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Centaurea iberica trevir. Ex spreng. Phytochemical content and evaluation of cytotoxicity, phytotoxicity, anti-inflammatory, larvicidal and anti-inflammatory potentials

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ABSTRACT

Due to their tremendous therapeutic ability to treat a wide range of illnesses, medicinal plants are employed as a rich source and nutraceuticals practically in all civilizations. The goal of the current study was to evaluate phytochemistry and biological potentials of medicinal plant *Centaurea iberica* as previously no research work has been reported. To prepare plant extracts, five different solvents, methanol, ethanol, n-hexane, ethyl acetate and chloroform were used. Total phenolic content of the investigated plant was recorded highest in the methanolic extract ranges from 91 ± 1.2 mg/g, total flavonoid content recorded 24 ± 1.1 mg/g. Moreover, maximum LC_{50} value (9.95 µg/mL) was recorded for methanolic extract using cytotoxicity assay. Radish seed germination phytotoxicity assay indicated the highest phytotoxic potential in n-hexane (55 % seed inhibition) extract of *C. iberica*. However, in anti-inflammatory assay less than 50 % inhibition was observed for methanolic extract and plant was found to be inactive against larvicidal activity. Based on the results of this study, it is recommended that more *in vitro* and in vivo research activities be done in the future, as well as chemical characterization to identify various compounds that may be utilized to treat various illnesses.

1. Introduction

Throughout human history, medicinal plants have been essential to traditional medical practises in many different societies. Since ancient times, people have used medicinal plants as a source of cures for a wide range of illnesses (Batool et al., 2019. The extensive use of medicinal herbs and the knowledge that has been passed down through the generations demonstrate their historical significance of medicinal plants, highlighting their global geographic significance as well as their role as the source of modern medicine (Bibi et al., 2024). The medicinal plants have specifically attracted scientific community and pharma industries due to their rich phytochemical composition such as alkaloids, flavonoids, saponins, tannins, vitamins and minerals etc which are used to cure different diseases (Iqbal et al., 2018, Abbasi et al., 2022, Ijaz et al., 2024a). This showed the continued importance and investigation of therapeutic herbs in modern science. The ethnobotanical study conducted in many locations provides valuable information about the practices surrounding the use of therapeutic herbs by the local people (Ijaz et al., 2023, Ijaz et al., 2024b). Further, Haile et al. (2022) focuses on use of therapeutic herbs in the therapy of respiratory disorders in Ethiopia during a twenty-year period, makes clear the significance of medicinal plants in addressing certain health concerns. Furthermore,

Abbreviations: CIE, Centaurea iberica ethanolic; CIA, Centaurea iberica acetate; CIH, Centaurea iberica n-hexane; CIC, Centaurea iberica chloroform; CIM, Centaurea iberica Methanolic.

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Abbasi et al. (2020) and Batool et al. (2020) highlight the long-term use of medicinal plants, noting that, despite the advent of evidence-based medical practises, the use of medicinal plants remains nearly universal, particularly in non-industrialized societies. This emphasises how pharmaceuticals and nutraceuticals are still derived from medicinal plants. To protect the diversity of medicinal flora, it is essential to record and preserve traditional knowledge about medicinal plants in order to protect the variety of medicinal plants for coming generations (Ali et al., 2023). Furthermore, the study by Gafna et al. (2017) emphasises the need for conservation efforts by highlighting the biodiversity of medicinal plants and the impact of human activities on the availability of traditional herbal medicine. In conclusion, a wealth of research, ethnobotanical studies, and reviews carried out worldwide demonstrate the historical and current significance of medicinal plants in traditional medicine systems. Medicinal plants continue to be important for addressing health issues and advancing pharmaceutical research because of their wealth of knowledge and therapeutic potential. The Asteraceae family wild herb *C. iberica* is primarily found in Western Asia and the Mediterranean region (Odeh et al., 2014). It has long been utilised for a variety of purposes as an alternative medicine. It has been widely used in Turkey to treat wound healing, insect or snake bites, and stomach pains (Koca et al., 2009). Similarly, it is well-known in Lebanon as a choleretic and appetite-boosting tonic (Koca et al., 2009, Gul et al., 2022). Furthermore, the northern regions of Pakistan are frequently home to it (Khan et al., 2011). *C. iberica* hypoglycemic effect and its demonstrated activity in insulin secretion demonstrate the herb's medicinal value (Thome et al., 2012, Izol et al., 2023). Nevertheless, *C. iberica* did not significantly alter plasma glucose levels in a screening study for anti-diabetic and anti-ulcer properties (Twaij & Al-Dujaili, 2014). The current study was aimed to emphasizes *C. iberica* potential as a valuable medicinal plant by highlighting its geographic distribution, traditional uses, and medicinal significance.

