristics of *Vicia faba* seeds, leaves, and stems. The data show significant differences across treatments compared to the control group. In terms of seed count, the Methanol treatment resulted in a significantly higher number of seeds ($p < 0.001$) compared to both the ethanol and control treatments. Similarly, the weight of the seeds was also significantly greater under the Methanol treatment ($p < 0.001$), while ethanol showed a moderate increase, albeit less

Table 6: Dunnett's comparison test of the effects of methanolic extract on *Citrus clementina* seeds (Root sizes) over the 5-day period (N=4).

Treatment	Day 00	Day 01	Day 02	Day 03	Day 04	Day 05	Mean ± SD (cm)
Control	1.125	1.175	1.825	2.1	2.4	2.45	1.85 ± 0.239
0.01	1.55	1.775	1.85	1.975	2	1.85	1.83 ± 0.066
0.02	1.55	1.8	1.95	2.05	2.25	2.25	1.98 ± 0.111
0.03	1.425	1.775	1.925	2.2	2.375	2.525	2.04 ± 0.167
0.05	1.6	1.825	2.4	3.2	3.5	3.85	2.73 ± 0.377*
0.1	1.525	1.525	1.625	1.75	1.95	2.225	1.77 ± 0.113

Note: The asterisk (*) indicates a statistically significant difference (p < 0.05). N= number of experimental repetitions per treatment.

pronounced. Regarding the number of leaves, Ethanol-treated plants exhibited significantly more leaves than the control ($p < 0.05$), but the methanol treatment did not significantly differ from the control. For seed size, the Methanol group showed a larger seed size than the other treatments, with a significant difference from the ethanol group ($p < 0.01$). In contrast, leaf size did not differ significantly across treatments, suggesting ethanol and methanol had a minimal effect on this parameter. Finally, stem size was significantly larger in the Methanol treatment compared to the control ($p < 0.05$), with no significant difference between ethanol and control treatments. Figure 2 illustrates the effects of various treatments on the size of *V. faba* seed sacs. The data show a significant increase in seed sac size across all treatments compared to the control group. Both methanol and ethanol treatments resulted in significantly larger seed sacs, with the ethanol treatment yielding the largest size, followed by the methanol treatment. Specifically, the ethanol treatment significantly enhanced the seed sac size compared to both the control ($p < 0.001$) and methanol treatments ($p < 0.001$). Methanol treatment also showed a significant increase in seed sac size compared to the control ($p < 0.001$). Overall, these results suggest that both ethanol and methanol treatments have a positive effect on the development of *Vicia faba* seed sacs, with ethanol being the most effective.

Table 7: Analysis of variance (Dunnett's comparison test) of the effects of ethanolic extract as a on *Citrus clementina* seeds growth (Root size) over the 5-day period (N=4).

Treatment	Day 00	Day 01	Day 02	Day 03	Day 04	Day 05	Mean ± SD (cm)
Control	1.12	1.17	1.82	2.10	2.40	2.45	1.85 ± 0.239
0.01	1.55	1.77	1.85	2.12	2.25	2.62	2.03 ± 0.157
0.02	1.55	1.80	1.95	2.07	2.25	2.55	2.03 ± 0.143
0.03	1.42	1.77	1.92	1.35	2.50	2.85	1,97 ± 0.243
0.05	1.10	1.20	1.25	1.62	2.37	2,5	1.68 ± 0.252
0.1	1.47	1.5	1.57	1.62	1.95	2,12	1.72 ± 0.106

Note: The asterisk (*) indicates a statistically significant difference ($p < 0.05$). N= number of experimental repetitions per treatment.

