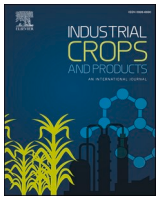
Kisiriko, M., Bitchagno-Mbahbou, G., Harflett, C., Noleto-Dias, C., Naboulsi, I., Anastasiadi, M., Terry, L. A., Sobeh, M., Beale, M. H. and Ward, J. L. 2025. Bioactivity screening of selected Moroccan medicinal and aromatic plants, and the chemical basis of the phytotoxicity of caper, Capparis spinosa L. *Industrial Crops and Products.* 233, p. 121355. https://doi.org/10.1016/j.indcrop.2025.121355

Industrial Crops & Products 233 (2025) 121355

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop



Bioactivity screening of selected Moroccan medicinal and aromatic plants, and the chemical basis of the phytotoxicity of caper, *Capparis spinosa* L.

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ARTICLE INFO

Keywords:
Capparis spinosa
Phytotoxic
Inhibitory
Oxylipins
Alkaloids
Loliolide
8-(1*H*-indol-3-yl-methyl)rutin

ABSTRACT

Plant natural products are potential sources of biostimulants that can help plants overcome the effects of stress. The adverse effects of soil salinity on wheat growth necessitate the exploration of alternative sustainable solutions, such as biostimulants from medicinal and aromatic plants, to enhance crop resilience and productivity. This study aimed to screen nine Moroccan medicinal and aromatic plant extracts for their effect on wheat growth under saline and non-saline conditions using a seed soaking treatment, in a completely randomised experiment. Except for *Marrubium vulgare* leaf and *Origanum compactum* extracts, which averagely improved root length by 25 % and 14 %, respectively, none of the other extracts had significant positive effects on wheat seedling growth. *Capparis spinosa* (caper) extracts inhibited wheat emergence and growth, with leaf extracts being more phytotoxic than the stem extracts. The leaf extracts of *C. spinosa* caused an average reduction of the leaf length, root length, shoot dry weight and root dry weight of the wheat seedlings by 31 %, 21 %, 92 % and 94 %, respectively, compared with the control. Further fractionation of the leaf crude extract and follow-up screening revealed that the phytotoxicity likely resulted from a synergy between compounds in different fractions. Chemical analysis of the most active fraction by UHPLC-MS and NMR revealed loliolide as the major compound, alongside oxylipins and indole alkaloid derivatives. Additionally, a previously undescribed compound, 8-(1*H*-indol-3-yl-methyl) rutin, was also identified. These compounds potentially contribute to the phytotoxicity. The results of this experiment show that although two extracts enhanced root length, overall biostimulant effects were minimal, with *C. spinosa* extracts being significantly toxic, indicating the need to prevent their application on wheat.

1. Introduction

Salt stress is a major abiotic factor that significantly affects crop growth, resulting in reduced yield and productivity (Mutlu-Durak and Yildiz Kutman, 2021; Sharma et al., 2019). Excessive concentrations of salts in the soil reduce the ability of roots to take up water by osmosis, causing physiological drought. The associated accumulation of ions, such as sodium and chloride ions, leads to nutrient imbalances and ion toxicity due to disruption of cellular ion homeostasis and nutrient uptake (Atta et al., 2023). These ionic effects compromise essential physiological functions such as photosynthesis, protein synthesis and enzyme activity. Salinity also triggers the generation of reactive oxygen species (ROS), which induces oxidative stress (Liu et al., 2024). These effects altogether lead to poor germination, stunted growth and in some cases plant death, causing significant yield losses. Plants respond to salt stress through a range of morphological, physiological and biochemical mechanisms, including reduction in photosynthesis, activation of enzymatic and non-enzymatic antioxidants to scavenge ROS, ion transport regulation, activation of signalling pathways involving hormones and expression of salt-responsive genes, among others (Liu et al., 2024; Atta et al., 2023; Rady et al., 2019). Several interventions can be taken to help plants increase their resistance to salt stress, and one such intervention is biostimulation. Biostimulation involves the application of substances derived from natural sources that have the potential to

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https://doi.org/10.1016/j.indcrop.2025.121355
Received 25 January 2025; Received in revised form 11 June 2025; Accepted 12 June 2025
Available online 26 June 2025

improve crop growth and yield by enhancing nutrient uptake and increasing the resistance of plants to stresses, including salinity (Kisiriko et al., 2021). The major biostimulants include microorganisms, seaweed extracts, humic and fulvic acids, protein hydrolysates, chitin and chitosan derivatives, and plant extracts (Kisiriko et al., 2021; Yakhin et al., 2017).

Plant extracts, particularly those derived from medicinal and aromatic plants, have been used as biostimulants due to the known abundance of bioactive secondary metabolites in their tissues. Exogenously applied extracts of such plants have been demonstrated to stimulate the growth of various crops at different stages, from seed germination to fruiting, through several mechanisms. These mechanisms include influencing phytohormone activity, modulating antioxidant enzyme activity, and enhancing beneficial metabolites, among other plausible processes (Kisiriko et al., 2021). However, in addition to the biostimulant effects, some plant extracts can be phytotoxic, significantly hindering seed germination and plant growth (Bashar et al., 2023). These extracts are primarily aqueous and may be applied through methods such as seed soaking, foliar application, irrigation of whole plants, or soil drenching. Soaking seeds in medicinal and aromatic plant extracts has been shown to improve the growth and productivity of several crops, including maize (Rehman et al., 2015), common beans (Rady et al., 2019), eggplants (Ali et al., 2019), and wheat (Ahmed et al., 2021; Mehta and Chowdhury, 2021), under both normal and stress conditions. Seed soaking provides a straightforward method for testing many extracts, involving a one-time treatment before planting the seeds.

In Morocco, medicinal and aromatic plants are abundant. Due to the aridity of most of the country, these plants experience significant drought and salt stress and potentially accumulate secondary metabolites in their tissues as a defence mechanism (Sharma et al., 2019). These plants could therefore be good sources of extracts and/or compounds with beneficial bioactivities, for use in agriculture, compared with species growing in non-stressed environments.

Wheat is an important crop in Morocco, being a major staple food and a source of livelihood for many families. In fact, it is second only to olives as the most important national crop (Achli et al., 2025). However, its yields have been significantly affected in recent years due to drought and the salinity of the soil owing to the irrigation with saline water (Achli et al., 2025; Manhou et al., 2024). One of the ways to mitigate the effect of salinity stress on wheat and improve productivity is often through the application of fertilisers. However, despite their benefits, the deleterious effects of many fertilisers and synthetic agrochemicals, including soil degradation, water and air pollution, plant damage from prolonged usage, as well as health risks in humans and animals, are well documented (Carvalho, 2017). Consequently, there is a need for scientific research to explore less harmful and more sustainable alternatives from natural sources, of which biostimulants from extracts of medicinal and aromatic plants are key candidates. These plant extracts have been shown to alleviate the effects of stress and improve seed germination and growth under saline conditions (Ahmed et al., 2021; Mehta and Chowdhury, 2021; Rady et al., 2019). These extracts can mitigate the adverse effects of salinity on crop growth through various mechanisms. They are usually rich in bioactive compounds such as phenolics, alkaloids, and terpenoids that act as natural antioxidants that scavenge harmful ROS produced under salt stress, and enhance the activity of key antioxidant enzymes such as superoxide dismutase, catalase and peroxidase (Ahmed et al., 2021; Kisiriko et al., 2021; Rady et al., 2019). The extracts can also improve salt tolerance by providing seeds with excess beneficial components, for instance sugars, amino acids, chlorophyll, carotenoids, proline and mineral nutrients. Proline and soluble sugars are osmoprotectants and their accumulation can influence hormonal balance, improving water retention, nutrient uptake and growth under saline conditions (Rady et al., 2019). Therefore, exploring more plant extracts for their potential conferment of salinity tolerance to plants is of profound importance.

There is a general lack of studies on the application of extracts derived from Moroccan medicinal and aromatic plants in mitigating salt stress in wheat. However, these plants are promising due to the secondary metabolites that may accumulate in their tissues as part of their defence mechanism against stress. Therefore, the objective of this study was to screen aqueous extracts of nine Moroccan medicinal and aromatic plants, including a mix of common culinary and wild species, for their effects on the growth of wheat under saline and non-saline conditions. This paper presents the results of the trials, including a phytotoxic extract from *Capparis spinosa*, and we explore the chemistry contributing to the observed phytotoxicity, which led to the characterisation of a compound with a novel chemical backbone.

2. Materials and methods

2.1. General instrumental analysis procedures

UHPLC–MS data were acquired on an LTQ-Orbitrap Elite mass spectrometer coupled to a Dionex UltiMate 3000 RS UHPLC system (Fisher Scientific) employing a reversed-phase Hypersil GOLD C18 selectivity HPLC column (3 μm, 30 × 2.1 mm i.d. Thermo Fisher Scientific) maintained at 35 °C. Data was collected and examined using Xcalibur v. 2.2 (Thermo Fisher Scientific). NMR data was obtained using a Bruker Avance 600 MHz NMR spectrometer (Bruker Biospin, Germany) operating at 600.05 MHz (^{1}H) and 150 MHz (^{13}C). Spectra were acquired at 300 K and the chemical shifts were referenced relative to d_4-3-(trimethylsilyl) propionic-2,2,3,3 acid (TSP-d_4) (Sigma-Aldrich). Data was analysed using MestreNova software (Mestrelab Research SL, Spain). Detailed methodology, further NMR and UHPLC-MS parameters as well as analysis conditions can be found in Noleto-Dias et al. (2019).

2.2. Plant material

The plant material was sourced from different regions in western Morocco. *Vachellia gummifera* leaves (Vgum-l) and stems, including the bark (Vgum-s) were harvested from two-year-old plants at the experimental farm (32.219731E, − 7.892268N) of Mohammed VI Polytechnic University in Ben Guerir, Morocco. Leaves of *Origanum compactum* (Ocom), *Thymus vulgaris* (Tvul) and dried seeds of *Ammodaucus leucotrichus* (Aleu) were sourced from Coopérative Arij Al Ghaba, a local certified co-operative in Marrakesh. Leaves of *Origanum majorana* (Omaj) were obtained from Coopérative Les Douceurs du Maroc, another local certified co-operative in Marrakesh. Seeds of *Peganum harmala* (Phar) were sourced from Ben Guerir. *Marrubium vulgare* leaves (Mvul-l) and stems (Mvul-s), *Capparis spinosa* leaves (Cspi-l) and stems (Cspi-s), and *Foeniculum vulgare* leaves (Fvul) were all sourced from Safi. The samples were air-dried in the dark at room temperature for 20 days before being transported to Rothamsted Research in the UK, where they were milled to a fine powder (Retsch Ultra Mill ZM200, Retsch, UK). The milled samples were stored at room temperature in the dark until analysis.

2.3. Extraction

The extracts were prepared according to a method modified from (Mutlu-Durak and Yildiz Kutman, 2021). Briefly, a 4 % extract was prepared for each plant. The milled plant material (4 g) was added to water (100 mL) in a round-bottomed flask. The mixture was stirred constantly for 30 min in a water bath maintained at 90 °C using a magnetic stirrer. The resulting extracts were cooled and filtered through a funnel plugged with cotton wool and then through a Buchner funnel using a vacuum. The filtered extracts were stored at − 20 °C until use. For fractionation on the Biotage Selekt®, 10 g of milled *C. spinosa* leaves was extracted in 250 mL of water/methanol (4:1) following the same procedure. The solvent in the resultant extract was reduced using a rotary evaporator (Buchi, Germany), and the extract was decanted. The extract was then loaded onto the Biotage Selekt® for fractionation.