2. Material and methods

2.1. Collection of plant and extracts preparation

The medicinal plant called *C. iberica* (*Asteraceae*) was collected in its flowering stage from the mountain range of Murree, Pakistani (33.9070° N, 73.3943° E). The plant was taxonomically identified by consulting with Flora of Pakistani and literature review. The *C. iberica* was surrounded by *Acacia modesta* and *Diosypros lotus* being the dominant surrounding flora. The research activities were performed at Plant Biochemistry and Molecular Biology Lab, QAU Islamabad. Following collection and identification, the entire plant was carefully cleaned under running tap water and allowed to dry in the shade. After that, a fine powder was made from the plant extract. Additionally, *C. iberica* extracts were prepared using five distinct solvents, (polar solvents: methanol and ethanol), and ethyl acetate, n-hexane, and chloroform (non-polar solvents). Every solvent was used in 250 mL to make the extracts. The extracts were then carefully filtered using Whatman filter papers and the resulting filtrates were placed in a fume hood to dry. The extracts were scraped off, moved into an eppendorf tubes and then kept at 4°C after drying for future use (Bibi et al., 2024). For additional quantitative and qualitative examination, the mixes were employed.

2.2. Qualitative phytochemical screening

For the evaluation of different bioactive components, various phytochemical tests were performed using standard methods; saponins (foam test), alkaloids (Mayer's rea-gent), tannins (Gelatin test), flavonoids (Alkaline reagent test), phenols (Ferric chloride test), quinone and coumarins.

2.3. Total phenolic content determination

Procedure narrated by Clarke et al. (2013) with few modifications was followed to check the total phenolic content. Plant sample solution of 20 µL was poured in 96 wells plate. Then diluted Folin-Ciocalteu reagent (90 µL) and 6 % solution of sodium carbonate was added into the wells. After this all the mixtures were incubated at room temperature for one and Optical density (OD) was taken at 630 nm using a microplate reader (biotech). Gallic acid which was taken as a positive control prepared in DMSO. Thus, total phenolic content (TPC) was articulated as Gallic acid equivalents mg/g of plant extract.

2.4. Total flavonoid content determination

A method outlined by Vishwarkarma et al. (2014) was followed to measure the total flavonoid content. Sample solution of 20 µL from each sample was taken and then carefully shifted into 96 wells plate. In the next step, 10 µL of aluminium chloride and 10 µL of potassium acetate were added into each well. After this, distill water was mix to make a volume up to 200 µL followed by incubation at 37°C for 30 min. Finally, the absorbance of the reaction mixture was recorded at 405 nm using microplate reader. Quercetin was taken as a standard in the assessment of total flavonoid content. Flavonoid contents were expressed as equivalent of quercetin.

2.5. Phytotoxicity assay

For phytotoxicity evaluation, radish seed assay which was described by Arzu and Camper (2002) was used. For this assay 10,000 µg/mL of each plant sample extract were used. The whole process was carried out under sterilized conditions. In each petri plate sterilized Whattman filter paper # 1 was placed. On the filter paper stock solution was poured and evaporated and then 5 mL distill water was added. The seeds were sterilized with 0.1 M HgCl2 for 30 s. After sterilization 15 seeds were placed at regular distances in each petri plate and placed in growth chamber at 25°C with 60 % humidity. Finally, on 6th day root length was measured and the percent of seed germination was recorded. The assay was per-formed in triplicates and ANOVA was applied.

