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PHYTOCHEMICAL COMPOSITION, CYTOTOXICITY AND PHYTOTOXICITY OF *EUPHORBIA DRACUNCULOIDES* LAM. FRUIT EXTRACT

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Abstract

Weed plants are a significant threat to agricultural growth and productivity, causing economic losses due to synthetic herbicides, environmental pollution, and harm to non-target organisms. To address this issue, the study focused on analyzing the phytochemical profile, cytotoxic properties, and effects of *Euphorbia dracunculoides* fruit methanolic extract on seed germination, and growth of radish, and canola. Major phytochemicals, including alkaloids, flavonoids, phenols, tannins, steroids, and catechin, were identified. The extract was evaluated using brine shrimp at concentrations ranging from 1.25 to 640 µg dry weight equivalent (DWE) /mL to determine cytotoxicity, comparing its LD50 value with the standard potassium dichromate. For phytotoxicity, concentrations of 2.5, 5, and 10 mg DWE/mL were used to assess their impact on seed germination and seedling growth in radish and canola. The concentrations causing 50% inhibition (IC50) for seed germination and seedling growth were determined. Results showed that the extract exhibited high sensitivity to both plant species, with brine shrimp displaying the highest mortality rate at 640 µg DWE/mL. It significantly inhibited seed germination and seedling growth in radish and canola, with recorded IC50 values for both species. The extract caused 50% growth inhibition of shoot length at concentrations of 5.16 and 4.75 mg DWE/mL and 50% growth inhibition of root length at concentrations of 4.39 and 5.50 mg DWE/mL, respectively. The methanolic extract of E. *dracunculoides* fruit demonstrates potential as a sustainable and cost-effective herbicidal agent, potentially reducing reliance on synthetic herbicides and mitigating herbicide-resistant weed species. However, further research is required to understand its mechanisms of cytotoxicity and phytotoxicity, assess its safety and effectiveness, and explore its application in integrated weed management strategies.

Key words: Allelochemicals, Bio herbicides, Radish, Canola, Weeds, *Artemia salina*.

Introduction

The threat of weeds to crop productivity is a major concern, with an estimated 1800 weed species causing a substantial 31.5% reduction in plant production, resulting in annual losses of 32 billion USD (Kubiak *et al.*, 2022). Weeds possess remarkable resilience, efficiently utilizing natural resources like soil moisture, nutrients, light, air, and space, surpassing cultivated crops in this aspect (Shekhawat *et al.*, 2020). Their rapid growth and extensive root systems make them formidable adversaries. Controlling weeds typically involves the use of synthetic herbicides, but this has led to the emergence of herbicide-resistant weed species, alongside environmental pollution and harm to non-target organisms (Kim *et al.*, 2017). Globally, over 500 cases of herbicide-resistant weeds have been reported (Heap, 1999). The scarcity of new synthetic herbicides targeting novel sites in the last two decades emphasizes the urgent need to explore natural compounds with fresh herbicidal potential (Dayan *et al.*, 2012). Addressing weed-related challenges involves exploring the phytotoxic potential of natural compounds. The diverse array of phytochemicals in plants presents a promising avenue for sustainable and cost-effective botanical herbicides, aiming to circumvent the development of herbicide-resistant weed species (Hossen *et al.*, 2023, Khan *et al.*, 2023, Kostina-Bednarz *et al.*, 2023). Various phytotoxic chemicals naturally produced by plants hinder the germination, growth, and development of other plants, including tannins, phenolic acids, coumarins, and terpenoids (Qasim *et al.*, 2019, Raclavská *et al.*, 2021, Khan *et al.*, 2023). Leveraging this diversity offers a promising path for botanical herbicides that are sustainable, cost-effective, and do not contribute to herbicide-resistant weed species (Hossen *et al.*, 2023, Khan *et al.*, 2023, Kostina-Bednarz *et al.*, 2023). A common method to assess phytotoxicity involves radish seed bioassays, observing the impact of compounds or plant extracts on germination and seedling growth. Radish was selected for phytotoxicity assay because of its fast germination and seedling establishment, and its sensitivity to toxic materials (Chung *et al*., 2017, Modlitbová *et al*., 2019). For instance, (Khan *et al.*, 2023) found significant inhibitory effects of organic extracts from *Chenopodium glaucum* on radish seed germination and growth at a concentration of 10 mg/mL. Cytotoxicity, crucial in this research, identifies compounds that inhibit metabolic processes or cause organismal harm. The brine shrimp lethality assay serves as a cost-effective preliminary screening method for cytotoxic compounds (Baravalia *et al.*, 2012, Kamanja *et al.*, 2018). *Euphorbia dracunculoides*, a prevalent weed in rain-fed regions, affects crops like chickpeas and wheat (Tanveer *et al.*, 2012). Extracts from this species exhibit medicinal

properties and allelopathic effects on chickpeas (Tanveer *et al.*, 2012, Khattak *et al.*, 2017). Rich in bioactive compounds like phenols, tannins, anthraquinones, alkaloids, saponins, and flavonoids, *E. dracunculoides* holds promise for various applications (Majid *et al.*, 2015). This research hypothesizes that *Euphorbia dracunculoides* fruit methanolic extract possesses cytotoxic and phytotoxic potential. Objectives include assessing cytotoxicity using the brine shrimp assay and investigating phytotoxic effects on radish and canola germination and growth. Despite its potential significance, systematic studies exploring the cytotoxicity and herbicidal capabilities of *E. dracunculoides* fruit extracts are notably lacking. This research aims to fill this gap by evaluating the cytotoxic potential against brine shrimp and assessing phytotoxic effects on radish and canola.

