International Journal of Environmental Studies and Researches (2023), 1(3) : 9-27

Phytochemistry and Biological Attributes of *Datura innoxia*

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Abstract

Herbal medicine is an imperative portion of both traditional and modern medicines. Medicinal plants have been used in healthcare since time immemorial. This study evaluated the phytochemical constituents and allelopathic potentiality as well as the anticancer activity of *Datura innoxia* Mill. Plant extracts were prepared to carry out a phytochemical screening. D. innoxia contains alkaloids, flavonoids, phenolics, terpenoids, saponins and amino acids which are mainly responsible for the biochemical and pharmacological activity resulting in high antioxidant activity of the plant. Germination and growth bioassays were conducted on two important crops (*Vicia faba and Triticum aestivum*) as recipient species. D. innoxia exhibited a strong allelopathic potential suppressing the initial growth and several growth parameters of the two recipient plants. The study inspected cytotoxic effects using four carcinoma cell lines. Plant ethanolic extract showed high anticancer activity especially against MCF-7. This study demonstrated that D. innoxia has high antioxidant activity, valuable potentiality as an anticancer agent as well as herbicidal activity against harmful weeds.

Keywords: Phytochemistry; Allelopathy; Anticancer potential; Antioxidant activity.

Introduction

Medicinal plants have been utilized in medications and healthcare all over the world. Several researches have been estimated for verification of their effectiveness which have managed to the manufacturing of plant-based remedies (**El-Darier *et al*., 2001; El-Darier and Abdelhady, 2017**). Plant bioactive constituents can be used directly as healing agents and also as raw materials for the manufacturing of drugs as models **(Cinelli and Jones, 2021)**.

Datura is a genus of medicinal herbs belongs to Solanaceae that is accredited with poisonous as well as medicinal properties. The different species of genus *Datura* especially *D. innoxia* exhibited several potent biological, medicinal and pharmacological activities which are referring to the occurrence of widespread varieties of bioactive ingredients which have been consumed possibly to heal plentiful of humanoid illness **(Kpaka *et al*., 2021; Sharma *et al*., 2021)**. **Ali *et al*. (2022)** reported that *D. innoxia* is an invasive species affected the native dominant naturalized species in several sites as it inhibited their seedling germination and reduced the weight as well as the length of seedlings due to its allelopathic effect that

results from the production of many phytochemical constituents as exudates in the natural environment. Cancer is still a major health issue and cause high mortality. There are numerous efforts all over the world have been growing exponentially to control cancer comprising several techniques as chemotherapy and radiotherapy. So, the progression of significant and highly effective chemical and biotechnological medications with fewer side effects is very essential **(Zhang *et al.*, 2018; Sung *et al.*, 2021)**. However, the development of new drugsfrom natural sources with fewer side effects becomes a promising field in cancer research. Plants are still an auspicious reservoir for a novel chemical compounds in this area **(Javed *et al.*, 2017; Rashed *et al.*, 2022)**. This study was carried out to evaluate the phytochemical constituents, antioxidant activity and allelopathic potential as well as the anticancer activity of *D. innoxia* growing in ruderal habitats.

The study species

Datura innoxia is a stout, erect annual to perennial herb to up to 2 m high, with swollen taproot, and a spreading crown up to 2m in diameter. Stems have dense and spreading glandular hairs **(Ibrahim and Al-Gifri, 2016).** Mature leaves broadly ovate and the lamina are up to 20cm long; almost entire or irregularly lobed towards the base. Inflorescence has solitary and bisexual flowers in the bifurcations of the branches; while peduncles are about 10mm and stout. Flowers have a pleasant smell and are found in 4 distinct colors which are yellow, red, violet or greenish-white **(Srivastava et al., 2012).** Calyx is persistent and very prominent; it is 5-10cm long, narrowly cylindrical and 3-6 lobed. Lobes are 13-20 mm long and sometimes incompletely separated **(Traore et al., 2019).** Corolla is white with green veins and a tube which is 15-16cm long. Stamens have anthers which are 8-10mm long. Style is 10-14 cm long and stigma is below anthers. The fruit is globose or ovoid spiny capsule with numerous slender spines, about 3-5 cm in diameter; spines are numerous, slender, sharp and all about are equal in length; 10 mm long **(Benabderrahim et al., 2019)**. The breaking of capsule occurred irregularly when ripe, releasing brown seeds which have D-shaped and 4-5 mm long asdemonstrated in **Plate 1 (Sell and Murrell, 2009).**

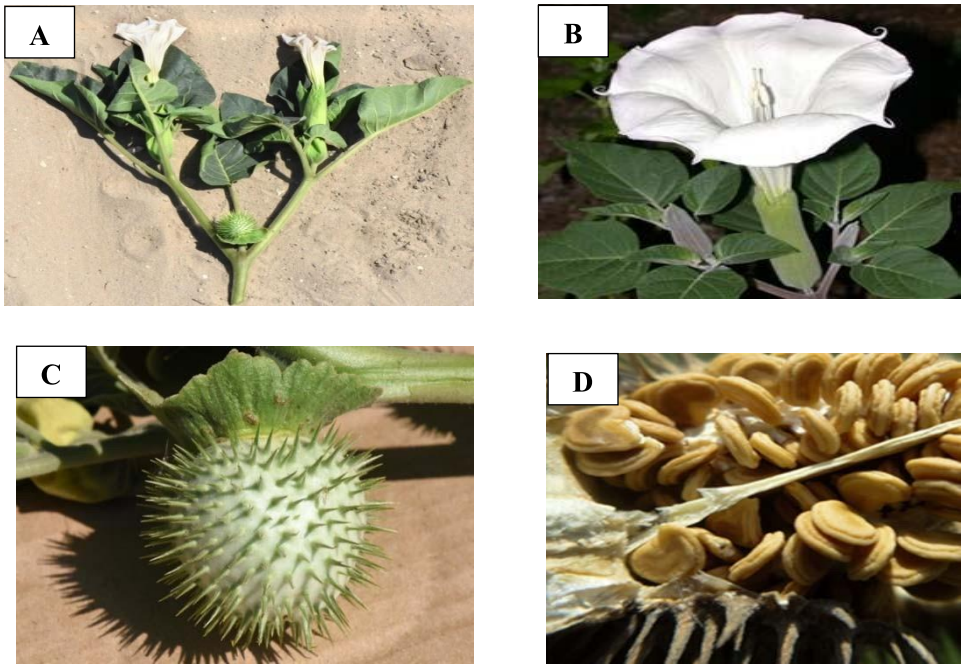

Plate 1. *Datura innoxia* Mill., **A**: Morphology of the whole plant, **B**: Morphology ofthe flower, **C**: Morphology of the fruit and **D**: Morphology of plant seeds. (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:316945-2).