2.4. Fractionations

2.4.1. Fractionation of the Capparis spinosa leaf crude extract

Fractionation of the crude extract was carried out on a Biotage Selekt® high-performance automated flash chromatography system equipped with a Sfär C18 column (Duo 100 Å, 30 µm, 120 g, Biotage, UK). Chromatographic separation was done using a flow rate of 25 mL/min of the mobile phases, water, and acetonitrile. The gradient was started at 2 % acetonitrile in water for 10 column volumes (CV). This was then increased in steps of 10 % until 50 % for 10 CV at each step. It was then increased from 50 % to 100 %, and kept at 100 % for 20 CV. Each CV was equivalent to 45 mL. Liquid loading was used for introducing the sample to the column and the eluent was automatically collected in individual glass tubes according to the UV pattern. The eluting compounds were monitored between the UV wavelengths, 200 and 400 nm. The contents of the glass tubes were systematically combined to form 10 fractions covering the regions shown in Fig. S1 (Supplementary information).

2.4.2. Isolation of compounds from fraction 10

Further fractionation of fraction 10 from the Biotage Selekt® was achieved using a Dionex UltiMate 3000, Thermo Fisher Scientific HPLC system equipped with an Ascentis C-18 column (5 µm, 5 × 250 mm, Supelco, UK). Separation was achieved using a constant flow rate of 1 mL/min of the mobile phases, water + 0.1 % formic acid (A) and acetonitrile + 0.1 % formic acid (B) (both Optima™ grade, Thermo Fisher Scientific, Germany). The gradient used was: 0–10 min, 5 % B; 10–50 min, 22 % B; 50–60 min, 37 % B; 60–75 min, 50 % B; 75–85 min, 70 % B; and finally, 85–90 min, 100 % B. Several injections (each 100 µL) were made, and the resultant subfractions were automatically collected by time into individual glass tubes. For each subsequent run, the system was programmed to automatically resume collection into the same glass tubes. The eluting compounds were monitored between the wavelengths, 200 and 800 nm. After collection, an aliquot (200 µL) of each subfraction was transferred into a glass vial and analysed by UHPLC-MS. The remainder of the subfraction was dried overnight using a Speedvac concentrator (Genevac, Suffolk, UK) and then dissolved in 700 µL of D_2O/CD_3OD (4:1 v/v, 1 mL) (Goss Scientific) containing 0.01 % TSP-d_4 for NMR analysis. From the 66 subfractions that were collected, compounds **1**–**23** were identified, with compound **16** being previously undescribed.

8-(1*H*-indol-3-yl-methyl)rutin (**16**). Colourless amorphous solid; UV λ_{max} 221, 271 nm; ^1H and ^{13}C NMR data (D_2O/CD_3OD (4:1), see Table 3: UHPLC-MS, $[M - H]^-$ ion at *m/z* 738.2026 (calcd for $C_{36}H_{36}NO_{16}^-$, *m/z* 738.2040).

2.5. Pot experiments

Wheat seeds (Cadenza cultivar) were obtained from the seed stock at Rothamsted Research. The seeds were surface sterilised by soaking them in 1 % sodium hypochlorite solution (Fisher Scientific) for 10 min. They were then thoroughly rinsed with water (at least 7 rinses) and after soaked in the plant extracts prepared at a concentration of 4 % as mentioned in Section 2.3 for 20 h at room temperature in the dark. Seeds for the control experiment were soaked in deionised water.

Six-inch pots were filled with 0.9 kg of Rothamsted prescriptive mix (RPM), a standard compost mix consisting of 75 % Medium grade (L&P) peat, 12 % screened, sterilised loam, 3 % Medium grade vermiculite and 10 % Grit (5 mm screened, lime free) (Scotts UK Professional, Ipswich, Suffolk). The experiment was carried out under both saline and non-saline conditions. For the saline set up, 100 mM NaCl (200 mL) (Sigma-Aldrich) was added to each pot, whereas for the non-saline set up, water (200 mL) was added before sowing the seeds. Twenty seeds were sown at a depth of 2.5 cm, approximately equidistant to each other in each pot, and the experiment was organised in a completely randomised design with three replicates. The experimental design considered two factors, namely the treatment (plant extract or control) and the salinity condition at two levels (non-saline and saline). The experiment, therefore, involved 13 treatments under each salinity condition, including the 12 extracts and the control, resulting in 26 treatment combinations and a total of 78 pots (experimental units) after replication. The experiment was conducted in a controlled environment growth chamber maintained at temperature/humidity conditions of 20 °C/65 % and 15 °C/5 % during the day and night respectively. The chamber was maintained at a light regime of 16 h light and 8 h dark with the light level set at 600 µmol. Until the 7th day, all pots were watered (100 mL) every two days. On the 7th day, the pots under saline conditions were supplemented with another 200 mL of 100 mM NaCl. The pots under non-saline conditions were correspondingly watered with 200 mL of water. No further watering was done until harvest. After 14 days, the seedlings were harvested, and the shoot and root lengths were measured. The seedling roots were thoroughly washed and separated from the shoots. The roots and shoots were dried for 48 h in an oven maintained at 80 °C, after which their dry weights were recorded.

2.6. Assay with Capparis spinosa leaf extracts and fractions

Cell trays (15 cells, 6.5 × 6.3 cm) were filled with RPM and watered. Wheat seeds were sterilised as described above. Each of the 10 fractions from the Biotage Selekt® and a crude *C. spinosa* leaf extract made with water/methanol (4:1) (crude-wat/met) were dried down and reconstituted in water maintaining the final concentration at 4 % w.r.t the starting plant material. The seeds were separately soaked in the reconstituted fractions, water (control) and another crude leaf extract made with water (crude-water) for 20 h at room temperature in the dark. Four seeds were sown per cell at a depth of 2.5 cm using four replicates in a randomised complete block design. They were grown in the same chamber under the same conditions as with the pot experiment and were watered every two days. The total daily emergence was recorded for 11 days.

2.7. Data and statistical analysis

The significance of the effect of the extract treatments on the growth parameters of the wheat seedlings was assessed using analysis of variance (ANOVA) with Genstat (22nd edition, VSN International Ltd., Hemel Hempstead, U.K.). Where necessary, data were transformed using the square root or $\log_{10}$ transformation to conform with ANOVA assumptions of normality and equality of variance. A comparison of means was done using Fisher's unprotected least significant difference (LSD) test at 5 % significance.

The cumulative emergence (CE), total emergence (TE), mean emergence time (MET) and emergence index (EI) were calculated according to these formulae adapted from Chen et al. (2023) and Lozano-Isla et al. (2019) and the references therein.

$$CE = \left(\frac{n_i}{N}\right) \quad \times 100$$

$$TE = \left(\frac{\sum_{i=1}^{k} n_i}{N}\right) \times \quad 100$$

$$MET = \left(\frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}\right)$$

$$EI = \sum \left(\frac{G_i}{D_i}\right)$$

Where n_i is the number of seedlings that emerged in the i^{th} time; N is

the total number of seeds in each replicate; k is the last day of the emergence evaluation; t_i is the time from the beginning of the experiment to the i^{th} observation; G_i is the number of seeds emerged on the i^{th} day, D_i is the day of emergence.

The data from the assays with the *C. spinosa* fractions was analysed using the GerminaR package in the R software. The data sets for MET and EI were subjected to an ANOVA followed by a comparison of means by the Student-Newman-Keuls test at 5 % significance.

3. Results and discussion

3.1. Initial screening of extracts

The effect of twelve water extracts from nine selected plants on the growth of wheat seedlings under saline and non-saline conditions was tested in a pot experiment using a seed soaking application. After 14 days, the leaf length, root length, shoot dry weight and root dry weight of the harvested seedlings were measured to assess the effect of the extracts. Under non-saline conditions, the highest values of the leaf length, root length, shoot dry weight and root dry weight were observed with Fvul (34.4 cm), Mvul-l (19.9 cm), Ocom (2.490 g) and Vgum-l (0.722 g), respectively. On the other hand, the lowest values for the same respective parameters were all observed with the Cspi-l extract being 24.8 cm, 12.4 cm, 0.221 g and 0.043 g. Under the saline conditions, the highest values of the leaf length, root length, shoot dry weight and root dry weight were observed with Mvul-l (32.0 cm), Mvul-l (15.5 cm), Mvul-s (2.049 g) and Vgum-l (0.526 g). The lowest values of the same respective parameters were also observed with Cspi-l (similar to the non-saline conditions) being 17.9 cm, 9.9 cm, 0.133 g and 0.025 g.

Generally, saline conditions negatively affected the growth of wheat seedlings as evidenced by the lower values of all the analysed growth parameters of the harvested seedlings in comparison to the non-saline conditions for all treatments (Fig. 1). Analysis of the results of the growth parameters under both saline and non-saline conditions using a two-way ANOVA showed that the effects of treating the seeds with the extracts and the salinity were independently significant on all parameters, but the interaction of both factors was not significant. Based on this result, we explored the main effect of the extract treatments through a comparison of means using Fisher's unprotected least significant difference test. Fig. 2 and Tables S1–S4 show the pairwise comparisons of the different extract treatments and their significance on the leaf and root length, as well as shoot and root dry weight. The comparisons are based on the combined average values for both the saline and non-saline conditions.

To assess how the extracts influenced the growth of the wheat seedlings, pairwise comparisons with the control were evaluated. Clearly, only Cspi-l and Cspi-s had a significant effect on the leaf length of the seedlings (Fig. 2A). Under both the saline and non-saline conditions, both treatments produced seedlings with smaller leaf lengths compared with the control, with Cspi-l causing a stronger negative effect than Cspi-s (Fig. 1A). Combining the results of the saline and non-saline conditions showed that treatment with Cspi-l and Cspi-s caused average decreases of 31 % and 12 % respectively of the seedling leaf lengths.

