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Original article

Phytochemical analysis and phytotoxic evaluation of *Chenopodium glaucum* L.



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ABSTRACT

Plant secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids with medicinal properties can be investigated as botanical herbicides. Therefore, we have investigated phytochemicals profile and phytotoxicity of aqueous, ethyl acetate, *n*-hexane and methanol extracts (5 mg/mL and 10 mg/mL) of *Chenopodium glaucum* L. (*C. glaucum*) on radish under laboratory conditions. Initial screening of the extracts showed the occurrence of several phytochemicals like alkaloids, flavonoids, anthraquinone, cardiac glycosides, saponins, tannins and terpenoids. Alkaloids and tannins content was higher in methanol extract followed by ethyl acetate and *n*-Hexane extracts. Elemental analysis revealed that the presence of minerals like magnesium 0.725 mg/100 g DW, calcium 2.25 mg/100 g DW, potassium 6.55 mg/100 g DW, sodium 0.375 mg/100 g DW, iron 225 mg/100 g DW, zinc 51 mg/100 g DW, copper 12 mg/100 g DW, and manganese 119 mg/100 g DW was found. In phytotoxicity assay, the extracts at 10 mg/mL showed inhibitory effects on the growth of radish. Maximum decrease in seed germination (%), seed germination index, seedling fresh and dry weight was caused by the methanolic extract at 10 mg/mL. The chlorophyll *a* content was decreased while chlorophyll *b* content was increased with increasing concentration of the extracts. Phenolics content of the seedlings was higher with the application of 10 mg/mL of the extracts. Powder microscopy of *C. glaucum* showed the presence of calcium oxalate crystal, pieces of trichome, fragments of cork cells, starch granules, phloem fibers and lignified xylem vessels with annular thickenings. Microscopic study of abaxial surface of leaf showed the presence of anisocytic type of stomata, surrounded by subsidiary cells. Stomatal size was measured 25.017 μm and the mean density was 570.66/mm². Findings of this study concluded that phytochemicals found in *C. glaucum* extracts were highly phytotoxic on seed germination and seedling growth of radish. It

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is therefore recommended to isolate and characterize such novel compounds from this plant species for the development of new biodegradable botanical herbicides.

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1. Introduction

Weeds in crops have been reported to minimize crop productivity up to 34 % of the total yield (Jabran et al., 2015). Application of synthetic herbicide is still the most effective way and frequently used to control weeds in cultivated crops (Elshamy et al., 2019; Espig et al., 2022). However, the use of synthetic herbicides have lead to several problems such as development of herbicide resistant species and can damage environment and human health (Landrigan and Benbrook 2015; Mominul-Islam and Kato-Noguchi 2014; Bhandari et al., 2014; Pathak et al., 2022). Extensive use of synthetic herbicides results in the loss of biodiversity and soil fertility (Mahmood et al., 2016; Sangiorgio et al., 2022). Therefore, in order to minimize the effect of synthetic herbicides on environment and crops, several weed management strategies have been tried and are getting interest. Exploring plants secondary metabolites can be a safe alternative to synthetic herbicides (Araniti et al., 2022). Therefore, reserachers are working on the isolation and charactization of novel compounds from plants with herbicidal properties (Pouresmaeil et al., 2020).

The secondary metabolites in plants have a diverse type of actions and phtotoxic effects (Lipkus et al. 2008; Araniti et al., 2022). These natural substances are biodegradable and harmLess (Bajwa et al., 2019). Plants are a major source of phytochemicals which have also been reported as phytotoxic in nature (Alvin et al. 2014; Shakya 2016; Franco et al., 2022). Therefore, medicinal plants are needed to be explored as a source of phytotoxic substances.

The chemical compounds responsible for the phytotoxic effects are called allelochemicals, used by plants for their survival under diverse environmental conditions. The allelochemicals can be therefore exploited as an alternatives to synthetic herbicides (Motmainna et al., 2021). Bio-herbicides are advantageous over chemical herbicides because they are non-toxic for the environment, they are highly specific to target weed species and are biodegradable. For searching bio-herbicidal potential of a plant species, phytotoxicity assay is commonly employed (Fukushima et al., 1998).

Radish seed bioassay has been extensively used previously for phytotoxicity evaluation of plants extracts (Terker and camper 2002, Inayatullah et al., 2007, Khan et al., 2011;). Radish seeds are commenly available, easily to germinate and very sensitive to test compounds (Einhellung and Rasmuseen 1978) and are a standard assay in phytotoxicity studies (Patterson 1986).

Correct identification is the main problem in herbal drugs as some plant materials in fresh as well as in dry powder form look too similar that they cannot be distinguished from one another (Springfield, 2005; Muyumba et al., 2021). The real danger in herbal drugs preparation and subscription is their adulteration (Opara, 2004; Monti et al., 2022). Observation of microscopic material of medicinal plants is necessary for identification of powder (WHO, 1998). Therefore, development of standardization is necessary for the identification of the important medicinal plants. Standardization of identification of powder material of medicinal plants is necessary for the prevention of adulteration. It is general observation that the more effective natural drugs are more exploited. The natural drugs are adulterated with low quality material easily to meet the growing demand (Chanda et al., 2013).