2.6. Cytotoxicity assay

For the assessment of cytotoxicity, brine shrimp lethality experiment was performed as outlined by Finney et al. (1952). The stock solution was used to make serial dilutions of 1000, 500, and 250 µg per mL. All the concentrations were taken in glass vials and the solutions were evaporated. Artificial sea water was used for hatching eggs (Artemia salina) in a bi-partitioned tray. For the preparation of sea water, 3.8 g sea salt was mixed with 1 L of distill water and it was dissolved by continuous stirring. Then a pinch of eggs was added one side of bipartition tray and covered with aluminium foil. After incubation for 24 hrs, eggs which were hatched nauplii moved towards light source. Then, 10 nauplii were collected via micro pipette and were shifted to each well along with 1 L of sea water with evaporated plant samples. Vincristine sulphate was used as positive control and methanol was used as negative control respectively. After incubation for 24 hrs dead nauplii were counted in all the wells and percent mortality was checked. Experiment was carried out in triplicate and IC50 values were calculated using Graph Pad Prism software.

2.7. Larvicidal and insecticidal assay of C. Iberica

For the determination of cytotoxicity of plant extract, Larvicidal and insecticidal activity of plant was performed following Ghosh et al. (2012) method. For the preparation of stock solution of 200 ppm, plant extract (100 mg) was mixed with 5 mL of ethanol. For test solution, 49.5 mL of distill water of 200 ppm was mixed with 0.5 mL from stock

solution. After this, the flask which contains plant solution ten larvae were moved into it. After 24 hrs, percentage mortality was determined using the formula below.

2.8. % Mortality = (% Survival in the untreated control − % survival in the untreated control % Survival in the untreated control) × 100 Anti-inflammatory activity

Luminol-enhanced chemiluminescence test for evaluating anti-inflammatory activity outlined by Helfand et al. (1982) was performed. For this assay, 25 µL of cell suspension of diluted whole blood HBSS was incubated with 25 µL of three other concentrations of ex-tracts (1 µg/mL, 10 µg/mL, 100 µg/mL). The experiment was performed in triplicates. For positive control ibuprofen was used. Experiment was performed in 96 wells plate and incubation was done for 15 min at 37°C in Luminometer. After incubation, serum opsonoized zymosan (SOZ) (25 µL) and intracellular reactive oxygen species detecting probe (25µL) were filled with the exception of blank wells. Using a luminometer, chemiluminescence peaks were measured in relative light units (RLU).

2.9. Statistics

Every experiment was run three times. With Microsoft Excel (2016), the descriptive statistics were applied. Using Statistics version 8.1, the results of the phytochemical analysis, biological assessment, and antioxidant were submitted with least significant difference (LSD) and analysis of variance (ANOVA). Graph Pad Prism version 5.01 was utilized to compute IC50 values, and Finney's 1952 probit analysis programme was employed to estimate LC50 values.

3. Results and discussion

3.1. Quantitative analysis on phytochemicals constituents

Quantitative phytochemical analysis is important for the phytochemical measurement, including total flavonoid content (TFC) and total phenolic content (TPC).

3.2. Test for total phenolic content (TPC)

The Folin-Ciocalteu technique was used to estimate the total phenolic content. TPC was articulated on the basis of Gallic acid equivalents (mg GAE / g of dry sample) (Fig. 1). For total phenolic content, the highest value was depicted by CIM (91 ± 1.2 mg/g). After CIM, CIE depicted excellent value (79 ± 0.8 mg/g), followed by CIA (59 ± 0.6 mg/g). CIC and CIH showed minimum value as compared to the others. CIC has 56 ± 1.0 mg/g and CIH has 55 ± 0.2 mg/g values for the total phenolic content.

3.3. Test for total flavonoid content (TFC)

In total flavonoid content, Quercetin was taken as standard. The standard equivalents were represented as Quercetin equivalents (mg QE / g of dry sample). The highest value of TFC was recorded for CIM with the value of 24 ± 1.1 mg/g, followed by CIA (18 ± 0.3 mg/g), CIE (17 ± 1.4 mg/g), CIC (16 ± 0.1 mg/g) as shown in Fig. 2 and the lowest value was noted for n-hexane (14 ± 0.1 mg/g).