Only Cspi-l, Mvul-l and Ocom significantly affected the root lengths (Fig. 2B). As with the leaf lengths, treatment with Cspi-l produced seedlings with smaller root lengths compared with the control, under both the saline and non-saline conditions (Fig. 1B). Treatment with Cspi-

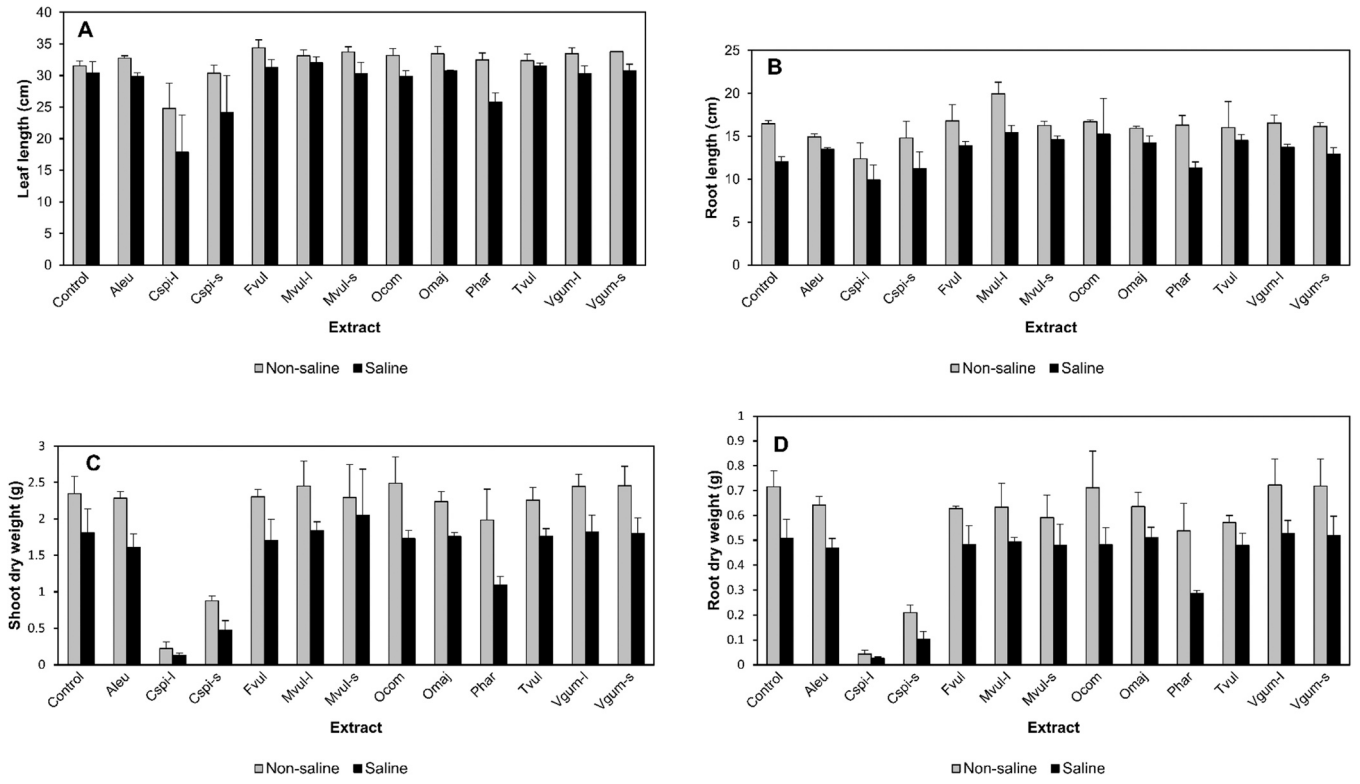

Fig. 1. Effect of soaking wheat seeds in different extracts on the leaf length (A), root length (B), shoot dry weight (C) and root dry weight (D) of the seedlings, under saline and non-saline conditions. Values are means of three independent replicates, each containing 20 seeds and the error bars represent the standard deviation. Two-way ANOVA indicated significant effects of salinity and extract treatment ($p < 0.05$), but no significant interaction between them. This figure provides a visual overview of the treatment responses and detailed statistical comparisons for main effects are presented in Fig. 2. Aleu – *Ammodaucus leucotrichus*, Cspi-l – *Capparis spinosa* (leaves), Cspi-s – *Capparis spinosa* (stems), Fvul – *Foeniculum vulgare*, Mvul-l – *Marrubium vulgare* (leaves), Mvul-s – *Marrubium vulgare* (stems), Ocom – *Origanum compactum*, Omaj – *Origanum majorana*, Phar – *Peganum harmala*, Tvul – *Thymus vulgaris*, Vgum-l – *Vachellia gummifera* (leaves), Vgum-s – *Vachellia gummifera* (stems).

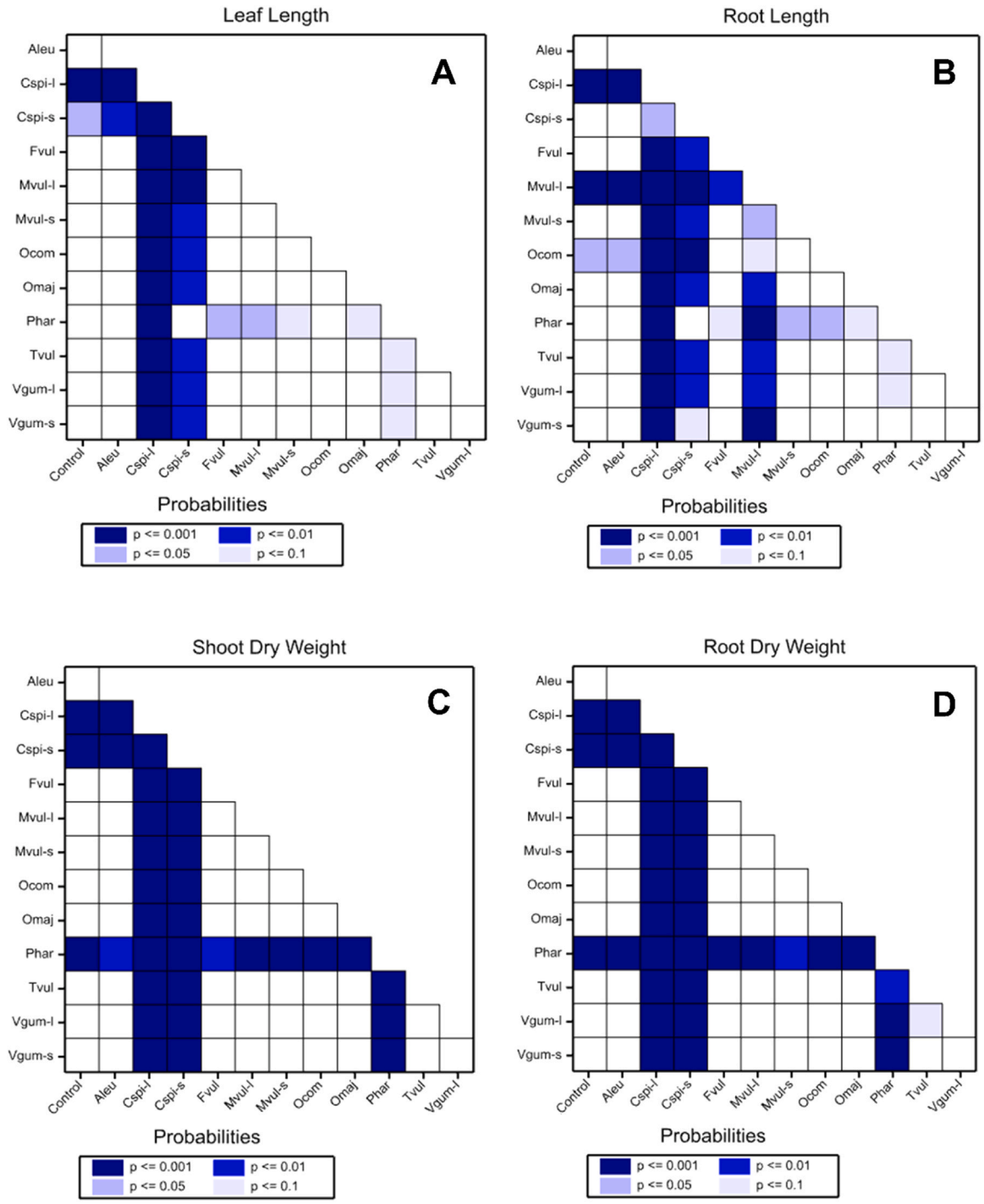

Fig. 2. Pairwise probability plots for the significance of the extract treatments on the leaf length (A), root length (B), shoot dry weight (C) and root dry weight (D) of wheat seedlings. The analysis was done using Fisher's unprotected Least Significant Difference test. Aleu – *Ammodaucus leucotrichus*, Cspi-l – *Capparis spinosa* (leaves), Cspi-s – *Capparis spinosa* (stems), Fvul – *Foeniculum vulgare*, Mvul-l – *Marrubium vulgare* (leaves), Mvul-s – *Marrubium vulgare* (stems), Ocom – *Origanum compactum*, Omaj – *Origanum majorana*, Phar – *Peganum harmala*, Tvul – *Thymus vulgaris*, Vgum-l – *Vachellia gummifera* (leaves), Vgum-s – *Vachellia gummifera* (stems).

l caused an average reduction in the root lengths by 21 %. Conversely, Mvul-l significantly improved wheat root length compared with the control and in fact produced seedlings with the longest root lengths of all the treatments under both salinity conditions. Treatment with Mvul-l improved the root lengths by an average of 25 %. Despite a minor stimulation under non-saline conditions, treatment with Ocom averagely increased the root lengths by 14 %. Cspi-l, Cspi-s and Phar significantly affected the shoot and root dry weights (Figs. 2C and 2D). A look at the mean shoot and root dry weights (Figs. 1C and 1D) shows that all three extracts markedly decreased both the shoot and root dry weights of the treated seedlings in comparison with the control under both conditions of salinity with the effect being in the order Cspi-l > Cspi-s > Phar. Treatment with Cspi-l, Cspi-s and Phar caused average decreases in shoot dry weights of 92 %, 68 % and 27 %, respectively. Similarly, the root dry weights were decreased by averages of 94 %, 75 % and 34 % by treatment with Cspi-l, Cspi-s and Phar, respectively.

In comparison with the control, Aleu, Fvul, Mvul-s, Omaj, Tvul,

Vgum-l and Vgum-s did not have a significant effect on any of the assessed parameters. Mvul-l and Ocom significantly improved only the root length. In a study by Dallali et al. (2017), aqueous extracts of *M. vulgare* leaves increased the root length of the weed species *Sinapis arvensis* L. under laboratory conditions, albeit the increase was not statistically significant. However, the same extract significantly increased the shoot length of the same test species. Interestingly, on the contrary, the same extract at the same concentration significantly inhibited both the root and shoot growth of *Lactuca sativa* L. seedlings under the same conditions (Dallali et al., 2017). Treatment with Phar significantly decreased the shoot and root dry weights. An experiment by Sodaeizadeh et al. (2009) showed that aqueous extracts of *P. harmala* leaves, stems and roots caused concentration- and species-dependent inhibition of the germination and seedling growth of the two weed species *Avena fatua* L. and *Convolvulus arvensis* L. The extract from the leaves was the most phytotoxic followed by the one from the stems, while the root extract was the least toxic. Seed extracts were however, not assessed in the experiment (Sodaeizadeh et al., 2009).

Treatment with Cspi-s significantly decreased all the assessed growth parameters except the root length. Perhaps most interestingly, treatment with Cspi-l significantly decreased all assessed growth parameters, indicating that it exerted the highest phytotoxicity and inhibition on the growth of wheat seedlings. It is worth noting that the effect on all assessed parameters was more severe on wheat treated with the leaf extract of *C. spinosa* than with its stem extract under both saline and non-saline conditions, indicating that its leaves contain more phytotoxic compounds than the stems. Other studies have also shown that leaf extracts of *C. spinosa* are more phytotoxic compared with extracts derived from other plant parts (El-Bakkosh et al., 2020; Ladhari et al., 2013). Aqueous leaf, root and fruit extracts of *C. spinosa* were tested in a Petri Dish assay for their effect on the growth of wheat. The leaf extract had the highest inhibitory effect on the growth parameters, including the plumule length and radicle length, followed by the root extract, with the least inhibition being observed with the fruit extract (El-Bakkosh et al., 2020).

