Volume 7, Issue 1: KUS: MariNEX 1508: xx-xx: 19102025: 2026

KHULNA UNIVERSITY
STUDIES

journal homepage: www.kus.ku.ac.bd

Research article

Solvent Effects on Yield and Toxicity of some Spice Extracts for Aquaculture

Most. Arfin Naher Eva, Rajdwip Sarkar, Nabonita Roy, Uttam Adhikary, Halima Tus Sadia and Alokesh Kumar Ghosh*

Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna-9208, Bangladesh

A B S T R A C T

Medicinal spices serve as antibiotic alternatives in aquaculture via immune-stimulating phytochemicals. The current study intends to assess the spice extraction yield and toxicity using the brine shrimp lethality test (BSLA) and the zebrafish larvae acute toxicity test (Zebrafish assay) in five different solvents including hexane (H), ethyl acetate (EA), ethanol (E), methanol (M), and water (W). Probit analysis was used to calculate the LC_{50} for zebrafish and brine shrimp assay. The highest extraction yield was obtained from cashew nut (49%), while piper chili showed the lowest yield (2.7%). Among the extracts, piper chili and black pepper exhibited the highest toxicity across nearly all solvents in both toxicity models, whereas mustard seed, poppy seed and fenugreek seed showed reduced toxicity levels, indicating their potential suitability for aquaculture applications. Probit regression analysis (p < 0.05) indicated that all water extracts of spices were non-toxic (LC_{50} > 1000 µg/mL). In terms of sensitivity, Zebrafish larvae exhibited a higher toxicity response than brine shrimp, as evidenced by significantly lower LC_{50} values in the zebrafish assay compared to BSLA. The results highlight the importance of careful solvent selection to optimize bioactive chemical extraction, avoiding toxicity, conserving compound integrity, and ensure the safe, effective, and sustainable use of spice extracts in aquaculture.

A R T I C L E   I N F O

Article timeline:
Date of Submission:
19 October, 2025
Date of Acceptance:
26 April, 2026
Article available online:
5 May, 2026

***Keywords*:**
Spices
Solvents
Toxicity
Aquaculture
Brine shrimp
Zebrafish
LC_{50}

Introduction

The growing dependence on antibiotics in aquaculture, fueled by disease outbreaks, has generated concerns about antibiotic resistance and environmental harm, leading to a shift toward organic alternatives featuring medicinal herbs and spices (Sumana et al., 2025; Ghosh et al., 2025). Antibiotics play a major role in Bangladesh's rapidly expanding finfish aquaculture industry. According to a national survey, 71% of inland and coastal farms stated that they utilized antibiotics at least once per production cycle, with oxytetracycline, ciprofloxacin, and amoxicillin being the most commonly used. This raises serious concerns about the development of resistance in culture ponds (Chowdhury et al., 2022). Spice antioxidants enhance fish health in aquaculture via phytochemicals, which are ascribed to their rich phytochemical makeup (Taştan and Salem, 2021). The phytochemicals (primarily phenolics, flavonoids, terpenes, and alkaloids) in spices have been shown in studies to improve fish antioxidant status and immunological response, making them ideal feed additions in aquaculture (Ahmmed et al., 2025; Ghosh et al., 2025; Guldiken et al., 2018). Though spices have been widely used, they vary in terms of concentration of the phytochemicals. This can also greatly vary depending on the extraction techniques and conditions and, hence, can considerably change their bioactive potential. The extraction yields of bioactive compounds (phenolics, flavonoids, terpenes, and alkaloids) are affected by a number of factors including solvent polarity, time of extraction, temperature, and sample-solvent ratio (Tripathi et al., 2025). Various approaches like solvent extraction, steam distillation, and supercritical fluid extraction can yield different spice extracts (Hossain et al., 2017). The differences may also have a profound impact on the potency of the phytochemicals, which explains why there is a need to optimize extraction processes to obtain consistent recovery of bioactive compounds.

While the health benefits of spice phytochemicals are well-documented, the safety of natural additives in aquaculture, particularly phytochemicals (phenolics, flavonoids, terpenes, and alkaloids), must be thoroughly assessed, as certain plant parts and extraction processes may be toxic despite their prevalent perception as innocuous natural products (Gabor et al., 2010; Guldiken et al., 2018). Toxicity in herbal extracts can be caused by contaminants or plant compounds, and assays, such as in

*Corresponding author: <alokesh.ghosh@fmrt.ku.ac.bd>

DOI: https://doi.org/10.53808/KUS.2026.SI.7.01.MariNEX1536-ls

vivo experiments on laboratory animals, are used to assess their potential harm (Hamidi et al., 2014). Acute toxicity investigations, particularly the assessment of median lethal concentration (LC_{50}), show significant variation in the toxicological effects of essential oils and plant extracts across species (Jacques Quignard et al., 2004). Brine shrimps serve as a model organism in toxicological assays. The ease of rearing millions of brine shrimp has significantly facilitated the assessment of various environmental pollutants' effects on these organisms under controlled experimental circumstances (Borja et al., 2016; Moshi et al., 2004). In toxicological research, the zebrafish is a dependable model because of its high fecundity, quick growth, and capacity to dissolve substances directly in embryos. Because of its transparency, developmental staging and non-invasive toxicological endpoint evaluation are made simple (Satpathy and Parida, 2020; Yumnamcha et al., 2015).

Although the effect of spices as antioxidants is not a new topic studied in the past, the gap in knowledge about the extraction efficacy and toxicity of the compounds in aquaculture is still apparent. Moreover, the study design using a comparative approach of the brine shrimp lethality test (BSLA) and zebrafish assays is novel and has not been widely used to measure the safety of spice extracts in aquaculture environment. Therefore, this study aims to evaluate the extraction efficiency of some selected spices in Bangladesh using several solvents and to assess their toxicity by using the BSLA and zebrafish assays in a comparative framework. This integrative approach seeks to identify safe and effective extraction conditions, thereby supporting to increase the awareness of sustainable and effective use of spice extracts in aquaculture and other areas.