Material and Methods

The semi-mature fruits of *E. dracunculoides* (Fig. 1) were collected from 100 healthy plants growing in the wild in Tehsil Takht-e-Nasrati (32.47° to 33.28° North and 70.30° to 71.30° East), District Karak Pakistan**.** All the fruits were washed with tap water and then kept in the shade for drying at room temperature. When all the fruits were dried, they were hand-broken into small pieces and ground into fine powder by using a grinding machine. After sieving through a sieve plate (5 mm pore size), the fine powder of 50 g was then extracted in 500 mL analytical grade methanol for 72 h (Sigma Aldrich Co Ltd.) at 35°C with continuous shaking in an incubator shaker. The fruit extract was filtered by using a Whatman No. 1 filter paper. The methanol present in the solution was evaporated at 40°C until the formation of a thick gummy extract.

Qualitative determination of phytochemicals: The extract was evaluated for the presence of phytochemicals like tannins, flavonoids, steroids, phenols, alkaloids, Cardiac Glycosides, Catechin, and Volatile oils (Sofowora, 1993, Harborne, 1973, Trease & Evans, 1983, Raad *et al*., 2020.

Tannins: The extract (0.5 g) was mixed with bromine water (10 mL). Presence of tannins was confirmed after discoloration of the bromine water.

Flavonoids: Flavonoids were detected by using Shinoda test. The extract was shaken with Mg ribbon and concentrated HCl. After a few minutes the appearance of pink color indicated the presence of alkaloids.

Steroids: The extract was mixed with 2ml of chloroform and concentrated sulfuric acid. The mixture was separated into two layers, a methanol layer and a lower chloroform layer. Red color was appeared in the lower chloroform layer, which indicated the presence of steroids**.**

Phenol: Drop by drop, 5% Glacial acetic acid was added to the extract (1 µL). After that, the mixture was treated with 5% $NaNO_2$. The formation of muddy brown color confirmed the presence of phenols.

Fig. 1. *E. dracunculoides*.

Alkaloids: Hager's test: Hager's test was used for the detection of alkaloids. The extract solution (2 mL) was added to a small amount of Hager's reagent. After mixing, a yellow color was appeared, which confirmed the presence of alkaloids. Mayer's test: A 2 mL of the extract was mixed with HCl (2 mL) and later several drops of Mayer's reagent were added to it. White precipitate of green color appearance after reaction is indicator of alkaloids in extract.

Cardiac glycosides (Keller-Kiliani test): The extract solution 2 mL was mixed with glacial acetic acid (1 mL) having several drops of $FeCl_3$. The mixture was then added with few drops of concentrated sulphuric acid which resulted in the production of blue green color. This appearance of blue green color indicated the presence of cardiac glycosides.

Catechin: The match stick was dipped in plant extract, dried, and then moistened with concentrated hydrochloric acid. When heated near a flame, the color of wood is changed into red or pink due to the presence of catechin.

Volatile oils: A little amount of diluted hydrochloric acid and sodium hydroxide were also added along with 2 mL of oil. The appearance of white precipitate showed the presence of volatile oils).

Cytotoxicity assay: Cytotoxicity of the extract was evaluated using the Brine Shrimp Lethality Test (Duke, 2002). A stock solution of the extract in DMSO at a concentration of 10 mg DWE/mL was prepared. For control, 10 mg/mL of potassium dichromate was prepared in distilled water. The stock solution was diluted with DMSO to obtain a range of concentrations, including 1.25, 2.5, 5, 10, 20, 40, 80, 160, 320 and 640 µg/mL DWE /mL. To obtain the same concentration range, distilled water was used to dilute the potassium dichromate stock solution (positive control). A reference negative control solution of DMSO was also prepared in distilled water (Meyer *et al.*, 1982). The culture dishes and flasks were cleaned and sterilized in order to get the test subjects ready for the assay. The brine shrimp eggs were transferred into 500 mL of sterile artificial seawater in a conical flask. The eggs in the flask were incubated for 48 hours at 25°C and 120 rpm in a laminar flow hood for hatching (McLaughlin *et al.*, 1998). For each concentration of the test chemical and the positive control, the Brine Shrimp Lethality Assay was conducted in triplicate. A 96-well plate with 100 µL of test and control samples was then filled with a known number of nauplii, followed by incubation under standard growth conditions for 24 hrs (McLaughlin *et al.*, 1998). By using a microplate reader, the number of surviving nauplii in each well was counted. The following formula was then used to calculate the percentage of survival in each well.

$$\text{Mortality (\%)} = \left(\frac{\text{Number of surviving nauplii}}{\text{Total number of nauplii}}\right) \times 100$$

Both the extract and the reference potassium dichromate LD50 value were determined using a probit analysis tool. The log concentration of the test drug and the positive control were plotted against the percentage mortality. The toxicity of the drug was established by comparing the LD50 value of the test compound to that of the positive control.

Phytotoxicity assay: Phytotoxicity of *E. dracunculoides* fruit extract was performed by using radish (variety Desi White) and canola (variety Rainbow) seed germination assay under *in vitro* conditions (Turker & Camper, 2002, Butler, 2004). The various concentrations of the fruit extract, i.e., 1, 5, and 10 mg DWE/mL, were prepared in methanol. Glass Petri dishes arranged with Whatman No. 1 filter papers (two filter papers per Petri dish) were autoclaved by using an autoclave machine (WiseClave Model WAC 60, Korea). Seeds of radish and canola were purchased from a local market in District Bannu and then sterilized by washing first with 10% Clorox solution and then with 95% ethanol. The filter papers in Petri dishes were moistened with 0.5 mL methanolic solutions of fruit extract separately. Later on, methanol was evaporated in an open-air oven. The 10 10 seeds of radish and canola were placed on filter papers in each Petri dish and moistened with 10 ml sterilized distilled water. For control, the filter papers were applied with only distilled water. The experiment had three replicas for each treatment. All the Petri dishes were kept in the dark at a room temperature of 25±1°C. After noting the seed germination data, all the Petri dishes were shifted to a growth chamber, and their covers were removed. The internal environmental conditions of the growth chamber were 16 hours of illumination, 8 hours of dark per 24-hour cycle, and 25°C temperature.

