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Ethno-phytotechnology of *Tribulus terrestris* L. (Zygophyllaceae)

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ETHNO-PHYTOTECHNOLOGY combines ethnobotany and biotechnology. This study evaluated the ethnobotanical role, anticancer potential, and allelopathy of *Tribulus terrestris* L. The ethnobotanical survey of twenty informants used an open-ended questionnaire. *T. terrestris* contains steroids, saponins, antioxidants, flavonoids, alkaloids, phenolics, proteins, and amino acids. The study investigated cytotoxic effects using six carcinoma cell lines. *Hordeum vulgare* and *Lepidium sativum* were used as recipient species in the allelopathy experiments. We found that 95% of the informants stated that *T. terrestris* is an aggressive species that injures livestock, reduces biodiversity, leads to soil dryness, consumes large amounts of space during the vegetative season, and affects soil pH and the absorption of minerals. Ethanolic extracts produced a significant effect on the prostate (**PC3**), breast (**MCF 7**), lung (**A549**), and liver (**HEP-G2**) carcinoma cell lines, with IC_{50} values of 19, 22, 33, and 33µg/mL, respectively. The intestinal carcinoma cell line (**CAco2**) had an IC_{50} 60µg/mL. The colon (**HCT**) carcinoma cell line had an IC_{50} value of 68 µg/mL. Water extracts inhibited the seed germination, plumule length, radicle growth, and fresh and dry matter production of the recipient species. This study demonstrated that *T. terrestris* is potentially valuable as an anticancer agent and an herbicide against harmful weeds.

Keywords: Allelopathy, Anticancer potentiality, Ethnobotany, Phytochemistry, *Tribulus terrestris*.

Introduction

The association between ethnobotany and biotechnology is termed ethno-phytotechnology (de la Parra & Quave, 2017). Ethnobotany can be defined as the scientific study of traditional botanical information and has led to identifying effective remedies (Faruque et al., 2018). The production, manipulation, and characterization of such remedies have been greatly improved by technological developments and analytical instrumentation (Atanasov et al., 2021).

Ethnobotany is an important aspect of ethnoecology, dealing with plants. Recently, there has been considerable interest in plants' consumption and methods for their preservation and management. Although multiple studies have advocated the advantages of participatory research in ethnoscience, few have provided solid contributions from case studies involving residents in all project phases (Rodrigues et al., 2020). Gaoue et al. (2017) reported that most ethnobotanists understand the interactions between humans and the environment and the use of plant resources to meet their cultural and physical requirements.

Most current methods for treating cancer include a combination of approaches, including chemotherapeutic agents, surgery, radiotherapy, and hormone therapy (Wang et al., 2018; Dewangan et al., 2019; Zhang et al., 2019; Yeh et al., 2020; El-Benhawy et al., 2021). Rashed et al. (2022) suggested that traditional medicine plays a valuable role in cancer treatment.

Cheng & Cheng (2015) defined allelopathy as a normal biological phenomenon in which one organism produces biochemicals that impact

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other organisms' growth, existence, progress, and reproduction. Kato-Noguchi & Kurniadie (2021) studied allelochemicals that have effects that may be favorable or unfavorable on neighboring organisms.

The genus *Tribulus* contains 25 species, various members of which are poisonous to grazing animals (Dighe et al., 2020). According to Boulos (2000) the genus *Tribulus* is represented by nine species in Egypt. *Tribulus terrestris* is a green-appressed-hairy annual with prostrate-branched stems, branched striate leaves, yellow flowers, and fruit 1.5–2.5cm in diameter. Each mericarp has four thick rigid spines. In traditional medicine, *T. terrestris* is well known to treat various diseases in many countries. It can be applied directly or used as the main ingredient in many medicines and food supplements (Hashim et al., 2014). Dighe et al. (2020) stated that the plant is used for healing purposes such as strengthening, nutrition, rejuvenation, diuretic, anti-inflammatory, or aphrodisiac agent, and renal calculi.

This study was carried out to assess the ethnobotanical role of *T. terrestris* and assess its anticancer potential and herbicidal activity to determine its allelopathic effects.

Materials and Methods

Ethnobotanical studies

Ethnobotanical data were collected during four field trips in 2020, from the Nubaria region, Egypt, by collecting information using the "open-ended" interview technique, based on a structured questionnaire in which specific questions were asked of a sample of adults more than 50 years old. The questionnaire included 36 questions in Arabic, asking the respondents to describe the plant in terms of its location, the parts used medicinally, methods of administration, and the extent of its impact on the neighboring vegetation. Informants gave extensive responses to a series of general questions. Some of them had been prepared in advance, and some arose naturally during the conversation. Sometimes, interviews were held with a local expert or key informants who had a profound knowledge of a particular aspect of local culture. These approaches, often referred to as informal or qualitative methods, yielded responses used to write up general ethnographic accounts of the community and its culture. These responses were coded and categorized before being interpreted. Open-ended interviews are essentially casual conversations that can reveal detailed life histories (Baines & Hviding, 1992).

Phytochemical screening

Shoot samples of *T. terrestris* were washed and dried in an oven at 45°C, then ground to a fine powder. To prepare *T. terrestris* shoot ethanolic extract (TTSEE), 200g of the powder was macerated in 1 L of 99.6% denatured ethanol for 24 h at room temperature, filtered, and then dried using a rotary evaporator under reduced pressure. According to Sasikala et al. (2014), qualitative and quantitative phytochemical screening was carried out to estimate the presence of saponins, alkaloids, phenolics, flavonoids, carbohydrates, protein, amino acids, glycosides, tannins, and terpenoids.

Estimation of anti-proliferative activity

TTSEE was tested using the method of Skehan et al. (1990) at the National Cancer Institute, Cairo, Egypt, by serial subculturing. The current study used six human cancer cell lines in the current study: A549 (Lung carcinoma), MCF 7 (Breast carcinoma), Hep-G2 (Liver hepatocellular carcinoma), CAco (Intestinal carcinoma), PC3 (Prostate carcinoma), and HCT (Colon carcinoma).

Growth inhibition percentage (GIP)

The growth inhibition percentage (GIP) was calculated according to the general equations of Mosmann (1983):

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

Preparation of T. terrestris shoot aqueous extract (TTSAE) for germination bioassays

One-hundred and fifty grams of shoot powder were soaked in 1L of distilled water for 24h. The solution was filtered to make different concentrations: 0.5, 1, 2, 4, 8, and 16%. Distilled water was used as a control (El-Darier et al., 2018).