Materials and Methods

Experimental spices and solvents

The experimental spices were purchased from Khulna City market in order to assess their extraction yield and potential for toxicity. The species' scientific name, common name, local name, and comprehensive details were recorded (Table 1). Five different solvents, including hexane (H), ethyl acetate (EA), ethanol (E), methanol (M), and water (W), were used to extract the spices. The name of the spices was collected from the website 'http://www.theplantlist.org'. The pharmacological properties presented in the Table 1 are derived from existing literature and should be regarded as suggestive of the potential advantages of the spices, rather than research results.

Extract preparation using small scale extraction

Yields were obtained in accordance with Panda et al. (2019) and Ghosh et al. (2024) after the small-scale extraction technique was completed. Each component was dried in an oven set to 35°C after the spices were cleaned in order to remove any last traces of moisture. A 500 µm particle size was achieved by grinding the dry materials into a fine powder using a mechanical grinder, and the powder's weight was recorded. To summarize, 10 mL of each solvent (hexane, ethyl acetate, ethanol, methanol and water, separately) and 1g of each powder were put into 15 mL sterile polypropylene tubes. In a sonicator water bath (Hwashin, Korea), extraction was performed at room temperature by sonicating the samples for four times for 15 minutes at 6-hour intervals (Panda et al., 2019). Following a 24-hour period, the tubes were centrifuged for 10 minutes at 3000 rpm, and the supernatant was then moved to tubes that had been previously weighed. A hood was used to remove the organic solvents, and a freeze dryer was used to remove the aqueous solvent. The residue from the water extract was cleaned with 70% ethanol to get rid of contaminants. The complete procedure was carried out three times in order to reduce experimental error. To achieve a final stock concentration of 100 mg/mL, the organic extracts were dissolved in DMSO and the water extract was dissolved in autoclaved water. Until further examination, all samples were stored at 4°C.

Toxicity Test of extracts using Brine shrimp (*Artemia* sp.) lethality assay

With a few minor adjustments, the toxicity test of spice extracts was carried out using Waghulde et al. (2019). In order to create artificial seawater, 17.5 g of sea salt was dissolved in 500 mL of distilled water, and the mixture was thoroughly mixed until the salt was completely dissolved. A hatching container containing artificial seawater was then filled with 1g of brine shrimp, and the container was incubated for 24 hours at 25–28°C with constant light and aeration to allow the brine shrimp to hatch. The nauplii (larvae) were separated from the unhatched egg by attracting them to a light source and collecting them with a pipette after hatching. The stock solution that was kept at 4°C (100 mg/mL of all samples) was utilized. The toxicity was subsequently assessed using the subsequent approach after a two-fold serial dilution (starting final concentration, 1000 µg/mL) (Rajabi et al., 2015). Each well containing test solutions (1000, 500, 250, 125 µg/mL, etc.) received ten brine shrimp nauplii in triplicate. As a control, artificial seawater containing DMSO was used. To avoid evaporation, the 96-well plate was covered and incubated for 24 hours at 25-28 °C with continuous illumination. Following incubation, mortality percentages for each concentration were computed by counting the number of live and dead nauplii under a microscope (Sarah et al., 2017).

$$\text{Mortality\%} = \frac{no.of\ dead\ nauplii}{no.of\ dead\ nauplii + no.of\ live\ nauplii} \times 100$$

The LC_{50} values were calculated by comparing the quantity of dead nauplii to an upward trend in spice extract concentration. The concentration that kills 50% of the nauplii (LC_{50}) was calculated using statistical software and the Probit method with a level of significance of 95%.

Toxicity test of spices extracts using Zebrafish Larvae

The toxicity assay methodology for zebrafish larvae was modified from Xiong et al. (2022). In accordance with guideline No. OECD TG 236 (e.g., controlled temperature, defined dilution water/vehicle and mortality-based endpoints), the aquarium fish Danio rerio (Zebrafish) was subjected to toxicity testing using spice extracts throughout the larval stages. At 2 dpf (days post fertilization), the eggs were placed in a light incubator, and

Table 1: The experimental spices (common name, scientific name family and pharmacological properties)

English name	Local Name	Scientific Name	Family	Used Part	Pharmacological properties
Cashew Nut	Hijolbadam	*Anacardium occidentale* L.	Anacardiaceae	Seed (nut)	antioxidant, anti-inflammatory, antimicrobial, hepatoprotective (Chen et al., 2023).
Mustard Seed	Shorisha	*Brassica juncea* (L.) Czern.	Brassicaceae	Seed	antimicrobial, antioxidant, antidiabetic, cardioprotective (Tian and Deng, 2020).
Cardamom	Elach	*Elettaria cardamomum* (L.) Maton	Zingiberaceae	Fruit (capsule)	antioxidant, antimicrobial, gastroprotective, (Abdullah et al., 2022).
Poppy Seed	Posta Dana	*Papaver somniferum* L.	Papaveraceae	Seed	analgesic, sedative, antioxidant, antispasmodic, anti-inflammatory (Muhammad et al., 2021).
Piper Chilli	Choi Jhal	*Piper chaba* Hunter	Piperaceae	Fruit	antimicrobial, antioxidant, gastroprotective, (Salehi et al., 2019).
Black Pepper	Golmorich	*Piper nigrum* L.	Piperaceae	Fruit (berry)	antioxidant, antimicrobial, hepatoprotective, antidepressant (Takooree et al., 2019).
Fenugreek Seed	Methi	*Trigonella foenum-graecum* L.	Fabaceae	Seed	antioxidant, antimicrobial, hypocholesterolemic, gastroprotective (Gavahian et al., 2024).
Ginger	Ada	*Zingiber officinale* Roscoe	Zingiberaceae	Rhizome	antioxidant, antimicrobial, antidiabetic (Mao et al., 2019).