After 24 hours, seed germination was started. Every seed having a 5 mm radial length was considered a germinated one. The seed germination data was recorded for five days unstill the last seed in control was grown.

$$\text{Seed germination (\%)} = \left(\frac{\text{Number of seeds germinated}}{\text{Total number of seeds grown}}\right) \times 100$$

After growth for 14 days' seedlings in each treatment group were weighed using a digital balance (UniBloc Model TX323L Shimadzu, Japan). Then, the seedlings were dried to constant weight in an oven (WiseVen DAIHAN Scientific Model 050, Korea) at 72°C for three days, and their dry weight was noted. The length of both shoot and root was measured using a measuring tape. The chlorophyll and carotenoid contents of Radish and Canola seedlings were measured using the and (Arnon, 1949, Kirk, 1967) techniques.

Data analyses

Mean values were determined for all the treatments, and standard error was calculated. Results tested via analysis of variance and differences among means were calculated by LSD test using Statistics version 8.1 (USA). The extract concentrations required for 50% growth inhibition (IC_{50} values) of the radish and canola were calculated using the Quest Graph™ IC_{50} Calculator.

Results

Qualitative evaluation of phytochemicals of the *E. dracunculoides* fruit extract: Preliminary screening of the extract revealed the presence of different phytochemicals (Table 1). All the significant phytochemicals like alkaloids, flavonoids, phenols, tannins, steroids, catechin, and volatile oils were present. Only cardiac glycosides were absent in the extract.

Table 1. Phytochemicals in *E. dracunculoides* fruit extract + indicates presence and – indicates absence of a phytochemical.

Phytochemical	Extract
Alkaloids	+
Flavonoids	+
Phenol	+
Tannins	+
Steroids	+
Volatile oil	+
Cardiac glycosides	-
Catechin	+

Cytotoxicity effects of the *E. dracunculoides* fruit extract on brine shrimps: The mortality (%) of the brine shrimps showed that the maximum mortality (%) was achieved at 640 µg/mL of the extract. At 640 µg DWE/mL of *E. dracunculoides* fruit extract, the mortality (%) was 100%. The standard drug potassium dichromate used as a positive control showed 100% mortality of brine shrimps at all the tested concentrations above 80 µg DWE/mL (Table 2).

The LD-50 values were determined for both the extract and standard potassium dichromate. The LD-50 value obtained for extract in brine shrimp lethality assay was 57.95 µg DWE /mL. At the same time, the LD-50 value calculated for the standard drug potassium dichromate was 6.087 µg DWE/mL (Table 3).

Phytotoxicity effects of the *E. dracunculoides* fruit extract on radish and canola

Seed germination: The seed germination (%) of both the radish and canola was significantly decreased by the extract at all three concentrations. However, the decreasing effect of the extract on seed germination (%) of both the plant species was significantly higher ($p>0.05$) at 10mg/mL concentration. In radish, the extract at 10mg DWE/mL showed a 46% decrease in seed germination (%) than the extract-free control. In canola, the extract at 10mg/mL concentration showed a 61% decrease in seed germination (%) as compared to its respective control (Fig. 1).

Growth parameters: All the concentrations of the extract showed a significant decrease in the shoot and root fresh weight of both the radish and canola. The extract at 2.5, 5, and 10 mg DWE/mL exhibited a 34%, 60%, and 62% decrease in shoot fresh weight of radish as compared to its extract-free control. All three concentrations (2.5, 5, and 10 mg DWE/mL) of the extract showed a 39%, 55%, and 79% decrease in shoot fresh weight of canola. The extract at 2.5, 5, and 10 mg/mL concentration showed a 65%, 65%, and 60% decrease in root fresh weight of radish as compared to its extract-free control. In canola, the extract showed 47%, 39%, and 65% decrease in root fresh weight of canola at 2.5, 5, and 10 mg DWE/mL concentration (Fig. 2a, 2b).

The shoot and root dry weight values of both the radish and canola were lower for treatment applied with extract as compared to respective extract-accessible controls. In radish, the extract at 10 mg DWE/mL showed a 65% and 64% decrease in shoot and root dry weight. Whereas in canola, the extract at 10 mg DWE/mL exhibited an 80% and 66% decrease in shoot and root dry weight as compared to its respective extract-free control (Fig. 3a, 3b).

The extract at all three concentrations (2.5, 5, and 10 mg DWE/mL) showed inhibitory effects on shoot elongation of both the radish and canola. The maximum decrease in shoot length of both the plant species was recorded due to the application of extract at 10mg/mL concentration. The extract at 10 mg DWE/mL showed a 45% decrease in the shoot length of radish and a 57% decrease in the shoot length of canola as compared to their respective extract-free extract (Fig. 4a).

Similarly, the root elongation of both the radish and canola was significantly decreased by extract application**.** The values of root length were lowest for treatments received at 10 mg DWE/mL concentration. At 10mg/mL concentration, the extract showed a 13% and 78% decrease in the root length of radish and canola, respectively (Fig. 4b).

Table 2. Mortality (%) of brine shrimps at various concentrations of the extract and standard drug potassium dichromate.

Concentration (µg/mL)	Number of nauplii used in the experiment	Number of dead nauplii		Mortality (%)	
		Extract	Potassium dichromate	Extract	Potassium dichromate
1.25	10	0	1	0^{g}	10^{e}
2.5	10	0	3	0^{g}	30^{d}
5	10	0	4	0^{g}	40^{d}
10	10	0	6	0^{g}	60^{c}
20	10	3	9	30^{f}	90^{b}
40	10	4	9	40^{e}	90^{b}
80	10	5	10	50^{d}	100^{a}
160	10	7	10	70^{c}	100^{a}
320	10	9	10	90^{b}	100^{a}
640	10	10	10	100^{a}	100^{a}

Table 3. LD_{50} results of *E. dracunculoides* fruit extract against brine shrimp.