Ten seeds of each recipient species (*Hordeum vulgare*; Poaceae and *Lipidium sativum;* Brassicaceae) were arranged in 9 cm diameter Petri dishes separately on discs of Whatman No.1 filter paper under normal laboratory conditions, with day temperatures ranging from 25°C to 30°C and night temperatures ranging from 20°C to 25°C. In the case of a mixed culture, five seeds from each recipient species were arranged in the same dish under the same conditions.

Ten milliliters of TTSAE from the different concentrations, and distilled water, were added every two days to three replicates for 15 days. Seeds were considered to have germinated when their radicle was nearly 2 mm in length. The radicle and plumule lengths were measured using a common ruler. The germination percentage (GP) was calculated according to the general equation:

Number of germinated seeds/total number of seeds X 100

Growth bioassays

In this experiment, shoots of *T. terrestris* were applied to the two recipient species as a powder (El-Darier et al., 2018). The soil was sterilized for three days in an oven at 90°C, and then 1000g of soil was placed in each pot. The powder was added to the soil at percentages 0.5, 1, 1.5, 2, 2.5, and 3% every two days, with three replicates of each treatment. Untreated plants were used as a control. After 21 days, the seedlings' fresh and dry weights, and shoot and root lengths, in both pure and mixed cultures were recorded.

For a pure culture, twenty seeds of a single recipient species were planted in each plastic pot, while for a mixed culture, ten seeds of each recipient species were planted in the same pot. The pots were exposed to sunlight and irrigated with approximately 200mL of water every two days. After thirty-four days, the seedlings were carefully collected, washed with tap water to remove adherent soil particles, and gently blotted with filter paper. The samples were separated into shoots and roots to determine some growth criteria for each individual; shoot length (cm), root length (cm), seedling fresh weight (g), and seedling dry weight (g). To determine dry weight, the samples were placed in an oven at a temperature of 55°C until they reached a constant weight.

Statistical analysis

One-way analyses of variance (ANOVA) were used to assess the effect of the ethanolic extract on six carcinoma cell lines, six human cell lines, and of the aqueous extract of *T. terrestris* on the GP, radicle length, and plumule length in pure and mixed cultures of *Hordeum vulgare* and *Lepidium sativum* (Kirkpatrick & Feeney, 2013). Pairwise comparisons of means were performed using Least Significant Differences at the 0.05 probability level.

Results

Ethnobotanical survey

The data in Table 1 indicate that all informants recognized *T. terrestris*, and knew it to be a summer annual. Among the informants, 35% confirmed its hepatoprotective effect, 30% its diuretic activity, 25% its removal of kidney stones, and 20% its analgesic and aphrodisiac actions and enhancement of sex hormones. Fifteen percent saw it as an antihypertensive, stomachic, and urinary antiseptic, and antibacterial herb, and 10% recognized it as having antitumor and immunomodulatory activity. Furthermore, 95% of the informants stated that *T. terrestris* could injure livestock and reduce plant biodiversity.

Phytochemical screening

Tables 2, 3 and Fig. 1 show that *T. terrestris* had high concentrations of total proteins and amino acids, phenolics, steroids, and antioxidants, while saponins and flavonoids were present at lower levels, followed by alkaloids.

Estimation of anti-proliferated activity

The *in vitro* cytotoxic activity of *T. terrestris* shoot ethanolic extract (TTSEE) was determined in lung (**A549**), colon (**HCT**), prostate (**PC3**), breast (**MCF 7**), liver (**HEP-G2**), and intestine (**CAco2)** carcinoma cell lines**.** The half-maximal inhibitory concentration (IC_{50}) value was determined from dose-response curves of percent growth inhibition against test concentrations. Visual observations indicated that the viability of cancer cell lines was reduced in a dose-dependent manner with extract concentration.

The extract showed a significant effect on prostate (**PC3**), breast (**MCF 7**), lung (**A549**), and liver (**HEP-G2**) carcinoma cell lines with IC_{50} values of 19, 22, 33, and 33µg/mL, respectively. However, the IC_{50} of the extract on the intestinal carcinoma cell line (**CAco2**) was 60µg/mL. The colon (**HCT**) carcinoma cell lines had IC_{50} values of about 68µg/mL (Fig. 2).

Growth inhibition

As shown in Fig. 3, the effect of TTSEE was studied after 48 hours at concentrations of control, 12.5, 25, 50, and 100µg/mL. The proliferation of the **PC3**, **MCF 7**, **A549** and **HEP-G2** carcinoma cell lines was significantly inhibited by TTSEE in a concentration-dependent manner over 48 h, with more than 75% suppression. In the case of the **CAco2** carcinoma cell line, proliferation started to be inhibited when the concentration of TTSEE reached 60%, with 40% suppression. TTSEE showed 32% suppression on the colon carcinoma cell line **(HCT).**

TABLE 1. The ethnobotanical description of *T. terrestris* in the Nubaria region, collected using an open-ended questionnaire in 2020

Informant code	Associated crops	Effects		Medicinal uses
		Beneficial	harmful	
1	Maize, cantaloupe, watermelon	-	Affects associated crop by drying the soil	Diuretic, to remove kidney stones
2	Abandonment fields	Fire ignition	-	Immunomodulatory, antibacterial
3	Cantaloup, Maize, and watermelon	-	Affects associated crop by drying the soil	Antihypertensive, Stomachic
4	Maize and cantaloup	-	Lead to soil dryness, affects the soil pH and absorption of minerals	Diuretics, enhance sex hormones
5	Maize and Squash	Fire ignition	Lead to soil dryness	To remove kidney stones
6	Maize	-	Lead to soil dryness	Stomachic
7	Maize, cantaloupe, and Squash	Eaten by cows and camels	Lead to soil dryness, affect the associated plants as it consumes large space to grow and affect the productivity of the associated crops	Antihypertensive, antitumor, antibacterial
8	Maize, cantaloupe, and Squash	-	Lead to soil dryness and affect the associated crop	Stomachic, diuretic, hepatoprotective
9	Garden weeds	Fire ignition	Lead to soil dryness	Enhance sex hormones -
10	Maize	-	Lead to soil dryness and affect the accompanied crop	Analgesic, diuretic, hepatoprotective
11	Maize	Eaten by cows and camels	Soil dryness	Diuretic, urinary antiseptic, to remove kidney stones-
12	Maize, cucumber	-	Soil dryness	Analgesic, hepatoprotective
13	Maize	-	Consume large space during vegetative season	Hepatoprotective, immunomodulatory
14	Cantaloup and tomato	-	Lead to soil dryness and affect associated crops	Hepatoprotective, enhance sex hormones, antitumor,
15	Abandonment fields	Eaten by cows	Lead to soil dryness	Analgesic, to remove kidney stones
16	Maize and fruit trees	-	Absorb minerals of the main cultivated plant	Diuretic, hepatoprotective, urinary antiseptic
17	Cantaloup and pea	-	Lead to soil dryness	Analgesic, to remove kidney stones,
18	Eggplant and cantaloup	Eaten by cows	Lead to soil dryness	To treat some liver disorders
19	Cantaloup, abandonment fields	-	Absorb large amount of water that dries the soil	Aphrodisiac, enhance sex hormones
20	Maize, pea	-	Consume large space during vegetative season	Antihypertensive, antibacterial, urinary antiseptic