3.4. Cytotoxicity analysis

It is one of the simple and convenient test to find out the pharmacological activities of extracts from therapeutic plants. Brine shrimp lethality assay was used to detect the toxicity of plant extracts which were made using five distinct solvents. The plant extracts were assessed at five different concentrations and the LC_{50} values was calculated. Plant extracts which has minimum LC_{50} represents stronger cytotoxic potential. CIM extract showed best result with lowest LC50 value (9.95 µg/mL), followed by CIH (16.82 µg/mL), CIA showed 23.71 µg/mL, CIE (23.87 µg/mL) and the highest value was recorded by CIC as compared to rest of the extracts with maximum LC_{50} (25.66 µg/ml) (Table 1).

3.5. Phytotoxicity analysis

Phytotoxic effect of the medicinally important plant *C. iberica* was evaluated to examine the allelopathic potential using all extracts prepared in five different solvents. Phytotoxicity was checked using radish seeds which are used for evaluation of allelopathic potential of medicinal plants. The results revealed that the maximum seed inhibition and minimum root length was shown by n-hexane (CIH) extract of *C. iberica* (seed inhibition: 55 %: root length: 8.33 mm), after n-hexane maximum seed inhibition and minimum root length was shown by ethyl acetate (CIA) of *C. iberica* (seed inhibition: 44 %: root length: 9.4 mm), followed by CIE (seed inhibition: 38 %: root length 8.66 mm) while, chloroform extract showed maximum values (seed inhibition: 33 %: root length: 8.33), followed by CIM (seed inhibition 27 %: root length 14.66 mm). Water was taken as a positive control (see inhibition 0 %: root length 40.99 mm) Details about *phytotoxicity analysis* are given in Fig. 3 and Fig. 4).

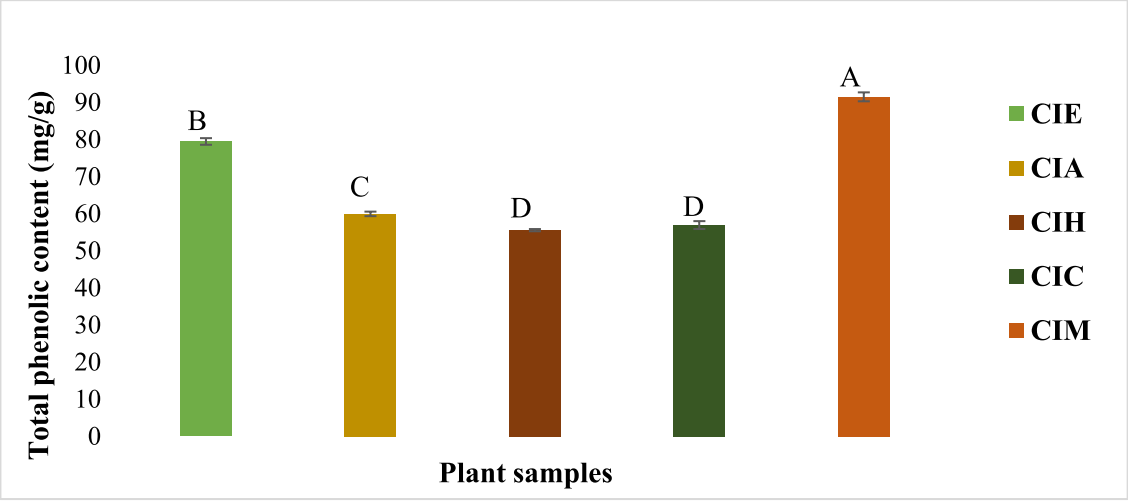

Fig. 1. Total phenolic content of different extracts of *Centaurea iberica*.

