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Research Article

Antioxidant potential of phytochemicals of *Merremia Quinquefolia* (L.) Hallier F. leaves extracts and their mitigation effect against imidacloprid-induced toxicity in zebrafish model

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ABSTRACT

In recent years, there has been growing interest in the role of free radicals and their impact on human health, particularly due to their ability to cause oxidative stress and cellular damage. Naturally occurring antioxidants, especially those derived from medicinal plants, are recognized for their potential to neutralize these harmful reactive oxygen species. *Merremia quinquefolia* (L.) Hallier f. (MQ) is one such medicinal plant, yet its phytochemical properties and antioxidant potential remain largely unexplored. This study aimed to investigate the phytochemical profile of MQ leaf extracts and quantify their total phenolic and antioxidant content. Furthermore, the study evaluated the protective effects of MQ leaf extract against imidacloprid-induced toxicity using Zebrafish as an in vivo model. The results of phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, tannins, carbohydrates, and saponins. Among the extracts, the acetone fraction exhibited the highest phenolic and antioxidant content, while ethyl acetate showed the lowest. In vivo analysis demonstrated that the antioxidant properties of MQ extract played a significant role in mitigating oxidative stress induced by pesticide exposure. These findings support the potential therapeutic application of MQ leaves as a natural antioxidant source for combating pesticide-related oxidative damage.

Keywords:
Merremia quinquefolia; Phytochemicals; Antioxidants; Zebrafish; Imidacloprid

1. INTRODUCTION

The respiration process generates certain potentially hazardous free radical intermediates known as reactive oxygen species (ROS). Although these ROS serve as signaling molecules, excessive ROS production can cause damage to cell metabolism by producing oxidative stress [1]. Therefore, it is important for normal cellular physiology to maintain a balance between these two opposing effects of ROS. In addition to the metabolic processes of the human body, external factors, including pollution, stress, ozone radiation, pesticides, and industrial chemicals, can also result in the production of free radicals [2]. Various acute and chronic disorders in humans, including cancer, diabetes, aging, atherosclerosis, immunosuppression, neurodegeneration, and cardiovascular diseases, are known to be influenced by free radical reactions [3,4].

Antioxidants are the compounds that have the capability to scavenge these harmful ROS and shield the human body by lowering the risk for acute and chronic disorders. High doses of synthetic antioxidants have been shown to disrupt the balance of ROS in proliferating cells, leading to DNA damage and the induction of premature cellular senescence[5]. This emphasizes the need of acquiring antioxidants from natural

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sources through a well-balanced diet, as they are generally safer and better suited to supporting the body's physiological activities without causing harmful side effects. Phenolic compounds, flavonoids, saponins, phytosterols, and polysaccharides are the most active sources of antioxidants. These are plant secondary metabolites, and their higher content is responsible for lots of biological activity of medicinal plants, including anti-inflammatory, antimicrobial, antidiarrheal, antiviral, anti-carcinogenic and, antiallergic activities [6].

Merremia quinquefolia (L.) Hallier f. (MQ), a medicinal plant belonging to the Convolvulaceae family, is a herbaceous climber predominantly found in the Indian states of Madhya Pradesh, Rajasthan, Gujarat, Odisha, and Maharashtra [7,8]. Traditionally, its leaves have been used to cure burns, scalds, and ulcers, while its roots are utilized as a natural oral cleanser. Gas chromatography-mass spectrometry (GC-MS) profiling of the plant's twigs revealed the presence of many pharmacologically active phytoconstituents, which are responsible for its potent antioxidant content [9]. However, to date, there is a lack of scientific data addressing the pharmacological activities of MQ leaves. Therefore, the present study aimed to investigate the phytochemical composition of various MQ leaf extracts, with a special emphasis on their total phenolic and antioxidant content. In addition, this study also aimed to assess the protective effects of MQ leaf extract against imidacloprid-induced toxicity after 96 h exposure period using Zebrafish (*Danio rerio*) as an in vivo model. Owing to the fact that approximately 70% of protein-coding genes in humans have orthologs in Zebrafish [10], this organism serves as a widely accepted model in biomedical research.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Ascorbic acid, ammonium molybdate, aluminium chloride, sodium phosphate, gallic acid, Folin-Ciocalteu phenol reagent, sodium hydroxide, sodium carbonate, and all other analytical grade solvents utilized during the experiment were obtained from Thermo Fisher Scientific India Pvt. Ltd.