The Cspi-l and Cspi-s extracts not only inhibited the growth of the seedlings as evidenced from the values of the growth parameters, but they also inhibited the emergence of the seedlings. Whereas the seeds soaked in all the other extracts, except Phar, achieved total emergence (TE) of over 95 % in both saline and non-saline conditions (like the control), seeds soaked in the Cspi-l extract only achieved TEs of 10 and 16 % in the saline and non-saline conditions, respectively. Seeds soaked in the Cspi-s extract achieved TEs of 42 and 53 % in the saline and non-saline conditions, respectively. The TE of seedlings in the Phar extract was slightly less than the control, with 85 and 77 % in the non-saline and saline conditions, respectively. A few studies have reported the germination inhibitory effect of *C. spinosa*. Extracts of *C. spinosa* showed a concentration-dependent as well as organ and target-specific inhibition of the germination of lettuce, radish, peganum and thistle seeds with the leaves being the most phytotoxic (Ladhari et al., 2013). At a similar concentration as tested in our study (4 %), the aqueous leaf extract completely inhibited the germination of all the species. Extracts of *C. spinosa* were reported to significantly reduce seed germination and seedling growth of wheat and alfalfa as well the weed species, *Amaranthus retroflexus* and *Chenopodium murale* (Qasem, 2002; Sodaeizade and Hakimi Maybodi, 2010).

Combining the above results for the seedling emergence and all assessed seedling growth parameters clearly showed that Cspi-l was the most bioactive extract exerting significant phytotoxicity and growth inhibition of the wheat seedlings. As such, we investigated the chemistry of the Cspi-l extract to identify the compound(s) that potentially contribute to the observed phytotoxicity. As the effect of the extract was the same under both non-saline and saline conditions, all the additional assays were conducted under non-saline conditions.

3.2. Comparison of the bioactivity of Capparis spinosa leaf extracts at 50 °C and 90 °C extraction

As 90 °C is a relatively high temperature compared with common extraction temperatures, the bioactivity of the *C. spinosa* leaf extract obtained at a lower temperature of 50 °C (Cspi-l-50) was tested alongside the extract obtained at 90 °C (Cspi-l). Extraction at 50 °C has been shown to be efficient in metabolomic extraction and analysis (Noleto-Dias et al., 2020; Corol et al., 2014). The same seed soaking treatment was applied using Cspi-l, Cspi-l-50, and a water control under non-saline conditions in a pot experiment. The results showed that the bioactivity profiles of both Cspi-l and Cspi-l-50 were the same with both extracts showing similar phytotoxicity (Fig. 3). Both extracts similarly inhibited the emergence of the seedlings compared with the control, with no emergence observed in any of the replicates with seeds soaked in Cspi-l and only one seedling emerging in one of the replicates with seeds soaked in Cspi-l-50.

3.3. Fractionation and screening of Capparis spinosa leaf extract fractions

To identify the potential compounds responsible for the observed phytotoxicity, the crude extract was fractionated to break it down into groups of compounds based on polarity. Each fraction was assayed to attempt to identify one that contained the bioactive compounds that could be elucidated by further analysis of the chemistry of that fraction. As the Cspi-l and Cspi-l-50 extracts had similar phytotoxicity, the Cspi-l-50 extract was considered for further analysis. A 4 % extract was prepared in water/methanol (4:1) following the same procedure and separated into 10 fractions on a Biotage Selekt® preparative HPLC. The decision to extract in water/methanol (4:1) was to follow already established and validated analytical procedures for metabolite analysis (Corol et al., 2014). The chemical profiles of the water and water/-methanol (4:1) extracts do not differ significantly as they have close polarities. However, the water/methanol (4:1) may have been expected to extract higher levels of less polar metabolites. The chemical profiles of the two extracts (Fig. S2) however were remarkably similar.

The cumulative emergence upon soaking the seeds in the 10 fractions, a water control, a crude leaf extract made with water (crude-water), and another crude extract made with water/methanol (4:1) (crude-wat/met) is shown in Fig. 4. The data for TE from the screening of the fractions was not subjected to a statistical analysis due to the lack of variability in the data as a result of the small number of seeds (4) used in each replicate. Like what was observed in the initial screening

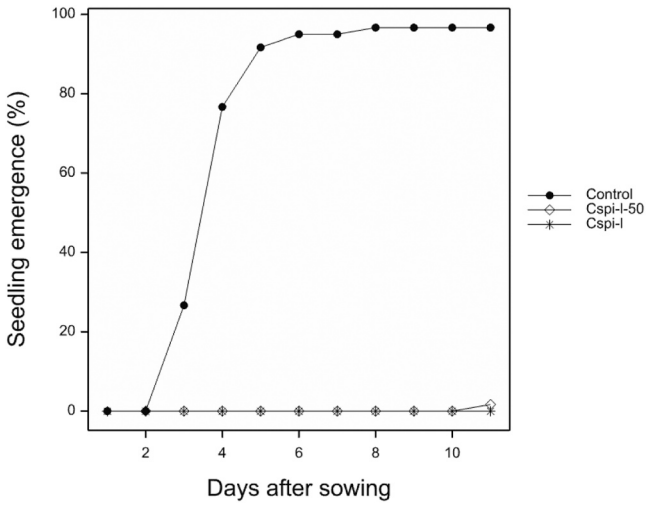

Fig. 3. Comparison of the effect of the *Capparis spinosa* leaf extract prepared at 90 °C (Cspi-l) and 50 °C (Cspi-l-50) on the cumulative emergence of wheat seedlings under non-saline conditions.

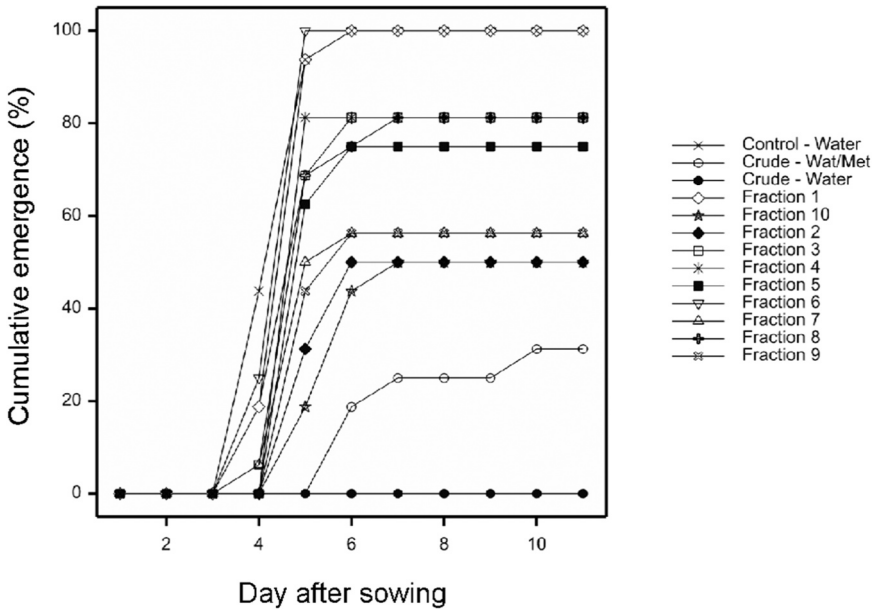

Fig. 4. Cumulative emergence of wheat seedlings soaked in water, *Capparis spinosa* crude extracts, and *Capparis spinosa* leaf fractions up to 11 days after sowing.

experiment, a TE of 100 % was observed with the control seeds. Additionally, the crude-water extract was 100 % phytotoxic as no seedling emergence was observed in all the replicates. The crude-wat/met extract delayed the emergence of the seedlings until 6 days after sowing and the TE was only 31.2 % on day 11. This result indicated that despite still being significantly phytotoxic to the seeds, the bioactivity of the crude-wat/met extract was slightly less than that of the crude-water extract. Similarly, considering the treatments with observed emergence, seedlings from the crude-wat/met extract had the highest MET (Table 1) which was also the only one significant from the control. This indicated that the seeds soaked in the crude-wat/met extract had the lowest speed of emergence. None of the fractions showed toxicity at a similar level to the crude-water and crude-wat/met extracts. Fractions 1 and 6 showed no toxicity as a TE of 100 % was observed, as with the control. A TE of 81.2 % was achieved with seeds soaked in fractions 3, 4 and 8. The TE for seeds soaked in fraction 5 was 75 % whereas seeds soaked in fractions 7 and 9 had a TE of 56.2 % at 11 days after sowing. The lowest emergence among the fractions was observed with fractions 2 and 10 which caused a TE of just 50 %. The EIs of these two fractions (2 and 10), and that of the crude-wat/met extract were significantly lower than the EI of the control treatment (Table 1) indicating that the percentage and rate of emergence of seedlings from these fractions were significantly lower compared with those from the control treatment.

Table 1
Effect of the crude extracts and fractions of *Capparis spinosa* leaves on the mean emergence time and emergence index of wheat seedlings.

Treatment	MET	EI
Control – Water	4.62 ± 0.433^{b}	0.88 ± 0.002^{a}
Crude – Wat/Met	6.75 ± 0.957^{a}	0.18 ± 0.004^{e}
Crude – Water	NE	NE
Fraction 1	4.88 ± 0.144^{b}	0.83 ± 0.000^{a}
Fraction 2	5.42 ± 0.500^{b}	0.30 ± 0.005^{d}
Fraction 3	5.04 ± 0.344^{b}	0.68 ± 0.040^{ab}
Fraction 4	5.00 ± 0.000^{b}	0.65 ± 0.017^{ab}
Fraction 5	5.17 ± 0.192^{b}	0.59 ± 0.004^{abc}
Fraction 6	4.75 ± 0.204^{b}	0.85 ± 0.000^{a}
Fraction 7	5.00 ± 0.000^{b}	0.67 ± 0.000^{ab}
Fraction 8	4.81 ± 0.747^{b}	0.80 ± 0.006^{a}
Fraction 9	5.38 ± 0.479^{b}	0.52 ± 0.003^{bc}
Fraction 10	5.67 ± 0.236^{b}	0.42 ± 0.009^{cd}

MET – Mean Emergence Time; EI – Emergence Index; NE – No emergence. The values within the column followed by different letters were significantly different at $p \leq 0.05$ according to the Student-Newman-Keuls test.

These results indicated that none of the fractions contained a compound or group of compounds that was solely responsible for the observed phytotoxicity of the crude extract. This suggests that the phytotoxicity is most likely a consequence of a synergistic interaction between compounds that are potentially spread across the different fractions. Separating these compounds across different fractions possibly lessened the synergistic interaction. This is not an uncommon result, as studies have shown that extracts and mixtures of compounds produce stronger phytotoxicity than singular compounds due to synergy (Masum et al., 2020; Macías et al., 2019; Nakano et al., 2004). However, as fractions 2 and 10 showed the highest toxicity out of all the fractions, it was reasonable to assume that some of the compounds in these two fractions could potentially be responsible for most of the phytotoxicity of the crude extract. The UHPLC-MS and ^{1}H NMR profiles of fraction 2 were similar to those of fraction 1, which showed no toxicity, except that fraction 2 contained a very high proportion of sugars, also evidenced by the syrupy appearance of the sample. The high proportion of sugars is reasonably due to the early position of this fraction in the reversed-phase chromatographic run, where the compounds with the highest polarity, including sugars elute. The phytotoxicity of fraction 2 was therefore possibly a consequence of its extreme sugar content that might have caused osmotic stress to the seeds and is known to delay seed germination (Zhu et al., 2009; Dekkers et al., 2004; Price et al., 2003). Based on this, we suspected that fraction 10 contained some of the potential phytotoxic compounds and hence performed further chemical analysis for their structural characterisation.