Sample	LD_{50}	Regression equation	R^2
Extract	57.95	y=1.4582x + 2.4305	0.9422
Potassium dichromate	6.087	Y=1.7787x +3.6152	0.9584

Photosynthetic pigments: The extract significantly inhibited the chlorophyll *a* and chlorophyll *b* content in the leaves of both the radish and canola. The decreasing effects of extract were dose-dependent such that chlorophyll values were lowest in leaves of treatment applied with extract at 10 mg DWE/mL concentration. The extract at 10 mg DWE/mL concentration showed a 78% and 79% decrease in leaf chlorophyll content of radish and canola as compared to their respective extract free control (Fig. 5a).

Like chlorophyll *a*, the chlorophyll *b* content was decreased in leaves of radish and canola due to the application of extract. The lowest value of chlorophyll *b* was recorded in the leaves of both the plant species treated with extract at 10 mg DWE/mL concentration. The extract at 10mg/mL concentration showed a 78% and 78% decrease in leaf chlorophyll *b* content as compared to their extract-free control (Fig. 5b).

Table 4. The concentration of *E. dracunculoides* fruit methanolic extract (mg DWE/mL) required for 50% growth inhibition (IC_{50}) of radish and canola.

Test plant species	Seed germination (%)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Shoot length (cm)	Root length (cm)	Chl 'a' (mg/g FW)	Chl 'b' (mg/g FW)
Radish	5.28	3.93	4.30	8.70	8.62	5.16	4.39	3.80	4.15
Canola	5.23	5.21	5.26	5.36	8.31	4.75	5.50	4.66	4.14

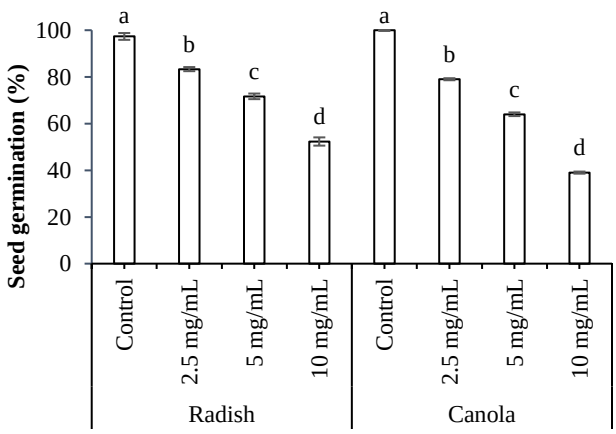

Fig. 1. Phytotoxic effect of *E. dracunculoides* fruit extract on seed germination of radish and canola.

***IC*$_{50}$ values of phytotoxic activity:** According to *IC*$_{50}$ values, it was evident that the extract was highly efficient in the growth inhibition of both the radish and canola. In the case of radish, *IC*$_{50}$ values of the extract for seed germination (%), shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, shoot length, root length, chlorophyll *a*, and chlorophyll *b* were 5.28, 3.93, 4.30, 8.70, 8.62, 5.16, 4.39, 3.80 and 4.15 mg DWE/mL respectively. Similarly, in canola *IC*$_{50,}$ values of the extract for seed germination (%), shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, shoot length, root length, chlorophyll *a*, and chlorophyll *b* were 5.23, 5.21, 5.26, 5.36, 8.31, 4.75, 5.50, 4.66 and 4.14 mg DWE/mL respectively (Table 4).

Discussion

Understanding the impact of *Euphorbia dracunculoides* fruit extract on both cytotoxicity and phytotoxicity (Sołtys-Kalina *et al.*, 2019, Abdelgaleil *et al.*, 2020) is crucial in evaluating its potential applications. This research delves into the qualitative evaluation of phytochemicals and the subsequent effects on brine shrimps, seed germination, growth parameters, and chlorophyll content in radish and canola. Each parameter provides insights into the potential applications and risks associated with the extract (Majid *et al.*, 2015). The main findings of our study include the preliminary screening identified a rich profile of phytochemicals in *E. dracunculoides* fruit extract, including alkaloids, flavonoids, phenols, tannins, steroids, catechin, and volatile oil. The extract exhibited significant cytotoxicity against brine shrimps, with a high mortality rate at 640 µg/mL. The LD-50 value of 57.95 µg DWE/mL suggests a potent cytotoxic effect comparable to the standard drug potassium dichromate (Meyer *et al.*, 1982, Nguta *et al.*, 2012). The cytotoxicity on brine shrimps suggests the presence of potent constituents, positioning the extract as a potential candidate for further exploration in anticancer research (Sharma *et al.*, 2014). The high cytotoxicity against brine shrimps correlates with the observed phytochemical richness, particularly phenols, alkaloids, and flavonoids. These bioactive compounds likely induce cell death by disrupting vital cellular functions. The LD-50 value, and cytotoxic effects align with previous studies on plant extracts with anticancer potential.