TABLE 2. Qualitative phytochemical screening of *T. terrestris* shoots in methanolic extract

Plant sample	Tannins	Total proteins and amino acids	Alkaloids	Cardiac glycosides	Flavonoids	Saponins	Phenolics
T. terrestris	++	+++	++	+	++	++	+++

(+++ Highly present, ++ moderately, + low)

Table 3. Qualitative phytochemical screening: presence (+) and absence (-) of compounds in leaves and fruits of *T. terrestris* in aqueous, methanolic, and ethanolic extracts

Phytochemicals	Leaves extract			Fruit extract		
	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol
Carbohydrates	+	+	-	+	+	-
Amino acids and Peptides	+	+	+	+	+	+
Glycosides	-	+	+	-	+	+
Tannins	+	+	+	+	+	+
Terpenoids	-	+	+	-	+	+
Phenols	-	+	+	-	+	+
Saponins	+	+	+	+	+	+
Alkaloids	-	+	+	-	+	+
Flavonoids	+	+	+	+	+	+

(Present: +, Absent: -)

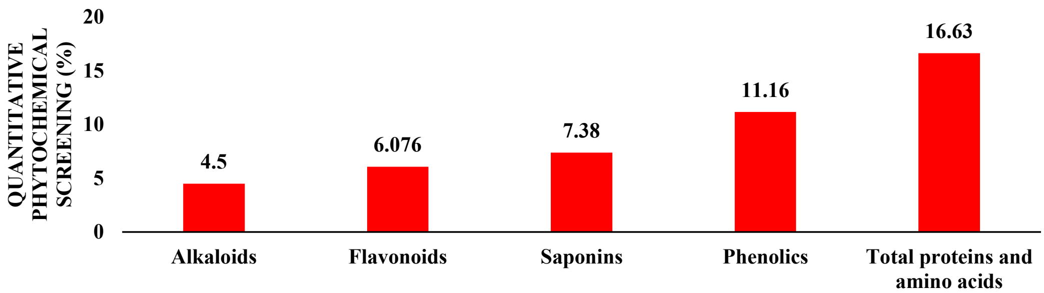


Fig. 1. Quantitative phytochemical screening (%) of *T. terrestris* shoot methanolic extract

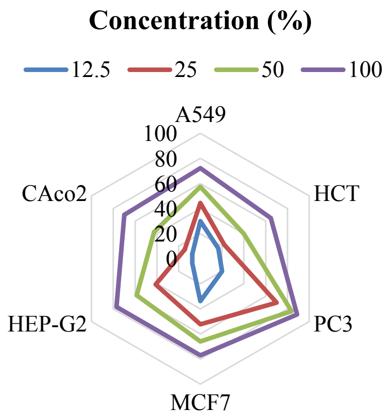
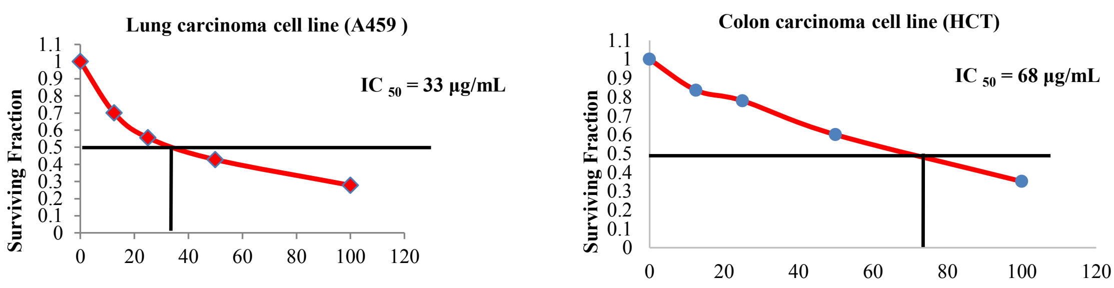


Fig. 2. Effect of different concentrations of *T. terrestris* shoot ethanolic extract (TTSEE) on six carcinoma cell lines