3.4. Chemical characterisation of fraction 10

Fraction 10 was further fractionated on reverse-phase preparative UHPLC yielding 66 subfractions. This instrument employs a smaller column operating at a lower flow rate and offering better separation of the compounds for easier characterisation. The resultant subfractions were analysed by UHPLC-MS and NMR. The structures of the compounds were elucidated by analysing their UHPLC-MS (Table 2) and NMR data from both 1D and 2D experiments and comparing them to the literature where necessary/available. Twenty-three (23) compounds were identified (Fig. 5 and Table 2). They belonged to three main compound classes *viz* oxylipins and related analogues, megastigmenes

Table 2
UHPLC-MS data of compounds identified from fraction 10 of the *Capparis spinosa* leaf extract.

Peak No.	RT (min)	Parent ion (*m/z*)	Ion	Δ (ppm)	Formula of ion	MS/MS product ions (*m/z*)	Identity
1	17.32	138.0196	$[M-H]^-$	− 0.61	$C_6H_4NO_3$	92.0265, 108.0215, 138.0194,	5-Formylpyrrole-2-carboxylic acid[a]
2	18.55	146.0600	$[M+H]^+$	− 0.17	C_9H_8NO	91.0543, 118.0653, 146.0603,	Indole-3-carboxaldehyde
3	18.59	162.0549	$[M+H]^+$	0.00	$C_9H_8NO_2$	91.0544, 116.0498, 118.0654, 134.0604, 144.0448, 162.0554	Indole-3-carboxylic acid
4	19.07	225.1485	$[M+H]^+$	− 5.24	$C_{13}H_{21}O_3$	99.0441, 107.0856, 109.0649, 149.0964,167.1070 183.1384	3-Hydroxy-5,6-epoxy-*β*-ionone
5	19.20	197.1172	$[M+H]^+$	− 0.07	$C_{11}H_{17}O_3$	107.0858, 133.1016, 135.1172, 161.0966, 179.1072	Loliolide
6	19.97	190.0509	$[M-H]^-$	6.72	$C_{10}H_8O_3N$	131.0374, 175.0273	6-Methoxyindole-3-carboxylic acid[a]
7	20.36	434.2084	$[M-H]^-$	0.85	$C_{25}H_{28}O_4N_3$	237.0915, 391.2027, 434.2080	Cadabicine
8	20.36	434.2084	$[M-H]^-$	0.85	$C_{25}H_{28}O_4N_3$	237.0915, 391.2027, 434.2080	Capparispine
9	20.54	176.0705	$[M+H]^+$	0.47	$C_{10}H_{10}O_2N$	117.0576, 125.0028, 133.0525, 148.0761	6-Methoxyindole-3-carboxaldehyde
10	22.31	187.0976	$[M-H]^-$	− 0.86	$C_9H_{15}O_4$	97.0658, 125.0971, 187.0977	Azelaic acid
11	24.80	243.1239	$[M-H]^-$	− 0.63	$C_{12}H_{19}O_5$	99.0089, 181.1231, 199.1337, 207.1023, 225.0019, 243.1235	Megastigmene[a]
12	25.52	263.1288	$[M-H]^-$	− 0.31	$C_{15}H_{19}O_4$	153.0918, 163.0762, 201.1282, 204.1152, 219.1388	Unknown
13	25.82	401.1453	$[M-H]^-$	0.02	$C_{18}H_{25}O_{10}$	144.0452, 171.0561	Oxylipin
14	26.33	201.1133	$[M-H]^-$	0.16	$C_{10}H_{17}O_4$	111.0816, 139.1129, 201.1134	Megastigmene[a]
15	26.73	343.2123	$[M-H]^-$	0.00	$C_{18}H_{31}O_6$	171.1024, 229.1442, 343.2123	Oxylipin
16	27.15	738.2026	$[M-H]^-$	− 0.46	$C_{36}H_{36}O_{16}N$	313.0538, 429.0860, 621.1463, 738.2044	8-(1*H*-indol-3-yl-methyl)rutin.
17	28.21	341.1970	$[M-H]^-$	− 0.11	$C_{18}H_{29}O_6$	83.0500, 139.0763, 201.1130, 209.1181, 235.1337	Oxylipin
18	28.21	327.2177	$[M-H]^-$	0.12	$C_{18}H_{31}O_5$	265.1808, 283.1913, 327.2176	Oxylipin
19	29.71	327.2174	$[M-H]^-$	− 0.87	$C_{18}H_{31}O_5$	171.1025, 183.1389, 211.1338, 229.1444, 291.1967, 327.2177	Oxylipin
20	30.76	329.2332	$[M-H]^-$	− 0.46	$C_{18}H_{33}O_5$	171.1024, 211.1336, 229.1442, 329.2331	Oxylipin
21	31.38	287.2225	$[M-H]^-$	− 0.75	$C_{16}H_{31}O_4$	241.2168, 269.2116, 285.2065, 287.2219	Dihydroxyhexadecanoic acid[a]
22	33.13	307.1912	$[M-H]^-$	− 0.89	$C_{18}H_{27}O_4$	97.0656, 121.0656, 185.1179, 211.1336, 235.1336	Corchorifatty acid B
23	34.31	305.1757	$[M-H]^-$	− 0.15	$C_{18}H_{25}O_4$	97.0657, 125.0969, 135.0813, 205.1595, 269.1547, 287.1651	(10*E*,12*E*,14*E*)-9,16-Dioxooctadeca-10,12,14-trienoic acid

[a] Compounds given putative identifications.

and alkaloids (Fig. 6).

The major peaks in the ^{1}H NMR of fraction 10 (Fig. 7) were the distinct angular methyl singlets at δ_H 1.75 (s, 3H), 1.45 (s, 3H) and 1.28 (s, 3H). These singlets matched those in the subfraction of compound **5**. The UHPLC-MS and NMR data of compound **5** was consistent with that of loliolide (Masum et al., 2020; Okada et al., 1994) as reported in the literature. Three additional megastigmene derivatives were isolated, but only 3-hydroxy-5,6-epoxy-*β*-ionone (**4**) could be identified unambiguously when its NMR data was compared with what is reported in the literature (Masum et al., 2020; D'Abrosca et al., 2004). The structures of the other two could not be conclusively identified due to the lack of exploitable ^{1}H NMR data. Besides loliolide, the other standout ^{1}H NMR peaks were the aliphatic methylenes and methyls at δ_H 2.32 (m, nCH_2), 2.07–2.01 (m, nCH_2), 1.63–1.53 (m, nCH_2), 1.36–1.28 (m, nCH_2) and 0.96–0.90 (m, nCH_3). Analysis of both the UHPLC-MS and ^{1}H NMR data of subfractions with matching peaks revealed that these signals belonged to oxylipins. The structures of some of the oxylipins could be solved by 1D and 2D NMR as they were isolated in a reasonable amount to allow NMR analysis. They were identified as corchorifatty acid B (**22**) (Yoshikawa et al., 1998) and (10*E*,12*E*,14*E*)-9,16-dioxooctadeca-10,12,14-trienoic acid (**23**) (Herz and Kulanthaivel, 1984). The structures of six other oxylipins could not be solved but their ^{1}H NMR exhibited clear aliphatic methylene and methyl signals attributable to fragments found in compound **22**. Their MS characteristics indicated either mono- or di-hydration, and in some cases mono- or di-hydrogenation of corchorifatty acid B. The dicarboxylic acid, azelaic acid (**10**) (Brenna et al., 2020) was also identified based on its NMR data.

The other main constituents of the fraction were indole-type and spermidine-type alkaloids. Both classes of alkaloids are known to occur in the *Capparis* genus. The spermidine-type alkaloids included cadabicine (**7**) and capparispine (**8**) and were both identified by comparing their NMR data to what has been reported in the literature. Cadabicine contains two *trans*-configured phenolic acyls and this was evidenced by the two pairs of olefinic signals at δ_H 7.61 (d, J = 15.8 Hz, 1H), 6.60 (d, J = 15.8 Hz, 1H) and 7.31 (d, J = 15.6 Hz, 1H), 5.86 (d, J = 15.6 Hz, 1H) in the NMR spectrum (Ahmad et al., 1985). Capparispine (**8**) on the other hand instead features *trans*-coumaroyl and *cis*-caffeoyl moieties that were shown by the olefinic pairs at 7.67 (d, J = 15.8 Hz, 1H), 6.70 (d, J = 15.7 Hz, 1H) and 6.47 (d, J = 12.8 Hz, 1H), 5.74 (d, J = 12.7 Hz, 1H) respectively (Fu et al., 2008). Capparispine, capparispine 26-*O*-*β*-D-glucoside and cadabicine 26-*O*-*β*-D-glucoside hydrochloride have been reported from the roots of *C. spinosa* (Fu et al., 2008). Similarly, two indole-type alkaloids namely indole-3-carboxaldehyde (**2**) (Que et al., 2022) and indole-3-carboxylic acid (**3**) (Que et al., 2022) were identified by comparing their NMR data to the literature. Additionally, their 6-methoxylated analogues namely 6-methoxyindole-3--carboxylic acid (**6**) and 6-methoxyindole-3-carboxaldehyde (**9**) were identified putatively and from the NMR data (List S1), respectively. Interestingly, however, the data additionally showed the presence of a new compound (**16**) derived from an unprecedented coupling of a flavonoid and an indole backbone.

Compound **16** was colourless upon drying. It was assigned a molecular formula of $C_{36}H_{37}NO_{16}$ based on its $[M-H]^-$ ion at *m/z* 738.2026 (calcd. for $C_{36}H_{36}NO_{16}^-$, *m/z* 738.2040). Its ^{1}H NMR spectrum showed aromatic resonances of an ABX spin system at δ_H 7.72 (d, J = 2.2 Hz, H-2'), 7.33 (dd, J = 8.4, 2.2 Hz, H-6') and 6.74 (d, J = 8.4 Hz, H-5') as well as an ABCD spin system at δ_H 7.65 (dd, J = 8.0, 1.0 Hz, H-4''), 7.06 (ddd, J = 8.1, 7.0, 1.0 Hz, H-6''), 6.94 (ddd, J = 8.0, 7.0, 1.0 Hz, H-5'') and 7.31 (brd, J = 7.7 Hz, H-7'') alongside two singlets at δ_H 6.88 (s, H-2'') and 6.39 (s, H-6). The assignment of the latter singlet to H-6 was based on the distinctive albeit weak ^4J coupling from the singlet, as well as from an AB quartet spin system at 4.24 (d, J = 14.8 Hz, H-10''a) and 4.20 (d, J = 14.8 Hz, H-10''b), to an oxygenated aromatic carbon at δ_C 157.9 in the HMBC spectrum. This chemical shift value recalls that of the

Table 3
^{1}H and ^{13}C NMR spectroscopic data of compound **16** (δ in ppm, J in Hz).