the larvae were collected at 4 dpf for acute toxicity testing. Zebrafish larvae (4 days post-fertilization) were subjected to different concentrations of a test solution (range from 1.95 to 1000 µg/mL) in a 12-well plate. Each well had 10 larvae and three replicates for each concentration. DMSO and water have served as controls. The plate was incubated under light for 24 hours, and dead larvae were counted utilizing a microscope. The mortality percentage for each concentration was then estimated using a standard formula (Xiong et al., 2022).

$$\text{Mortality\%} = \frac{no.of\ dead\ nauplii}{no.of\ dead\ nauplii + no.of\ live\ nauplii} \times 100$$

Probit analysis was used to calculate the LC_{50} for zebrafish at 24 hours post-immersion (hpi) with a 95% confidence level.

Data analysis

The data was analyzed using Microsoft Excel 2024. The yields obtained with different solvents were compared. Extraction yield was calculated using the formula: $\frac{W_2 - W_1}{W_0} \times 100$ (Anokwuru et al., 2011). Where, W_1 = The weight of the Eppendorf alone, W_2 = The weight of the extract and the Eppendorf and W_0 = The weight of the initial dried sample. Every experiment was compared to its corresponding negative control. Probit Regression Analysis ($p < 0.05$) was used in Microsoft Excel to determine median lethal concentrations (LC_{50}) in order to express toxicity (Finney, 1971).

Results

Extraction yield of spices in different solvents

The extraction yield of eight distinct spices was tested using five different solvents (hexane, ethyl acetate, ethanol, methanol, and water). The findings are shown in Figure 1, which shows significant differences in extraction efficiency based on the solvent polarity and the spice under consideration. Among the individual spices *Anacardium occidentale* had the greatest extraction yield (49.3%) in ethyl acetate, while the *Piper chaba* exhibited the lowest extraction yield (2.7%) in hexane. Most spices had the lowest extraction efficiency when extracted with methanol, yield ranged from 5.7% for ginger to 22% for poppy seed. In terms of overall solvent performance, the extraction yield was highest with ethyl acetate, followed by water, hexane, ethanol, and methanol.

Table 2: Degree of Toxicity (Clarkson et al., 2004)

LC_{50} Value (µg/mL)	Toxicity Range
>1000	Non-Toxic
500 - 1000	low toxic
100 - 500	medium toxic
0 - 100	highly toxic

Toxicity bioassay of brine shrimp and zebrafish using Probit analysis

The results of the mortality test for Brine Shrimp nauplii and Zebrafish larvae were considered valid since the control sample had a death rate of 0%. The LC_{50} values, which were deemed harmful by the Clarkson's toxicity criterion (Clarkson et al., 2004), were estimated using Probit analysis and a statistical comparison (ANOVA) was performed to assess the significant differences among the solvents. The findings showed the significant differences across solvents for different spices.

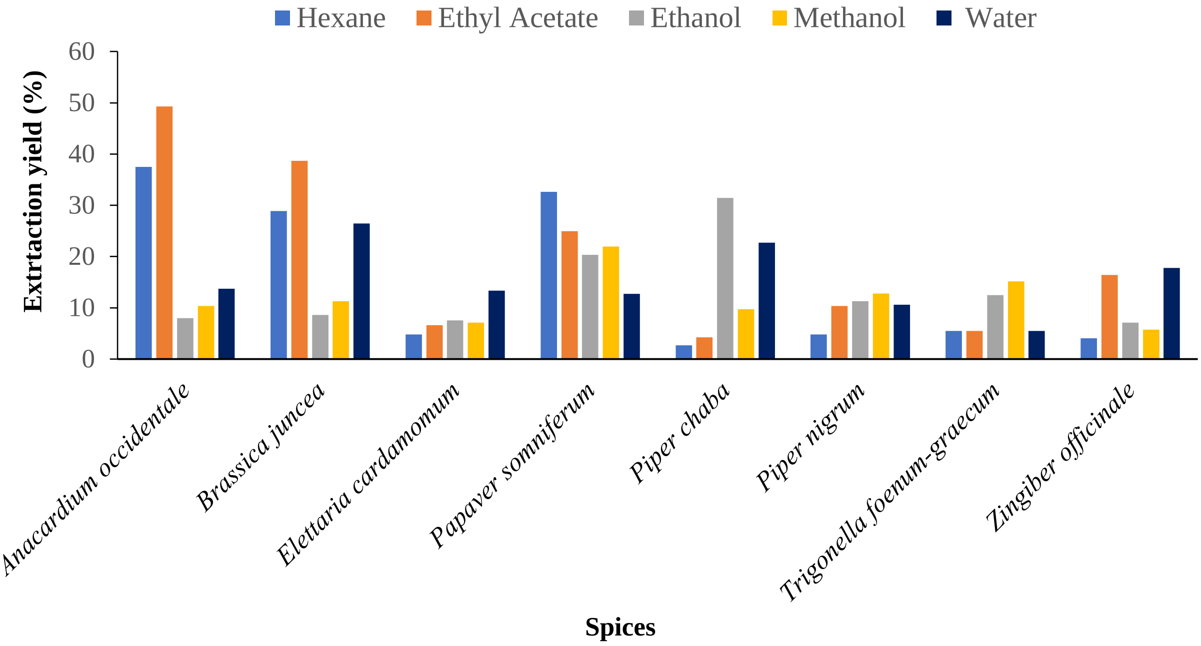

Figure 1: Yield (%) of 8 spices with respect to five solvents

Determination of the LC_{50} in the Brine shrimp lethality assay

The LC_{50} values (µg/mL) are summarized in Table 2 with LC_{50} values ranging from 15 µg/mL (hexane) to 54 µg/mL (ethyl acetate); black pepper (*Piper nigrum*) showed the highest toxicity of any spice in all solvents. However, it showed medium toxicity in water, with LC_{50} values of 326 µg/mL. Piper chilli also showed highest toxicity across all solvents except water ranging between 18 µg/mL (ethyl acetate) to 80 µg/mL (ethanol). Ginger and cashew nut also demonstrated notable toxicity in methanol (17 µg/mL) and ethanol (32 µg/mL), respectively.