Materials and methods

Datura innoxia shoots were collected from different locations at Nubaria region (65 km south west of Alexandria), Egypt and composite samples were organized in order to carry out the present study.

Phytochemical screening

Plant samples of *D. innoxia* were washed and dried in an oven at 45°C, then ground to a fine powder. The plant extracts (aqueous and ethanolic) were prepared according to **Sasikala *etal*. (2014**). Fine powder of shoots was soaked in sterile distilled water (10% w/v) separately in Erlenmeyer flask to prepare the aqueous extract. The flasks were placed on orbital shaker for 24 hours for extraction process. The extract was then filtered using Whatman No.1 filter paper and stored in labeled sterile bottles and kept at 4°C. On the other hand, ethanolic extract was prepared by soaking 200 g of powder into 96% ethanol for 48 hours at room temperature on orbital shaker then dried under a reduced pressure at 40°C using rotary evaporator. The dried extract was stored in sterile bottles until further use **(Baoduy *et al*., 2015)**. Then, qualitative phytochemical screening was performed; as following: carbohydrates, proteins, amino acids, phenols, saponins and alkaloids were detected according to **Rajendrabhai (2017);** tannins and terpenoids were distinguished according to **Ramya *et al*. (2019);** while, flavonoids were identified according to **Baoduy *et al*. (2015).** In the quantitative screening; protein content was determined according to **Horwitz (2000),** saponins according to **Majinda (2012),** alkaloids andflavonoids according to **Khalifa *et al*. (2017)** and finally determination of phenols was according to **Hussain *et al*. (2011).**

Determination of antioxidant activity (DPPH)

Methanolic extract was prepared by soaking 5 mg of powder in 5ml 80% methanol overnight then filtered and the filtrates were completed up to 5ml with 80% methanol. **Bandoniene *et al.* (2002)** evaluated the antioxidant activity of phenol extracts through the utilization of the stable 2, 2-diphenyll–picrylhydrazyl radical (DPPH). As the radical scavenging activities of the examined samples, expressed as percentage inhibition of DPPHand calculated according to the formula recommended by **Molyneaux (2004)**:

$$A = (A\ \text{control} - A\ \text{sample})/A\ \text{control} * 100$$

Where, $\mathbf{A}_{\mathbf{control}}$ is the absorbance at 515nm of the blank sample at time t =0 min and $\mathbf{A}_{\mathbf{sample}}$ is the final absorbance of the test sample at 515 nm.

Preparation of *Datura innoxia* shoots aqueous extract (DISAE) for germination and growth bioassays

Two hundred grams of powdered shoots were soaked in one liter of distilled water for 24 hrs. The solution was filtered to make different concentrations DISAE (0.5, 2, 4, 8, 16, and 32%) as well as the control (distilled water) for germination experiment. On the other hand, shoots of the donor species (DISP) were applied to the recipient species [*Vicia faba* L. cv. Nubaria 1 (Fabaceae) and *Triticum aestivum* L**.** cv. Misr 1 (Poaceae)] as a powder for growthexperiment **(El-Darier *et al*., 2018)**.

Germination bioassay

Ten seeds from each recipient species were arranged in 9-cm diameter petri-dishes separately on disc of Whatman No.1 filter paper under normal laboratory conditions with day temperature ranging 25-30°C and night temperature ranging 20-25°C. Ten mL of DISAE (0.5, 2, 4, 8, 16, and 32%) and distilled water as control were added every two days to three replicates for 6 days. The Petri-dish lid was removed just at initiation of seed germination and seedlings emergence. Seeds were considered to be germinated when their radicle was nearly 2 mm. The radicle and plumule lengths were measured using a common ruler. The germination percentage (**GP**) was calculated according to the general equation:

GP= Number of germinated seeds/total number of seeds X 100

Growth bioassay

The soil applied for cultivation was sterilized for three days in the oven at (90º C), and then 750 g of this soil was placed in each pot with addition of 1, 2, 4, and 8% of DISP and the distilled water (as control) every two days to three replicates. Twenty seeds of each of the recipient species were planted in each plastic pot separately. The pots were well exposed to sunlight and were irrigated with every two days with approximately 200 ml of water. After thirty-four days, the homogenous seedling were carefully collected then washed with tap water. The samples were separated into shoots and roots for determination of growth parameters; seedling fresh and dry weights as well as shoot and root lengths.

Extraction and estimation of chlorophyll and carotenoid contents

Chlorophylls and carotenoids were determined according to the method described by **Lichtenthaler *et al*. (1987)**. One hundred mg fresh weight of *V. faba* and *T. aestivum* leaves were soaked in 5 mL of di-methyleformamide in dark over night for complete extraction. Absorbance was recorded at 646.8 nm and 663.2 nm for chlorophylls assay and 453 nm for carotenoids assay (in the supernatant) by a UV-Visible spectrophotometer (JENWAY, 6305, UK). The content of pigments was calculated according to the following formulae:

Chlorophyll *a* = (12.25 A 663.2– 2.79 A 646.8),

Chlorophyll *b* = (21.21 A646.8– 5.1 A 663.2)

Carotenoids = 4.2 A 453 - (0.0264 Chlorophyll a + 0.426 Chlorophyll b).

