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RESEARCH ARTICLE

Bioactive Potential of *Cicer arietinum* L. Extracts Evaluated by Cytotoxicity, Larvicidal and Piscicidal Activity under Laboratory Conditions

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Abstract

Extracts of chickpea *Cicer arietinum* L. with petroleum ether (PE), $CHCl_3$, and CH_3OH were tested for cytotoxicity against brine shrimp *Artemia salina* L. nauplii, larvicidal activity against mosquito *Culex quinquefasciatus* Say larvae, and piscicidal activity against guppy *Poecilia reticulata* Peters. All chickpea extracts exhibited cytotoxicity, with the $CHCl_3$ extract proving the most effective at a minimum LC_{50} of 7.27 ppm, followed by the CH_3OH and PE extract, with LC_{50}s of 22.05 and 27.33 ppm, respectively, after 48 h of exposure. Against mosquito larvae, the PE extract showed the highest larvicidal activity with an LC_{50} value of 38.59 ppm at 48 h, while the CH_3OH and $CHCl_3$ extracts gave LC_{50} values of 66.01 and 72.22 ppm after 48 h of exposure. Against guppies, only the $CHCl_3$ and CH_3OH extracts of the tested plant showed favorable piscicidal activity. The $CHCl_3$ extract proved more active, with an LC_{50} of 5.35 ppm, compared to 23.91 ppm for the CH_3OH extract, after 48 h of exposure. The PE extract showed no lethality to the fish studied. Based on the intensity of sensitivity of the three test organisms to the chickpea extracts, the tested agents could be ranked in descending order of effectiveness as follows: *P. reticulata* ($CHCl_3$; LC_{50} = 5.35 ppm) > *A. salina* nauplii ($CHCl_3$; LC_{50} = 7.27 ppm) > *C. quinquefasciatus* larvae (PE; LC_{50} = 38.59 ppm).

Keywords: *Artemia*, *Cicer*, *Culex*, Cytotoxicity, Larvicidal, Piscicidal activity, *Poecilia*.

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Introduction

The chickpea *Cicer arietinum* L., also known as Bengal pea or Indian pea, is an edible legume in the Fabaceae family. It is one of the oldest cultivated vegetables and is rich in protein (Grasso et al. 2022, Arora et al. 2023, Begum et al. 2023). It is primarily grown as a winter crop in Asia, Europe, Australia, and North America; Southeast Asia accounts for over 80% of the global supply, with India being the leading producer (Shukla et al. 2012, Rachwa-Rosiak et al. 2015). Although it thrives in temperate regions, this legume is an annual plant well-suited to mild, dry climates and tolerates heat well if the soil is sufficiently moist (Wallace et al. 2016, Bulbula and Urga 2018).

The chickpea has numerous branches extending from its base and erect, angular or winged stems, as well as densely pubescent and glandular leaves. Its proteins are highly digestible and exhibit high bioavailability (48–89.01%) (Kou et al. 2013, Ahmed et al. 2020, Zhang et al. 2024). In chickpeas, globulins constitute 57% of the storage proteins, followed by glutelins (18%), albumins (12%), and prolamins (3%) (Singh and Jambunathan 1982). Chickpea seeds vary in size, shape, and color (Singh et al. 1991). They are classified into two varieties based on these variations: Kabuli and Desi (Bibi et al. 2007). Kabuli-type seeds have a thin, white to cream-colored seed coat and weigh 28 to 70 g per 100 seeds. Desi variety chickpea seeds have a thicker, irregularly shaped shell that can range in color from light beige to black and weigh up to 28 g per 100 seeds (Segev et al. 2011).