Position	δ_H	δc
1		
2		161.5, C
3		137.3, C
4		181.6, C
4a		107.7, C
5		162.3, C
6	6.39, s	101.8, CH
7		165.3, C
8		110.5, C
8a		157.9, C
1'		125.2, C
2'	7.72, d (2.2)	120.3, CH
3'		147.5, C
4'		151.6, C
5'	6.74, d (8.4)	117.9, CH
6'	7.33, dd (8.4, 2.2)	125.6, CH
1''		
2''	6.88, s	125.6, CH
3''		116.9, C
4''	7.65, dd (8.0, 1.0)	121.8, CH
5''	6.94, ddd (8.0, 7.0, 1.0)	121.5, CH
6''	7.06, ddd (8.1, 7.0, 1.2)	124.3, CH
7''	7.31, br d (7.7)	114.2, CH
8''		140.0, C
9''		130.5, C
10''	4.24, d (14.8)	21.0, CH_2
	4.20, d (14.8)	
1'''	5.04, d (7.7)	106.5, CH
2'''	3.49, dd (9.3, 7.8)	77.9, CH
3'''	3.43, m	79.8, CH
4'''	3.30, m	79.2, CH
5'''	3.30, m	74.0 – 75.0, CH
6'''	3.76, (10.5)	70.6, CH_2
	3.37, m	
1''''	4.50, d (1.7)	104.3, CH
2''''	3.64, dd (3.5, 1.7)	74.0, CH
3''''	3.55, dd (9.6, 3.5)	74.4, CH
4''''	3.30, m	75.8, CH
5''''	3.43, m	71.8, CH
6''''	1.10, d (6.2)	19.8, CH_3

Data collected at 600 MHz for ^{1}H and 150 MHz for ^{13}C NMR in D_2O:CD_3OD (4:1). Spectra were referenced to TSP-d_4 (0.01 % w/v) at δ_H 0.00.

carbon C-8a when positions C-6 and C-8 in flavonoids are occupied by electron-neutral groups like proton or methyl (Agrawal, 1989). In addition, the singlet at δ_H 6.39 (H-6) showed a cross peak to the carbonyl C-4 (δ_C 181.6) which also helps to distinguish between H-6 and H-8 in flavonoids as H-6 is most likely to show a strong HMBC interaction with C-4. The HMBC spectrum also exhibited further cross peaks from H-2', H-5' and H-6' to quaternary carbons at δ_C 161.5 (C-2), 151.6 (C-4'), 147.5 (C-3') and 125.2 (C-1'), and then from the singlet H-6 to carbons at δ_C 165.3 (C-7), 162.3 (C-5) and 107.7 (C-4a), consistent with resonances of quercetin substituted at C-8 as judged by additional cross peaks from H-6 and H-10'' to an upfield carbon at δ_C 21.0 (C-10''). Further HMBC interactions from H-2'' to C-10''; from the ABCD protons H-4'', H-5'' and H-6'' to quaternary carbons at δ_C 140.0 (C-8'') and 130.5 (C-9''); from H-7'' to C-9''; from H-2'' and H-4'' to C-3'' (δ_C 116.9) and from H-2'' to C-10'' allowed the identification of the appended group as a 3-methyleneindole. Ultimately, the data demonstrated the occurrence of a methylene bridged 1*H*-indole-quercetin hybrid in compound **16**, unprecedented in nature. The MS/MS of the parent ion at *m/z* 738.2026 showed a product ion at *m/z* 621.1463 ($C_{28}H_{29}O_{16}^-$) from the loss of *m/z* 117.0572 (C_8H_7N) corresponding to the indole group. Additionally, the presence in the ^{1}H NMR spectrum of two anomeric protons at δ_H 5.04 (d, $J = 7.7$ Hz, H-1''') and 4.50 (d, $J = 1.7$ Hz, H-1'''') together with their associated signals confirmed the presence of a β-glucopyranose and α-rhamnopyranose (Table 3). The sugars were determined to be attached to the 3-position of the aglycone based on the HMBC interaction from H-1''' to C-3. The sugar sequence was defined based on the HMBC interaction from H-6''' protons at δ_H 3.76 (d, $J = 10.5$ Hz, H-6a''') and 3.37 (m, H-6b''') to C-1'''' (δ_C 104.3), supporting the identity of the flavonoid backbone in **16** as rutin. Thus, compound **16** was newly characterized as 8-(1*H*-indol-3-yl-methyl) rutin.

Indeed, the indole moiety in compound **16** is believed to arise from the breakdown of glucobrassicin (Fig. 8), one of the glucosinolates known to occur in *C. spinosa* (Matthäus and Özcan, 2002) and also detected in our bulk extract (data not shown). As proposed in the literature, the degradation of glucobrassicin starts with an enzymatic hydrolysis catalyzed by myrosinase (Bones and Rossiter, 2006; Wagner and Rimbach, 2009). At near neutral pH values, skatylisothiocyanate is initially formed upon the hydrolysis, which can lose a thiocyanate ion to form a 3-methylene-*3H*-indolium cation. This cation then reacts with

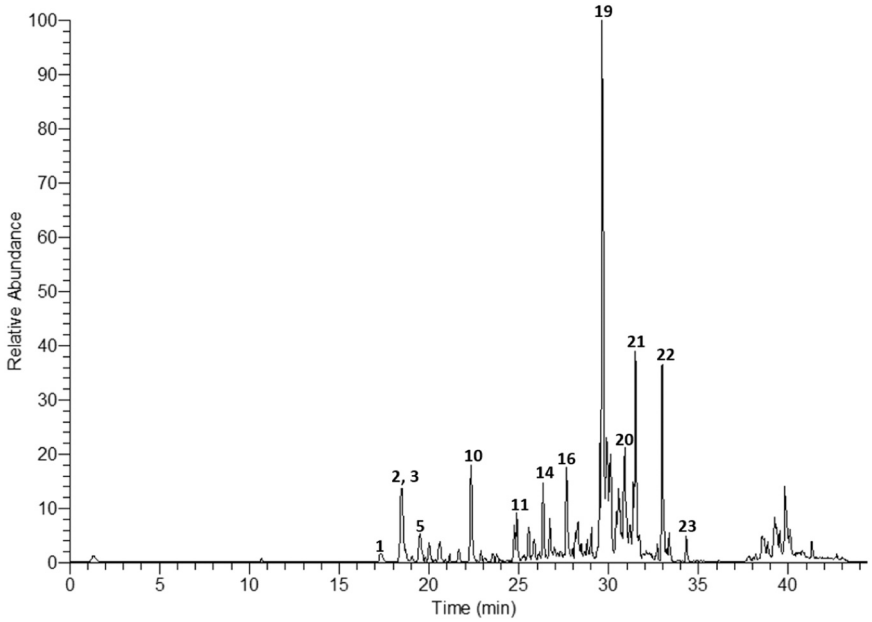

Fig. 5. Total ion chromatogram (negative ion mode) of fraction 10 from the *Capparis spinosa* leaf extract.

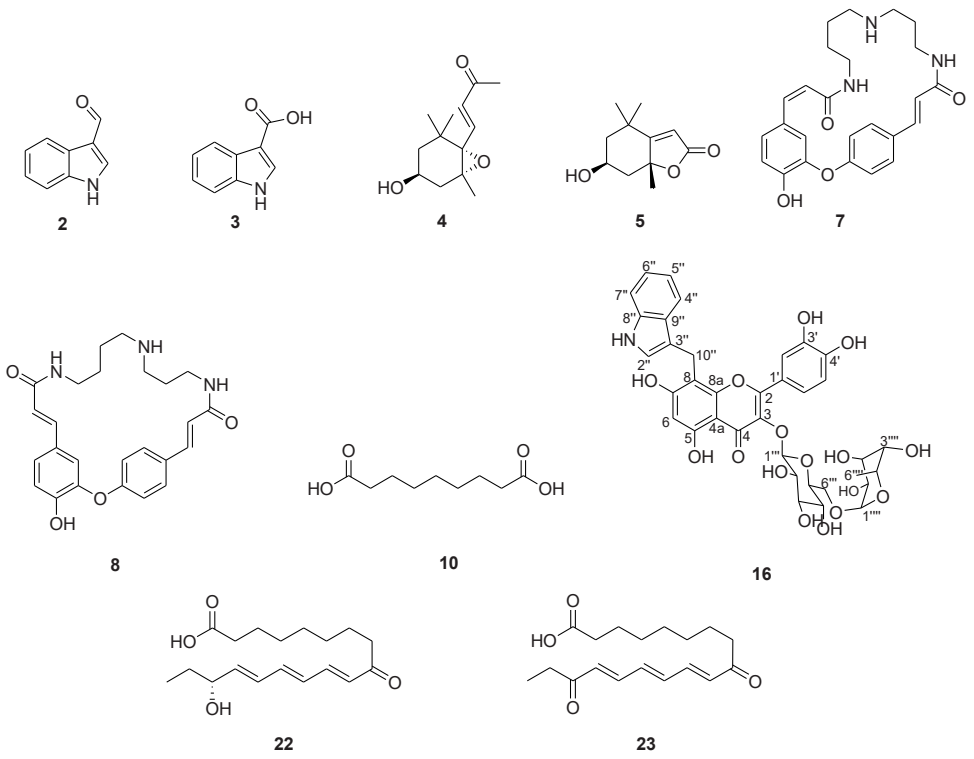

Fig. 6. Structures of compounds isolated from fraction 10 of *Capparis spinosa* leaf extract and validated by NMR.

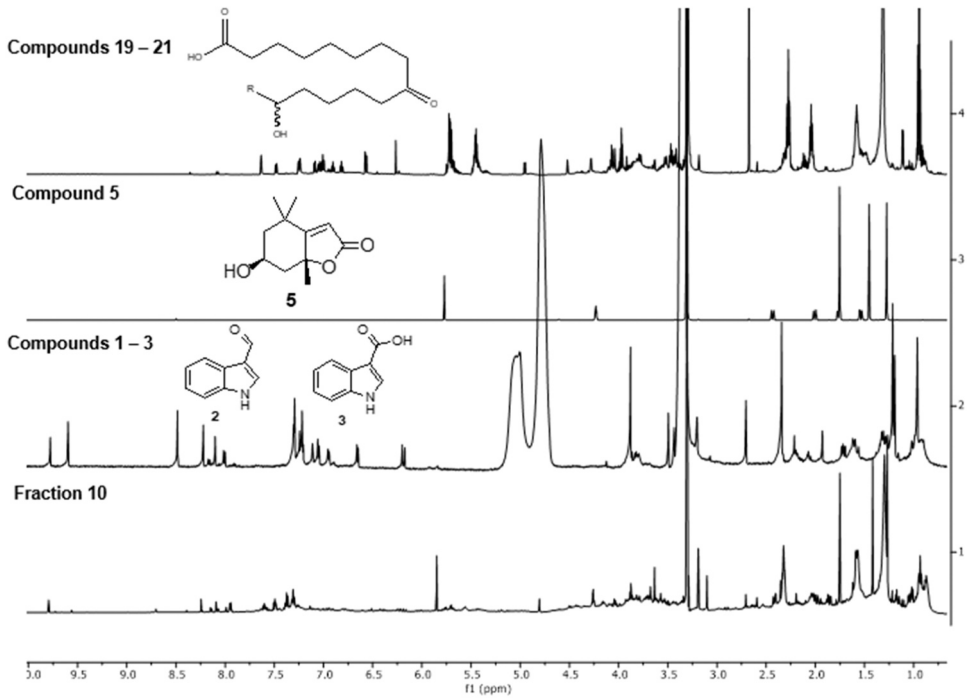

Fig. 7. Stacked ^{1}H NMR spectra highlighting major constituents in the active fraction 10. Spectrum of compounds **1**–**3** includes traces from oxylipins in the upfield region and the spectrum of compounds **19**–**21** contains traces of compound **16.**

rutin to form compound **16**. Under acidic pH, the process could occur *via* a 3-indolylacetonitrile intermediate. The same type of condensation has been observed in the building up of ascorbigen, in *Brassica* vegetables (Wagner and Rimbach, 2009). As the decay of glucobrassicin in Brassicaceae is induced by plant tissue disruption during processing and culinary procedures, and influenced by the hydrolysis conditions, including temperature, pH, metal ion solutions and some proteins (Bones and Rossiter, 2006), one could question the natural occurrence of the new indole-flavonoid skeleton. It is most likely that as much as compound **16** is a natural compound, it does not have a genetically programmed biosynthesis and its formation is somewhat artefactual.