Chenopodium glaucum belongs to family Chenopodiaceae. The members of this family are found in Australia, New Zealand and Asia. Many members of this family like Salsola and Sueda prefer saline soils. Salsolin and salsolidin used in hypertensive medications are obtained from the members of this family (Akopian et al., 2020). The plant is annual herb and occurs as a weed on road sides, gardens and along cultivated fields. The *C. glaucum* grows up to 5–20 cm height and is usually glabrous (Laghari et al., 2010; Ahmad et al., 2003). Due to environmental risks associated with synthetic herbicides we carried out these studies to explore phytoxicity of *C. glaucum* by using radish seed bioassay with perspectives of botanical herbicides.

2. Materials and methods

2.1. Collection of plant material

Arial parts from 100 plants of *C. glaucum* were collected from District Bannu, Pakistan and shade dried. The *C. glaucum* was identified by comparing its characteristics with flora of Pakistan and deposited in the herbarium Department of Botany University of Science and Technology Bannu, Pakistan assigning it voucher number Cg-C-1. The coordinates of the collection area are; Latitude: 32.88041 N 32°52'49.31089" Longitude: 70.58595 E 70° 35'9.03027" Elevation 342.58 m. All the dried aerial parts were converted to fine powder with a particle size 5 mm. Crude extracts were prepared by soaking 50 g of the plant powder in 1L of distilled water, *n*-hexane, ethyl acetate and methanol separately. The extracts were filtered separately through Whatman No.1 filter paper and dried by evaporating the solvents at 40 °C with a rotary evaporator (Model, RE301, Japan). The yield of methanolic extract was 9.3 g, aqueous 7 g, ethyl acetate 3.8 g and *n*-hexane 1.3 g.

3. Phytochemicals analysis

3.1. Qualitative determination of phytochemicals

The qualitative assessment of extracts for diverse groups of phytochemicals was performed according to standard available protocols (Harbone, 1973; Trease and Evans, 1985; Congesta et al., 2005; Ngameni et al., 2013).

3.2. Assessment of alkaloids

First the Mayer's reagent was prepared for which distilled water (60 mL) was taken to dissolve 335 mg of HgCl₂. The 5 g of KI was mixed in 20 mL of distilled H₂O. Both solution were mixed in distilled water and volume was made up to 100 mL.

For the qualitative assessment of alkaloids, the 2 mg of each crude extract was separately mixed with concentrated H₂SO₄. The mixture was further mixed with the Mayer's reagent. Formation of white precipitation indicated presence of alkaloids in the samples.

3.3. Assessment of flavonoids

The 1 mg of each crude extract was mixed with 1 mL of 2 N NaOH solution. Yellow color formation confirmed flavonoids in the extract samples.

3.4. Assessment of coumarins

Each crude extract (1 mg/mL) was mixed with 1 mL of NaOH (10 %) in a test tube. Yellow color formation is the indication of the presence of coumarins in the test sample.

3.5. Assessment of saponins

The 2 mg of extract was taken (aqueous, ethyl acetate, methanol and *n*-hexane) and mixed with 2 mL of distilled water by shaking continuously. A soapy layer formations signs the presence of Saponin in the extract samples.

3.6. Assessment of tannins

The 1 mg of each crude extract was mixed with two mL of 5 % ferric chloride. The formation of the dark blue color is the sign of the presence of tannins.

3.7. Assessment of terpenoids

Chloroform (2 mL) and concentrated H₂SO₄ (2 mL) were mixed with 0.5 mg of each aqueous, ethyl acetate, methanol and *n*-hexane crude extract. Red brown color appearance in the middle of the two layer is the indication of terpenoids.

3.8. Assessment of anthraquinone

The 1 mg of each crude extract was mixed with 2 mL of diluted 2 % HCL. Development of red color is the indication of anthraquinone.

3.9. Quantitative determination of phytochemicals

3.9.1. Condensed tannins

By using the method of Sun et al. (1998) total content of condensed tannins in various extracts of *C. glaucum* was estimated. This method measures the content proanthocyanidins in plant samples. For samples preparation the 0.5 μL extract was mixed with 3 mL vanillin solution (4 %) and concentrated HCl (1.5 mL). After 15 min of incubation at normal room temperature (25 ± 1) the absorbance of all the samples was noted at 500 nm using a VIS-Spectrophotometer (VIS-721 Cangzhou, China). The concentration of total condensed tannins was expressed as mg Catechins/g D. W of a plant sample. All the samples were prepared and analyzed in triplicate.

3.10. Alkaloids

Alkaloids content in various extracts of *C. glaucum* was determined (Harborne 1984). The 200 mL acetic acid (10 %) in ethanol was mixed with 5 g of the extracts separately. The samples were allowed to stand at room temperature for 4 hrs. All the sets of samples were first filtered and then concentrated to one quarter of the total volume in water bath. All the samples were added with concentrated ammonium hydroxide solution until the completion of precipitation was formed. The precipitate was carefully transferred to a separate conical flask and carefully washed with diluted ammonium hydroxide solution. After filtration, the alkaloid's containing precipitate was collected subsequently dried and weighed.