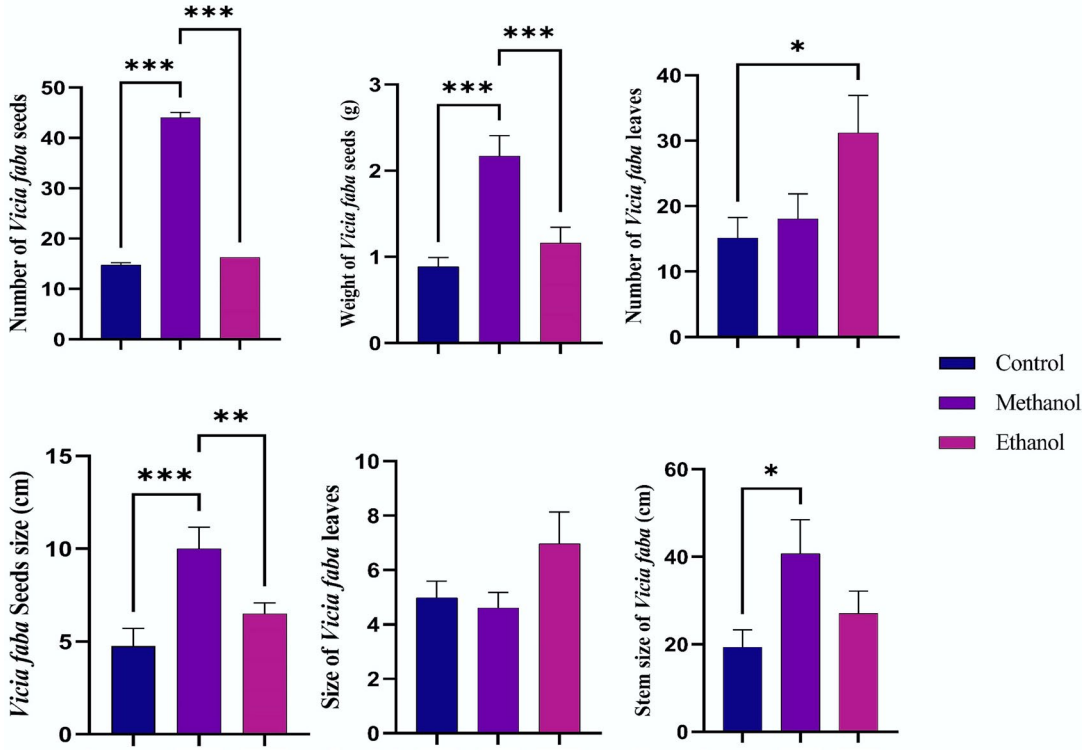

Figure 1: Effects of methanol and ethanol treatments on growth parameters and morphological characteristics of *Vicia faba* L. (seed count, weight, and dimensions; leaf and stem morphometrics).

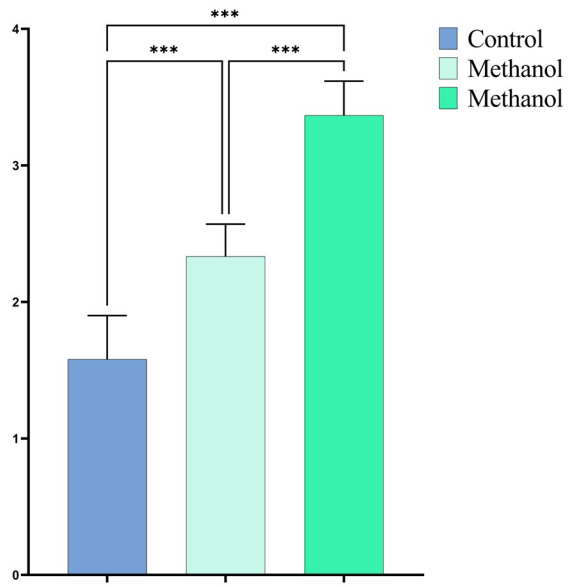

Figure 2: Effects of treatments on the size of *Vicia faba* seed sacs.

Figure 3 demonstrates that the control group displayed normal developmental progression, marked by significant stem elongation and enlarged leaf shape, corresponding with the typical growth patterns of this species. Conversely, Ethanol-treated plants exhibited modest growth, as shown by increased stem height, leaf area, and a postponed progression to advanced vegetative phases. Methanol-treated plants exhibited significant morphological changes, including considerable stem stunting and chlorotic leaf coloring, indicating a more pronounced impact on growth relative to the control group.