The results showed that loliolide (**5**) was the major compound in the

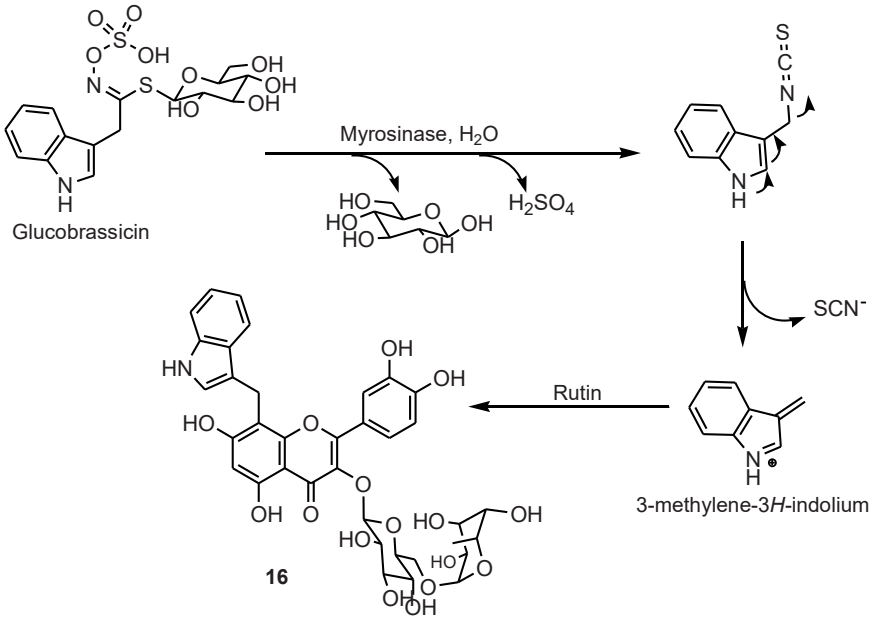

Fig. 8. Plausible mechanism for the formation of compound **16** through hydrolysis of glucobrassicin mediated by myrosinase.

most active fraction followed by the oxylipins taken together and the indole alkaloid derivatives. Loliolide is arguably the most ubiquitous lactone and has been isolated and identified as one of the major phytotoxic components in several allelopathic plant extracts (Khatun et al., 2023). Loliolide and 3-hydroxy-5,6-epoxy-β-ionone (**4**) isolated from the straw of the Bangladeshi indigenous rice variety 'Goria' inhibited the *in vitro* growth of *Echinochloa oryzicola* and *Lepidium sativum* L. seedlings with 3-hydroxy-5,6-epoxy-β-ionone showing stronger inhibition compared with loliolide when tested separately. However, a stronger synergistic inhibition was observed when applied as a mixture (Masum et al., 2020). The two compounds isolated from the algae *Spirulina platensis* similarly significantly inhibited the seed germination, shoot growth and root growth of Chinese amaranth seeds in an *in vitro* assay with stronger inhibition from 3-hydroxy-5,6-epoxy-β-ionone (Charoenying et al., 2022). Oxylipins are derivatives of unsaturated fatty acids and are important signalling molecules that contribute to growth, development, and defence in plants (Savchenko et al., 2014). Studies that have assessed the phytotoxicity of plant-derived oxylipins are very limited. However, several oxylipins isolated from *Cestrum parqui* were shown to inhibit the germination as well as root and shoot growth of *Lactuca sativa* with structure-dependent phytotoxicity (Fiorentino et al., 2008). Alkaloids also play a defensive role in plants and their phytotoxicity is well known. They have been shown to inhibit the growth of several model weed species and other plants (Lei et al., 2021; Macías et al., 2019; Nakano et al., 2004). Therefore, it can be concluded that these same compounds were partly responsible for the observed phytotoxicity of the *C. spinosa* leaf extract on wheat. Due to the low yield of the isolated compounds, individual compounds could not be tested in further analysis.

Interestingly, in the study by Ladhari et al., (2013), the flavonoids quercetin-3-O-β-D-glucopyranoside, quercetin and kaempferol-3-O-β-D-glucopyranoside were implicated in the phytotoxicity of aqueous *C. spinosa* leaf extracts on the germination of lettuce, radish, peganum and thistle seeds (Ladhari et al., 2013). This may seem to suggest that compound **16** whose structure includes a flavonoid glycoside substructure may be partly responsible for the observed phytotoxicity. However, further assays with the pure compound are needed to confirm this speculation.

4. Conclusion

In this study, we sought to find extracts with biostimulant effects that could improve the emergence and growth of wheat under salinity stress. *M. vulgare* leaf and *O. compactum* extracts improved the root length, whereas none of the other extracts showed any significant positive effects on the growth of wheat. However, extracts of *P. harmala* and *C. spinosa* showed significant phytotoxicity towards the growth of wheat, with the latter being more potent, a result that has previously been shown in other target species. The phytotoxicity observed for *C. spinosa* extracts on wheat could be exploited by testing the effect of the extracts on grasses and weeds. If they confer the same phytotoxicity, this could prove their potential for use as a bioherbicide and could hence act as a much-needed ecologically friendly alternative to conventional synthetic herbicides. However, studies would be needed to test the extracts alongside known conventional selective herbicides to compare their efficacy and selectivity, as well as assessment using other application methods like foliar spraying or even incorporating the plant biomass in the soil. Chemical characterisation of the most active fraction of the leaf extract of *C. spinosa* has led to the identification of 8-(1*H*-indol-3-yl-methyl)rutin, a previously undescribed compound with a novel backbone. Further work is needed to scale up the subfractionation of fraction 10 so that its components can be tested for emergence inhibition to attempt to further elucidate the active compounds that could be responsible for the phytotoxicity of the extract. Additionally, further analysis is necessary to elucidate the mechanisms underlying the phytotoxic effects observed. At this stage, it can be hypothesised that the observed activity can probably be attributed to the identified main compounds, including loliolide, oxylipins and alkaloids, including 8-(1*H*-indol-3-yl-methyl)rutin. The findings of this study open new avenues for understanding the role of secondary metabolites in plant responses to salt stress.

CRediT authorship contribution statement

Ward Jane Louise: Writing – review & editing, Supervision, Project administration, Conceptualization. **Beale Michael Henry:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **KISIRIKO Musa:** Writing – original draft, Visualization, Investigation, Formal analysis. **Mansour Sobeh:** Writing – review & editing. **Terry**

Leon Alexander: Writing – review & editing, Supervision. **Maria Anastasiadi:** Writing – review & editing, Supervision, Formal analysis. **Claudia Harflett:** Investigation. **Bitchagno Gabin Thierry Mbahbou:** Writing – review & editing, Visualization, Investigation, Formal analysis. **Imane Naboulsi:** Investigation. **Clarice Noleto-Dias:** Writing – review & editing, Supervision, Formal analysis.

Funding

This research paper is part of the PhD project "Agricultural Bioproducts from Medicinal and Aromatic Plants" and sits within the FP05 project (Bioproducts for African Agriculture), which is funded by OCP Morocco. FP05 is a collaboration between Mohammed VI Polytechnic University, Rothamsted Research and Cranfield University.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Musa Kisiriko reports financial support was provided by OCP Group. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to acknowledge Charlotte Lomax and Gianluca Ruvo for their assistance in the collection of UHPLC-MS and NMR data, respectively.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2025.121355.

Data availability

Data will be made available on request.

References

Achli, S., Ongoma, V., Epule, T.E., Dhiba, D., Salih, W., Ousayd, L., Chehbouni, A., 2025. Exploring the key drivers of crop yields in Morocco – a systematic review. Front. Agron. 7, 1515938. https://doi.org/10.3389/fagro.2025.1515938.

Agrawal, P.K. (Ed.), 1989. Carbon-13 NMR of Flavonoids. Elsevier Science Publishers B. V.

Ahmad, V.U., Amber, A.-u-R., Arif, S., Chen, M.H.M., Clardy, J., 1985. Cadabicine, an alkaloid from *Cadaba farinosa*. Phytochemistry 24 (11), 2709–2711. https://doi.org/10.1016/S0031-9422(00)80700-1.

Ahmed, T., Abou Elezz, A., Khalid, M.F., 2021. Hydropriming with Moringa leaf extract mitigates salt stress in wheat seedlings. Agriculture 11, 1254. https://doi.org/10.3390/agriculture11121254.

Ali, M., Hayat, S., Ahmad, H., Ghani, M.I., Amin, B., Atif, M.J., Cheng, Z., 2019. Priming of *Solanum melongena* L. seeds enhances germination, alters antioxidant enzymes, modulates ROS, and improves early seedling growth: indicating aqueous garlic extract as seed-priming bio-stimulant for eggplant production. Appl. Sci. 9, 2203. https://doi.org/10.3390/app9112203.

Atta, K., Mondal, S., Gorai, S., Singh, A.P., Kumari, A., Ghosh, T., Roy, A., Hembram, S., Gaikwad, D.J., Mondal, S., Bhattacharya, S., Jha, U.C., Jespersen, D., 2023. Impacts of salinity stress on crop plants: improving salt tolerance through genetic and molecular dissection. Front. Plant Sci. 14, 1241736. https://doi.org/10.3389/fpls.2023.1241736.

Bashar, H.M.K., Juraimi, A.S., Ahmad-Hamdani, M.S., Uddin, M.K., Asib, N., Anwar, M. P., Rahaman, F., Haque, M.A., Hossain, A., 2023. Evaluation of allelopathic effects of *Parthenium hysterophorus* L. methanolic extracts on some selected plants and weeds. PLoS One 18 (1), e0280159. https://doi.org/10.1371/journal.pone.0280159.

Bones, A.M., Rossiter, J.T., 2006. The enzymic and chemically induced decomposition of glucosinolates. Phytochemistry 67 (11), 1053–1067. https://doi.org/10.1016/j.phytochem.2006.02.024.

Brenna, E., Colombo, D., Di Lecce, G., Gatti, F.G., Ghezzi, M.C., Tentori, F., Tessaro, D., Viola, M., 2020. Conversion of oleic acid into azelaic and pelargonic acid by a chemo-enzymatic route. Molecules 25, 1882. https://doi.org/10.3390/molecules25081882.

Carvalho, F.P., 2017. Pesticides, environment, and food safety. Food Energy Secur. 6 (2), 48–60. https://doi.org/10.1002/fes3.108.