Estimation of anti-cancer activity

Datura innoxia shoots ethanolic extract (DISEE) was tested using the method of **Skehan *et al*. (1990)** at the National Cancer Institute, Cairo, Egypt by serial sub culturing. Four human carcinoma cell lines were used in the current study; A549 (Lung carcinoma), MCF-7 (Breast carcinoma), HepG2 (Liver carcinoma) and HCT116 (Colon carcinoma). Growth inhibition percentage (**GIP**) was calculated according to the general equations estimated by **Mosmann (1983)**:

GIP= [100- (Treated survival cells /control cells) * 100]

Statistical analysis

Some data of the present study were subjected where appropriate to standard one-way analysis of variance (ANOVA). Other data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Shapiro-Wilk test was used to verify the normality of distribution Quantitative data was described using mean, standard deviation and standard error. Significance of the obtained results was judged at the 5% level. F-test (ANOVA) was used for normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Duncan) for pairwise comparisons **(Kirkpatrick and Feeney, 2013).**

Results

Phytochemical Composition and Antioxidant Activity (DPPH)

Table 1 and 2 showed the qualitative and quantitative phytochemical screening of *D. innoxia*. Notably, all the examined phytochemicals mentioned before were present in the ethanolic extract of the study species except steroids were absent. Carbohydrates contribute with the highest value followed by ash content. In addition, fat, protein and moisture achieved moderate records. Furthermore, the concentration level of total phenolics exhibited higher values in both aqueous and ethanolic extracts, but the total flavonoids that were achieved the lower values in both extracts. Whereas the data in Table 3 presented the variation of *D. innoxia* DPPH scavenging activity (%) in response to the different concentrations of methanolic shoot extract.

Table 1. Qualitative phytochemical screening (presence-absence) of *Datura innoxia* leaves ethanolic extract.

Constituent	Presence-Absence
Alkaloids	+
Flavonoids	+
Amino acids	+
Tannins	+
Saponins	+
Steroids	-
Carbohydrates	+
Terpenoids	+

(Present +, Absent -)

Table 2. Quantitative biochemical composition and phytochemical screening of *Datura innoxia* shoot aqueous and ethanolic extracts.

		Constituent	Concentration
Extract	Aqueous	Ash content [a]	15.59
		Carbohydrates [a]	22.15
		Crude fiber [a]	5.55
		Fat [a]	14.52
		Moisture [a]	11.00
		Protein [a]	12.90
		Atropine [b]	0.83
		Scopolamine [b]	0.04
		Total Phenolic [c]	482.8
		Total Flavonoids [d]	96.9
	Ethanolic	Total Phenolics [c]	452.5
		Total Flavonoids [d]	258.3

a: % b: (µg/mg) c: (mg GAE/g) d: (mg Quercetin/g)

Table 3. Variation in the antioxidant activity (DPPH scavenging activity) (%) of *Datura innoxia* methanolic shoot extract. Values are means ± SD based on triplicate independent determinations and different letters means significant difference as evaluated by Duncan's multiple comparison test.

Concentration (µg/mL)	Antioxidant activity	
	Mean±SD	SE
12.5	17.31^{f} ± 2.03	1.17
25	30.65^{e} ± 2.98	1.72
50	45.31^{d} ± 3.01	1.74
100	67.79^{c} ± 5.02	2.90
200	83.23^{b} ± 3.02	1.74
400	94.32^{a} ± 4.02	2.32
F	228.422*	
P	<0.001*	

SD: Standard deviation SE: Standard Error

F: F for One-way ANOVA test, pairwise comparison between each 2 groups were done using Post Hoc Test (Duncan) p: p value for comparing between the different studied conc.

*: Statistically significant at $p \leq 0.05$

Allelopathic PotentialGermination bioassay

This bioassay was accomplished to evaluate the effect of *D. innoxia* shoot aqueous extract (DISAE) on germination percentage as well as plumule and radicle lengths of the two recipient species.

Germination percentage

As shown in **Figure 1**, After 6 days of sowing; firstly, the GP of *V. faba* recorded the highest value (100%) at concentrations (0.5, 2 and 8%) while the lowest value was estimated at 16 % concentration level. Secondly, the GP of *T. aestivum* improved by increasing the concentration level of DISAE, until it reached the peak value (87%) at concentration of 4%, after that it declined till it achieved the lowermost value (33%) at 16% concentration level.

Plumule (PL) and radicle (RL) lengths (cm)

Figure 2 demonstrates both plumule and radicle lengths of *V. faba*. After 6 days of sowing, the maximum records were achieved for both lengths at concentration 2%. However, the lowest values were recorded for both lengths at 32% concentration level. As well, after 6 days of sowing, plumule length of *T. aestivum* attained the highest value at 4%, after that the length decreased gradually by increasing the concentration till reached the lowest value at 32% concentration level. Furthermore, the radicle length achieved the maximum value at control and then declined progressively by increasing the concentration level of DISAE until reached the minimum value at the highest concentration 32% **(Figure 3).**

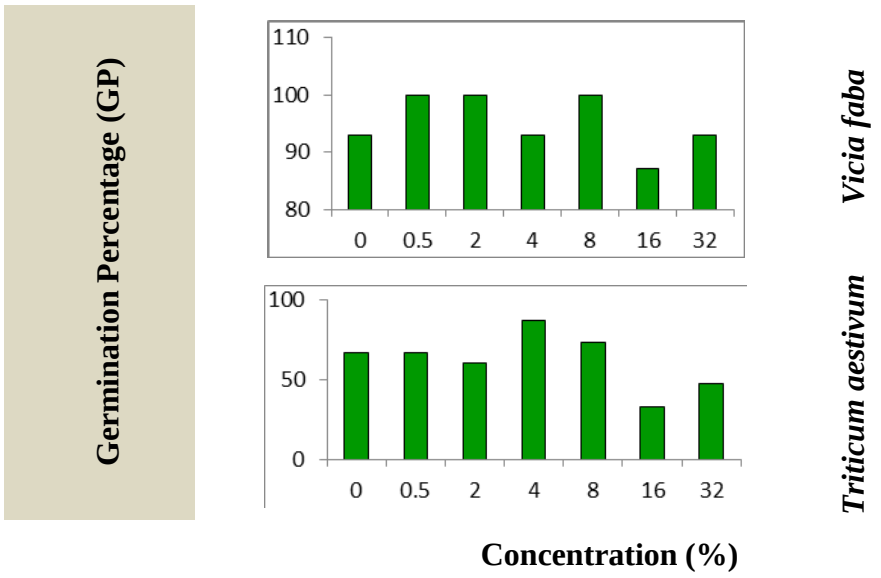

Figure 1. Variation in germination percentage (GP) of *Vicia faba* and *Triticum aestivum* seeds as affected by *Datura innoxia* shoots aqueous extract (DISAE). Data are means of three replicates.