Biostimulation assay on *Citrus clementina*

The results presented in the charts highlight the effects of methanolic and ethanolic extracts on various growth parameters of *C. clementina*, including the number of leaves, leaf size, number of branches, and pruning branches Figure 4. A comparative analysis with a control group provides insight into the influence of these treatments on plant growth. Figure 5 illustrates the comparative effects of methanol and ethanol treatments on the growth and morphological characteristics of the studied organism.

Figure 3: Growth stages of *Vicia faba* under different treatments: (A) Control, (B) Ethanol, (C) Methanol.

Figure 4: Progress of *Citrus clementina* after 10 weeks of treatment (A) Control. (B) Ethanol. (C) Methanol.

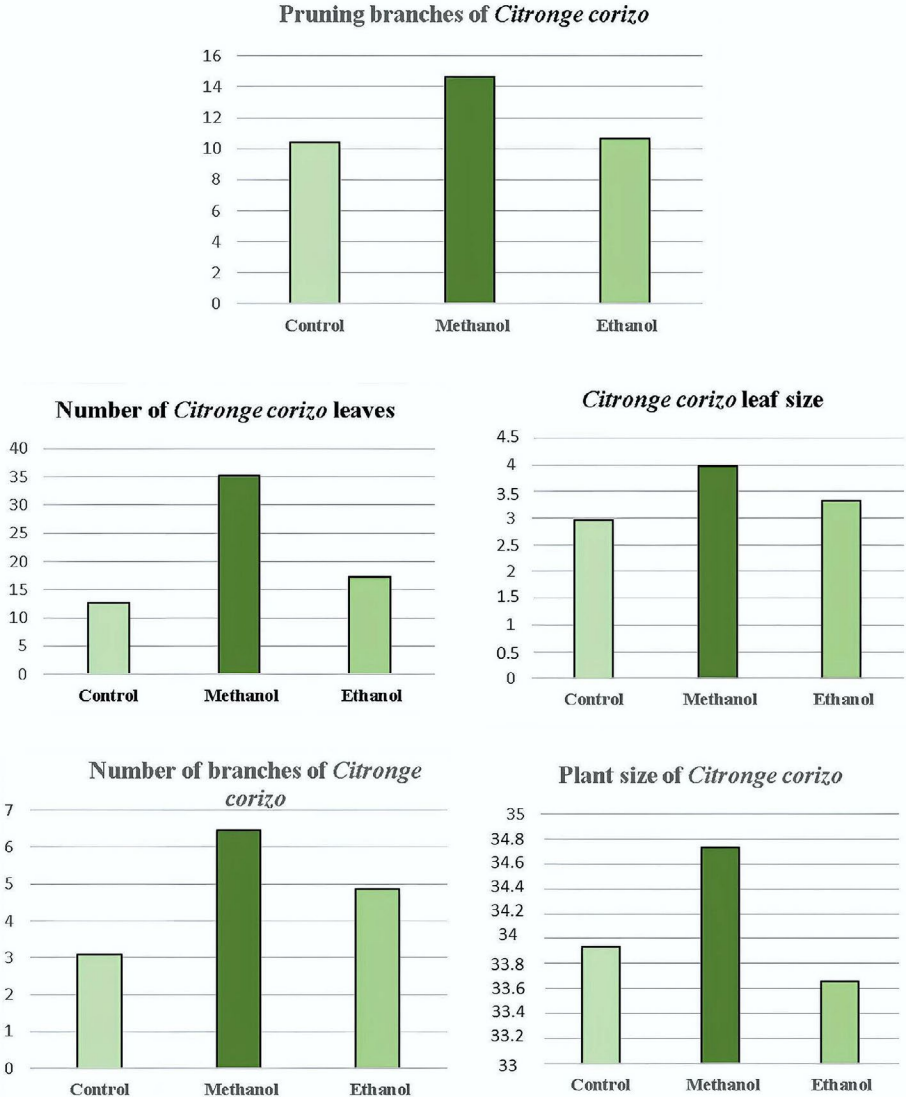

Figure 5: Effects of methanol and ethanol treatments on the growth and morphology of *Citrus clementina*.