In contrast, mustard seed and poppy seed exhibited non-toxicity across all solvents with an LC_{50} > 1000 µg/mL, except in ethanol (with LC_{50} of 620 µg/mL and 818 µg/mL, respectively). Water produced non-toxic effects for most of the spices extracts except black pepper (326 µg/mL) and fenugreek seed (814 µg/mL), while non-polar solvent hexane exhibited moderate toxicity for most of the spice extracts.

Determination of the LC_{50} in the zebrafish model

Table 2 shows the LC_{50} values of zebrafish model tends to be more sensitive compared to BSLA. *Piper chaba* and *P. nigrum* were the most toxic spices with the LC_{50} values of between 5 and 41 µg/mL in all solvents, which makes them very toxic. Ginger was extremely toxic in all solvents, with LC_{50} values lower than 100 µg/mL. The non-polar solvent hexane gave the widest high-toxicity range, with LC_{50} values ranging between 5 and 134 µg/mL on most spices, except mustard seed with the LC_{50} of more than 1000 µg/mL. Ethyl acetate provided mixed responses: ginger, black pepper, *Piper chaba*, and cardamom were very toxic and the mustard seed, poppy seed and fenugreek were non-toxic. Most spices were highly toxic to extracts of ethanol or methanol exception being fenugreek, cardamom and poppy seed. The polarity of the solvent has a strong effect on toxicity because polar solvents (ethanol, methanol) were shown to extract more toxic compounds overall.

Comparison of toxicity between two bioassay model

Figure 2 shows the comparative toxicity of two models, namely the zebrafish larvae model and the brine shrimp fatality test. As evidenced by a higher percentage of extract falling into the extremely toxic group (LC_{50} between 0-100 µg/mL) for the zebrafish model, the results indicated that zebrafish larvae were more sensitive to the spice extracts than brine shrimp, particularly for the polar solvents. In hexane 75% of spice extracts exhibited higher toxicity to zebrafish, on the other hand only 25% showed high toxicity to brine shrimp. In semi-polar solvent (ethyl acetate) it showed 50% for zebrafish and 25% for brine shrimp. The figure 2 shows that zebrafish larvae were more toxic to most spice extracts, especially when extracted with polar solvents (ethanol, methanol), where toxicity levels were consistently higher than those found in the brine shrimp model. This comparative visual analysis clarifies the numerical data shown in Tables 2 and 3, displaying the overall trend and highlighting major discrepancies.

Discussion

The selection of solvents and the extraction process is greatly dependent on the extraction process of plants and herbs, which in turn has a direct impact on the bioactivity

Table 3: LC_{50} of Different spices against Different solvent in BSLT

Brine Shrimp Lethality Assay						
Spices		LC_{50}				
Common Name	***Scientific Name***	**Hexene**	**EA**	**Ethanol**	**Methanol**	**Water**
Cashew nut	*Anacardium occidentale*	151 ± 2.89 (MT)	614 ± 4.55 (LT)	32 ± 1.7 (HT)	22 ± 1.24 (HT)	>1000 (NT)
Mustard Seed	*Brassica juncea*	>1000 (NT)	>1000 (NT)	620 ± 0.72 (LT)	>1000 (NT)	>1000 (NT)
Cardamom	*Elettaria cardamomum*	322 ± 5.78 (MT)	257 ± 1.30 (MT)	347 ± 5.14 (MT)	154 ± 3.7 (MT)	>1000 (NT)
Poppy Seed	*Papaver somniferum*	>1000 (NT)	>1000 (NT)	818 ± 6.18 (LT)	>1000 (NT)	>1000 (NT)
Piper chilli	*Piper chaba*	42 ± 3.62 (HT)	18 ± 0.83 (HT)	80 ± 0.92 (HT)	20 ± 2.13 (HT)	>1000 (NT)
Black Pepper	*Piper nigrum*	15 ± 0.78 (HT)	54 ± 2.99 (HT)	19 ± 2 (HT)	18 ± 0.9 (HT)	326 ± 2.61 (MT)
Fenugreek Seed	*Trigonella foenum-graecum*	348 ± 4.19 (MT)	>1000 (NT)	>1000 (NT)	>1000 (NT)	814 ± 6.18 (LT)
Ginger	*Zingiber officinale*	163 ± 3.10 (MT)	106 ± 4.87 (MT)	37 ± 2.04 (HT)	17 ± 0.75 (HT)	>1000 (NT)

of extracts (Lezoul et al., 2020). The present study indicated that there was a total of five solvents (hexane, ethyl acetate, ethanol, methanol, water) used in solubilizing the compounds in eight spices and these solvents had different extraction efficiency. In earlier works, it has been shown that polar solvents are more efficient when dealing with hydrophilic compounds; meanwhile, non-polar solvents are more efficient when it comes to extracting lipophilic compounds (Correia et al., 2023). These findings are also consistent with this study and it is observed that the highest extraction yields were obtained with methanol and in many cases with hexane and water, which gave the highest yields. With a moderate level of polarization, ethyl acetate displayed a higher level of extraction efficiency than hexane, extracting 49% of cashew nuts and 39% of mustard seeds. This goes in line with the hypothesis that moderately polar solvents are very efficient in extracting both polar and non-polar compounds. Further, this observation also demonstrates the unique role of ethyl acetate in the extraction of lipophilic compounds such as cashew and mustard seeds, because it is more polar and, accordingly, these compounds are more similar to this solvent. Conversely, non-polar hexane demonstrated different levels of efficiency in different spices with a maximum of 32.6% yield in poppy seed and a relatively low yield in mustard seed (28.9%).