3.11. Phytotoxicity assay

The 500 mg of aqueous, *n*-hexane, ethyl acetate and methanol plant extracts were dissolved separately in 50 mL of the respective solvent for making a stock solution of 10 mg/mL. Stock solutions of the extracts (aqueous, methanol, *n*-hexane, ethyl acetate) were further diluted to make 5 mg/mL solutions. Respective solvents and sterilized distilled water were used negative and as positive control respectively. Phytotoxicity assay was performed according to the method of Atta-ur-Redman (1991) and Turker and Camper (2002). *Raphanus sativus* L. (Radish) seeds were used for the determination of seed germination indices, seedling fresh weight and dry weight, photosynthetic pigments and total phenolics contents.

The seed surface sterilization solution having 0.1 % HgCl₂ was made in a beaker. Rapidly washing the radish seeds with the sterilizing solution for 2 to 3 min followed by 3–4 washings with autoclaved distilled water. The wet seeds were dried with a sterilized blotting paper before using in the assay. Petri plates were sterilized in an autoclave and fitted with two Whatman No.1 filter paper. The 0.5 mL of the 5 mg/mL and 10 mg/mL of the extract was dropped on each filter paper in a Petri plate. The solvent in each Petri plate was evaporated and sprinkled with 5 mL of autoclaved distilled water. Each of the extract treatment had three replicates. For control, 0.5 mL *n*-hexane, ethyl acetate and methanol added to the plate, vacuum evaporated and then 5 mL autoclaved distilled water added in each Petri plate. Three replicates were prepared for each control. Radish seed (sterilized) 10 in number were cultivated with forceps at suitable distance in each Petri plate. Petri plates were incubated at 25 °C in partial darkness. After the seeds were germinated (100 % germination in control) the seedlings were transferred to a growth chamber (16 hrs dark and 8 hrs illumination, 25 ± 1 °C temperature) for further two weeks and supplied with respective solutions when required. After two weeks all the seedlings were carefully collected and analyzed for different growth traits.

Seed germination (%).

$$\text{Germination \%} = \frac{\text{Total seeds germinated}}{\text{No of seeds grown}} \times 100 \quad (1)$$

Seed germination index.

$$\text{Germination index} = \frac{\text{Germinted seeds on day 1st}}{\text{NoNo. of days}} + \frac{\text{seeds germinated on day 2nd}}{\text{NoNo. of days}} + \frac{\text{No. seeds germinated at day 15}}{\text{No. of days from sowing}} \quad (2)$$

3.12. Fresh weight and dry weight of raddish seedling.

The weight of fresh seedlings in all the treatments was measured by using a digital balance. For dry weight, the seedlings were dried in an oven at 72 °C for 72 h and then their weight was determined.

3.13. Determination of photosynthetic pigments

Arnon (1949) and Kirk (1968) method was used for the determination of chlorophyll and carotenoids content of radish seedlings. Leaf extract was prepared by grinding 0.01 g green tissues in 80 % acetone (5 mL). After centrifugation (4000 × g) for 15 min the supernatant obtained was carefully taken in glass test tubes and used for the estimation of leaf pigments content. The absorbance of all the test samples was noted at 645 nm and 663 nm for Chl *a* and Chl *b* whereas, the absorbance for carotenoids

was recorded at 480 nm using a spectrophotometer (Hitachi's U-5100 Japan).

3.14. Total phenolic contents determination

Quantification of soluble phenolic was made according to the method of Swain and Hillis (1959). A 100 mg of powdered leaves were extracted in methanol, water, ethyl acetate and *n*-hexane. 50 μ L of the supernatant was diluted to a volume of 0.5 mL by adding methanol, water, ethyl acetate and *n*-hexane respectively. Folin-Ciocalteu reagent (50 % v/v) in amount of 0.25 mL was added in a mixture and kept for 3 min. 7.5 % sodium carbonate solutions (500 μ L) was added in the mixture and kept in dark for one hour at 25 °C. The absorbance was taken at 765 nm by using spectrophotometer (Hitachi's U-5100 Japan). Gallic acid in concentration of 0 to 32 μ g/mL was used for plotting a standard curve for comparison of gallic acid from the measured samples.

3.15. Elemental analysis

Procedure outlined by Zafar et al. (2010) was adopted for elemental analysis. The *C. glaucum* powder (0.25 g) was taken in a 50 mL flask. 6.5 mL of mixed acids solution was added in the flask. The mixed acids solution contained HNO₃, H₂SO₄ and HClO₄ in the ratio of 5:1:0.5. The sample was boiled on hot plate (MS-300HS, Korea) until digestion was completed and white fume was formed. The digested material was transferred to another flask and filtration was made with Whatmann No. 42 filter paper. The filtrate was collected in labelled bottles and the mixture was analyzed for elements sodium (Na⁺), potassium (K⁺), magnesium (Mg⁺²), calcium (Ca⁺²), iron (Fe⁺²), zinc (Zn⁺²) and copper (Cu⁺³) by using atomic absorption spectrophotometer (Shimadzu AA-670 w).