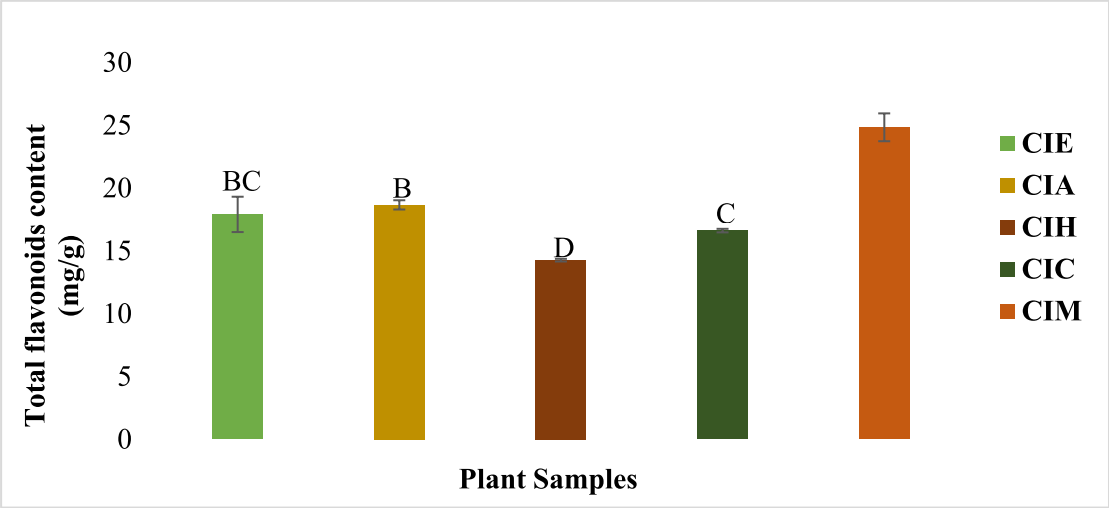

Fig. 2. Total flavonoid contents of different extracts of *C. iberica*.

Table 1
Brine shrimp mortality rate as a percentage at five distinct *C. iberica* extract concentrations, along with corresponding LC_{50} values.

Plant extracts	Percentage Mortality at different doses (µg/ml)					LC_{50} (µg/ml)	95 % Confidence Interval
	6	12	25	50	100		
CIE	13.33	30.00	40.00	63.33	96.66	23.87	15.62–36.48
CIA	26.66	30.00	36.66	63.33	90.00	23.71	13.45–41.80
CIM	46.66	50.00	60.00	80.00	90.00	9.95	4.738–20.89
CIH	30.00	40.00	53.33	73.33	90.00	16.82	9.309–30.39
CIC	13.33	23.33	40.00	66.66	93.33	25.66	16.51–39.89

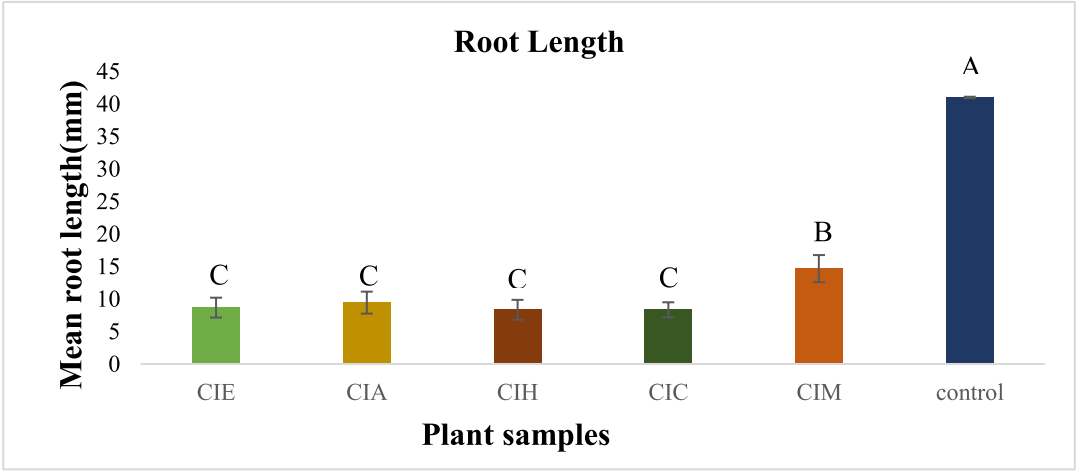

Fig. 3. Phytotoxicity (Mean root length) of different extracts of *C. iberica*.

3.6. Insecticidal and larvicidal assay

Testing of *C. iberica*'s methanolic extract was done against dengue fever and three different insects were used to determine larvicidal and insecticidal potential of the plant extract. Permethrin was employed as a standard that revealed 100 % inhibition. However, plant extracts did not show any mortality against all examined organisms. Hence, it can be suggested that CIM was inactive against selected insects as well as larva causing the dengue fever (Table 2).