The extract demonstrated dose-dependent phytotoxic effects on both radish and canola, affecting seed germination, growth parameters, and chlorophyll content. The IC50 values reflected efficient growth inhibition (McLaughlin *et al.*, 1998, Ayatollahi *et al.*, 2010). The comprehensive phytochemical profile underscores the potential of *E. dracunculoides* fruit extract as a source of bioactive compounds. The concentration-dependent decrease in seed germination, particularly at 10 mg DWE/mL, points to the extract's phytotoxicity. The rich phenolic content may disrupt enzymatic processes involved in germination (Tanveer *et al.*, 2012, Majid *et al.*, 2015, Zeb *et al.*, 2016). The extract-induced reductions in shoot and root fresh weights, as well as shoot and root dry weights, indicate a hindrance in overall plant growth. Phenolic compounds, known for herbicidal properties, likely interfere with cellular processes, impacting biomass accumulation (Majid *et al.*, 2015, Zeb *et al.*, 2016, Enneb *et al.*, 2023). Inhibitory effects on shoot and root elongation, especially at 10 mg DWE/mL, suggest interference with secondary metabolites. Compounds present in the extract may impede cell division and affect photosynthetic processes crucial for plant elongation (Begum *et al.*, 2022). Concentration-dependent reduction in chlorophyll a and chlorophyll b content implies oxidative stress induction (Chaparzadeh & Hosseinzad-Behboud, 2015). Phenolic compounds, prevalent in the extract, may inhibit enzyme activity and gibberellin synthesis, impacting chlorophyll pigments (Boutoub *et al.*, 2021). In phytotoxicity, the presence of phenolic compounds and tannins in the extract provides insight into the mechanisms of action. These compounds, known for herbicidal properties, may interfere with enzymatic processes, cell division, and photosynthesis, leading to the observed inhibitory effects on seed germination, growth parameters, and chlorophyll content (Majid *et al.*, 2015). The presence of phenols and tannins correlates with herbicidal properties reported in the literature. The dose-dependent phytotoxicity on radish and canola further emphasizes the potential applications of the extract as a source of herbicidal compounds (Perotti *et al.*, 2020). The IC50 values highlight the extract's selectivity in inhibiting shoot growth over root growth in both radish and canola. This differential impact on chlorophyll a and chlorophyll b content indicates a species-specific response, a phenomenon reported in other plant extract studies (Hossen *et al.*, 2021, 2023).

The observed effects on seed germination, growth parameters, and chlorophyll content have implications for potential agricultural applications. However, the study urges caution in the ethnomedicinal use of *E. dracunculoides* fruit extract due to its cytotoxicity. In conclusion, this research sheds light on the multifaceted effects of *E. dracunculoides* fruit extract, offering valuable insights into its cytotoxic and phytotoxic potential. Further exploration of the underlying mechanisms and isolation of active compounds holds promise for future applications in medicine and agriculture.

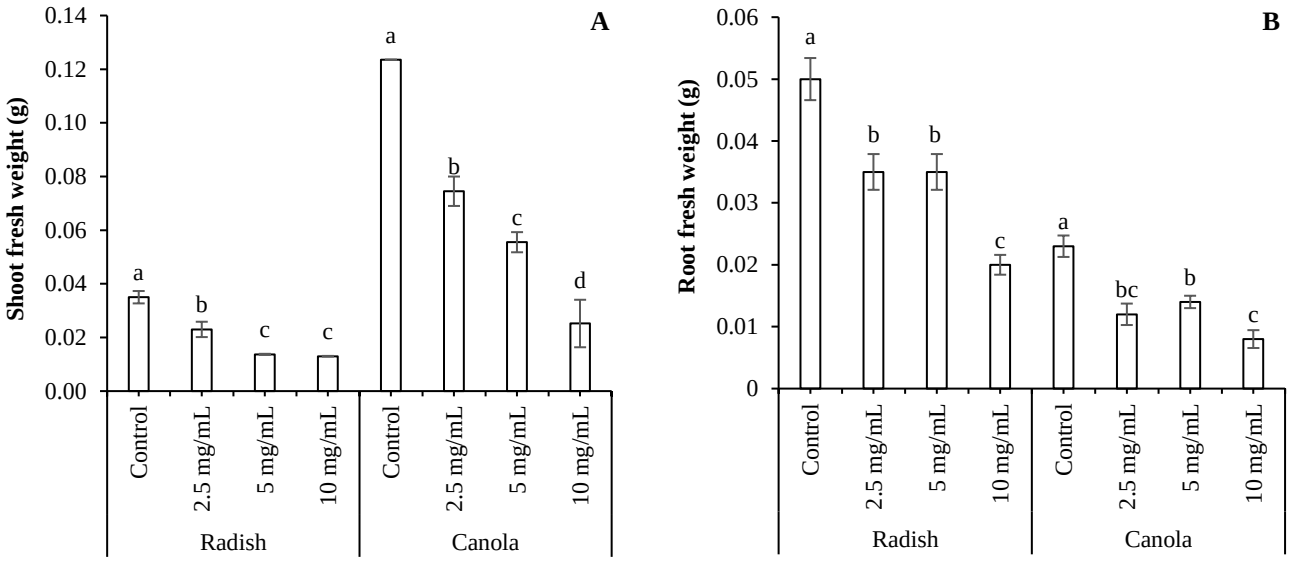

Fig. 2. Phytotoxic effect of *E. dracunculoides* fruit extract on shoot (a) and root (b) fresh weight of radish and canola.