Across all parameters, the methanolic extract of *D. viscosa* exhibited the most pronounced effects on plant growth, outperforming both the ethanolic extract and the control. This suggests that methanol may be a more efficient solvent for extracting bioactive compounds that positively influence *C. clementina* growth. The ethanolic extract also demonstrated growth-promoting effects but was consistently less effective than the methanolic extract. The control group, which received no extract treatment, showed the lowest values across all measured parameters.

The methanol extract, rich in polyphenols, flavonoids, and tannins, demonstrates significant potential as a biostimulant for enhancing plant development, primarily owing to its potent antioxidant capabilities and diverse roles in plant defense and structural reinforcement. The extract's notable antioxidant capacity, evidenced by DPPH and ABTS assays, correlates with its elevated concentrations of polyphenols and flavonoids (Hayat et al., 2020; Rai, Kumari, & Han, 2023). These compounds are crucial for neutralising reactive oxygen species (ROS), which accumulate owing to environmental stressors such as UV radiation, dehydration, or pathogen invasion (Gu et al., 2019). The extract likely preserves cellular integrity by mitigating oxidative damage, allowing plants to allocate resources to growth rather than repair, a process consistent with the findings of Cheynier et al. (2017), which emphasized the significance of polyphenols in scavenging reactive oxygen species and adapting to stress.

Polyphenols, particularly flavonoids, bolster plant defense by acting as antibacterial agents and ultraviolet protectants (Cheynier et al., 2017; Hayat et al., 2020). Flavonoids absorb harmful UV wavelengths, reducing DNA damage, while tannins strengthen cell walls by crosslinking with polysaccharides, so enhancing mechanical resistance to pathogens and abiotic stresses (Rai, Kumari, & Han, 2023). The dual role of antioxidant activity and structural fortification may collaboratively improve plant resilience, hence indirectly promoting growth in suboptimal conditions. Plants exposed to these extracts may experience reduced metabolic costs associated with stress responses, hence promoting enhanced biomass accumulation or root growth.The efficacy of methanol solvent in extracting these advantageous compounds, as noted by Vundać, Brantner and Plazibat (2007), underscores its utility. Nonetheless, while *in vitro* investigations like DPPH provide valuable insights into antioxidant capability, applying these results to *in vivo* systems requires caution. Environmental variables, bioavailability, and interactions with soil microorganisms may affect the extract's potency. Future study must validate these findings in practical settings and across other plant species to assess their broader applicability. The divergent functions of plant secondary metabolites, from growth-enhancing antioxidants to phytotoxic allelochemicals, highlight the intricacy of plant-plant interactions and their consequences for agricultural systems. Methanol extracts abundant in polyphenols and flavonoids promote plant growth by alleviating oxidative stress (Cheynier et al., 2017; Hayat et al., 2020), whereas species like *D. viscosa* utilize allelopathic mechanisms to inhibit competitors, demonstrating the dualistic properties of these bioactive compounds.

D. viscosa produces sesquiterpenoids, including α-costic acid, which induce oxidative stress and cellular damage in neighboring plants, evidenced by chlorosis, lesions, and cell death in tomato seedlings (Zanotti et al., 2024). This phytotoxicity parallels oxidative damage caused by environmental stress, albeit through chemical mediation, highlighting the function of secondary metabolites as ecological weapons. Volatile organic compounds (VOCs) from *D. viscosa* hinder seed germination and root elongation in lettuce by altering water status and inducing oxidative damage (Araniti et al., 2017). These findings align with the antigerminative actions of its extracts on cress, radish, and fungal pathogens, suggesting significant application as a natural herbicide (Pane et al., 2023).

The distinction between growth promotion and inhibition depends on concentration, bioavailability, and ecological environment. Polyphenols in methanol extracts scavenge reactive oxygen species (ROS), enhancing stress resilience and directing resources towards development (Gu et al., 2019; Rai, Kumari, & Han, 2023). Conversely, the α-costic acid and VOCs of D. viscosa intensify ROS buildup in target plants, resulting in metabolic failure. This paradox underscores the necessity for meticulous treatment tactics to either increase crop vigour or inhibit weed growth.