3.16. Microscopic study of *C. Glaucum* powder

Powder microscopy was carried out according to the protocol of WHO (1998). The dried stem and leaf of *C. glaucum* were grinded into coarse powder form. The powder material was mounted on a slide by dipping the moistened tip of needle in the powder and then gently tapping on slide. The slide already contained a drop of phloroglucinol in concentrated HCl, chloral hydrate and iodine solution.

3.17. Stomatal density and size

Leaf stomatal density (S_D) and stomata size (S_S) of *C. glaucum* were calculated according to the method of Volenikova & Ticha (2001). Clear nail polish was used to prepare a clear cast of leaf lower surface. A strip of clean sticky tape was placed over the lower surface on nail polish area and pressed gently. The sticky tape was pulled off in such a way that the layer of nail polish also come off with it. The sticky tape was put on the slide and observed at 400x. The stomata were count at 3 field of view (FOVs). Stomata density was calculated according to the formula.

Stomata density = Number of stomata/ Area (mm²).

Where area is calculated as $A = \pi r^2$

3.18. Statistical analysis

The data of phytochemicals and phytotoxic assay were analyzed by using analysis of variance technique. Least significant difference test was performed for comparison of treatment means by using Statistix-9 (Steel and Torrie 1997). The standard error value was recorded for replicates of treatments by using excel sheet.

4. Results

4.1. Phytochemicals

Methanol, aqueous, *n*-hexane and ethyl acetate extract of *C. glaucum* were analysed for the presence of different phytochemicals such as flavonoids, anthraquinone, alkaloids, cardiac glycosides, tannins, terpenoids and saponins (Table 1). Anthraquinone was found absent in methanol extract, alkaloids and anthraquinone were absent in aqueous extract, anthraquinone and cardiac glycosides were absent in *n*-hexane extract and anthraquinone and cardiac glycoside were absent in ethyl acetate extract.

The results for quantitative analysis of extracts is shown in Table 2. Alkaloids were significantly higher ($p < 0.05$) in methanol extract (5.15 %), next in *n*-hexane (3.45 %) and ethyl acetate (3.03 %). Alkaloids were absent in aqueous extract. Significantly higher amount of tannins was present in methanol extract (3.33 mg/g) followed by ethyl acetate extract (2.29 mg/g), aqueous extract (1.98 mg/g) and *n*-hexane extract (1.78 mg/g).

4.2. Elemental analysis

Elemental analysis of *C. glaucum* is shown in below (Table 3). The elemental analysis showed the presence of calcium 2.25 mg, potassium 6.55 mg, magnesium 0.725 mg, sodium 0.375 mg, iron 225 mg, copper 12 mg, zinc 51 mg, and manganese 119 mg in 100 g.d.w.

5. Phytotoxicity of *C. Glaucum*

5.1. Phytotoxicity of *C. Glaucum* extracts on seed germination indices of radish

A reduction in seed germination (%) and seed germination index was recorded in all the extracts; however, higher concentration of the extracts were significantly more toxic on radish seed germination (Figs. 1 and 2). The 100 percent germination was recorded in control treatments. The treatment of 10 mg/mL of the extracts showed severe decreases in germination (%). The methanol, aqueous, *n*-hexane and ethyl acetate extracts showed 37 %, 34 %, 25 % and 14 % decrease in seed germination percentage. Like seed germination (%), the seed germination index values were lower in treatments having 10 mg/mL of the extracts. However, *n*-Hexane

Table 1
Qualitative phytochemical analysis of *C. glaucum*.

	Methanol	Aqueous	<i>n</i> -hexane	Ethyl acetate
Alkaloids	+	-	+	+
Flavonoids	+	+	+	+
Anthraquinone	-	-	-	-
Cardiac glycosides	+	+	-	-
Saponin	+	+	+	+
Tannins	+	+	+	+
Terpenoids	+	+	+	+

Table 2
Alkaloids and condensed tannins content.

Extract	Alkaloids (%)	Total condensed tannins (mg/g DW)
Aqueous	0.000 ± 0.00 ^d	1.98 ± 0.37 ^b
Ethyl acetate	3.03 ± 0.05 ^c	2.29 ± 0.17 ^b
Hexane	3.45 ± 0.04 ^b	1.78 ± 0.08 ^b
Methanol	5.15 ± 0.08 ^a	3.33 ± 0.28 ^a

and methanol extracts were significantly more toxic on seed germination index of radish.

5.2. Phytotoxicity of *C. Glaucum* extracts on fresh and dry weight of radish seedling

Fresh and dry weights of radish seedling were significantly decreased in treatments having aqueous, ethyl acetate, methanol and *n*-hexane extracts (5 mg/mL and 10 mg/mL) as compared to its control (Figs. 3 and 4). The most significant decrease was observed in *n*-hexane and methanolic extracts of *C. glaucum* (10 mg/mL).