Polar solvents like ethanol and methanol gave lower extraction values of most spices. More research ought to be done to explain why ethyl acetate produces more extract than other solvents. This might include looking at the impact of polarity when extracting particular lipophilic or hydrophilic compounds; particularly concerning the profile of each spice. The study in the future therefore needs to involve the matching of the solvent polarity with bioactive compounds found in spices. Aqueous extraction is more effective for isolating polar compounds like flavonoids and phenolics from plants such as *Moringa oleifera* and *Craterostigma plantagineum*, while ethanol targets non-polar compounds like alkaloids and triterpenoids, potentially leading to lower yields for certain phytochemicals compared to water (Maina et al., 2023; Palaiogiannis et al., 2023).

In the case of toxicity assays, ethanol and methanol are polar solvents, which generated more toxicity compared to non-polar solvents such as hexane. These findings suggest that polar solvents are likely to extract larger biologically active substances that might augment toxicity (Puthongking et al., 2023; Dimitrova et al., 2024). The dose-response curve between toxicity and suitability should be studied. The toxicity is high and can be controlled, and does not imply that these extracts cannot be used in aquaculture. Therefore, the toxicity should have been considered in the context of dose-response, which we intend to investigate in the experiments that we are going to conduct. Examples include research that would establish the best extract doses that would not cause toxicity but retain bioactivity, thereby enhancing their use in aquafeeds. This would entail the determination of the impact of various extract concentrations on fish health, including the immune responses as well as growth. Piper chilli and black pepper are extremely toxic due to their high amount of piperine and capsaicin, both of which have significant bioactive characteristics (Meghwal and Goswami, 2013; Srinivasan, 2007). Notably, aqueous extracts of all spices exhibited non-toxicity (LC_{50} > 1000 µg/mL) except black pepper and fenugreek seed. This highlights that water cannot extract harmful lipophilic compounds, making it the safest extraction medium for toxicity and more appropriate for aquaculture.

Table 4: LC_{50} of Different spices against Different solvent in Zebrafish Larvae

Zebrafish Larvae						
Spices		**LC_{50}**				
Common Name	***Scientific Name***	**Hexene**	**EA**	**Ethanol**	**Methanol**	**Water**
Cashew nut	*Anacardium occidentale*	134 ± 3.84 (MT)	552 ± 9.58 (LT)	3 ± 0.21 (HT)	67 ± 2.05 (HT)	>1000 (NT)
Mustard Seed	*Brassica juncea*	>1000 (NT)	>1000 (NT)	33 ± 0.21 (HT)	11 ± 2.51 (HT)	>1000 (NT)
Cardamom	*Elettaria cardamomum*	31 ± 0.43 (HT)	59 ± 1.54 (HT)	74 ± 5.98 (HT)	137 ± 1.65 (MT)	>1000 (NT)
Poppy Seed	*Papaver somniferum*	29 ± 0.69 (HT)	>1000 (NT)	26 ± 0.7 (HT)	903 ± 3.25 (LT)	>1000 (NT)
Piper chilli	*Piper chaba*	5 ± 0.58 (HT)	12 ± 0.62 (HT)	5 ± 0.87 (HT)	2 ± 0.01 (HT)	>1000 (NT)
Black Pepper	*Piper nigrum*	5 ± 0.48 (HT)	6 ± 0.92 (HT)	41 ± 2.42 (HT)	14 ± 0.56 (HT)	>1000 (NT)
Fenugreek Seed	*Trigonella foenum-graecum*	46 ± 2.11 (HT)	>1000 (NT)	>1000 (NT)	67 ± 3.15 (HT)	>1000 (NT)
Ginger	*Zingiber officinale*	28 ± 1.75 (HT)	5 ± 0.94 (HT)	13 ± 0.87 (HT)	9 ± 1.12 (HT)	>1000 (NT)

Zebrafish larvae assay was found to be much sensitive as compared to the brine shrimp lethality assay, as shown in Figure 2. Higher proportions of extracts were in the highly toxic group in zebrafish in comparison to the BSLA. This distinction highlights the benefits of zebrafish which have vertebrate relevant metabolic and developmental pathways, behavior and neurotoxicity, making them more mechanistically informative and better matched with mammalian hazard profiles than brine shrimp (Modarresi Chahardehi et al., 2020; Von Hellfeld et al., 2023). It thus can be used to predict fish species in a better way than the invertebrate BSLA. Although BSLA is a fast, low-cost initial screen, its reduced sensitivity prevents extrapolation to aquaculture species.

Upon aquaculture applications, water extracts of mustard seed, poppy seed, and others, including cashew in water, reveal themselves as promising candidates of immunostimulants or functional feed supplements, of non-toxicity or low-toxicity. Their suitability is due to their low acute toxicity and possible bioactive effects (e.g., phenolics, glucosinolates). Toxic spices such as *Piper chaba*, *P. nigrum* and *Zingiber officinale* that contain high concentrations of piperine or capsaicin can be antiparasitic. Nevertheless, the dose-response optimization is necessary to prevent the negative effect at the feed-inclusive levels (Shin et al., 2023; Ruiz et al., 2024). Toxicity does not necessarily imply inappropriateness. Safe windows can be available at low levels where immune stimulatory effects take place without being fatal or growth impairing. These opportunities are conjectural until specific in vivo feeding experiments determine growth performance, feed ratio, immunity (e.g. lysozyme, phagocytic activity), and disease resistance in target aquaculture.