The roots and seeds of this plant possess numerous therapeutic properties (Prasad 2009). Its acidic and astringent leaves stimulate taste and appetite, while also treating bronchitis and reducing flatulence (Vadnere et al. 2012). Furthermore, the seeds of *C. arietinum* exhibit anti-inflammatory (Sharma et al. 2020) and antihyperglycemic (Goyal et al. 2011) properties; the wall of the mature pod has antioxidant properties (Vadnere

et al. 2012); the root has antidiarrheal (Sharma et al. 2020), antibacterial (Kumar et al. 2014), and antipyretic (Dalal et al. 2011) properties; and the aerial parts have antifungal properties (Bajwa et al. 2006). One study indicated that *C. arietinum* contributed to the treatment of diabetes, cardiovascular diseases, and certain types of cancer (Qureshi et al. 2006). Brine shrimp, *Artemia salina* L., belongs to a very ancient genus of crustaceans (crayfish) called Anostraca. The genus Anostraca includes several species, but A. salina is relatively easy to rear and, due to its high hatching rate, is used as a model organism in toxicology laboratories worldwide (Ruebhart et al. 2008).

Female brine shrimp can lay their eggs by mating or parthenogenesis. Nauplii about 0.5 mm long emerge from the eggs. These eggs can remain dormant as cysts if they are not fertilized. The cysts can persist for several years and hatch upon contact with salt water (Gajardo et al. 2006. Gazardo and Beardmore 2012). Another model organism, *Culex quinquefasciatus*, is a mosquito species found worldwide. This mosquito has been observed breeding in shallow ponds located in streams (Derraik 2005, Derraik and Selany 2005), as well as in artificial environments such as drains and cesspools (Burkot et al. 1984), septic tanks, rainwater harvesting systems, and tires. At maturity, *C. quinquefasciatus* generally measures between 3.96 and 4.25 mm (Lima et al. 2003).

They lay their eggs in rafts on the water's surface, which do not withstand desiccation (Weinstein et al. 1997). Most eggs hatch into larvae (larvae) in 2 to 3 days, and their movements allow them to swim. The filarial nematode *Wuchereria bancrofti*, avian malaria, and arboviruses such as St. Louis encephalitis virus, Zika virus, Western equine encephalitis virus, and West Nile virus are transmitted by *C. quinquefasciatus*. In this study, the guppy, Poecilia reticulata, was also used as a test organism. The guppy has been recognized as a useful model for toxicological research by the Organisation for Economic Co-operation and Development (OECD) and others because of its high sensitivity to environmental contaminants and its ease of handling and rearing in aquariums (Dorrington et al. 2012, Rocha et al. 2015).

Guppies consume various foods found in their environment, including benthic algae and mosquito larvae, which helps limit the spread of malaria. However, they can harm local fish populations under certain circumstances. This test organism is viviparous and has a short reproductive cycle. The objective of this research was to evaluate the bioactive potential of the test plant *C. arietinum* by its cytotoxic activity against *A. salina* nauplii, its larvicidal activity against *C. quinquefasciatus* larvae, and its piscicidal activity against *P. reticulata* adults.

Materials and Methods

Collection and processing of plant materials

Fresh chickpea *C. arietinum* plants were collected on the Rajshahi University campus and identified by the Department of Botany, which also maintained a control specimen in its herbarium. The leaves and stems were first separated, and then the roots were cleaned of any impurities without being washed. The leaves, roots, and stems were then cut and chopped into small pieces, dried in a well-ventilated room, and ground using an electric grinder (taking care not to overheat). They were then weighed and stored separately in Erlenmeyer flasks for the addition of solvent.

Extraction procedure

The solvent was added at a rate of 300 mL per 100 g of dust and filtered twice over a 48-hour period. After complete removal of the previous solvent, the next solvent was added in the same manner, and this process was repeated until extracts were obtained in petroleum ether, $CHCl_3$, and CH_3OH. Filtration was performed in the same flask using Whatman filter paper every 24 hours, and the extracts were then evaporated until only residues remained. These extracts were then transferred to glass flasks and stored in a refrigerator at 4°C, after appropriate labelling.