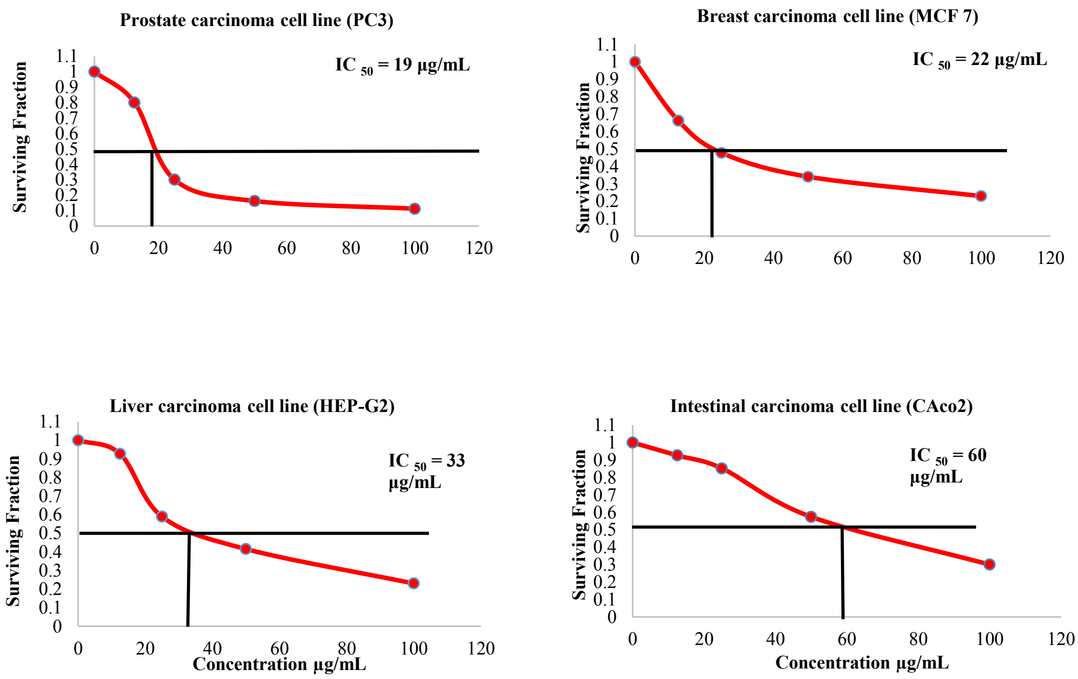


Fig. 3. *In vitro* cytotoxicity of *Tribulus terrestris* shoot ethanolic extracts on six human cell lines using SRB assays with the radar method [A549: Lung carcinoma cell line, MCF 7: Breast carcinoma cell line, Hep-G2: Liver hepatocellular carcinoma cell line, CAco: Intestinal carcinoma cell line, PC3: Prostate carcinoma cell line, and HCT: Colon carcinoma cell line]

A radar chart is also known as a spider chart in Excel or a Web or polar chart. It is used to display two or more data series in two dimensions. The axes start on the same point of the radar chart.

Allelopathic effects

Bioassays were performed to evaluate the effect of TTSAE on GP, plumule, and radicle lengths of the recipient species.

Germination bioassays

One-way ANOVA tests were used to analyze the original data of germination percent, radicle length, and plumule length of the recipient species, *H. vulgare,* and *L. sativum*.

a. *Germination percentages: H. vulgare* in pure culture after 15 days of sowing (Fig. 4), had the highest GP (100%) at a TTSAE concentration of 2%. The GP decreased with increasing TTSAE concentration. In mixed culture (Fig. 5), the highest value of GP (100%) was recorded at 0% concentration of TTSAE (control), and also decreased with increasing TTSAE concentration. For *L. sativum* in pure culture (Fig. 6), the GP was high (100%) at concentrations of 0 and 1% TTSAE, then decreased with increasing concentration. In mixed culture (Fig. 7) GP was 100% at concentrations 0.5, 1, and 2% TTSAE.

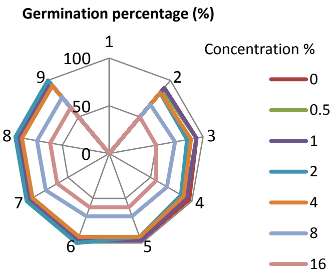


Fig. 4. Variation in germination percentage (GP) of *Hordeum vulgare* seeds as affected by *Tribulus terrestris* shoot aqueous extracts (TTSAE) in pure culture [Data are means of three replicates]

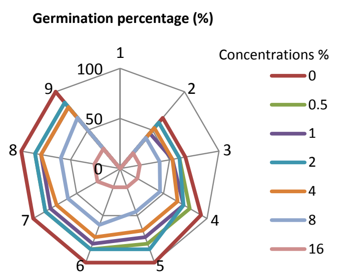


Fig. 5. Variation in germination percentage (GP) of *Hordeum vulgare* seeds as affected by

Tribulus terrestris **shoot aqueous extracts (TTSAE) in mixed culture with *Lepidium sativum*** **[Data are means of three replicates]**

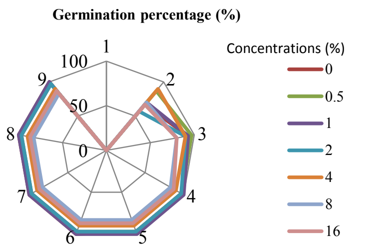


Fig. 6. Variation in germination percentage (GP) of *Lepidium sativum* seeds as affected by *Tribulus terrestris* shoot aqueous extracts (TTSAE) in pure culture [Data are means of three replicates]

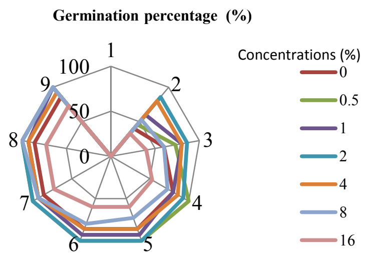


Fig. 7. Variation in germination percentage (GP) of *Lepidium sativum* seeds as affected by *Tribulus terrestris* shoot aqueous extracts (TTSAE) in mixed culture with *Hordeum vulgare* [Data are means of three replicates]

b. *Radicle length*

The radicle length of *H. vulgare* was strongly affected by different concentrations of TTSAE (Figs. 8 and 9). The radicles were longest (6.89 and 4.47 cm) in both pure and mixed cultures at concentrations of 0.5 and 0 % (control), respectively, and then decreased with increasing extract concentrations. The radicle lengths of *L. sativum* were highest (2.45 cm) and (2.98 cm) at the control and 0.5 % in pure and mixed cultures, respectively, and decreased gradually with increasing TTSAE concentrations (Figs. 10 and 11).

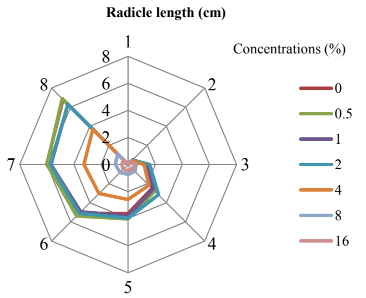


Fig. 8. Variation in radicle length (cm) of *Hordeum vulgare* seedlings as affected by *Tribulus terrestris* shoot aqueous extracts (TTSAE) in pure culture [Data are means of three replicates]

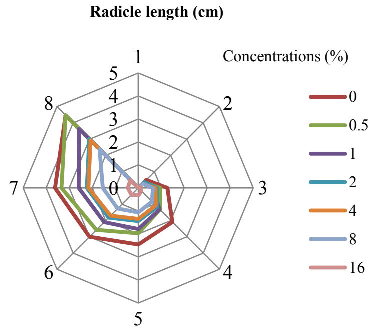


Fig. 9. Variation in radicle length (cm) of *Hordeum vulgare* seedlings as affected by *Tribulus terrestris* shoot aqueous extracts (TTSAE) in mixed culture with *Lepidium sativum* [Data are means of three replicates]