3.7. Anti-inflammatory activity

Helfand et al. (1982) described the luminol-enhanced chemiluminescence assay, which was used to determine the anti-inflammatory activity. Plant extracts, whole blood diluted in Hanks balanced salt solution (a solution that contains calcium and magnesium chlorides), and a Luminometer, SOZ (serum opsonized zymosan), The crystalline solid luminol, which ranges in colour from white to pale yellow and is soluble in most polar organic solvents but insoluble in water, Ibuprofen, and ROS (reactive oxygen species). Experiment was performed in 96 wells plate and incubation was done for 15 min at 37 °C in Luminometer. After the incubation, serum opsonized zymosan (SOZ) (25 µL) and Except for blank wells, each well received a 25 µL intracellular reactive oxygen species detection probe. A luminometer was used to record the peaks of chemiluminescence in terms of relative light units (Table 3).

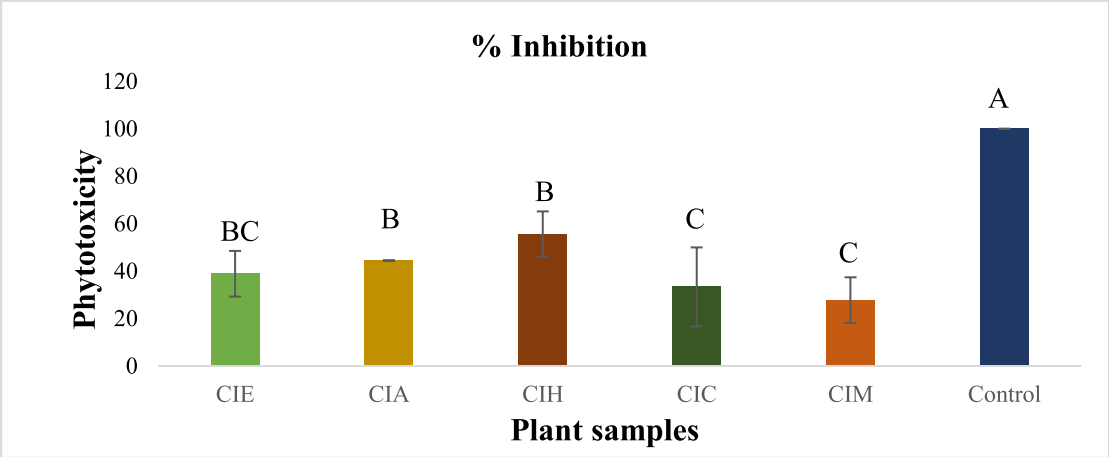

Fig. 4. Phytotoxicity (Percentage germination inhibition) of different extratcs of *C. iberica*.

Table 2
Insecticidal and Larvicidal activity of C. iberica (CIM).

Bioassays	Name of insects	% mortality			
		Positive control	Negative Control	Plant Extract (CIM)	Standard (Permethrin)
Insecticidal assay	*Tribolium castaneum*	100 %	0 %	0 %	100 %
	Sitophilus oryzae	100 %	0 %	0 %	100 %
	Rhyzopertha dominica	100 %	0 %	0 %	100 %
Larvicidal assay (Dengue fever)		100 %	0 %	0 %	100 %

Key: CIM; *C. iberica* methanolic extract; **standard**: Permethrin.

Table 3
Anti-inflammatory assay of C. iberica (CIM).

Anti-inflammatory assay	
% inhibition	IC_{50}
45.6 %	–
73.2 %	–

Key: CIM; *Centaurea iberica* methanolic extract.

4. Discussion and conclusion

This study's objective was to look into the biological potential of *C. iberica*. The anti-inflammatory, phytotoxic, cytotoxic, Insecticidal and Larvicidal activity of five distinct *C. iberica* extracts (methanol, chloroform, n-hexane, ethyl acetate, and ethanol) were examined. The spectroscopic examination showed the amount of phenol was found in higher concentrations than flavonoids. Highest phenolic contents were detected in CIM (91.489 ± 1.203 mg GAE/g) and CIE (79.458 ± 0.894 mg GAE/g) high levels of flavonoids were also noted in CIM (24.839 ± 1.108 mg QE/g). Many research investigations have also indicated that the use of various solvents has a significant impact on TPC and TFC values (Uddin et al., 2018; Zohra et al., 2019). Our study correlates with the earlier findings of Erel et al. (2011), Khan et al. (2011) and Tekeli et al. (2010) who demonstrated the existence of flavones, steroids, fatty acids, volatile constituents, lignans glycosides and nitrogenous compounds in *C. iberica* extracts. The primary function of phenolic and flavonoid chemicals is to preserve living organisms by stabilising free radicals. (Karmakar et al., 2019). As a result of the current investigation, it is possible that the biological potential of *C. iberica* extracts is attributable to the existence of these secondary metabolites.