Hatching of Artemia Salina cysts

Fresh artemia cysts were purchased from the Mirpur aquarium market in Dhaka, Bangladesh. In the aquarium, the eggs hatched in 1 liter of 1 M NaCl saline (pH 8.5). The eggs were incubated under fluorescent lighting for 48 h, and the nauplii hatched in 24 to 36 h at a temperature of 30 to 35°C. After hatching, the nauplii were transferred to test tubes containing ten nauplii each. Each tube was filled with 2 ml of sodium chloride (NaCl) solution.

Hatching of *C. quinquefasciatus* larvae

C. quinquefasciatus egg masses were collected from various sewers on the Rajshahi University campus. The eggs were then placed in a new 500 ml beaker containing 250 ml of ordinary pond water and kept in the dark in the laboratory for 24 hours until hatching. After hatching, the larvae were transferred to test tubes, each containing 10 newly hatched larvae (Imam et al. 2014).

Reproduction of *P. reticulata*

P. reticulata guppy fries were purchased from a pet shop in Rajshahi City. After collection, the fish were placed in a glass rearing tank filled with ordinary pond water at the Aquaculture Laboratory of the Department of Zoology at Rajshahi University. The temperature was maintained between 22 and 25°C and the water was continuously aerated by an aerator. Five fish of equal size were transferred into each 500 ml beaker during the experiment (Shahjahani et al. 2013).

Cytotoxicity test

Samples at different concentrations were prepared in test tubes by adding a precise amount of DMSO (dimethyl sulfoxide) (Asif et al. 2023). Water was then pipetted to fill the pre-labeled test tubes (up to 10 mL). Nauplii were visually counted and transferred to test tubes containing 10 mL of water. The test tubes, along with a control group, were stored at room temperature. Mortality was recorded after 6, 12, 18, 24, 30, 36, 42, and 48 h of exposure. Plant extract solutions at different concentrations of the three C. arietinum extracts in petroleum ether (300, 200, 100, and 50 ppm), in $CHCl_3$ (80, 60, 40, 20, 10, and 5 ppm), and in choline chloride (80, 60, 40, 20, 10, and 5 ppm), as well as in CH_3OH (200, 150, 100, 50, 25, and 10 ppm), were applied to artificial seawater containing 1% DMSO (v/v) for this test. Ten nauplii were used in each test tube, with three replicates for each concentration.

Subsequently, 1 ml of NaCl solution was added to each test tube. The number of surviving and dead nauplii was counted using a stereomicroscope after 6, 12, 18, 24, 30, 36, 42, and 48 hours of incubation, and the percentage mortality (%M) was calculated for each trial relative to the control. DMSO (1%) served as the negative control, and its final concentration in the test volume was always kept below 1% to avoid any adverse effects due to its toxicity. The LC_{50} represents the sample concentration required to kill half (50%) of the artemia population and was calculated by plotting the percentage inhibition (%) against the logarithm of the extract concentration. According to Meyer et al. (1982), an LC_{50} less than 1 mg/ml is considered toxic, while an LC_{50} greater than 1 mg/ml is considered non-toxic (Abbott 1925).

Larvicidal test

In the lethality test, the *C. arietinum* extract was tested on one-day-old *C. quinquefasciatus* larvae. Samples at different concentrations, considered doses, were prepared in test tubes by adding a predetermined amount of DMSO to make them hydrophilic before adding water to each tube. Then, using a pipette, water was added until the pre-graduated test tube was full (up to 10 ml). Ten freshly hatched (one-day-old) larvae were placed in each test tube containing different concentrations of the indicated doses, along with a control group. The larvae were visually counted 6 hours after application and then every 6 hours for the following 48 hours. The doses selected for the C. arietinum extract against mosquito larvae in petroleum ether were 300, 200, 100, 50 and 25 ppm in $CHCl_3$; and 250, 200, 150, 100 and 50 ppm in CH_3OH for the final experiment (Asif et al. 2023).