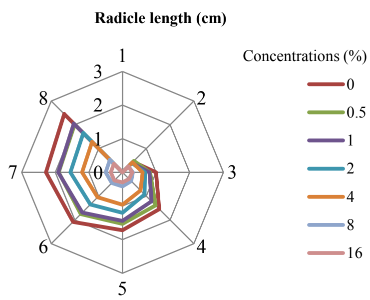


Fig. 10. Variation in radicle length (cm) of *Lepidium sativum* seedlings as affected by *Tribulus terrestris* shoot aqueous extracts (TTSAE) in pure culture [Data are means of three replicates]

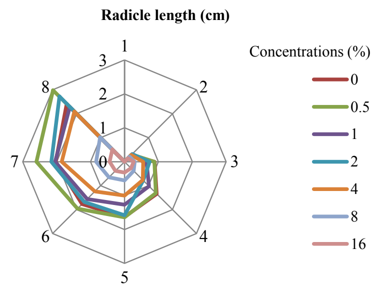


Fig. 11. Variation in radicle length (cm) of *Lepidium sativum* seedlings as affected by *Tribulus terrestris* shoot aqueous extracts (TTSAE) in mixed culture with Hordeum vulgare [Data are means of three replicates]

c. *Plumule length*

The plumule lengths of H. vulgare (Figs. 12 and 13) were significantly affected by different concentrations of TTSAE in both pure and mixed cultures. In pure culture, the lengths increased with increasing TTSAE concentration until they reached 12.01 cm at 2%, after which they decreased gradually. In mixed culture, the length decreased with increasing TTSAE concentrations until it reached the lowest value (1.5 cm) at 16%. In the case of *L. sativum* (Figs. 14 and 15), the plumule length was adversely affected by TTSAE concentration level either in pure or mixed cultures, as it attained the lowest values (0.39 and 0.31 cm, respectively) at the highest concentration (16%).

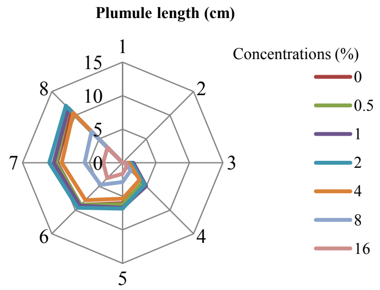


Fig. 12. Variation in plumule length (cm) of *Hordeum vulgare* seedlings as affected by *Tribulus terrestris* shoot aqueous extracts (TTSAE) in pure culture [Data are means of three replicates]

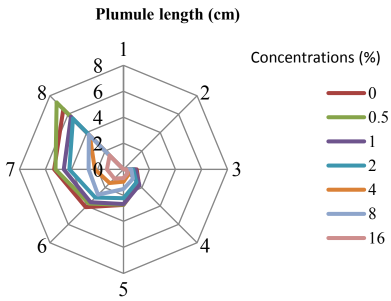


Fig. 13. Variation in plumule length (cm) of *Hordeum vulgare* seedlings as affected by *Tribulus terrestris* shoot aqueous extracts (TTSAE) in mixed culture with Lepidium sativum [Data are means of three replicates]

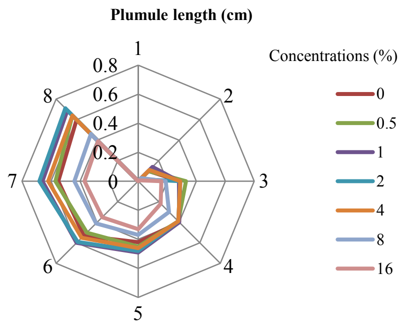


Fig. 14. Variation in plumule length (cm) of *Lepidium sativum* seedlings as affected by *Tribulus terrestris* shoot aqueous extracts (TTSAE) in pure culture [Data are means of three replicates]

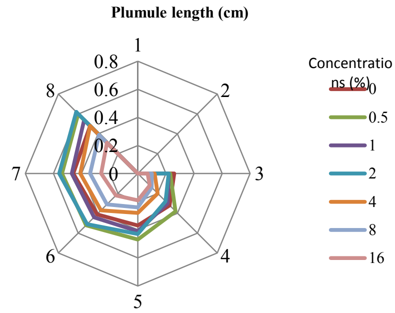


Fig. 15. Variation in plumule length (cm) of *Lepidium sativum* seedlings as affected by *Tribulus terrestris* shoot aqueous extracts (TTSAE) in mixed culture with *Hordeum vulgare* [Data are means of three replicates]

Phytochemistry of the recipient species after treatments with Tribulus terrestris shoot powder (TTSP)

In pure and mixed cultures of *H. vulgare*, the total antioxidant content increased with increasing concentrations of *T. terrestris* shoot powder (TTSP) (Fig. 16). The caffeic and ferulic acid content was high at high concentrations of TTSP. In contrast, phenolics and total flavonoids decreased with increasing concentrations of the extract.

In pure cultures of *L. sativum,* the total antioxidants, (+)-catechin, caffeic, p-coumaric, and ferulic acids, were high at a 2% concentration level of TTSP (Fig. 17). However, the total antioxidants were the highest recorded at a concentration of 1.5% TTSP in mixed culture. Phenolics exhibited variations in their content with the different concentration levels. Even though the total flavonoids in the two cultures were different, they were highest at lower concentration levels of TTSP and decreased with increasing concentration levels.

Discussion

El-Darier et al. (2021) defined traditional medicine as the use of plant-based preparations to maintain well-being, treat, identify, and avoid disease. *Tribulus terrestris* is an annual herb widely distributed in field crops and orchards in the Noubaria region, 60–70km southwest of Alexandria (EL-Darier & Youssef, 2017). Our study confirmed that information about the study species was not indigenous to the study area. Most informants were not born in the region; they came from some governorate of Delta, where the plant species are not distributed. The whole plant was used for its phytochemical and pharmacological effects, such as antihypertensive, antibacterial, diuretic, immunomodulatory, aphrodisiac, anti-urolithic, antihyperlipidemic, antidiabetic, anticancer, anthelmintic, hepatoprotective, analgesic, and anti-inflammatory activities (Chhatre et al., 2014; Tungmunnithum et al., 2018).