The dried biomass of *D. viscosa* markedly suppresses weed germination when incorporated into soil (Boari et al., 2021), offering a sustainable alternative to synthetic pesticides. Its allelochemicals can be included into integrated weed management (IWM) systems, reducing reliance on herbicides that foster resistance. The phytotoxic dangers to non-target plants necessitate careful formulation, including localised application or delayed treatments to avert crop damage.

Conclusions

D. viscosa demonstrates strong potential as a natural agro-biochemical, with methanolic extracts rich in polyphenols, flavonoids, and tannins significantly enhancing root growth in *Vicia faba* and *Citrus clementina* at optimal concentrations (0.05 mg/mL). In contrast, ethanolic extracts, though lower in total phenolics, showed superior antioxidant activity. These solvent-dependent differences underscore the need for tailored extraction methods. *D. viscosa* offers a sustainable alternative to synthetic agrochemicals, but careful dose evaluation is essential to avoid allelopathic effects and fully harness its agricultural benefits.

Author contributions

Conceptual idea: Aouzal, B.; Oudjane, F.; Souilah, N. Methodology design: Aouzal, B.; Laib, D.E. Data collection: Aouzal, B.; Bourouh, L. Data analysis and interpretation: Souilah, N.; Bourouh, L.; Benkherbache, N.; Boufahja, F.; Bendif, H. Writing and editing: Aouzal, B.; Oudjane, F.; Laib, D.E.; Benkherbache, N.; Boufahja, F.; Bendif, H.

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References

Abraham-Diego, J. et al. (2024). Experimental intensification and modeling of solid–liquid extraction of polyphenols from rice husk waste using organic solvents. *International Journal of Chemical Engineering*, 7145617, 16 pages.

Altemimi, A. et al. (2020). Optimization of ultrasonicated kaempferol extraction from *Ocimum basilicum* using a Box–Behnken design and its densitometric validation. *Foods*, *9*(10):1379.

Antony, A., & Farid, M. (2022). Effect of temperatures on polyphenols during extraction. *Applied Sciences*, 12(4), Article 2107. https://doi.org/10.3390/app1204210

Aouzal, B. et al. (2024). LC-MS/MS phytochemical analysis of *Ruta montana* and the hepatic preventive effects in male rats exposed to tebuconazole. *Global NEST Journal*, *26*(10):06877.

Araniti, F. et al. (2017). Allelopatic potential of *Dittrichia viscosa* (L.) W. Greuter mediated by VOCs: A physiological and metabolomic approach. *PLoS ONE*, *12*(1):e0170161.

Ben Mrid, R. et al. (2022). *Dittrichia viscosa* L. leaf: A valuable source of bioactive compounds with multiple pharmacological effects. *Molecules*, *27*(7):2108.

Bashir, S., & Al-Habib, A. (2020). Evaluation the activity of *Petroselinum crispum* aqueous extract as promoter rooting for stem cuttings of some plants. *Al-Mustansiriyah Journal of Science*, *31*(1):22-30

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*:1199-1200.

Boari, A. et al. (2021). Evaluation of *Dittrichia viscosa* (L.) Greuter dried biomass for weed management. *Plants*, *10*(1):147.

Bouzana A. et al. (2023). Phytochemical analysis by LC-MS/MS and in vitro antioxidant activity of the Algerian endemic plant *Dianthus sylvestris* subsp. *aristidis* (Batt.) Greuter & Burdet. *Global NEST Journal*, *25*(7):113-119.

Chang, C. C et al. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, *10*(3):178-182.

Cheynier, V. et al. (2017). Foreword focus on polyphenols. *Planta*, *246*:183.

Corwin, D. L., Rhoades, J. D., & Šimůnek, J. (2007). Leaching requirement for soil salinity control: Steady-state versus transient models. *Agricultural Water Management*, 90(3):165-180.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*(10):7313-7352.