The use of BSLA as a preliminary screen is a major weakness. Although it is associated with cytotoxicity in certain experiments, there is no consensus with fish or zebrafish toxicity because of the difference in metabolism, routes of exposure, and toxicity. Zebrafish has greater vertebrate relevance, but has to be validated against real farmed fish. Besides, solvents left in extracts particularly organic ones like ethyl acetate or methanol may confound toxicity unless they are completely evaporated. This may over estimate effects or be artificially present in aquaculture uses. The solvent removal should be verified and the safety of the residue determined according to feed standards in future research.

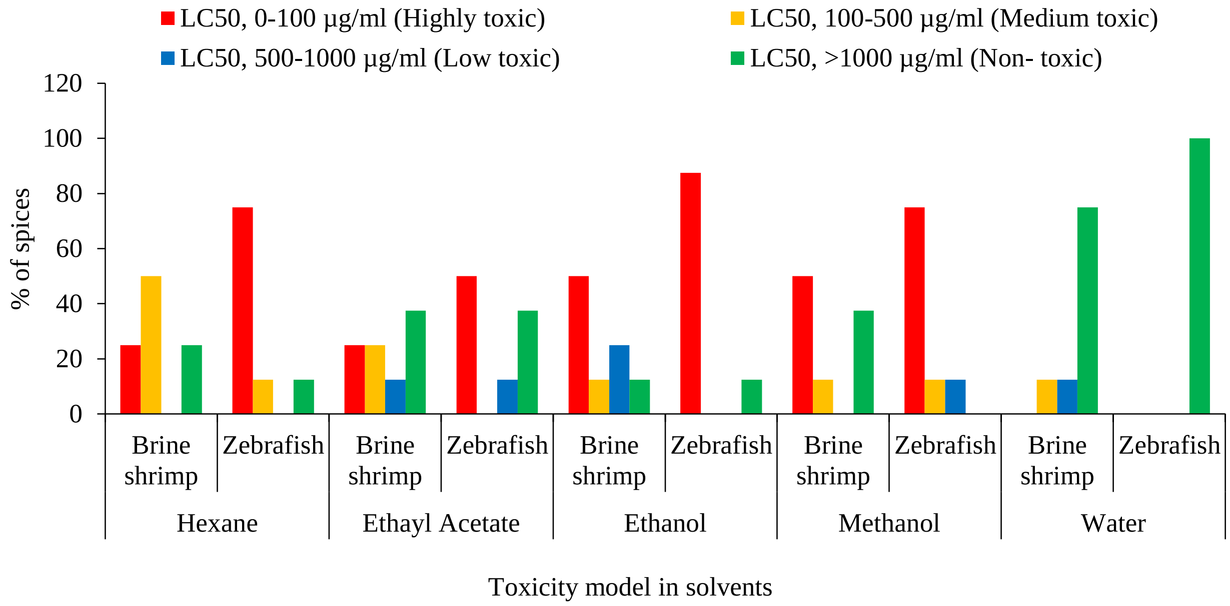

Figure 2: Comparison of toxicities between two models concerning spice extract

Conclusion

The findings indicate that the polarity of the solvent affects the yield of extraction and the toxicity of the spice extracts. Polar solvents (like methanol) have high polar bioactives yield but are more toxic whereas nonpolar solvents such as hexane are more appropriate to extract lipophilic compounds with less toxicity. Ethyl acetate is a fairly toxic solvent; it offers a compromise between the effectiveness of the extraction process and safety. These results are significant in the context of solvent choice to be used in certain applications, like toxicity testing, food, and pharmaceuticals. Nonetheless, the evidence of the direct use of these solvents in aquaculture is not entirely supported by experimental data, and more studies are required to prove these results in aquaculture. Future research should aim at identifying the bioactivity compounds that cause toxicity and determine the potential of the spice extracts as natural feed additives with aquaculture experiments that can be used to determine their effect on fish growth, immunity and resistance to diseases.

Ethical Approval

Ethical approval for this study was obtained from the Ethical Review Committee of the 'Research and Innovation Center' at Khulna University. The ethical code is KUAEC-2025-06-23.

Funding

The research was funded by the ‘Research and Innovation Centre’ (SL: KURIC-C-RGP-96-2023/24), Khulna University, Khulna, Bangladesh.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

The Head of the Fisheries and Marine Resource Technology Discipline at Khulna University in Bangladesh is to be thanked for providing the lab facilities. For the technical support, Md. Sumon Rahman is appreciated.

Authors’ contribution

Most. Arfin Naher Eva: Methodology, Data curation, Software, Writing-original draft preparation; **Rajdwip Sarkar:** Methodology, Data curation, Software, Writing-original draft preparation; **Nabonita Roy:** Methodology, Data curation; **Uttam Adhikary:** Methodology, Data analysis; **Halima Tus Sadia:** Methodology, Data curation, review; **Alokesh Kumar Ghosh**: Supervision, Fund acquisition, Writing - review & editing.

References

Abdullah, Ahmad, N., Tian, W., Zengliu, S., Zou, Y., Farooq, S., ... & Xiao, J. (2022). Recent advances in the extraction, chemical composition, therapeutic potential, and delivery of cardamom phytochemicals. *Frontiers in Nutrition*, *9*, 1024820.

Ahmmed, S. S., Sadia, H. T., Nasrin, F., Adhikary, U., Sarower, M. G., & Ghosh, A. K. (2025). Enhancing Growth and Immune Responses in Giant Freshwater Prawn (Macrobrachium rosenbergii) via Oral Administration of Fennel (Foeniculum vulgare) Extract against Vibrio parahaemolyticus. *Comparative Immunology Reports*, 200247.