Piscicidal activity test

To test the piscicidal activity against *P. reticulata*, extracts of *C. arietinum* were poured into 500 ml beakers and made hydrophilic by adding a small amount of DMSO. The beakers, previously labeled (up to 300 ml), were then filled with water. Five guppies were placed in each beaker and exposed for 48 hours. The number of dead fish was counted visually after 6 hours of exposure, then every 6 hours, up to 48 hours, and the results were compared to the control. The following concentrations of *C. arietinum* extract were used as final concentrations for the piscicidal activity test on guppies: in CHCl3: 16.67, 13.33, 10, 6.67, and 3.33 ppm; and in CH3OH 83.33, 66.67, 50, 33.33 and 16.67 ppm (Ferdous et al. 2018).

Statistical analysis

The lethality values of *C. arietinum* on brine shrimp nauplii, mosquito larvae, and guppies were corrected according to Abbott's formula (1925): Pr = (Po - Pc / 100 - Pc) × 100; where Pr represents the corrected mortality, Po the observed mortality, and Pc the mortality of the control group (Meyer et al. 1982). The data were then subjected to a probit analysis according to Finney (1947) and Busvine (1971) using software developed at Newcastle University (UK). The median lethal concentration (LC50) of the tested compounds was used to express the lethality relationship.

Results

Cytotoxicity of *C. arietinum* on Artemia salina

The cytotoxic activity of *C. arietinum* extracts on *Artemia* is shown in Table 1. This extract demonstrated promising cytotoxic potential against A. salina nauplii (Fig. 1a-f: Regression lines) for all three solvents. The PE extract of *C. arietinum* gave LC50 values of 933.67, 482.95, 213.48, 95.11, 62.39, 46.85, and 27.33 ppm after 12, 18, 24, 30, 36, 42, and 48 h; The $CHCl_3$ extract yielded LC_{50} values of 124.37, 105.39, 49.04, 28.57, 28.55, 15.08, 11.08, and 7.27 ppm; and the CH_3OH extract yielded LC50 values of 308.84, 287.83, 115.10, 74.54, 51.08, 37.42, 25.95, and 22.05 ppm after 6, 12, 18, 24, 30, 36, 42, and 48 h of exposure, respectively. The extracts of the plant tested against *A. salina* nauplii could be ranked in decreasing order of activity: $CHCl_3$ extract > CH_3OH extract > PE extract.

Table 1: LC_{50} values of *C. arietinum* extract on *A. arietinum* nauplii salina, *C. quinquefasciatus* larvae and *P. reticulata* fish.

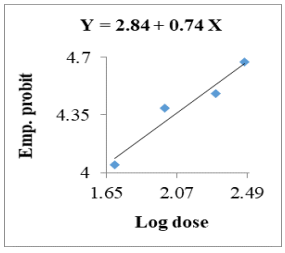
Plant name	Name of test agents	Solvent of extraction	LC50 values (ppm) at different exposure hours (h)							
			6	12	18	24	30	36	42	48
C. arietinum (Whole plant)	*A. salinanauplii*	Pet. ether	-	933.67	482.95	213.48	95.11	62.39	46.85	27.33
		CHCl3	124.37	105.39	49.04	28.57	28.55	15.08	11.08	7.27
		CH3OH	308.84	287.83	115.10	74.54	51.08	37.42	25.95	22.05
	C. quinquefasciatus larvae	Pet. ether	-	608.97	541.72	369.50	276.47	135.50	57.66	38.59
		CHCl3	704.62	670.91	589.77	361.60	199.78	141.82	84.97	72.22
		CH3OH	628.12	610.22	527.26	458.33	255.02	120.54	81.19	66.01
	P. reticulata fish	Pet. ether	Not active							
		CHCl3	39.31	30.72	18.41	15.50	11.53	9.92	6.54	5.35
		CH3OH	196.61	171.88	155.66	130.47	94.50	53.46	33.45	23.91