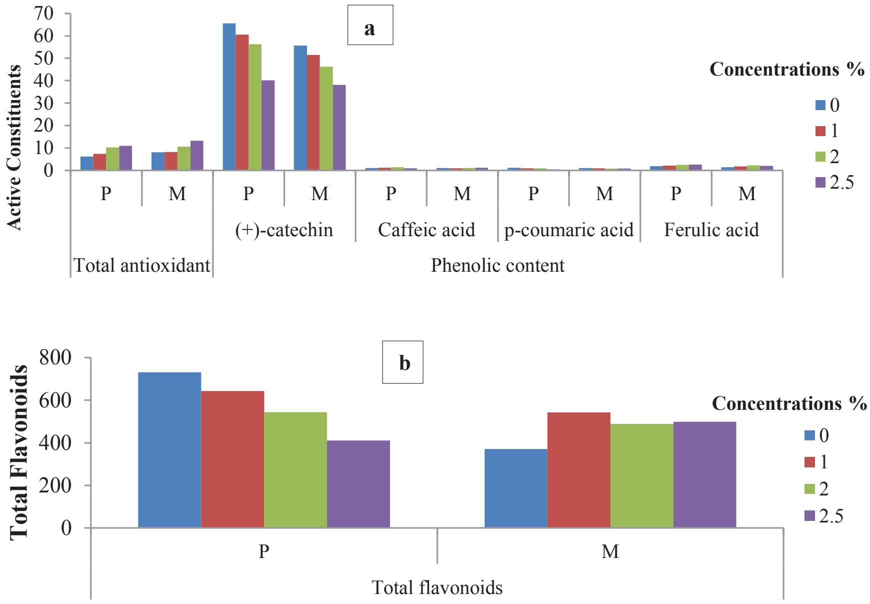


Fig. 16. Allelopathic effect of different concentrations (%) of *Tribulus terrestris* shoot powder (TTSP) on some phytochemical constituents a) total antioxidants, phenolics, and b) total flavonoids of *Hordeum vulgare* in pure (P) and mixed (M) cultures of *Lepidium sativum*

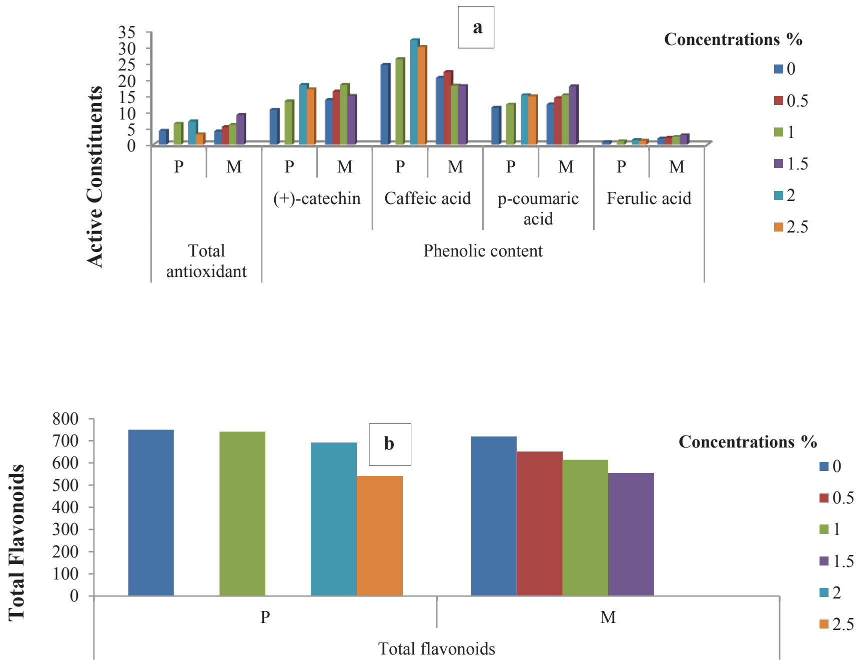


Fig. 17. Allelopathic effect of different concentrations (%) of *Tribulus terrestris* shoots powder (TTSP) on some phytochemical constituents a) total antioxidants, phenolics, and b) total flavonoids on *Lepidium sativum* in pure (P) and mixed (M) cultures with *Hordeum vulgare*

The present study indicated that the allelochemicals of the species, such as steroids, saponins, antioxidants, flavonoids, alkaloids, phenolics, proteins, amino acids, carbohydrates, glycosides, tannins, and terpenoids, could be responsible for its biological activities such as anticancer potential, and its interference with crop plants (allelopathy). Angelova et al. (2013) reported that the pharmacological value of the herb could be dependent upon the quantity of the active ingredients, which diverge significantly dependent on the habitat in which the plant grows, and the part used.

Antitumor research usually aims to discover active constituents that can suppress cancer cells' growth and/or proliferation. New compounds can be identified by studying the antitumor effects of naturally occurring substances in plant extracts or their pure fractions, which have been previously known and used for treatment in traditional medicine (Khan et al., 2019). The current study investigated the cytotoxic and anti-proliferative effects of *T. terrestris* shoot ethanolic extract on six human cell lines. Lee et al. (2005) and Tong et al. (2011) concentrated on studying the development of chemotherapeutic agents for improving anticancer activity and estimated that the steroidal saponins are potential candidates. These steroidal saponins are also responsible for the biological activity of many products from *T. terrestris*; consequently, this biological activity depends on the concentration and configuration of these active saponins (Ganzera et al., 2001; Dinchev et al., 2008).

EL-Darier et al. (2019) suggested that allelopathic interference, demonstrated to be valuable in the competition for resources, can modulate plant community function and dynamics. Consequently, it is crucial to assess the relative importance of these two plant interference mechanisms—resource competition and allelopathy—in experiments, even if it is difficult and often unrealistic to separate these interactions in complex ecosystems. Rudov et al. (2020) described that *T. terrestris*, a ruderal C_4 plant, is a medicinal herb usually grown in ruderal habitats and agricultural areas in temperate, tropical, and desert regions worldwide. It is an aggressive species with possible harmful effects on livestock.