5.3. Phytotoxicity of *C. Glaucum* extracts on photosynthetic pigments of radish

Chlorophyll *a* content of radish seedling was significantly reduced by aqueous, *n*-hexane, ethyl acetate and methanolic extract of *C. glaucum* (Fig. 5). The 10 mg/mL of the extract showed greater reduction in the chl *a* content but lower concentration (5 mg/mL) also remained significant. The highest reduction in chl *a* contents observed in 10 mg/mL of *n*-hexane extract.

Increase in Chl *b* content was recorded with increasing concentrations of the extracts (Fig. 6). Aqueous extract (5 mg/mL and

Table 3
Elemental analysis of *C. glaucum*.

Micro elements (mg/100 g.d.w.)	Magnesium	Calcium	Potassium	Sodium	Iron	Zinc	Copper	Manganese
	0.725	2.25	6.55	0.375	250	51	12	119

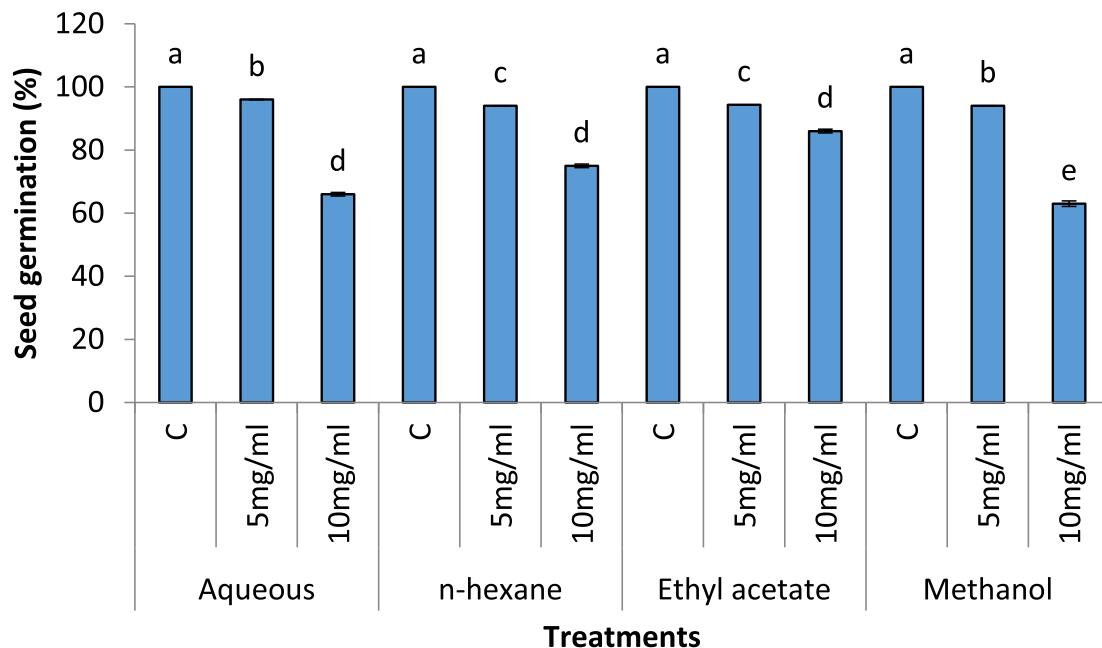


Fig. 1. Effect of different concentration of aqueous, *n*-Hexane, Ethyl acetate and Methanolic extract on seed germination (%) of *Raphanus sativus*.

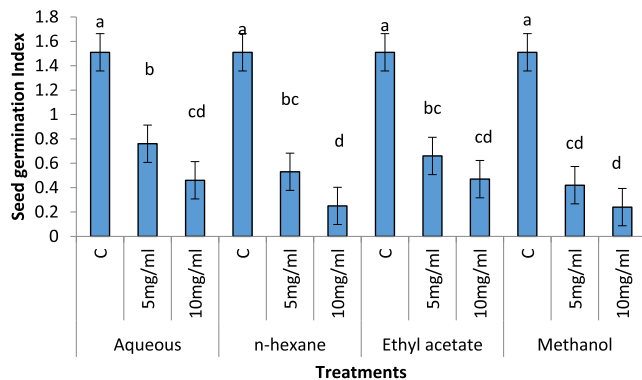


Fig. 2. Effect of different concentration of aqueous, *n*-Hexane, Ethyl acetate and Methanolic extract on seed germination index of *Raphanus sativus*.

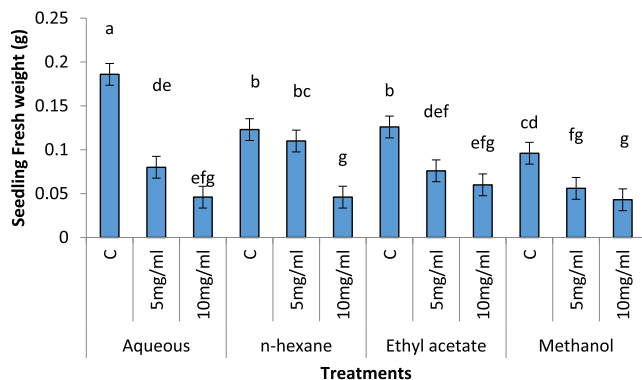


Fig. 3. Effect of different concentration of aqueous, *n*-Hexane, Ethyl acetate and Methanolic extract on fresh weight of *Raphanus sativus*.