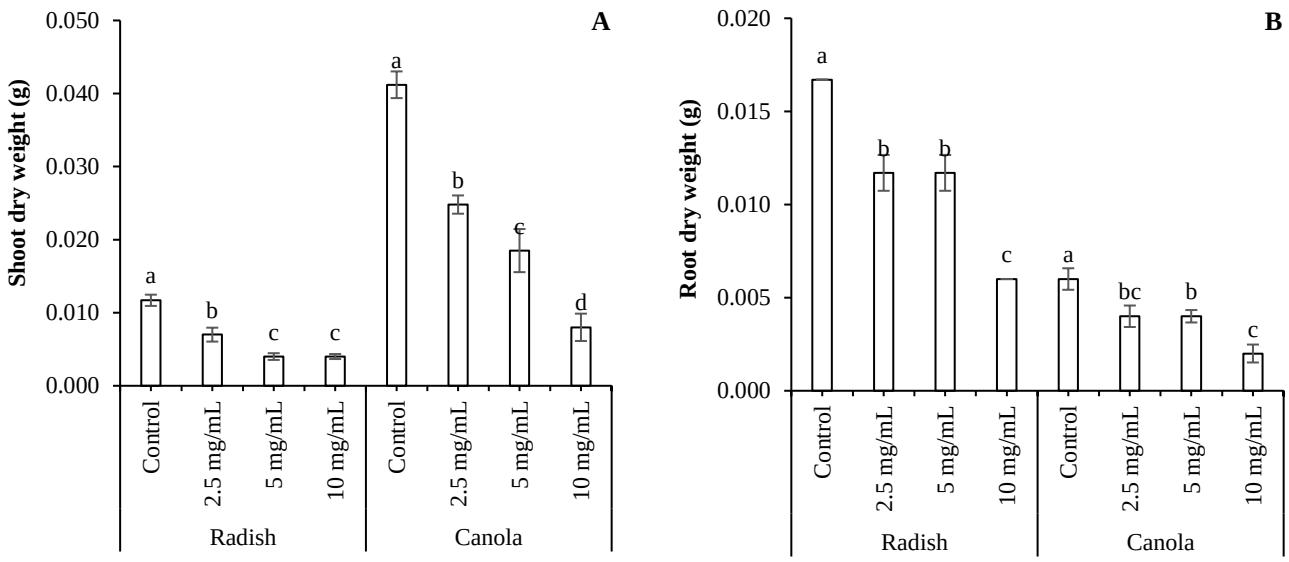

Fig. 3. Phytotoxic effect of *E. dracunculoides* fruit extract on shoot (a) and root (a) dry weight of radish and canola.

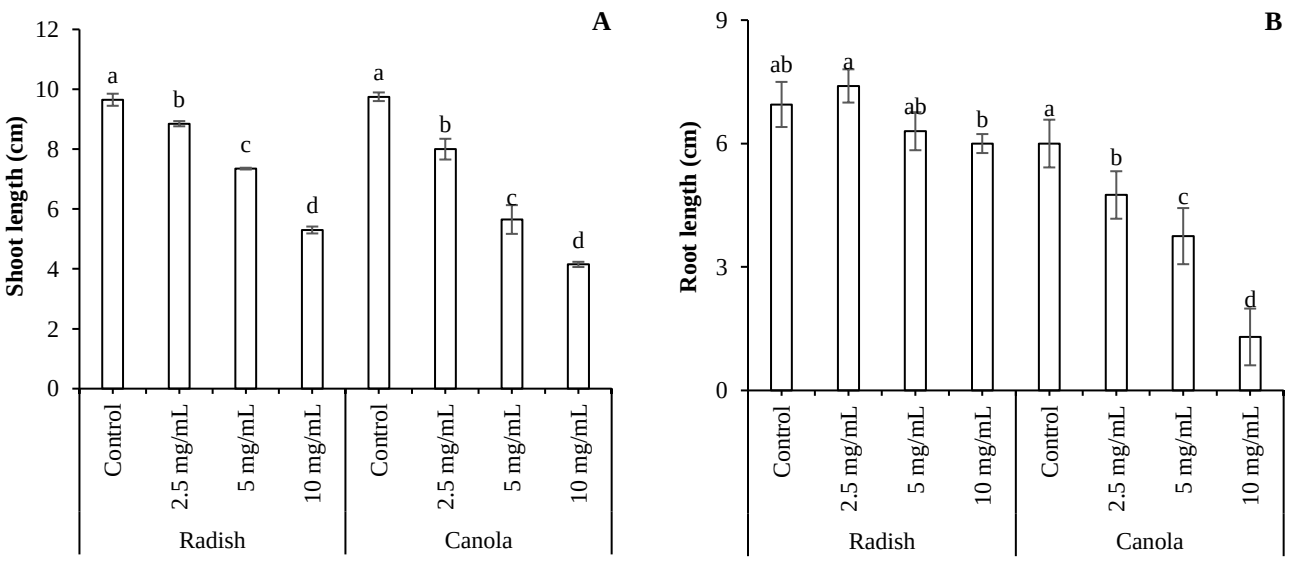

Fig. 4. Phytotoxic effect of *E. dracunculoides* fruit extract on shoot (a) and root (b) length of radish and canola.

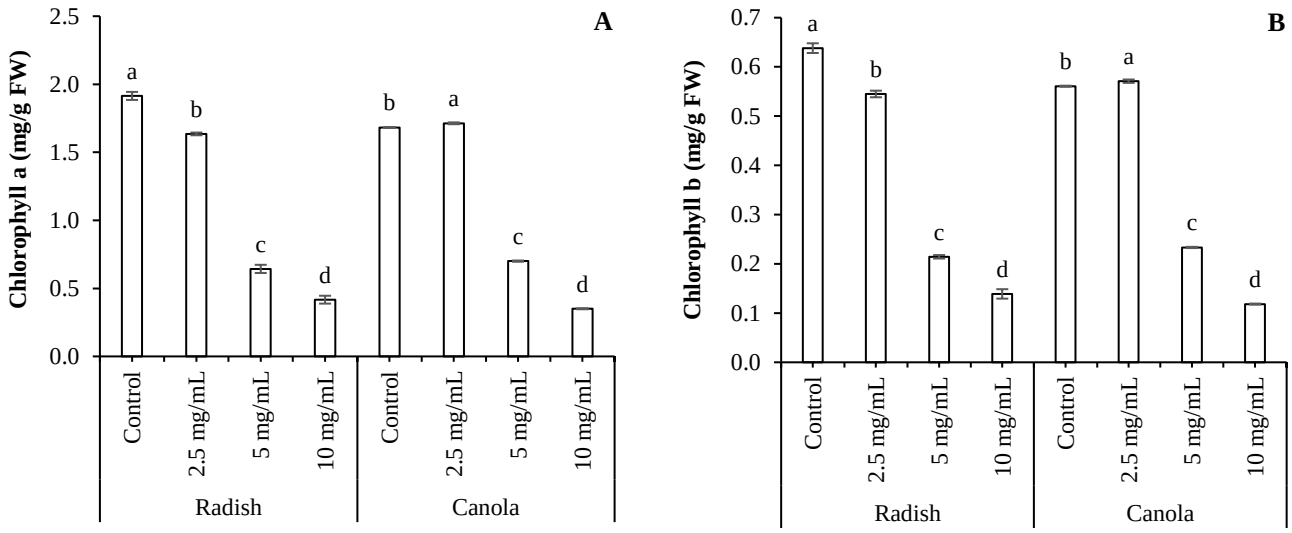

Fig. 5. Phytotoxic effect of *E. dracunculoides* fruit extract on Chlorophyll 'a' (a) and 'b' (b) of radish and canola.