De Paolis, A. et al. (2022). Evaluation of *Dittrichia viscosa* aquaporin *Nip1.1* gene as marker for arsenic tolerant plant selection. *Plants*, *11*(15):1968.

Duke, S. O. (1985). *Weed physiology*. Volume II: Herbicide physiology. CRC Press, 272p.

Duke, S. O., & Powles, S. B. (2009). Glyphosate: A once-in-a-century herbicide. *Pest Management Science*, *65*(4):474-480.

Google. (2024). 36°59'19.9"N 6°30'19.9"E [Mapa]. *Google Maps*. Available in: https://www.google.com/maps/place/36%C2%B059'19.9%22N+6%C2%B030'19.9%22E

Grauso, L. et al. (2020). Exploring *Dittrichia viscosa* (L.) greuter phytochemical diversity to explain its antimicrobial, nematicidal, and insecticidal activity. *Phytochemistry Reviews*, *19*(3):659-689.

Grossmann K. (2003). Auxin herbicides: Current status of mechanism and mode of action. *Pest Management Science*, *59*(2):135-145.

Gu, C. et al. (2019). LC-ESI-QTOF/MS characterisation of phenolic acids and flavonoids in polyphenol-rich fruits and vegetables and their potential antioxidant activities. *Antioxidants*, *8*(9):405.

Hayat, J. et al. (2020). Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of *Marrubium vulgare* L. from 2 different localities of Northeast of Morocco. *Heliyon*, *6*(11):e05609.

Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *The Journal of Nutritional Biochemistry*, *13*(10):572-584.

Holmsen, J., & Hess, D. (1984). Growth inhibition and disruption of mitosis by DCPA in oat (*Avena sativa*) roots. *Weed Science*, *32*(6):732-738.

Hossen, K., & Kato-Noguchi, H. (2020). Determination of allelopathic properties of *Acacia catechu* (L.f.) Willd. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *48*(4):2050-2059.

Jantasorn, A., Pongsupap, P., & Oiuphisittraiwat, T. (2021). In vitro effect of *Caesalpinia sappan* and *Crateva magna* extracts in enhancing seed germination and seedling growth of kdml105 rice variety. *Indian Journal of Agricultural Research*, *55*(4):458-462.

Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, *48*(4):412-422.

Khazaei, H. et al. (2021). Recent advances in faba bean genetic and genomic tools for crop improvement. *Legume Science*, *3*(3):e75.

Kulkarni, M. et al. (2006). Stimulation of rice (*Oryza sativa* L.) seedling vigour by smoke-water and butenolide. *Journal of Agronomy and Crop Science*, *192*(5):395-398.

Leather, G. R., & Einhellig, F. A. (1985). Mechanism of allelopathic action in tree of heaven (*Ailanthus altissima*). *Journal of Chemical Ecology*, *11*(5):635-646.

Li, H. B. et al. (2007). Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry*, *102*(3):771-776.

Makkar, H. P. S. (2003). Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research*, *49*(3):241-256.

Maksimović, T., Markovic, L., & Hasanagić, D. (2021). Influence of *Ambrosia artemisiifolia* extract on germination and growth of *Psum sativum* L. and *Phaseolus vulgaris* L. seedlings. *Zemljiste Biljka*, *70*(2):33-41.

Marschner, H. *Marschner's mineral nutrition of higher plants*. Academic Press; 2012. 651p.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, *7*(9):405-410.

Omezzine, F. et al. (2011). Allelopathic potential of *Inula viscosa* L. leaves and roots. *Journal of Food, Agriculture & Environment*, *9*(2):676-681.

Ounissi, I. et al. (2024). Phytochemical profile by LC-MS/MS, total phenolic content, and antioxidant properties of *Erodium guttatum* from Algeria. *Global NEST Journal*, *26*(10):06464.

Ounoughi, A. et al. (2020). Chemotypes and antibacterial activities of *Inula viscosa* essential oils from Algeria. *Biodiversitas Journal of Biological Diversity*, *21*(4):1504-1517.