The current work showed the allelopathic effect of TTSAE and TTSP, which have considerable suppressive effects on the germination percentage, plumule, radicle lengths, and shoot and root lengths of the recipient species *H. vulgare* and *L. sativum.* Pacanoski et al. (2014) suggest that the management of *T. terrestris* can be achieved by herbicide application and mechanical and biological control methods. Allelopathic activity is believed to be the joint action of several secondary metabolites. These metabolites exist in all plant tissues, including leaves, flowers, fruits, stems, roots, rhizomes, and seeds (Shinde & Salve, 2019). In keeping with the present results, Moosavi et al. (2011) stated that allelopathic effects could occur because some secondary metabolites counter each other. Weeds differ from other plants in being more aggressive and having specific characteristics that make them more competitive (Gomaa, 2012). They are reported to be one of the main factors that limit agricultural production systems (Radicetti et al., 2013).

Conclusions

All the informants questioned in this study revealed that they were aware of the study plant, its medicinal values, and its detrimental effects. TTSEE produced significant effects on six cancer cell lines that fluctuated from low to medium to strong. The GP and radicle and plumule lengths and shoot and root lengths of the recipient species *H. vulgare* and *L. sativum* were significantly affected by different concentrations of TTSAE and TTSP.

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References

Angelova, S., Gospodinova, Z., Krasteva, M., Antov, G., Lozanov, V., Markov, T., et al. (2013) Antitumor activity of Bulgarian herb *Tribulus terrestris* L. on human breast cancer cells. *Journal of BioScience and Biotechnology*, **2**(1), 25-32.

Atanasov, A.G., Zotchev, S.B., Dirsch, V.M. (2021) Natural products in drug discovery: advances and opportunities. *Reviews*. T*he International Natural Product Sciences Task force and Claudiu T. Supuran*, **20**, 201-216.

Boulos, L. (2000) "*Flora of Egypt Volume 2*". Cairo: Al Hadara publishing. 352p.

Baines, G., Hviding, E. (1992) Traditional environmental knowledge from the Marovo area of the Solomon Islands. In: "*Lore: Capturing Traditional Environmental Knowledge*", Johnson, M. (Ed.), pp. 91-110. Dene Cultural Institute, Fort Hay, Canada.

Cheng, F., Cheng, Z. (2015) Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in Plant Science*, **6**(1), 1020.

Chhatre, S., Nesari, T., Somani, G., Kanchan, D., Sathaye, S. (2014) Phytopharmacological overview of *Tribulus terrestris*. *Pharmacognosy Reviews*, **8**(15), 45-51.

de la Parra, J.D., Quave, C.L. (2017) Ethno-phytotechnology: harnessing the power of ethnobotany with biotechnology. *Trends in Biotechnology*, **35**(9), 802-806.

Dewangan, J., Srivastava, S., Mishra, S., Divakar, A., Kumar, S., Rath, S.K. (2019) Salinomycin inhibits breast cancer progression via targeting HIF-1α/ VEGF mediated tumor angiogenesis *in vitro* and *in vivo*. *Biochemical Pharmacology*, **164**(1), 326-335.

Dighe, S.U., Jadhav, R.S., Vikhe, D.N. (2020) Biological potential of *Tribulus terrestris*. *Journal of Drug Delivery and Therapeutics*, **10**(3), 262-264.

Dinchev, D., Janda, B., Evstatieva, L., Oleszek, W., Aslani, M.R., Kostova, I. (2008) Distribution of steroidal saponins in *Tribulus terrestris* from different geographical regions. *Phytochemistry*, **69**(1), 176-186.

El-Benhawy, S., El-Darier, S., Ebeid, S., Elblehi, S., Hammoury, S., El-Sheikh, M. (2021) *Fagonia arabica* extract exerts antitumor effect on mice bearing Ehrlich carcinoma. *Middle East Journal of Cancer*. doi: 10.30476/mejc.2021.86945.1377.

El-Darier, S.M., Youssef, R.S. (2017) Does salinity enhance allelopathic effects of *Tribulus terrestris* L. in *Citrullus vulgaris* Schrad.? agroecosystems at Nobaria, Egypt. *Direct Research Journal of Agriculture and Food Science*, **5**(1), 1-8.

El-Darier, S.M., El-Kenany, E.T., Abdellatif, A.A., Abdel Hady, E.F. (2018) Allelopathic prospective of *Retama raetam* L. against the noxious weed *Phalaris minor* Retz. growing in *Triticum aestivum* L. fields. *Rendiconti Lincei. Scienze Fisiche e Naturali*, **29**(1), 155-163.

El-Darier, S.M., El Kenany, E.T., Fawzy, E.E. (2019) Assessment of contribution of allelospoly and allelopathy to interference of *Medicago sativa* with *Raphanus sativus*. *The Egyptian Journal of Experimental Biology (Botany)*, **15**(2), 205-215.

El-Darier, S.M., Rashed, S.A., Fayez, A., Hassanein, S.S., Sharaby, M.R., Tawfik, N.M., Mansour, H., Adel, M. (2021) Medicinal plant-derived compounds as potential phytotherapy for COVID-19: future perspectives. *Journal of Pharmacognosy and Phytotherapy*, **13**(3), 68-81.

Faruque, M.O., Uddin, S.B., Barlow, J.W., Hu, S., Dong, S., Cai, Q., Li, X., Hu, X. (2018) Quantitative ethnobotany of medicinal plants used by indigenous communities in the Bandarban District of Bangladesh. *Frontiers in Pharmacology*, **9**, 40.

Ganzera, M., Bedir, E., Khan, I.A. (2001) Determination of steroidal saponins in *Tribulus terrestris* by reversed-phase high-performance liquid chromatography and evaporative light scattering detection. *Journal of Pharmaceutical Sciences*, **90**(11), 1752-1758.

Gaoue, O.G., Coe, M.A., Bond, M., Hart, G., Seyler, B.C., McMillen, H. (2017) Theories and major hypotheses in ethnobotany. *Economic Botany*, **71**(3), 269-287.

Gomaa, N.H. (2012) Composition and diversity of weed communities in Al-Jouf province, northern Saudi Arabia. *Saudi Journal of Biological Sciences*, **19**(3), 369-376.

Hashim, S., Bakht, T., Marwat, K.B., Jan, A. (2014) Medicinal properties, phytochemistry and pharmacology of *Tribulus terrestris* L. (Zygophyllaceae). *Pakistan Journal of Botany*, **46**(1), 399-404.

Kato-Noguchi, H., Kurniadie, D. (2021) Allelopathy of *Lantana camara* as an invasive plant. *Plants*, **10**(5), 1028.