Growth bioassay

Figure 4 and 5 showed the overall growth of the different parts of *V. faba* and *T. aestivum* that were affected by the allelopathic potentiality of *D. innoxia* shoot powder (DISP). For instance, in case of *V. faba*; fresh and dry weights of both shoot and root and also the water content attained the maximum records at concentration 8%. On the other hand, *T. aestivum* fresh and dry weights of both shoot and root exhibited the maximum values at the concentrations level 4%. Consequently, the water contents (g) for shoot and root were attained the maximum values at the concentrations level 4% and control, respectively. As revealed in Figure 6, the lengths of the different parts of *V. faba* and *T. aestivum* were positively affected by the allelopathic potentiality of DISP. The shoot and root were attained the peak average lengths at the concentration level (8%).

Chlorophyll content

Data presented in **Table 4 and 5** showed the effect of different concentrations of *D. innoxia* on the chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *a*+*b*) and (Chl *a/b*) aswell as carotenoid contents of *V. faba* and *T. aestivum*.

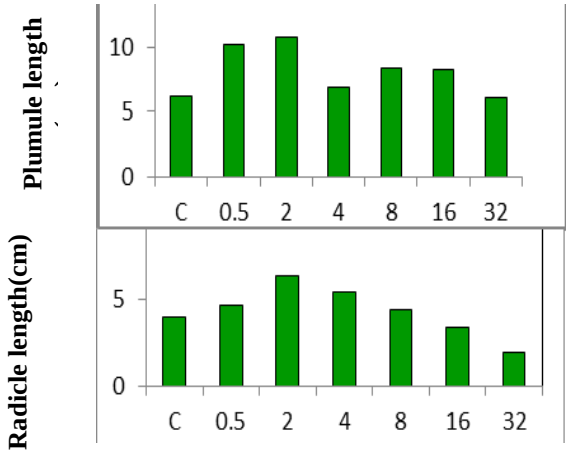

Figure 2. Variation in plumule and radicle lengths (cm) of *Vicia faba* seedlings as affected by *Datura innoxia* shoots aqueous extract (DISAE). Data are means of three replicates.

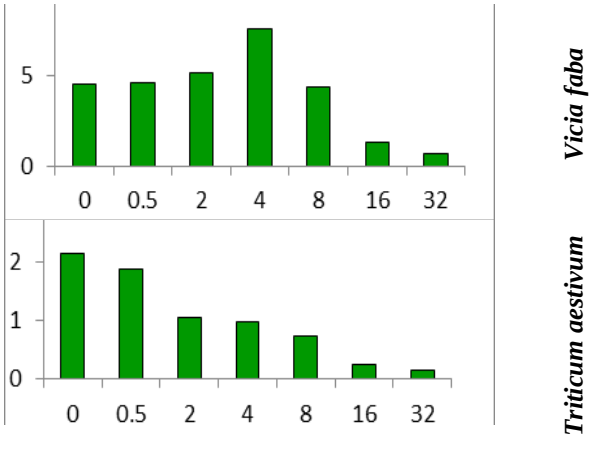

Figure 3. Variation in plumule and radicle lengths (cm) of *Triticum aestivum* seedlings as affected by *Datura innoxia* shoots aqueous extract (DISAE). Data are means of three replicates.

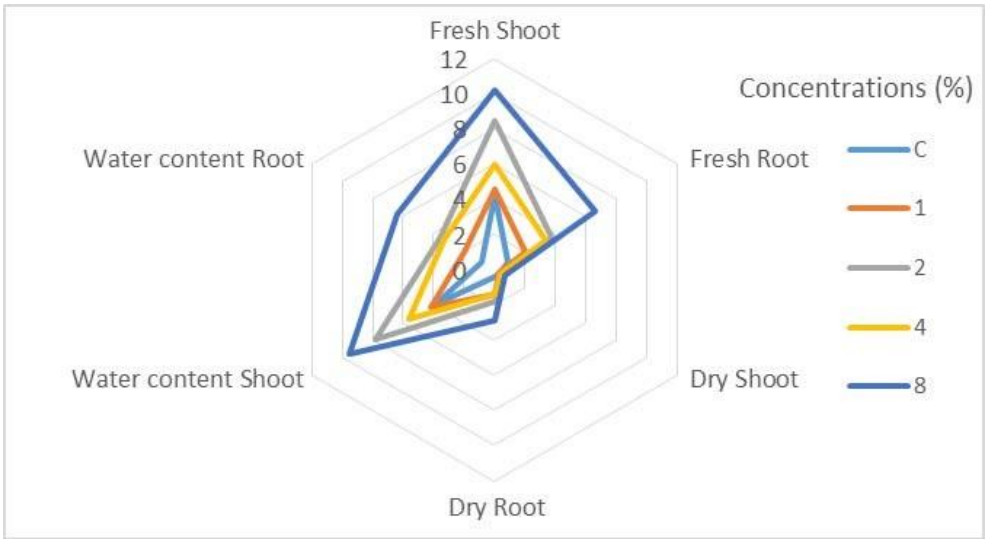

Figure 4. Variation in fresh and dry weights as well as water content (g) of *Vicia faba* seedlings as affected by *Datura innoxia* shoot powder (DISP). Data are means of three replicates.