Conclusions

The intriguing aspect of this research lies in the potent cytotoxic and phytotoxic effects of *Euphorbia dracunculoides* fruit extract, showcasing its rich phytochemical arsenal. These findings hold practical implications for potential applications in developing natural herbicides and exploring anticancer properties. Future research should delve into isolating and characterizing specific compounds responsible for these effects, enhancing our understanding of their mechanisms. Overcoming limitations, such as focusing on a broader range of target organisms and conducting field trials, will pave the way for more robust applications in sustainable agriculture and healthcare.

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References

Abdelgaleil, S.A.M., M.M.G. Saad, N.R. Ariefta and Y. Shiono. 2020. Antimicrobial and phytotoxic activities of secondary metabolites from Haplophyllum tuberculatum and Chrysanthemum coronarium. *S. Afr. J. Bot.*, 128: 35–41.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. polyphenoloxidase in beta vulgaris. *Plant Physiol.*, 24: 1–15.

Ayatollahi, A.M., M. Ghanadian, S. Afsharypuor, S. Siddiq and S.M. Pour-Hosseini. 2010. Biological screening of Euphorbia aellenii. *Iran. J. Pharm. Res.*, 9: 429.

Baravalia, Y., Y. Vaghasiya and S. Chanda. 2012. Brine shrimp cytotoxicity, anti-inflammatory and analgesic properties of Woodfordia fruticosa Kurz flowers. *Iran. J. Pharm. Res.*, 11: 851.

Begum, H.A., S.M. Ali, W. Khan, T. Yassen, D.A. Jones and K. Ali. 2022. Effects of Stem Extract of Euphorbia royleana Boiss on Seed Germination of brassica campestris L., Trifolium repens L. and Trigonella foenum-graecum L. *Proc. Natl. Acad. Sci. India Sect. B Biol. Sci.*, 92: 589–594.

Boutoub, O., S. El-Guendouz, A. Manhita, C.B. Dias, L.M. Estevinho, V.B. Paula, J. Carlier, M.C. Costa, B. Rodrigues, S. Raposo and others. 2021. Comparative study of the antioxidant and enzyme inhibitory activities of two types of Moroccan Euphorbia entire honey and their phenolic extracts. *Foods*, 10: 1909.

Butler, M.S. 2004. The role of natural product chemistry in drug discovery. *J. Nat. Prod.*, 67: 2141-2153.

Chaparzadeh, N. and E. Hosseinzad-Behboud. 2015. Evidence for enhancement of salinity induced oxidative damages by salicylic acid in radish (*Raphanus sativu*s L.). *J. Plant Physiol. Breed.*, 5: 23-33.

Chung, H.S, Y.J. Lee, M.M. Rahman, A.M. Abd El-Aty, H.S. Lee, M.H. Kabir, S.W. Kim, B.J. Park, J.E. Kim, F. Hacımüftüoğlu, N. Nahar, H.C. Shin and J.H. Shim. 2017. Uptake of the veterinary antibiotics chlortetracycline, enrofloxacin, and sulphathiazole from soil by radish, *Sci. Total Environ.*, 605-606:322-331.

Dayan, F.E., D.K. Owens and S.O. Duke. 2012. Rationale for a natural products approach to herbicide discovery. *Pest Manag. Sci.*, 68: 519-528.

Duke, J.A. 2002. Handbook of medicinal herbs. CRC press.

Enneb, H., K. Athmouni, R. Thabet and H. Ayadi. 2023. Phytochemical Compounds of Euphorbia bivonae Extract and Their Cytotoxicity Effects on the Lethality of Brine Shrimp Artemia salina and Embryonic Kidney (HEK293) Cells. *Chem. Biodiv.*, e202201135.

Harborne, J.B. 1973. Methods of plant analysis. In: Phytochemical Methods. Chapman and Hall London.

Heap, I.M. 1999. International survey of herbicide-resistant weeds: lessons and limitations. *In*: *1999 Brighton Crop Protection Conference: Weeds. Proceedings of an International Conference, Brighton, UK, 15-18 November* 1999. 769-776.

Hossen, K., Y. Asato, T. Teruya and H.K. Noguchi. 2023. Identification of four allelopathic compounds including a novel compound from Elaeocarpus floribundus Blume and determination of their allelopathic activity. *J. Environ. Manag.*, 326: 116728.

Hossen, K., A. Iwasaki, K. Suenaga and H.K. Noguchi. 2021. Phytotoxic activity and growth inhibitory substances from *Albizia richardiana* (Voigt.) King & Prain. *Appl. Sci.*, 11: 1455.

Kamanja, I.T., J.M. Mbaria, P.K. Gathumbi, M. Mbaabu, J.D. Kabasa and S.G. Kiama. 2018. Cytotoxicity of selected medicinal plants extracts using the brine shrimp lethality assay from Samburu county, Kenya.