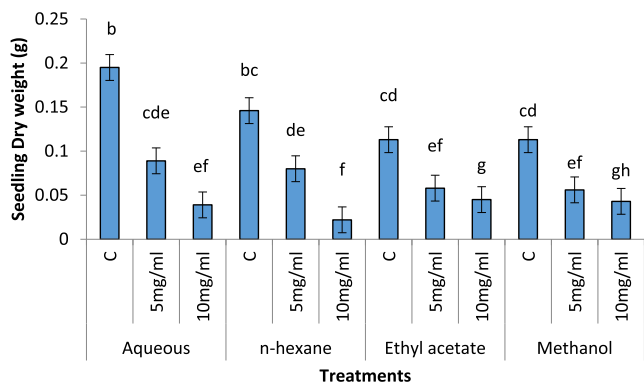


Fig. 4. Effect of different concentration of aqueous, n-Hexane, Ethyl acetate and Methanolic extract on dry weight of *Raphanus sativus*.

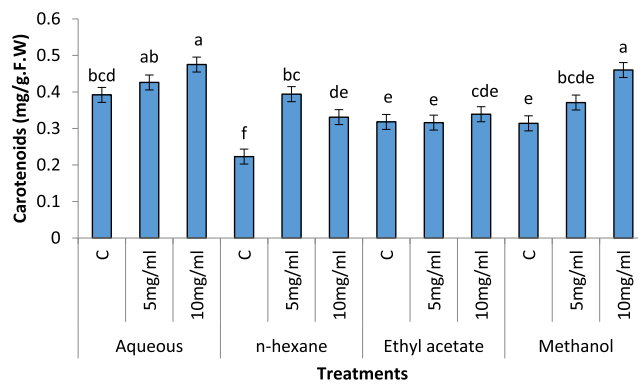


Fig. 7. Effect of different concentration of aqueous, n-Hexane, Ethyl acetate and Methanolic extract on Carotenoids content of *Raphanus sativus*.

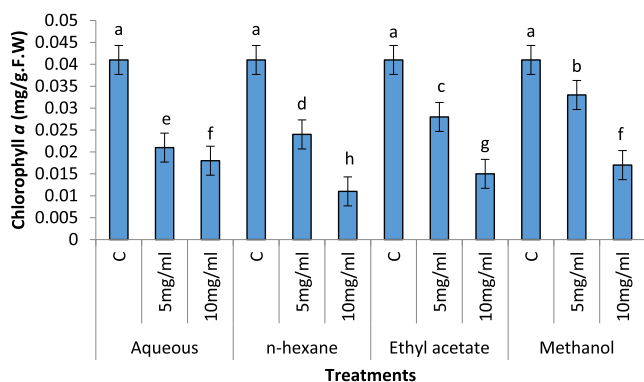


Fig. 5. Effect of different concentration of aqueous, n-Hexane, Ethyl acetate and Methanolic extract on Chl a content of *Raphanus sativus*.

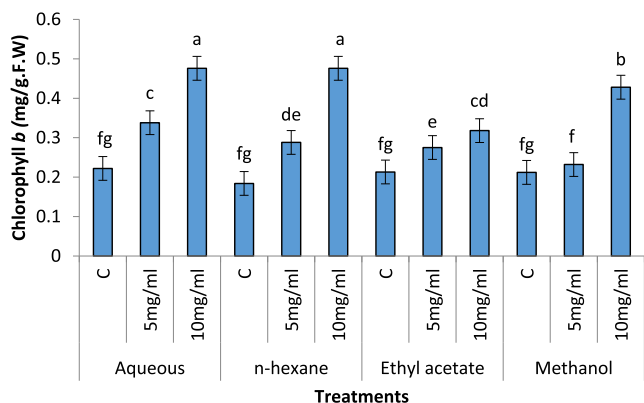


Fig. 6. Effect of different concentration of aqueous, n-Hexane, Ethyl acetate and Methanolic extract on Chl b content of *Raphanus sativus*.

10 mg/l) caused a tremendous increase in chl b content of radish seedling. The 10 mg/mL of the aqueous extract remained more significant as compared to 5 mg/mL. Carotenoids contents of radish seedling significantly increased in 10 mg/mL of aqueous, n-hexane, ethyl acetate and methanolic extract of *C. glaucum* (Fig. 7). However 5 mg/mL of the extract remained non-significant in aqueous, methanol and ethyl acetate and remained significant in n-hexane extract.