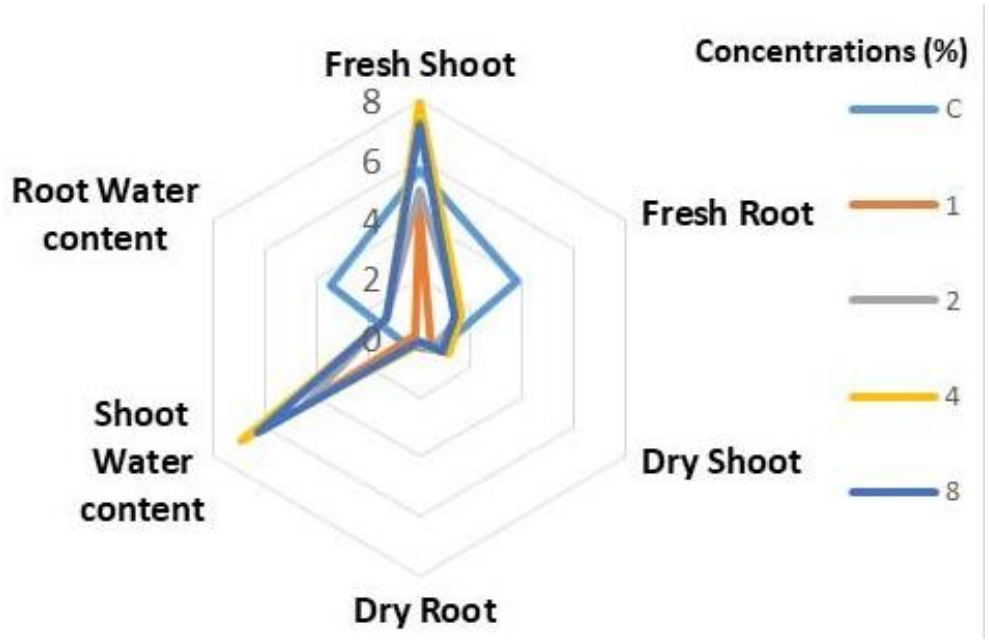

Figure 5. Variation in fresh and dry weights as well as water content (g) (value x10) of *Triticum aestivum* seedlings as affected by *Datura innoxia* shoot powder **(DISP). Data are means of three replicates.**

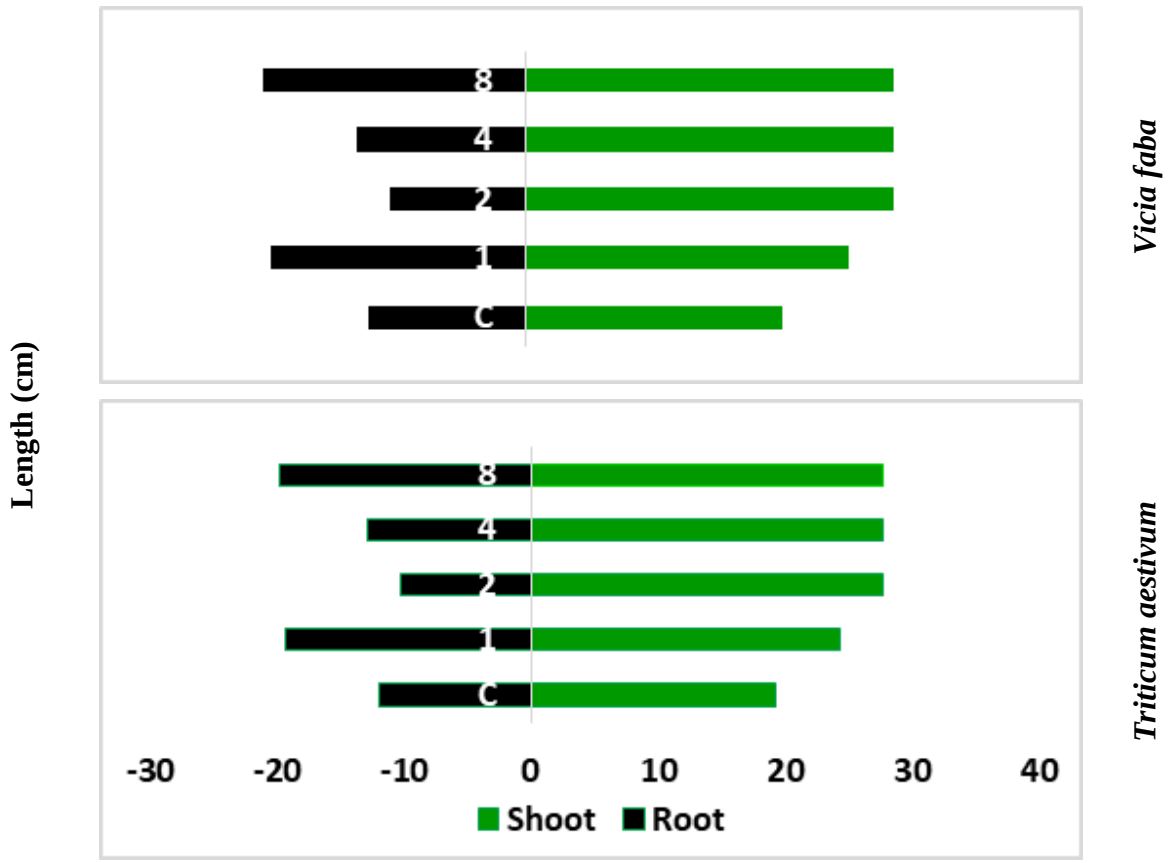

Figure 6. Variation in shoot and root lengths (cm) of *Vicia faba* and *Triticum aestivum* seedlings as affected by *Datura innoxia* shoots powder (DISP). Data are means of three replicates.

Table 4. Variation in chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *a+b*), (Chl *a/b*) and carotenoid contents of *Vicia faba*. Values are means ± SD based on triplicate independent determinations, and different letters between columns indicate significant difference as evaluated by Duncan's multiple comparison test.