Khan, T., Ali, M., Khan, A., Nisar, P., Jan, S.A., Afridi, S., Shinwari, Z.K. (2019) Anticancer plants: a review of the active phytochemicals, applications in animal models, and regulatory aspects. *Biomolecules*, **10**(1), 47.

Kirkpatrick, L.A., Feeney, B.C. (2013) A Simple Guide to IBM SPSS Statistics for version 20.0. Student ed. Cengage Learning, Wadsworth, Belmont, Calif.

Lee, M.S., Yuet-Wa, J.C., Kong, S.K., Yu, B., Eng-Choon, V.O., Nai-Ching, H.W., Chung-Wai, T.M., Fung, K.P. (2005) Effects of polyphyllin D, a steroidal saponin in Paris polyphylla, in growth inhibition of human breast cancer cells and in xenograft. *Cancer Biology and Therapy*, **4**(11), 1248-1254.

Moosavi, A., Afshari, R.T., Asadi, A., Gharineh, M.H. (2011) Allelopathic effects of aqueous extract of leaf stem and root of *sorghum bicolor* on seed germination and seedling growth of *Vigna radiata* L. *Notulae Scientia Biologicae*, **3**(2), 114-118.

Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, **65**(1-2), 55-63.

Pacanoski, Z., Týr, Š., Vereš, T. (2014) Puncturevine (*Tribulus terrestris* L.): noxious weed or powerful medical herb. *Journal of Central European Agriculture*, **15**(1), 11-23.

Rashed, Sh.A., Saad, T.I., El-Darier, S.M. (2022) Potential aptitude of four olive cultivars as anticancer and antioxidant agents: Oleuropein content. *Rendiconti Lincei. Scienze Fisiche e Naturali*, **33**(1), 195-203.

Radicetti, E., Mancinelli, R., Campiglia, E. (2013) Impact of managing cover crop residues on the floristic composition and species diversity of the weed community of pepper crop (*Capsicum annuum* L.). *Crop Protection*, **44**(1), 109-119.

Rodrigues, E., Cassas, F., Conde, B.E., da Cruz, C., Barretto, E.H.P., dos Santos, G., et al. (2020) Participatory ethnobotany and conservation: a methodological case study conducted with Quilombola communities in Brazil's Atlantic Forest. *Journal of Ethnobiology and Ethnomedicine*, **16**(1), 2.

Rudov, A., Mashkour, M., Djamali, M., Akhani, H. (2020) A Review of C4 plants in southwest Asia: an ecological, geographical, and taxonomical analysis of a region with high diversity of C4 eudicots. *Frontiers in Plant Science*, **11**(1), 546518.

Sasikala, S., Kannan, E., Brindha, D. (2014) Qualitative characterization of solvent and cooked extracts of *Tribulus terrestris* L. fruit. *European Journal of Medicinal Plants*, **4**(8), 907-919.

Shinde, M.A., Salve, J.T. (2019) Allelopathic effects of weeds on *Triticum aestivum*. *International Journal of Engineering Science and Computing*, **9**(2), 19873-19876.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., Boyd, M.R. (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute*, **82**(13), 1107-1112.

Tong, Q.Y., Qing, Y., Shu, D., He, Y., Zhao, Y.L., Li, Y., Wang, Z.L., et al. (2011) Deltonin, a steroidal saponin, inhibits colon cancer cell growth *in vitro* and tumor growth *in vivo* via induction of apoptosis and antiangiogenesis. *Cellular Physiology and Biochemistry*, **27**(3-4), 233-242.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A., Yangsabai, A. (2018) Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, **5**(3), 1-16.

Wang, Z., Qi, F., Cui, Y., Zhao, L., Sun, X., Tang, W., Cai, P. (2018) An update on Chinese herbal medicines as adjuvant treatment of anticancer therapeutics. *BioScience Trends*, **12**(3), 220-239.

Yeh, M., Chiu, H., Wu, M., Koo, M., Lin, N., Liao, K., Yeh, C., Li, T. (2020) Integrated Chinese herbal medicine and western medicine on the survival in patients with colorectal cancer: a retrospective study of medical records. *Evidence-Based Complementary and Alternative Medicine: eCAM*, **1**, 1-10.

Zhang, P., Meng, X., Tang, X., Ren, L., Liang, J. (2019) The effect of a Coix seed oil injection on cancer pain relief. *Supportive Care in Cancer*, **27**(2), 461-465.

التكنولوجيا النباتية والمعرفية لنبات الحسك الفصيلة الرطراطية

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قد استطاعت الدراسات النباتية والمعرفية أن تقيم الدور المعرفي للنباتات، وإمكانياتها لمكافحة السرطان، والأبعاد التضادي لنبات الحسك. ولقد تم إثبات أن هذا النبات يحتوي على المنشطات، والصابونين، ومضادات الأكسدة، والفلافونويد، والقلويدات، والفينولات، والبروتينات، والأحماض الأمينية. تناولت الدراسة التأثيرات السامة للخلايا باستخدام ستة خطوط من الخلايا السرطانية. تم استخدام نبات حب الرشاد والشعير كأنواع متلقية في تجارب الأبعاد التضاي. ووجد أن 95٪ من المستوطنين ذكروا أن نبات الحسك هو نوع عدواني يصيب الماشية، ويقلل من التنوع البيولوجي، ويؤدي إلى جفاف التربة، ويستهلك مساحات كبيرة خلال الموسم الخضري، ويؤثر على درجة حموضة التربة وامتصاص المعادن. أنتجت المستخلصات الإيثانولية تأثيرًا كبيرًا على سلالات خلايا سرطان البروستاتا (PC3) والثدي (MCF 7) والرئة (A549) والكبد (HEP-G2)، بقيم IC_{50} تبلغ 19، 22، 33 و 33 ميكروغرام/ مل ، على التوالى. يحتوي خط خلايا سرطان الأمعاء (CAco2) على 60 IC_{50} ميكروغرام / مل. كان لخط خلايا سرطان القولون (HCT) قيمة IC_{50} تبلغ 68 ميكروغرام / مل. المستخلصات المائية حالت دون إنبات البذور، وطول الريشة، ونمو الجذور، وإنتاج المادة الطازجة والجافة للأنواع المتلقية. أظهرت هذه الدراسة أن نبات الحسك يحتمل أن يكون ذا قيمة كعامل مضاد للسرطان ومبيد أعشاب ضد الأعشاب الضارة.