Larvicidal activity of *C. arietinum* on *C. quinquefasciatus*

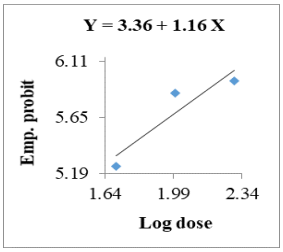
The larvicidal activity of the *C. arietinum* extract is presented in Table 1. The tested extract of this plant showed promising lethal effects against *C. quinquefasciatus* larvae (Fig. 2a-f: Regression lines) in three different solvents. The petroleum ether extract of *C. arietinum* showed LC_{50} values of 608.97, 541.72, 369.50, 276.47, 135.50, 57.66, and 38.59 ppm after 12, 18, 24, 30, 36, 42, and 48 h; in $CHCl_3$: 704.62, 670.91, 589.77, 361.60, 199.78, 141.82, 84.97, and 72.22 ppm; and in CH_3OH: 628.12, 610.22, 527.26, 458.33, 255.02, 120.54, 81.19, and 66.01 ppm after 6, 12, 18, 24, 30, 36, 42, and 48 h of exposure, respectively. Based on the plant's susceptibility to mosquito larvae, the extract can be ranked in descending order as follows: PE extract>CH_3OH extract >$CHCl_3$ extract.

Piscicidal activity of *C. arietinum* on *P. reticulata*

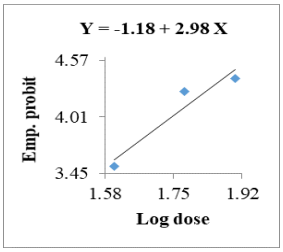
The piscicidal activity of *C. arietinum* is also presented in Table 1. The $CHCl_3$ and CH_3OH extracts of the tested plant showed promising toxic potential against the fish *P. reticulata* (Fig. 3a-d: Regression lines). The *C. arietinum* extract in $CHCl_3$ gave LC_{50} values of 39.31, 30.72, 18.41, 15.50, 11.53, 9.92, 6.54, and 5.35 ppm; The concentrations of chloroform hydrochloride ($CHCl_3$) and methyl hydroxide (CH_3OH) were 196.61, 171.88, 155.66, 130.47, 94.50, 53.46, 33.45, and 23.91 ppm, respectively, after 6, 12, 18, 24, 30, 36, 42, and 48 h of exposure. However, the petroleum ether extract of *C. arietinum* showed no toxic potential towards guppies (*P. reticulata*). Based on the degree of sensitivity of the tested plant to guppies, the extracts could be ranked in descending order as follows: $CHCl_3$ extract >CH_3OH extract. The corresponding regression lines of the CL_{50} values from Table 1 are shown in Figs. 1 (a-f), 2 (a-f) and 3 (a-d) are given below.



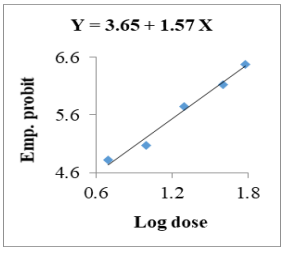
a) Pet. ether extract after 12 h of exposure.



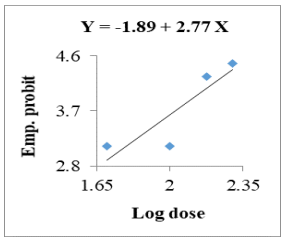
b) Pet. ether extract after 48 h of exposure



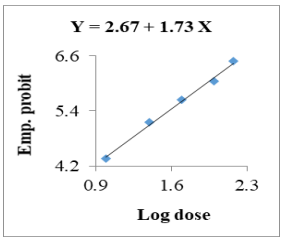
c) CHCl3 extract after 6 h of exposure.



d) CHCl3 extract after 48 h of exposure.

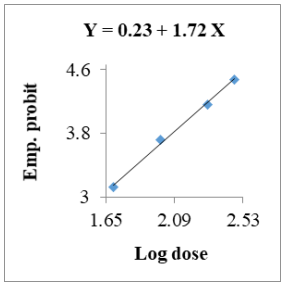


e) CH3OH extract after 6 h of exposure.