	C	1	2	4	8
Chl *a*	$129.0^{a} \pm 7.94$	$116.0^{a} \pm 7.0$	$103.0^{a} \pm 19.08$	$143.0^{a} \pm 24.58$	$116.0^{a} \pm 10.39$
Chl *b*	$35.0^{a} \pm 6.08$	$37.0^{a} \pm 14.73$	$37.0^{a} \pm 11.27$	$46.0^{a} \pm 12.77$	$43.0^{a} \pm 11.0$
Chl *a+b*	$164.0^{b} \pm 14.73$	$153.0^{bc} \pm 12.12$	$140.0^{c} \pm 12.49$	$190.0^{a} \pm 10.0$	$160.0^{bc} \pm 0.0$
Chl *a/b*	$37.0^{a} \pm 6.08$	$31.0^{a} \pm 0.0$	$28.0^{a} \pm 6.08$	$31.0^{a} \pm 9.0$	$27.0^{a} \pm 6.08$
Carotenoids	$31.0^{a} \pm 8.54$	$30.0^{a} \pm 0.0$	$27.0^{a} \pm 5.57$	$33.0^{a} \pm 1.0$	$26.0^{a} \pm 5.20$

Table 5. Variation in chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *a+b*), (Chl *a/b*) and carotenoid contents of *Triticum aestivum*. Values are means ± SD based on triplicate independent determinations, and different letters between columns indicate a significant difference as evaluated by Duncan's multiple comparison test.

	C	1	2	4	8
Chl *a*	77.0^{b} ± 20.42	211.0^{a} ± 16.46	212.0^{a} ± 8.0	205.0^{a} ± 19.0	197.0^{a} ± 49.87
Chl *b*	18.0^{b} ± 4.36	69.0^{a} ± 26.85	68.0^{a} ± 19.70	76.0^{a} ± 31.43	60.0^{a} ± 0.0
Chl *a+b*	95.0^{b} ± 29.82	280.0^{a} ± 26.46	280.0^{a} ± 43.59	281.0^{a} ± 51.10	256.0^{a} ± 19.29
Chl *a/b*	43.0^{a} ± 2.0	31.0^{a} ± 0.0	31.0^{a} ± 4.58	27.0^{a} ± 11.27	33.0^{a} ± 11.36
Carotenoids	20.0^{b} ± 0.0	33.0^{a} ± 8.54	34.0^{a} ± 6.08	28.0^{ab} ± 4.36	36.0^{a} ± 3.46

Anti-proliferative assay

The *in vitro* cytotoxic activity of *D. innoxia* shoots ethanolic extract (DISEE) was determined on Lung (A549), Breast (MCF-7), Liver (HepG2) and Colon (HCT116) carcinoma cell lines. The half-maximal inhibitory concentration IC50 value (concentration of active compound needed to reduce the cell viability to 50%) was determined from dose-response curves constructed for SRB method of percent growth inhibition against test concentrations. Accordingly, the obtained results in Figure 7 indicated that the incubation of *D. innoxia* significantly inhibited the cell proliferation of (MCF-7) with IC50= 9.8 µg/mL. However, the cell proliferation of HepG2 and HCT116 cell lines were moderately suppressed with IC50 values 10 and 36.5 µg/mL, respectively. While, (A549) exhibited the lowest inhibition record with IC50= 50µg/mL.

Growth inhibition percentage

The effect of DISEE was studied as a dose-response experiment after 48 hours at concentrations of 12.5, 25, 50 and 100µg/mL. The proliferation of A549, MCF-7, HepG2 and HCT116 carcinoma cell lines were significantly inhibited by DISEE in a concentration-dependent manner in 48 hrs. with more than 80% suppression at concentration 100% **(Figure 8).**

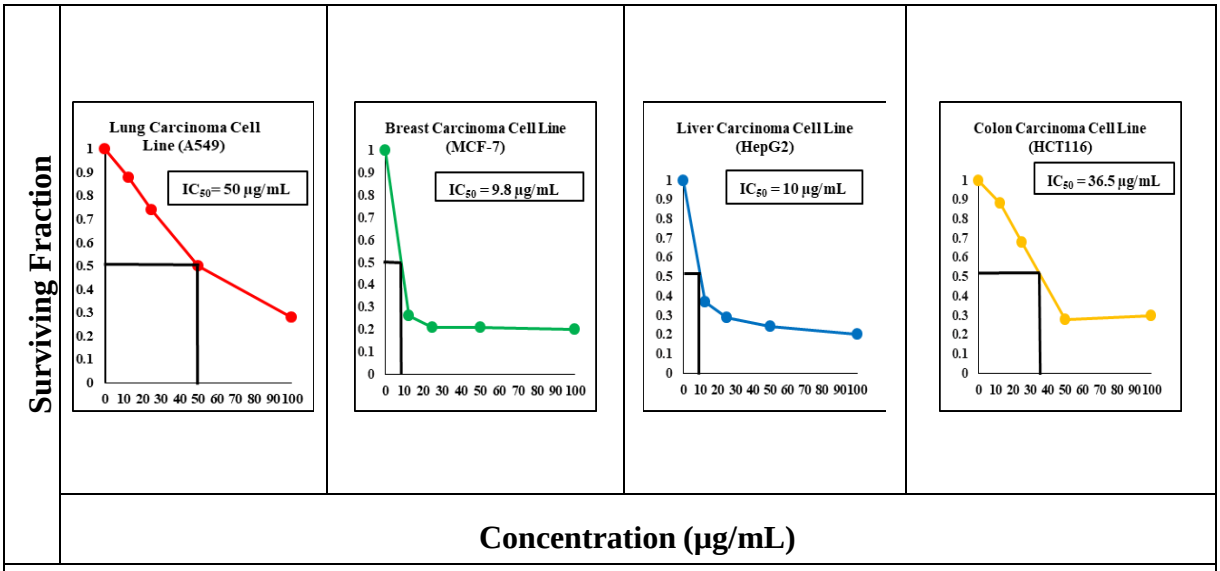

Figure 7. Effect of different concentrations of *Datura innoxia* shoots ethanolic extract (DISEE) on Lung (A549),Breast (MCF-7), Liver (HepG2) and Colon (HCT116) carcinoma cell lines.