Charoenying, P., Laosinwattana, C., Chotsaeng, N., 2022. The allelopathic activity of extracts and isolated from *Spirulina platensis*. Molecules 27, 3852. https://doi.org/10.3390/molecules27123852.

Chen, H., Wang, L., Guo, S., Li, M., Tian, Z., Han, B., Tang, X., Liu, B., 2023. Effects of light intensity on seedling emergence and early growth of *Liquidambar formosana* hance. Forests 14, 867. https://doi.org/10.3390/f14050867.

Corol, D.I., Harflett, C., Beale, M.H., Ward, J.L., 2014. An efficient high throughput metabotyping platform for screening of biomass willows. Metabolites 4, 946–976. https://doi.org/10.3390/metabo4040946.

D'Abrosca, B., DellaGreca, M., Fiorentino, A., Monaco, P., Oriano, P., Temussi, F., 2004. Structure elucidation and phytotoxicity of C13 nor-isoprenoids from *Cestrum parqui*. Phytochemistry 65 (4), 497–505. https://doi.org/10.1016/j.phytochem.2003.11.018.

Dallali, S., Rouz, S., Aichi, H., Ben Hassine, H., 2017. Phenolic content and allelopathic potential of leaves and rhizosphere soil aqueous extracts of white horehound (*Marrubium vulgare* L.). J. New Sci. Agric. Biotechnol. 39, 2106–2120.

Dekkers, B.J.W., Schuurmans, J.A.M.J., Smeekens, S.C.M., 2004. Glucose delays seed germination in *Arabidopsis thaliana*. Planta 218 (4), 579–588. https://doi.org/10.1007/s00425-003-1154-9.

El-Bakkosh, A.M., Shaieb, F.M., Idrs, A.M., 2020. Phytotoxicity and antifungal activity of *Capparis spinosa* L. EPH Int. J. Appl. Sci. 6 (1). https://doi.org/10.53555/eijas.v6i1.103.

Fiorentino, A., D'Abrosca, B., DellaGreca, M., Izzo, A., Natale, A., Pascarella, M.T., Pacifico, S., Zarrelli, A., Monaco, P., 2008. Chemical characterization of new oxylipins from *Cestrum parqui*, and their effects on seed germination and early seedling growth. Chem. Biodivers. 5 (9), 1780–1791. https://doi.org/10.1002/cbdv.200890167.

Fu, X.P., Wu, T., Abdurahim, M., Su, Z., Hou, X.L., Aisa, H.A., Wu, H., 2008. New spermidine alkaloids from *Capparis spinosa* roots. Phytochem. Lett. 1 (1), 59–62. https://doi.org/10.1016/j.phytol.2008.01.001.

Herz, W., Kulanthaivel, P., 1984. Ent-pimaranes, ent-kauranes, heliangolides and other constituents of three *Helianthus* species. Phytochemistry 23 (7), 1453–1459. https://doi.org/10.1016/S0031-9422(00)80485-9.

Khatun, M.R., Tojo, S., Teruya, T., Kato-Noguchi, H., 2023. The allelopathic effects of *Trewia nudiflora* leaf extracts and its identified substances. Plants 12, 1375. https://doi.org/10.3390/plants12061375.

Kisiriko, M., Anastasiadi, M., Terry, L.A., Yasri, A., Beale, M.H., Ward, J.L., 2021. Phenolics from medicinal and aromatic plants: characterisation and potential as biostimulants and bioprotectants. Molecules 26, 6343. https://doi.org/10.3390/molecules26216343.

Ladhari, A., Omezzine, F., Dellagreca, M., Zarrelli, A., Haouala, R., 2013. Phytotoxic activity of *Capparis spinosa* L. and its discovered active compounds. Allelopath. J. 32 (2), 175–190.

Lei, L., Zhao, Y., Shi, K., Liu, Y., Hu, Y., Shao, H., 2021. Phytotoxic activity of alkaloids in the desert plant *Sophora alopecuroides*. Toxins 13, 706. https://doi.org/10.3390/toxins13100706.

Liu, C., Jiang, X., Yuan, Z., 2024. Plant responses and adaptations to salt stress: a review. Horticulturae 10 (11), 1221. https://doi.org/10.3390/horticulturae10111221.

Lozano-Isla, F., Benites-Alfaro, O.E., Pompelli, M.F., 2019. GerminaR: an R package for germination analysis with the interactive web application "GerminaQuant for R". Ecol. Res. 34 (2), 339–346. https://doi.org/10.1111/1440-1703.1275.

Macías, F.A., Mejías, F.J.R., Molinillo, J.M.G., 2019. Recent advances in allelopathy for weed control: from knowledge to applications. Pest Manag. Sci. 75 (9), 2413–2436. https://doi.org/10.1002/ps.5355.

Manhou, K., Moussadek, R., Yachou, H., Zouahri, A., Douaik, A., Hilal, I., Ghanimi, A., Hmouni, D., Dakak, H., 2024. Assessing the impact of saline irrigation water on durum wheat (cv. Faraj) grown on sandy and clay soils. Agronomy 14 (12), 2865. https://doi.org/10.3390/agronomy14122865.

Masum, S.M., Akamine, H., Hossain, M.A., Sakagami, J.I., Ishii, T., Gima, S., 2020. Assessment of the allelopathic potential and identification of the phytotoxic substances from the straw of Bangladeshi indigenous rice variety 'Goria. Appl. Ecol. Environ. Res. 18 (4), 5547–5560. ⟨https://aloki.hu/pdf/1804_55475560.pdf⟩.

Matthäus, B., Özcan, M., 2002. Glucosinolate composition of young shoots and flower buds of capers (*Capparis* species) growing wild in Turkey. J. Agric. Food Chem. 50 (25), 7323–7325. https://doi.org/10.1021/jf020530+.

Mehta, A., Chowdhury, P., 2021. Exogenous application of *Chenopodium album* aqueous extracts on salt-stressed wheat seedling. Biosci. Biotechnol. Res. Commun. 14 (3). https://doi.org/10.21786/bbrc/14.3.44.

Mutlu-Durak, H., Yildiz Kutman, B., 2021. Seed treatment with biostimulants extracted from weeping willow (*Salix babylonica*) enhances early maize growth. Plants 10, 1449. https://doi.org/10.3390/plants10071449.

Nakano, H., Nakajima, E., Hiradate, S., Fujii, Y., Yamada, K., Shigemori, H., Hasegawa, K., 2004. Growth inhibitory alkaloids from mesquite (*Prosopis juliflora* (Sw.) DC.) leaves. Phytochemistry 65 (5), 587–591. https://doi.org/10.1016/j.phytochem.2004.01.006.

Noleto-Dias, C., Wu, Y., Bellisai, A., Macalpine, W., Beale, M.H., Ward, J.L., 2019. Phenylalkanoid glycosides (non-salicinoids) from wood chips of *Salix triandra* × *dasyclados* hybrid willow. Molecules 24, 1152. https://doi.org/10.3390/molecules24061152.

Noleto-Dias, C., Harflett, C., Beale, M.H., Ward, J.L., 2020. Sulfated flavanones and dihydroflavonols from willow. Phytochem Lett. 35, 88–93. https://doi.org/10.1016/j.phytol.2019.11.008.

Okada, N., Shirata, K., Niwano, M., Koshino, H., Uramoto, M., 1994. Immunosuppressive activity of a monoterpene from *Eucommia ulmoides*. Phytochemistry 37 (1), 281–282. https://doi.org/10.1016/0031-9422(94)85042-9.

Price, J., Li, T.-C., Kang, S.G., Na, J.K., Jang, J.-C., 2003. Mechanisms of glucose signaling during germination of *Arabidopsis*. Plant Physiol. 132 (3), 1424–1438. https://doi.org/10.1104/pp.103.020347.

Qasem, J.R., 2002. Allelopathic effects of selected medicinal plants on *Amaranthus retroflexus* and *Chenopodium murale*. Allelopath. J. 10 (2), 105–122.

Que, Y., Huang, D., Gong, S., Zhang, X., Yuan, B., Xue, M., Shi, W., Zeng, F., Liu, M., Chen, T., Yu, D., Yan, X., Wang, Z., Yang, L., Xiang, L., 2022. Indole-3-carboxylic acid from the endophytic fungus *Lasiodiplodia pseudotheobromae* LPS-1 as a synergist enhancing the antagonism of jasmonic acid against *Blumeria graminis* on wheat. Front. Cell. Infect. Microbiol 12. https://doi.org/10.3389/fcimb.2022.898500.

Rady, M.M., Desoky, E.S.M., Elrys, A.S., Boghdady, M.S., 2019. Can licorice root extract be used as an effective natural biostimulant for salt-stressed common bean plants? S. Afr. J. Bot. 121, 294–305. https://doi.org/10.1016/j.sajb.2018.11.019.

Rehman, H. u, Iqbal, H., Basra, S.M.A., Afzal, I., Farooq, M., Wakeel, A., Wang, N., 2015. Seed priming improves early seedling vigor, growth and productivity of spring maize. J. Integr. Agric. 14 (9), 1745–1754. https://doi.org/10.1016/S2095-3119(14)61000-5.

Savchenko, T.V., Zastrijnaja, O.M., Klimov, V.V., 2014. Oxylipins and plant abiotic stress resistance. Biochemistry 79 (4), 362–375. https://doi.org/10.1134/S0006297914040051.

Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M., Zheng, B., 2019. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. Molecules 24 (13), 2452. https://doi.org/10.3390/molecules24132452.

Sodaeizade, H., Hakimi Maybodi, M.H., 2010. Allelopatic effects of *Capparis spinosa*, *Herttia angustifolia* and *Peganum harmala* on germination and seedling growth of wheat and alfalfa. J. Agric. Sci. Sustain. Prod. 20 (1), 181–189.

Sodaeizadeh, H., Rafieiolhossaini, M., Havlik, J., Van Damme, P., 2009. Allelopathic activity of different plant parts of *Peganum harmala* L. and identification of their growth inhibitor substances. Plant Growth Regul. 59 (3), 227–236. https://doi.org/10.1007/s10725-009-9408-6.

Wagner, A.E., Rimbach, G., 2009. Ascorbigen: chemistry, occurrence, and biologic properties. Clin. Dermatol. 27 (2), 217–224. https://doi.org/10.1016/j.clindermatol.2008.01.012.

Yakhin, O.I., Lubyanov, A.A., Yakhin, I.A., Brown, P.H., 2017. Biostimulants in plant science: a global perspective. Front. Plant Sci. 7, 2049. https://doi.org/10.3389/fpls.2016.02049.

Yoshikawa, M., Murakami, T., Shimada, H., Yoshizumi, S., Saka, M., Yamahara, J., Matsuda, H., 1998. Medicinal Foodstuffs. XIV. On the bioactive constituents of Moroheiya. (2): new fatty acids, corchorifatty acids A, B, C, D, E, and F, from the leaves of *Corchorus olitorius* L. (Tiliaceae): structures and inhibitory effect on NO production in mouse peritoneal macrophages. Chem. Pharm. Bull. 46 (6), 1008–1014. https://doi.org/10.1248/cpb.46.1008.

Zhu, G., Ye, N., Zhang, J., 2009. Glucose-induced delay of seed germination in rice is mediated by the suppression of ABA catabolism rather than an enhancement of ABA biosynthesis. Plant Cell Physiol. 50 (3), 644–651. https://doi.org/10.1093/pcp/pcp022.