Anokwuru, C. P., Esiaba, I., Ajibaye, O., & Adesuyi, A. O. (2011). Polyphenolic content and antioxidant activity of Hibiscus sabdariffa calyx.

Borja, K. D. B., Buhion, V. T. L., & Canalita, E. E. (2016). Toxicity test on different brands of food seasonings using brine shrimp (Artemia salina) lethality test. *Biol Chem Res*, *3*, 227-33.

Chen, Y. Y., Li, N. Y., Guo, X., Huang, H. J., Garcia-Oliveira, P., Sun, J. Y., ... & Liu, C. (2023). The nutritional and bio-active constituents, functional activities, and industrial applications of cashew (Anacardium occidentale): A review. *Food Frontiers*, *4*(4), 1606-1621.

Chowdhury, S., Rheman, S., Debnath, N., Delamare-Deboutteville, J., Akhtar, Z., Ghosh, S., ... & Chowdhury, F. (2022). Antibiotics usage practices in aquaculture in Bangladesh and their associated factors. *One Health*, *15*, 100445.

Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G., ... & Folb, P. I. (2004). In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *Journal of ethnopharmacology*, *92*(2-3), 177-191.

Correia, V. T. da V., Silva, V. D. M., Mendonça, H. de O. P., Ramos, A. L. C. C., Silva, M. R., Augusti, R., De Paula, A. C. C. F. F., Ferreira, R. B., Melo, J. O. F., & Fante, C. A. (2023). Efficiency of Different Solvents in the Extraction of Bioactive Compounds from *Plinia cauliflora* and *Syzygium cumini* Fruits as Evaluated by Paper Spray Mass Spectrometry. *Molecules, 28*(5), 2359.

Dimitrova, S., Dragomanova, S., & Kehayova, G. (2024). Intravenous Lipid Emulsions in Anticonvulsants' Toxicity . *Scientia Pharmaceutica*, *92*(3), 37.

Finney, D. J. (1971). Probit analysis, Cambridge University Press. *Cambridge*, *UK*.

Gabor, E. F., Şara, A., & Barbu, A. (2010). The effects of some phytoadditives on growth, health and meat quality on different species of fish. *Scientific Papers Animal Science and Biotechnologies*, *43*(1), 61-61.

Gavahian, M., Bannikoppa, A. M., Majzoobi, M., Hsieh, C. W., Lin, J., & Farahnaky, A. (2024). Fenugreek bioactive compounds: A review of applications and extraction based on emerging technologies. *Critical Reviews in Food Science and Nutrition*, *64*(28), 10187-10203.

Ghosh, A. K., Dewaele, P., Hu, H., Panda, S. K., & Luyten, W. (2024). Antimicrobial efficacy of select medicinal plant extracts from Bangladesh against food-borne bacterial pathogens. *Journal of Food Safety*, *44*(3), e13147.

Ghosh, A. K., Islam, S. S., & Hasanuzzaman, A. F. M. (2025). Exploring the Potential of Medicinal Plants on Fish Resilience to Aeromonas hydrophila Infection: A Comprehensive Study of Immunology, Biochemistry, Growth and Survival. *Aquaculture, Fish and Fisheries*, *5*(3), e70084.

Guldiken, B., Ozkan, G., Catalkaya, G., Ceylan, F. D., Yalcinkaya, I. E., & Capanoglu, E. (2018). Phytochemicals of herbs and spices: Health versus toxicological effects. *Food and Chemical Toxicology*, *119*, 37-49.

Hamidi, M. R., Jovanova, B., & Panovska, T. K. (2014). Toxicological evaluation of the plant products using Brine Shrimp (Artemia salina L.) model. *Macedonian Pharmaceutical Bulletin/Makedonsko Farmacevtski Bilten*, *60*(1).

Hossain, M. B., Barry-Ryan, C., Martin-Diana, A. B., & Brunton, N. P. (2017). Optimisation of accelerated solvent extraction of antioxidant compounds from rosemary (*Rosmarinus officinalis* L.), marjoram (*Origanum majorana* L.) and oregano (*Origanum vulgare* L.) using response surface methodology. *Food Chemistry*, *116*(1), 35-44.

Jacques Quignard, E. L., Nunomura, S. M., Pohlit, A. M., Alecrim, A. M., da Silva Pinto, A. C., Portela, C. N., ... & Silva, S. G. (2004). Median lethal concentrations of Amazonian plant extracts in the brine shrimp assay. *Pharmaceutical Biology*, *42*(3), 253-257.

Lezoul, N. E. H., Belkadi, M., Habibi, F., & Guillén, F. (2020). Extraction Processes with Several Solvents on Total Bioactive Compounds in Different Organs of Three Medicinal Plants. *Molecules*, *25*(20), 4672.

Maina, G. J., Magak, N., Domnic, M., & Murugi, K. A. (2023). Phytochemicals Extraction in Craterostigma Plantagineum Hoscht: Aqueous and Ethanol Solvents. *Scholars International Journal of Anatomy and Physiology.*

Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Beta, T., & Li, H. B. (2019). Bioactive compounds and bioactivities of ginger (Zingiber officinale Roscoe). *Foods*, *8*(6), 185.

Meghwal, M., & Goswami, T. K. (2013). Piper nigrum and piperine: an update. *Phytotherapy Research*, *27*(8), 1121-1130.

Modarresi Chahardehi, A., Arsad, H., & Lim, V. (2020). Zebrafish as a successful animal model for screening toxicity of medicinal plants. *Plants*, *9*(10), 1345.