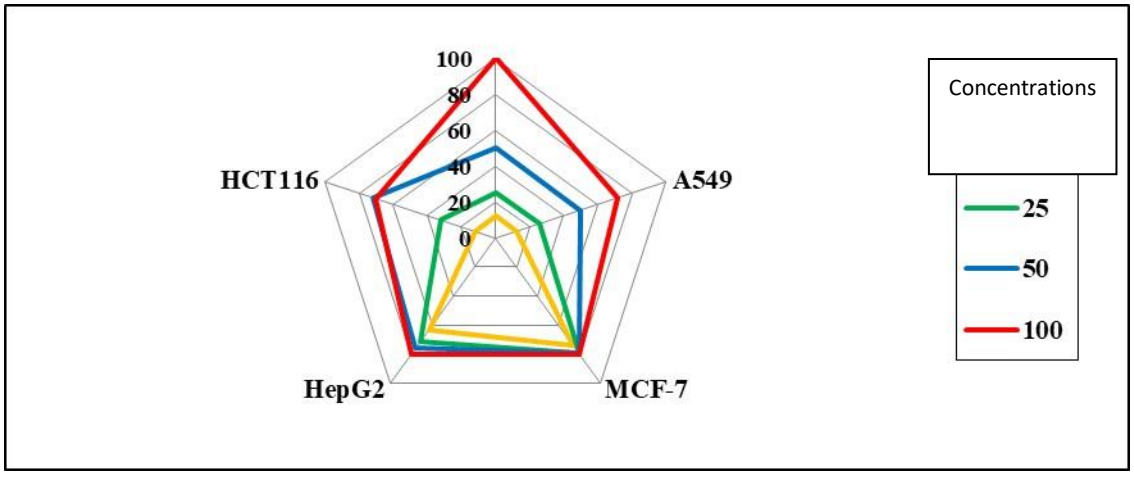

Figure 8. *In vitro* cytotoxicity of *Datura innoxia* shoots ethanolic extract (DISEE) against four human cell lines in the SRB assay using radar method on Lung (A549), Breast (MCF-7), Liver (HepG2) and Colon (HCT116) carcinoma cell lines.

Discussion

Medicinal plants are reservoir of diverse biological active constituents that exert therapeutic properties and also play vital roles in growth and development of plants as well as they affect the diverse physiological processes by inhibition or stimulation **(Mukim *et al*., 2022)**. **Sharma *et al*. (2021)** demonstrated that *Datura innoxia* is one of these medicinal plants. This study also revealed the screening and quantification of phytochemicals of both aqueous and ethanolic shoot extract of *D. innoxia* which showed relatively the same constituents of fat, protein, moisture, and crude fiber but with higher amounts for carbohydrates and ash content. Compared with the current study; carbohydrates contributed with the highest value followed by the ash content while fat, protein and moisture achieved moderate values. **Togola *et al*. (2019)** performed DPPH test to elucidate the scavenging activity of leaves and seeds ethanolic extracts. It was proved that leaves extract possessed higher antioxidant activity than seeds; this may have ascribed to the high levels of total flavonoids and phenolic compounds in the leaf that are twice as high as those of seeds. Because of the relatively antioxidant activity of *D. innoxia,* it can be utilized as a prospective source of natural antioxidants. In the current study *D. innoxia* DPPH scavenging activity (%) of shoot increased by increasing the plant extract concentration.

El-Darier (2002) defined allelopathy as an old and natural phenomenon that can be very operative in techniques of weed control and nowadays it attained the interest of researchers all over the world. Allelochemicals can stimulate or inhibit seed germination and seedling growth of plants and this is considered as prospective approach in drug industries and production of eco-friendly bio-herbicides. **Zheng and Li (2008)** proved that low concentrations of *D. innoxia* extract (<0.10 mg/mL) can stimulate the seed germination of *Zea mays* and the germination is inhibited by increasing the concentration. The present study declared that after six days of sowing the highest inhibition was achieved by applying 16% extract concentration. However, it was found that the highest GP was attained for *V. faba* at 0.5, 2, and 8% concentrations; while for *T. aestivum* at 4%. **Mahmoud (2015)** stated that there was a significant difference in germination of both *Vicia faba* and *Triticum aestivum* against *Amaranthus cruentus*, *Sinapis arvensis*, *Sisymbrium irio* and *Sonchus oleraceus* weed extracts concentrations 2.5, 5, 10 and 20%, with no significant difference in germination compared to the control at lowest concentration (2.5 %); these results are in consistence with those obtained by **Edrisi and Farahbakhsh (2011)** who recognized that *Sisymbrium irio* water extract had a great inhibitory effect on germination of *T. aestivum*. Moreover, the highest extract concentration (20%) provided germination nil in both *A. cruentus* and *S. oleraceus* in *T. aestivum* as well as *S. oleraceus* in *V. faba*. Thus, it was to be reported that extract concentrations was inversely proportional to germination. **Bonea (2019)** performed a study to assess the allelopathic effect of *D. innoxia* on the germination and initial growth of *Zea mays* and the extract exhibited significant deterioration in germination and seedling growth of *Z. mays*. The inhibitory effect of the extract on germination ranged from 23.2% to 57.1%, on root length- from 19.8% to 92.6% and on shoot length- from 33.2% to 69.6%. In another work, **Bagewadi *et al*. (2019)** examined the effect of *D. innoxia* when it was grown in mixed cultures with herbaceous spp. including *Pennisetum americanum*, *Brassica campestris* and *Setaria italica* in pot and field trials. Aqueous extracts from *D. innoxia* had also an inhibitory effect on seed germination and germination percentage of all the recipient species. Radicle growth of the recipient species was also suppressed by the volatiles of *D. innoxia*. In laboratory tests, volatiles from detached shoots of *D. innoxia* inhibited germination of *P. americanum* only but inhibited radicle growth of *B. campestris*, *S. italica* and *P. americanum*. Additionally, the extract of *D. innoxia* reduced the fresh and dry weight of all recipient species.