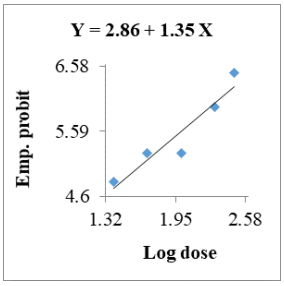


f) CH3OH extract after 48 h of exposure.

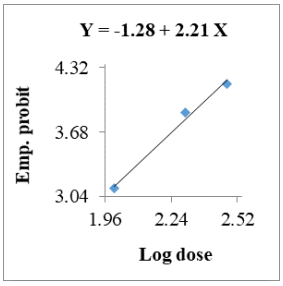
Fig. 1 (a-f): Regression lines of *C. arietinum* extracts *versus A. salina* nauplii at minimum and maximum exposure times.



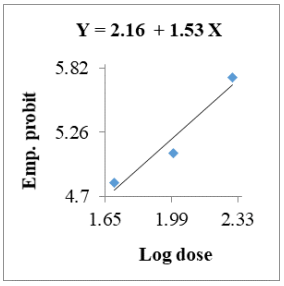
a) Pet. ether extract after 12 h of exposure.



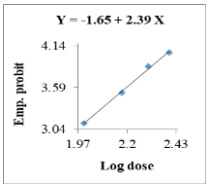
b) Pet. ether extract after 48 h of exposure.



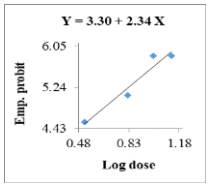
c) CHCl3 extract after 6 h of exposure.



d) CHCl3 extract after 48 h of exposure.

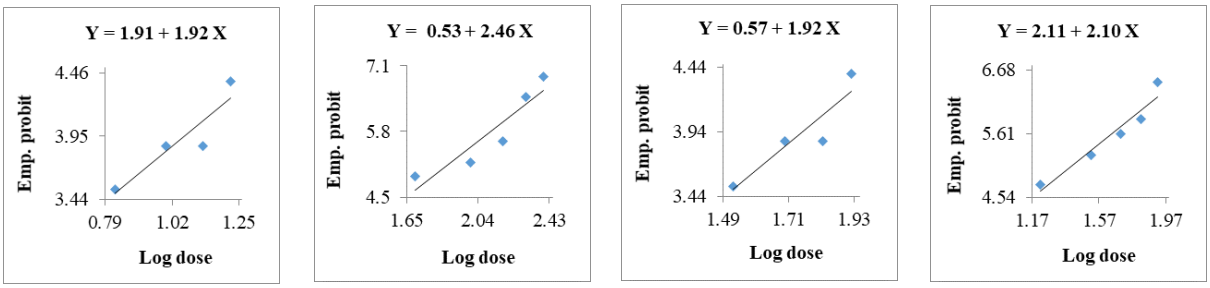


e) CH3OH extract after 6 h of exposure.



f) CH3OH extract after 48 h of exposure.

Fig. 2 (a-f): Regression lines of *C. arietinum* extracts against *C. quinquefasciatus* larvae at minimum and maximum exposure times.



a) $CHCl_3$ extract after 6 h of exposure.

b) $CHCl_3$ extract after 48 h of exposure.

c) CH_3OH extract after 6 h of exposure.

d) CH_3OH extract after 48 h of exposure.

Fig. 3(a-d): Regression lines of *C. arietinum* extracts versus *P. reticulata* fish at minimum and maximum times of exposure.

Discussion

According to the present study, the selected plant, *C. arietinum*, contains compounds with cytotoxic, larvicidal, and piscicidal effects. These results are corroborated by experiments conducted on *C. arietinum* by previous researchers. Kumar et al. (2014) isolated a novel antifungal protein, C-25, from *C. arietinum* and purified it by gel filtration. This protein acts as an antifungal and antiproliferative agent on human oral cancer cells (Kumar et al. 2014, Gonfa et al. 2020). The results of Rao et al. (2014) revealed that this plant extract produced anti-inflammatory and analgesic effects in rats (Segev et al. 2010). However, a study by Segev et al. (2010) showed that isolated hulls from a colored chickpea variety contain high levels of polyphenols and flavonoids, conferring strong antioxidant activity (Waili et al. 2018). Furthermore, Waili et al. (2018) extracted homogeneous peptides from chickpeas in the laboratory and tested their antimicrobial activity against *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus* (Kansal et al. 2008), which corroborates the results of the present study.