Moshi, M. J., Cosam, J. C., Mbwambo, Z. H., Kapingu, M., & Nkunya, M. H. (2004). Testing beyond ethnomedical claims: brine shrimp lethality of some Tanzanian plants. *Pharmaceutical Biology*, *42*(7), 547-551.

Muhammad, A., Akhtar, A., Aslam, S., Khan, R. S., Ahmed, Z., & Khalid, N. (2021). Review on physicochemical, medicinal and nutraceutical properties of poppy seeds: a potential functional food ingredient. *Functional Foods in Health and Disease*, *11*(10), 522-547.

Palaiogiannis, D., Chatzimitakos, T., Athanasiadis, V., Bozinou, E., Makris, D. P., & Lalas, S. (2023). Successive Solvent Extraction of Polyphenols and Flavonoids from Cistus creticus L. Leaves. *Oxygen 3*(3), 274–286.

Panda, S. K., Mohanta, Y. K., Padhi, L., & Luyten, W. (2019). Antimicrobial activity of select edible plants from Odisha, India against food-borne pathogens. *Lwt*, *113*, 108246.

Puthongking, P., Ratha, J., Panyatip, P., Datham, S., Siriparu, P., & Yongram, C. (2023). The Effect of Extraction Solvent on the Phytochemical Contents and Antioxidant and Acetylcholinesterase Inhibitory Activities of Extracts from the Leaves, Bark and Twig of Dipterocarpus alatus. *Tropical Journal of Natural Product Research*, *7*(12).

Rajabi, S., Ramazani, A., Hamidi, M., & Naji, T. (2015). Artemia salina as a model organism in toxicity assessment of nanoparticles. *DARU Journal of Pharmaceutical Sciences*, *23*(1), 20.

Ruiz, A., Sanahuja, I., Andree, K. B., Furones, D., Holhorea, P. G., Calduch-Giner, J. A., ... & Gisbert, E. (2024). Supplementation of gilthead seabream (Sparus aurata) diets with spices as a functional strategy to control excess adiposity through lipid, cholesterol and bile acid metabolism, and to induce an immunomodulatory intestinal regulation. *Aquaculture*, *581*, 740378.

Salehi, B., Zakaria, Z. A., Gyawali, R., Ibrahim, S. A., Rajkovic, J., Shinwari, Z. K., ... & Setzer, W. N. (2019). Piper species: A comprehensive review on their phytochemistry, biological activities and applications. *Molecules* , *24*(7), 1364.

Sarah, Q. S., Anny, F. C., & Misbahuddin, M. (2017). Brine shrimp lethality assay. ||| *Bangladesh Journal of Pharmacology*|||, *12*(2), 186-189.

Satpathy, L., & Parida, S. P. (2020). Acute Toxicity Assessment and Behavioral Responses Induced by Kandhamal Haladi in Adult Zebrafish (Danio rerio). *Biointerface Research in Applied Chemistry*, *11*(1), 7368-81.

Shin, J., Yun, K. S., Gunathilaka, B. E., Hasanthi, M., Ko, D., Lim, H., ... & Lee, K. J. (2023). Piperine supplementation in diet improves growth, feed efficiency, innate immunity, digestibility and disease resistance of Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture Reports*, *29*, 101490.

Srinivasan, K. (2007). Black pepper and its pungent principle-piperine: a review of diverse physiological effects. *Critical reviews in food science and nutrition*, *47*(8), 735-748.

Sumana, S. L., Xue, T., Hu, H., Abdullateef, M. M., Shui, Y., Ayana, G. U., ... & Xiaojun, J. (2025). Medicinal Plants as Ecological Solutions for Fish Growth and Immunostimulatory Effects in Aquaculture. *Aquaculture Research*, *2025*(1), 9778623.

Takooree, H., Aumeeruddy, M. Z., Rengasamy, K. R., Venugopala, K. N., Jeewon, R., Zengin, G., & Mahomoodally, M. F. (2019). A systematic review on black pepper (Piper nigrum L.): from folk uses to pharmacological applications. *Critical reviews in food science and nutrition*, *59*(sup1), S210-S243.

Taştan, Y., & Salem, M. O. A. (2021). Use of phytochemicals as feed supplements in aquaculture: A review on their effects on growth, immune response, and antioxidant status of finfish. *Journal of Agricultural Production*, *2*(1), 32-43.

Tian, Y., & Deng, F. (2020). Phytochemistry and biological activity of mustard (Brassica juncea): a review. *Cyta-journal of Food*, *18*(1), 704-718.

Tripathi, S., Singh, S., Mishra, N., & Mishra, N. (2025). The impact of solvent polarity on the phenolic and antioxidant capacity of green coffee beans (Robusta species) extracts. *Current Research in Nutrition and Food Science Journal*, *13*(2).

Von Hellfeld, R., Gade, C., Baumann, L., Leist, M., & Braunbeck, T. (2023). The sensitivity of the zebrafish embryo coiling assay for the detection of neurotoxicity by compounds with diverse modes of action. *Environmental Science and Pollution Research*, *30*(30), 75281-75299.

Waghulde, S., Kale, M. K., & Patil, V. (2019, November). Brine shrimp lethality assay of the aqueous and ethanolic extracts of the selected species of medicinal plants. In *Proceedings* (Vol. 41, No. 1, p. 47). MDPI.

Xiong, Y., Chen, X., Li, F., Chen, Z., & Qin, Z. (2022). Zebrafish larvae acute toxicity test: A promising alternative to the fish acute toxicity test. *Aquatic Toxicology*, *246*, 106143.

Yumnamcha, T., Roy, D., Devi, M. D., & Nongthomba, U. (2015). Evaluation of developmental toxicity and apoptotic induction of the aqueous extract of Millettia pachycarpa using zebrafish as model organism. *Toxicological & Environmental Chemistry*, *97*(10), 1363-1381.