In the same manner, the current study proved that *D. innoxia* has an inhibitory effect on the seedling growth of *V. faba* and *T. aestivum*. For instance; the root and shoot lengths of both recipient species were affected negatively by applying different concentrations of DISAE. In case of *V. faba*; the inhibitory effect of the extract on root length achieved 21% and on shoot reached 76%. Whereas, in case of *T. aestivum*; the plumule length increased firstly by increasing the concentration level of DISAE as it attained the highest value (7.66 cm) at 4%, after that the length decreased gradually till reached the lowest value (0.67 cm) at 32%. However, the radicle length was inhibited by increasing the concentrations of DISAE and the inhibitory effect of the extract on root length achieved 13%. Additionally, the shoot and root fresh and dry weights of *V. faba* were positively affected by *D. innoxia* shoot powder (DISP). Consequently, the water content also was not negatively affected by DISP. While, *T. aestivum* was harmfully affected by DISP. For the shoot and root of *T. aestivum*, at first by increasingthe concentration of DISP they were increased then they were decreased gradually. Therefore, the water contents exhibited the same manner.

El-Darier and El-Mogaspi (2009) suggested that production of alkaloids can be considered as plant defense mechanism against microorganisms, insects, mammals or any other enemy; this is concerned in allelopathy. Current surveys recommended that these compounds could interfere with other plants and cause disruption of its biological processes which is analogousto one more usually associated with animals **(Levitt and Lovett, 2012)**. **Shi *et al*. (2022)** reported that *D. innoxia* has a crucial role in suppressing the seed germination and plant growthof weeds and against several plant diseases as well as insects. **Gniazdowska and Bogatek (2005)** reported that allelochemicals released by plants possess significant effects on plant photosynthesis and chlorophyll content by blocking its biosynthesis **(Singh and Chaudhary, 2011)**.

Leaf chlorophyll content has been used as one of the elemental parameters in understanding the response of the plant to the environmental stress in which it inhabits**.** Therefore, it is likely that healthy plants which are able to grow well are also expected to have larger quantity of chlorophyll than unhealthy ones **(Schlemmer *et al.*, 2005; Wu *et al.*, 2008). Elisante *et al*. (2013)** and **Glab *et al*. (201**7) showed the effects of *D. innoxia* on several growth parameters of grass and legume species (*Cenchrus ciliaris* and *Neonotonia wightii*). Several parameters such as leaf chlorophyll content root and shoot lengths as well as fresh and dry weights were investigated. It was demonstrated that the total chlorophyll content was significantly reduced. Thus, high inhibitory effects were exhibited at higher concentrations particularly in 100% concentration. The root and shoot lengths, fresh and dry weights of both test species were significantly reduced by aqueous extracts of *D. innoxia* (seed and leaf). This is accomplished with results of the current study, as chlorophyll and carotenoids contents of *V. faba* and *T. aestivum* were negatively affected by *D. innoxia* shoot aqueous extracts.

Cancer is still a major health issue, and the incidence of cancer related deaths is increasing around the globe **(Bray *et al.*, 2018)**. Around the world, the most commonly diagnosed typesof cancer are colorectal, lung and breast that are related to death **(Sung *et al.*, 2021)**. Plants are still a promising reservoir for a novel chemical compound in the cancer research area and production of new anticancer drugs such as paclitaxel and camptothecins **(Rashed *et al.*, 2022)**.

Al-Zharani *et al*. (2021) examined the cytotoxic abilities of *D. innoxia* ethanolic extract against colon (A549), colorectal (LoVo), breast (MCF-7) and M.D. Anderson - Metastatic Breast 231 (MDA-MB-231) cancer cell lines, thereafter half maximal inhibitory concentration (IC50) values were calculated for each cell line. After 48 h treatment, the values

were obtained by using a dose-response inhibition curves. It was evidenced that ethanolic extract of *D. innoxia* was cytotoxic against all treated cell lines, while LoVo colon cancer cell line was the most sensitive with IC50 value (10µg/mL).

In the current work, *D. innoxia* was ethanolic extracted to attain the maximum amount and diversity of biologically active phytochemicals. The *in vitro* cytotoxic activity of *D. innoxia* shoots ethanolic extract exerted a cytotoxic effect on various carcinoma cell lines; lung (A549), breast (MCF-7), liver (HepG2) and colon (HCT116). Then, IC50 values were determined from dose-response curves. The results estimated that the cell proliferation of all cancer cell lines were significantly inhibited with lowest IC50 values ranged from 9.8 µg/mL (in case of MCF- 7) to 50 µg/mL (in case of A549). DISEE had high potent activity against breast (MCF-7) cell line.

The medicinal abilities of *D. innoxia* extract could be primarily accredited to its secondary metabolites, that perform synergistically rather than as a single compound **(Al-Zharani *et al.*, 2021)**. As above-mentioned, *D. innoxia* extract contains a combination of several phytochemical compounds that exhibit anticancer effects, so it can be concluded that the anticancer effects observed in the extract are associated with the presence of these compounds.

In conclusion, the phytochemical screening of *D. innoxia* showed the existence of imperative pharmacological bioactive constituents that resulting in high antioxidant activity. These constituents incorporated in the high anticancer potentiality of *D. innoxia* against severalhuman cancer types especially breast cancer MCF-7. *D. innoxia* has strong allelopathic potential which suppressed the seed germination percentage, the initial growth and several growth parameters of two recipient plant species *Vicia faba* and *Triticum aestivum* that are considered as very crucial economic crops. Finally, the study recommended exposing an integrated *D. innoxia* management strategy to stop further spread of this alien weed into the cultivated areas by maximum exploitation of its uses.

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