The cytotoxicity test on brine shrimp is widely employed to evaluate the cytotoxic capacity of related compounds and extracts from medicinal plants. (Hussain et al., 2019). The findings showed a concentration-dependent response, wherein brine shrimp mortality increases with increasing extract concentration and mortality decreases with decreasing plant extract concentration (Wakawa et al., 2017). Previously, Conforti et al. (2008) Granger and et al. (2009) also reported significant brine shrimp cytotoxic assay in the methanol extracts of *C. polyclada* and *C. centaurium*. Similarly, radish seeds are used to evaluate the allelopathic potential of selected plant extracts. Water was taken as a positive control which showed 0 % seed inhibition and 40.99 mm root length. In present studies, CIM showed significant results for phytotoxicity (27 % seed inhibition). It can be concluded from the present study that among all extracts, methanol extracts revealed maximum biological potential as compared to other extracts. To the best of our knowledge, this is the first study to examine the phytotoxicity and cytotoxicity of extracts from *C. iberica*.

In the commenced research, CIM extract evaluated for the anti-inflammatory activity, which revealed 45.6 % inhibition. Current results are similar with the earlier study of Koca et al. (2009), who reported maximum anti-inflammatory activity in the methanol extract of *C. iberica*. Our study significantly correlates with the study of Karamenderes, et al. (2007), who reported the anti-inflammatory potential of chloroform extract of *C. hyalolepis*, *C. athoa*, *C. aphrodisea*, *C. iberica* and *C. polyclada*. The insecticidal (*T. castaneum*, *S. oryzae* and *R. dominica*) and larvicidal (dengue fever) activity of CIM extract was carried out. However, the results show that CIM is not an appropriate choice for insecticidal and larvicidal activity. The study's overall conclusions point to *C. iberica*'s high content of secondary metabolites, which makes it effective in combating conditions like cancer and inflammation (Koca et al., 2009; Ochwang et al. (2009).

5. Conclusion

Centaurea iberica, a member of the Asteraceae family, has been utilized for centuries to treat a variety of aliments including rheumatoid arthritis, cancer, high fever, headaches, wound healing, and inflammation. The cytotoxicity, phytotoxicity, anti-inflammatory and larvicidal effects of five *C. iberica* extracts were investigated in the current study.

When compared to other extracts, the methanolic extract also shown anti-inflammatory activity. In the cytotoxicity experiment, methanolic extract had the highest LC50 value (9.95 ppm). As a result of biological processes occurring in the chosen plant, it can be concluded that it contains considerable phyto-compounds that may be used successfully for defensive reasons against various infections. In the future, It is important to do in vivo research to validate The effectiveness of bio-efficacy of chosen plant extracts, and chemical characterisation will aid in the isolation of numerous new compounds that may be employed for the treatment of various ailments.

Ethics approval

Not Applicable.

7. Consent to participate

All authors consent to participate in this manuscript.

8. Consent for publication

All authors consent to publish this manuscript in Journal of King Saud University – Science.

9. Code availability

Not Applicable.

CRediT authorship contribution statement

Haleema Bibi: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Javed Iqbal:** Writing – review & editing, Writing – original draft, Software, Conceptualization. **Banzeer Ahsan Abbasi:** Writing – review & editing, Software, Formal analysis. **Sobia Kanwal:** Writing – review & editing, Software, Formal analysis. **Mahboobeh Mahmoodi:** Writing – review & editing, Software, Formal analysis. **Mohammad Raish:** Writing – review & editing, Software, Formal analysis. **Tariq Mahmood:** Writing – review & editing, Supervision, Resources.

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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