Ozkan, E. et al. (2019). Promising medicinal plant *Inula viscosa* L.: Antiproliferative, antioxidant, antibacterial and phenolic profiles. *Progress in Nutrition*, *21*(2):652-661.

Pane, C. et al. (2023). Phytochemical extracts of *Dittrichia viscosa* (L.) Greuter from agroecological systems: Seed antigerminative properties and effectiveness in counteracting *Alternaria* leaf spot disease on baby-leaf spinach. *Biology*, *12*(6):790.

Paradiso, A., Durante, M., & Caretto, S. (2024). Establishment of *Dittrichia viscosa* L. hairy roots and improvement of bioactive compound production. *Plants*, *13*(22):3236.

Perdomo, E. C. et al. (2020). Development of new genetic resources for faba bean (*Vicia faba* L.) breeding through the discovery of gene-based SNP markers and the construction of a high-density consensus map. *Scientific Reports*, *10*:6790.

Price, M. L., Van Scoyoc, S., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry*, *26*(5):1214-1218.

Prisa, D. (2020). Biostimulant based on *Inula viscosa* L. (*Dittrichia viscosa* L.), algae and microorganisms in the growth and defense of *Spinacia oleracea* L. and *Lactuca sativa* L. *International Journal of Scientific Research in Multidisciplinary Studies*, *6*(11):1-6.

Qneibi, M. et al. (2021). *Inula viscosa* (L.) Greuter, phytochemical composition, antioxidant, total phenolic content, total flavonoids content and neuroprotective effects. *European Journal of Integrative Medicine*, *42*:101291.

Rai, A., Kumari, K., & Han, S. (2023). Polyphenolic profiling of *Victoria Amazonica* using MRM LC-MS/MS: A comparative analysis of various plant parts. *Scientia Horticulturae*, *320*:112206.

Re, R. et al. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9-10):1231-1237.

Rhimi, W. et al. (2018). *Dittrichia viscosa* L. leaves lipid extract: An unexploited source of essential fatty acids and tocopherols with antifungal and anti-inflammatory properties. *Industrial Crops and Products*, *113*:196-201.

Rice, E. L. *Allelopathy*. Academic Press; 1984. 368 p.

Sillmann, T., & Mattiuz, C. Growth inhibition of potted begonia via ethanol treatment. *Ornamental Horticulture*, *30*:e242675 2024.

Sriti Eljazi, J. et al. (2018). Essential oil composition, phenolic compound, and antioxidant potential of *Inula viscosa* as affected by extraction process. *International Journal of Food Properties*, *21*(1):2309-2319.

Sutariya, S. et al. (2022). Fourier transform infrared spectroscopy (FTIR) analysis, antioxidant and anti-inflammatory activities of leaf and fruit extracts of *Gymnosporia montana*. *Materials Today: Proceedings*, *73*:134-141.

Sladonja, B. et al. (2021). *Dittrichia viscosa*: native-non native invader. *Diversity*, *13*(8):380.

Vuko, E. et al. (2021). Not only a weed plant biological activities of essential oil and hydrosol of *Dittrichia viscosa* (L.) Greuter. *Plants*, *10*(9):1837.

Vundać, V., Brantner, A., & Plazibat, M. (2007). Content of polyphenolic constituents and antioxidant activity of some *Stachys* taxa. *Food Chemistry*, *104*(3):1277-1281.

Waterhouse, A. L. (2011). *Folin-Ciocalteu micro method for total phenol in wine*. Department of Viticulture and Enology, University of California, Davis, 2011. Available in: https://waterhouse.ucdavis.edu/folin-ciocalteau-micro-method-total-phenol-wine.

Xiaohong, Y. et al. (2012). Allelopathic activity of the extract from *Phytolacca americana* an invasive alien plant. *Journal of Ecology and Rural Environment*, *28*(2):139-145.

Zanotti, C. et al. (2024). α-costic acid, the main sesquiterpenoid isolated from *Dittrichia viscosa* (L) Greuter, induces oxidative stress and autophagy in tomato. *Plant Biology*, *26*(7):1213-1222.