Khan, S.U., F. Ullah, M. Hussain, M.Z. Ihsan, S. Mehmood, L. Ali, S. Saud, S. Fahad, S. Hassan, M. Zeeshan and others. 2023. Phytochemical analysis and phytotoxic evaluation of Chenopodium glaucum L. *J. King Saud Univ.*, 35: 102571.

Khattak, U., R. Ullah, S.A. Khan, S. Ullah and others. 2017. Pharmacognostic evaluation and analgesic efficacy of ethanolic extract of *Euphorbia dracunculoides* L. *Pharmacog., J.*, 9.

Kim, K.-H., E. Kabir and S.A. Jahan. 2017. Exposure to pesticides and the associated human health effects. *Sci. Total Environ.*, 575: 525–535.

Kirk, J.T.O. 1967. Studies on the dependence of chlorophyll synthesis on protein synthesis in Euglena gracilis, together with a nomogram for determination of chlorophyll concentration. *Planta*, 78: 200–207.

Kostina-Bednarz, M., J. Płonka and H. Barchanska. 2023. Allelopathy as a source of bioherbicides: challenges and prospects for sustainable agriculture. *Rev. Environ. Sci. Bio-Technol.*, 1–34.

Kubiak, A., A. Wolna-Maruwka, A. Niewiadomska and A.A. Pilarska. 2022. The problem of weed infestation of agricultural plantations vs. the assumptions of the European biodiversity strategy. *Agronomy*, 12: 1808.

Majid, M., M.R. Khan, N.A. Shah, I.U. Haq, M.A. Farooq, S. Ullah, A. Sharif, Z. Zahra, T. Younis and M. Sajid. 2015. Studies on phytochemical, antioxidant, anti-inflammatory and analgesic activities of Euphorbia dracunculoides. *BMC Compl. Altern. Med.*, 15: 1-15.

Modlitbová, A, A. Hlaváček, T. Švestková, P. Pořízka, L. Šimoníková, K. Novotný and J. Kaiser. 2019. The effects of photon-upconversion nanoparticles on the growth of radish and duckweed: Bioaccumulation, imaging, and spectroscopic studies. *Chemosphere.,* 225: 723-734

McLaughlin, J.L., L.L. Rogers and J.E. Anderson. 1998. The use of biological assays to evaluate botanicals. *Drug Inf. J.*, 32: 513–524.

Meyer, B.N., N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E.J. Nichols and J.L. McLaughlin. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med.*, 45: 31–34.

Nguta, J.M., J.M. Mbaria, D.W. Gakuya, P.K. Gathumbi, J.D. Kabasa and S.G. Kiama. 2012. Cytotoxicity of antimalarial plant extracts from Kenyan biodiversity to the brine shrimp, Artemia salina L.(Artemiidae). *Drugs Ther. Stud.*, 2: e12--e12.

Perotti, V.E., A.S. Larran, V.E. Palmieri, A.K. Martinatto and H.R. Permingeat. 2020. Herbicide resistant weeds: A call to integrate conventional agricultural practices, molecular biology knowledge and new technologies. *Plant Sci.*, 290: 110255.

Qasim, M., Y. Fujii, M.Z. Ahmed, I. Aziz, K.N. Watanabe and M. Ajmal Khan. 2019. Phytotoxic analysis of coastal medicinal plants and quantification of phenolic compounds using HPLC. *Plant Biosyst. Int. J. Deal. with all Asp. Plant Biol.*, 153: 767–774.

Raad, B., Ali, S.S. Rehman, K.U. Akhtar, N. Ullah, B. Wali, S. 2020. Phytochemical screening and biological activities of *Aloe vera* (L.) Burm. F. *Pure and Applied Biology (PAB)*, 10: 360-367.

Raclavská, H., J. R\uužičková, D. Juchelková, M. Šafá\vr, H. Brt'ková and K. Slamová. 2021. The quality of composts prepared in automatic composters from fruit waste generated by the production of beverages. *Bioresour. Technol.*, 341: 125878.

Sharma, N., K.W. Samarakoon, R. Gyawali, Y.-H. Park, S.-J. Lee, S.J. Oh, T.-H. Lee and D.K. Jeong. 2014. Evaluation of the antioxidant, anti-inflammatory, and anticancer activities of Euphorbia hirta ethanolic extract. *Molecules*, 19: 14567–14581.

Shekhawat, K., S.S. Rathore and B.S. Chauhan. 2020. Weed management in dry direct-seeded rice: A review on challenges and opportunities for sustainable rice production. *Agronomy*, 10: 1264.

Sołtys-Kalina, D., Z. Murawska, D. Strzelczyk-Żyta, I. Wasilewicz-Flis and W. Marczewski. 2019. Phytotoxic potential of cultivated and wild potato species (Solanum sp.): Role of glycoalkaloids, phenolics and flavonoids in phytotoxicity against mustard (*Sinapis alba* L.). *Acta Physiol. Plant.*, 41: 55.

Sofowora, A.E. 1993. Medicinal Plants and Traditional Medicines in Africa. 2nd edition. Spectrum Books, Ibadan, Nigeria.

Tanveer, A., M.K. Jabbar, A. Kahliq, A. Matloob, R. Abbas and M.M. Javaid. 2012. Allelopathic effects of aqueous and organic fractions of Euphorbia dracunculoides Lam. on germination and seedling growth of chickpea and wheat. *Chil. J. Agric. Res.*, 72: 495–501.

Trease GE, Evans WV. Pharmacognosy. 12th ed. London: Bailliere Tindall; 1983. pp 735-738.

Turker, A.U. and N.D. Camper. 2002. Biological activity of common mullein, a medicinal plant. *J. Ethnopharmacol.*, 82: 117–125.

Zeb, M.A., S. Mehmood, F. Ullah and T. Iqbal. 2016. Allelopathic potential of Euphorbia dracunculoides root aqueous extract on seed germination and early seedling growth of chickpea. *Pak. J. Weed Sci. Res.*, 22:3, 381–394.

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