However, according to Kansal (2008), the trypsin inhibitor from chickpea has demonstrated inhibitory activity against Helicoverpa armigera, both *in vitro* and *in vivo* (Kansal et al. 2008). Chickpea is also an important source of B vitamins (riboflavin, thiamine, niacin, and pyridoxine) as well as micronutrients, including iron (Fe), zinc (Zn), calcium (Ca), magnesium (Mg), potassium (K), copper (Cu), and phosphorus (P) (El-Adawy 2002, Arcan and Yemenicioğlu 2007, Thavarajah 2012). A study conducted by Arcan and Yemenicioğlu (2007) examined the thermal stability of antioxidant protein extracts (water-soluble) obtained from cooked chickpeas (121°C/20 min) (Chino et al. 2017). The development of new anticancer agents from natural sources such as chickpea seeds could represent a viable alternative for cancer prevention and treatment (Girón-Calle et al. 2004, Marcela et al. 2016, Chalamaiah et al. 2018). Furthermore, Girón-Calle et al. (2004) showed that $CaCO_2$ colon cancer cells exhibited an 80% reduction in proliferation after 4 to 6 days of treatment with an ethanol-acetone soluble fraction extracted from a chickpea protein concentrate (Girón-Calle et al., 2004).

However, Ye et al. (2002) extracted two peptides, cicerin (8.2 kDa) and arietin (5.6 kDa), from chickpea seeds. Both peptides showed antifungal activity against *Mycosphaerella arachidicola*, *Fusarium oxysporum*, and *Botrytis cinerea*. Interestingly, arietin exhibited stronger activity than cicerin (Arisawa et al. 1985). The results of the present study are also corroborated by those of Arisawa et al. (1985), who demonstrated the anti-ulcer, antibacterial, antifungal, and antitumor properties of tocotrienols, tocopherols, and steroids present in chickpea oil. Kan et al. (2010), researchers studied chickpea seed extracts and found antibacterial activity against Gram-negative bacteria such as Escherichia coli (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 10145), and *Klebsiella pneumoniae* (RSKK 574), with a minimum inhibitory concentration (MIC) ranging from 16 to 64 µg/mL (Kokoszko et al. 2017). Chickpeas contain a significant amount of iron, which contributes to red blood cell synthesis and can thus reduce fatigue and nausea (Fernando et al. 2010, Xu and Chang 2012). Finally, the results of Xu and Chang (2012) revealed that hydrophilic extracts of chickpea flour had antiproliferative activity on squamous cell carcinoma cells (CAL27), gastric adenocarcinoma cells (AGS), colorectal adenocarcinoma cells (SW 480), mammary adenocarcinoma cells (MCF-7) and leukemic cells (HL-60), with IC_{50} values of 2.31, 3.23, 5.71, 3.36 and 3.23 mg/ml, which also supports the results of the present study.

Conclusion

Analysis of cytotoxicity test results against A. salina, larvicidal activity against *C. quinquefasciatus*, and piscicidal activity against *P. reticulata* concluded that *C. arietinum* contains potent bioactive compounds with potential applications in the pharmaceutical industry, vector control, and aquatic pest management. These plant-derived, natural components are biodegradable, safe, environmentally friendly, and commercially viable. However, further research is needed to precisely identify the active principles and mechanisms responsible for this activity.

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Author's contribution: TM, KMM and AI collected samples and data, conducted experiments, writing the draft, analysis was done statistically. NI and MN designed the conceptualization and supervised the study, writing review and editing the manuscript. All authors have read and approved the final manuscript.

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