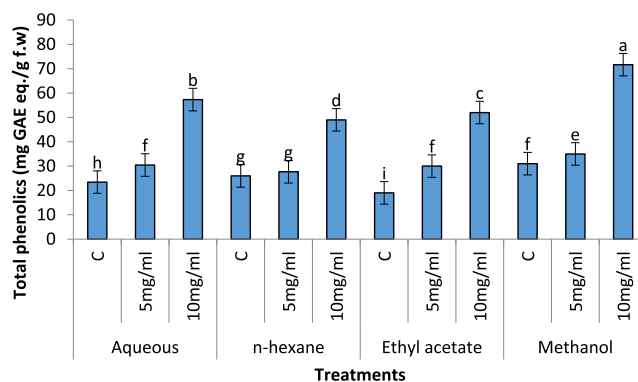


Fig. 8. Effect of different concentration of aqueous, n-Hexane, Ethyl acetate and Methanolic extract on total phenolics contents of *Raphanus sativus*.

5.4. Phytotoxicity of *C. Glaucum* extracts on total phenolics contents of radish seedling

Increase in phenolics contents of radish seedlings was observed with increasing concentration of the extracts (Fig. 8). Aqueous extract of *C. glaucum* in both the concentration (5 mg/mL and 10 mg/mL) significantly increased the phenolics contents of radish seedling. The 5 mg/mL of the extract of n-hexane remained insignificant while 10 mg/mL of the extract significantly increased the phenolics contents of radish seedling. Ethyl acetate and methanolic extract in both the concentrations (5 mg/mL and 10 mg/mL) also significantly increased the phenolic contents of radish seedling.

5.5. Powder microscopy

Crude powder drugs are adulterated with low quality of powder. Correct identification of the powder drug is necessary. Powder microscopy is a useful technique for identification of powder drug. Stem powder microscopy of *C. glaucum* showed the presence of calcium oxalate crystals. Broken pieces of trichome were also present in the powder. Fragments of cork cells and cortex cells were also present in the powder. Starch granules, phloem fibers and lignified xylem vessels with annular thickening were present as shown in (Fig. 9).

5.6. Microscopic study of abaxial surface of *C. Glaucum* leaf

Microscopic evaluation of leaf abaxial surface is a useful anatomical tool used for accurate identification of a plant species.

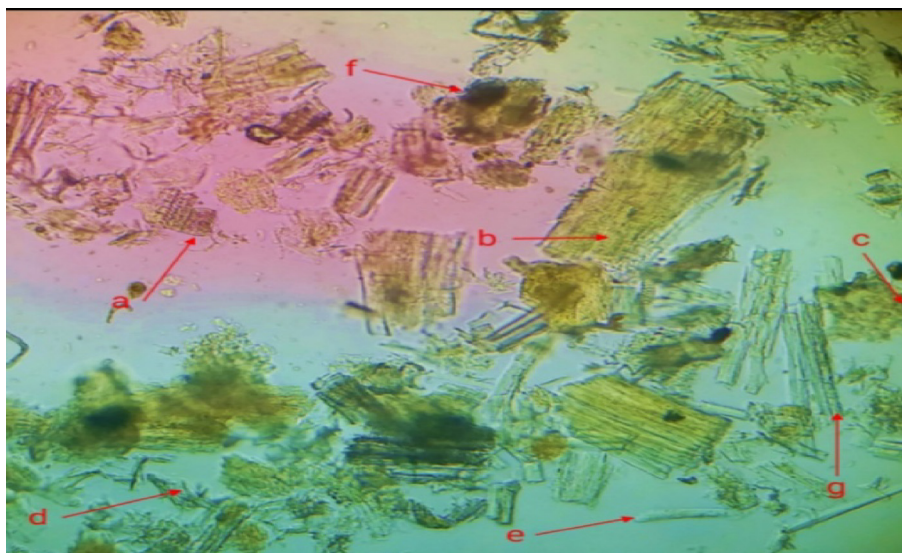


Fig. 9. Powder microscopy of *C. glaucum*. (a) Annular xylem vessels (b) Fragment of cork cell (c) Cortex cells (d) Trichome (e) Calcium oxalate crystals (f) Starch granules (g) Phloem fibers.

Microscopic study of abaxial surface of leaf showed the presence of anisocytic type of stomata, surrounded by subsidiary cells (Fig. 10).

5.7. Stomata density and stomata size of *C. glaucum* leaf

The stomatal density of *C. glaucum* was calculated in three different field of views. Results showed that stomatal density in three different field of views was 560/mm², 568/mm² and 584/mm² with a mean density of 570.66/mm² (Table 4). Size of stomata was measured 25.017 μm through image j software.



Fig. 10. Microscopic study of leaf stomatal number and density of *C. glaucum*. Anisocytic stomata (b)- Subsidiary cells (c)- epidermal cells.

Table 4
Stomatal density (S_b) of lower surface of leaf of *C. glaucum*.

Sample name	Magnification (ocular × objectives)	Surface	FOVs	Number of stomata in entire FOV	Stomatal Density Stomata/mm ²
<i>C. glaucum</i> .	400x	Lower	1	70	560
			2	71	568
			3	73	584
Mean					570.66

FOV means field of view.

6. Discussion

Various groups of phytochemicals such as flavonoids, alkaloids, anthraquinone, cardiac glycosides, tannins, terpenoids and saponins were found in the extracts of *C. glaucum*. A variety of biological activities like antimicrobial, antioxidant, anti-inflammatory, antiplasmodial, anticancer and phytotoxic activities are reported for these phytochemicals (Malaver et al., 2019; Wang et al., 2022a, 2022b). This study confirmed that methanolic extract was rich in alkaloids and condensed tannins which was also highly phytotoxic on growth of radish. These results are in accordance with those of Hashmi et al., (2021) that tannins and alkaloids were higher in methanolic extracts of *Lepidium pinnatifidum*.

The results showed that phytotoxic effect of *C. glaucum* extracts (aqueous, *n*-hexane, ethyl acetate and methanol) on radish are concentration dependent. Among the various extracts methanolic and ethyl acetate extracts were more phytotoxic than aqueous and *n*-hexane extracts which can be credited to their phytochemicals composition. Plant species *Euphorbia wallichii* and *Bergenia ciliata* enriched in tannins showed greater phytotoxicity in comparison to those with lower concentration of tannins (Ullah et al., 2015). Similarly, Khan et al. (2011) and Lopez-Rodríguez et al. (2022) reported that phytotoxicity of plants was closely related with their tannins and alkaloids content. The reduction in germination percentage and delayed germination by methanolic extract could be due its effects on rate of imbibition, delayed initiation of germination, cell division and elongation (Black, 1989; Ullah et al., 2014; Yan et al., 2022). Germination is hindered as the radicle penetrates the seed coat (Koodkaew et al., 2018; Dordevic et al., 2022). Toxic substances from the aqueous leaf extract of different plant species inhibited the seed germination of *Alfa alfa* (Chon et al., 2004). Chon et al., (2003) reported several phytochemicals

from Lettuce plant extract as the causative agents of seed germination inhibition. The greatest inhibition of seed germination was observed in methanol extract of *Lactuca sativa* (Chon et al., 2005). Studies as reported by Pérez et al. (2015) showed that phytotoxicity of plants was significantly correlated with their secondary metabolites content. Moreover, Uddin et al. (2007) reported that primary effects of phytotoxins on target plant was their inhibition of radical elongation.

Our study proved that *C. glaucum* methanolic and *n*-hexane extracts were highly phytotoxic on seedling growth of radish. This indicated that phytochemicals occurring in these extracts are phytotoxic in nature and can be exploited for testing as botanical herbicides. In radish phytotoxicity assay seedling fresh and dry weight was reduced by a number of medicinal plants in which *Woodfordia fruticosa* methanolic extract was most effective when applied at 10 mg/mL concentration (Khan et al., 2011).

The reduction in photosynthetic pigments of radish seedlings may be due to the stress of phytochemicals. The reduction in photosynthetic pigments of plants by phytochemicals have been reported (Huang et al., 2010; Ismail and Siddique, 2011; Han et al., 2012; Wang et al., 2022a). The reduction in Chl *a* and increase in Chl *b* may be due to damage of pigments systems by the extracts. In stress condition damage of photosystem I and an increase of Chl *b* around photosystem II has been mentioned (Kitajima and Hogan, 2003; Wang et al., 2022b). Phenolics compounds also showed increasing trends with increasing concentration of the extracts which may be due to the increased stress induced by the high concentration of the phytochemicals.

While analyzing mineral composition it was found that Fe content of *C. glaucum* is high (250 mg/100 g) as compared to the value recorded for highly medicinal plant *Ipomoea batatas* (16 mg/100 g) (Antia et al., 2006). The Zn value is also suitable as compared to early reported values of vegetables (Ibrahim et al., 2001). This indicated that *C. glaucum* as source of botanical herbicide could be beneficial for crop plants if tested extensively for its selective effects against weeds. Moreover, the various extracts of *C. glaucum* could be potential source of various kinds of minerals for human consumption.

Standardization and quality control of herbal drugs is necessary because adulteration is found in market herbal medicines. Microscopic analysis is among the cheapest of methods to correctly identify the particular herbal drug in powder form (Akbar et al., 2014; Uza & Dastagir, 2022). Powder microscopy and anatomical features provide useful knowledge about quality control of medicinal powder drugs (Nisar et al., 2021). Therefore, we have established standards for controlling adulteration in this plant powder for future use. Powder microscopy of different and highly medicinal plants like *Jasminum sambac* and *Nigella sativa* has been reported (Gowdhami et al., 2015; Rashid et al., 2018).

7. Conclusions

The *C. glaucum* possessed useful phytochemicals such as flavonoids, alkaloids, terpenoids, tannins, saponins, and cardiac glycosides. Moreover, its various extracts particularly methanolic and *n*-hexane were highly phytotoxic on radish and could be exploited for testing as botanical herbicides against noxious weeds. The elemental analysis revealed that *C. glaucum* is rich in zinc and iron increasing its value for human consumption as well. Powder microscopy showed useful structures that can be helpful in correct identification and quality control of this plant species. Findings of this study indicated the possible use of *C. glaucum* extracts as a source of natural herbicides in weed management. Moreover, further studies are recommended for the isolation of novel compounds from this plant species as herbicides.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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