2.2 Plant collection and authentication

The Plant (*Merremia quinquefolia* (L.) Hallier f.) collection has been done from the village Erich in the Jhansi district of Uttar Pradesh, India and identified by Dr. J. P. Tripathi (Retired Associate Professor) of the Department of Botany, Bipin Bihari College Jhansi, Uttar Pradesh, India. A voucher specimen (No. 3289) has been deposited in the department of herbarium, Bipin Bihari College, Jhansi (UP).

2.3. In vitro phytochemical study of MQ leaves

2.3.1. Preparation of leaf extracts of MQ for in vitro study

The leaves of *Merremia quinquefolia* (L.) Hallier f. (MQ) were separated, thoroughly cleaned under running water, and allowed to air dry in the shade. Using a grinder, the dried leaves were ground into a fine powder and kept for later use in an airtight box. Seventy five grams of the dried and powdered leaves of MQ were extracted first with ether for the removal of fats, then with non-polar to polar solvents such as ethyl acetate, acetone, ethanol, methanol, and distilled water using a soxhlet apparatus. Following this procedure, each extract was filtered through Whatman filter paper number 42, evaporated to yield crude extracts, and then refrigerated until needed.

2.3.2. Preliminary phytochemical test

The stock solutions (1 mg/mL) of ethyl acetate, acetone, ethanol, methanol, and aqueous crude extracts of MQ leaves were prepared by dissolving each crude extract in its own mother solvent, and the obtained stock solutions were subjected to preliminary phytochemical testing using standard procedures [11,12].

2.3.3. Determination of total phenolic content

The Folin-Ciocalteu method was applied to determine the total phenolic contents of each leaf extract by using gallic acid as a standard. In brief, 1 mL of Folin-Ciocalteu phenol reagent was combined with an aliquot of 1 mL of leaf extract (1 mg/mL) or a standard gallic acid solution (50, 100, 150, 200, 250, and 300 µg/mL). with 5 minutes, the mixture was given a thorough mixing with the addition of 10 mL of a 7% sodium carbonate solution and 13 mL of distilled water. Then the solution mixture was allowed to incubate at room temperature for 90 minutes in the dark. After that, a UV spectrophotometer was used to measure the absorbance of the solution against the blank at 765 nm. The total phenolic content of all the extracts was calculated by preparing a standard calibration curve of gallic acid, and the values were represented as mg of gallic acid equivalent (GE) per gram of each crude extract [9].

2.3.4. Determination of total antioxidant content

The phosphomolybdate assay was used to assess the total antioxidant activity of each leaf extract,

with ascorbic acid serving as the standard. In a test tube, an aliquot of 0.3 mL of the sample (1 mg/mL) or standard ascorbic acid solution (50, 100, 150, 200, 250, and 300 µg/mL) was taken out and added to 3 mL of reagent solution (28 mM sodium phosphate, 4 mM ammonium molybdate, and 0.6 M sulphuric acid in a 1:1:1 ratio). After capping, the test tubes were placed in a water bath for the incubation process at 95 to 98 °C for 90 minutes. A UV spectrophotometer was used to measure the absorbance of the solution mixture at 695 nm against a blank once the temperature was brought down to room temperature. The total antioxidant content of all the extracts was calculated using a standard calibration curve, and the results were expressed as mg of ascorbic acid equivalent (AAE) per gram of each crude extract [9].

2.4. In vivo effect of MQ extract against imidacloprid

2.4.1. Zebrafish Collection and maintenance

Zebrafish (*Danio rerio*), the test organisms, were bought from a local store in Jhansi, Uttar Pradesh, India. A total of 100 fish, measuring 2.5–3 cm in length and 2–2.5 grams in weight, were collected and brought to the lab to be maintained in a glass aquarium (200 L) that was filled with tap water. Fish were treated with a 0.2% KMnO4 solution for 1-2 minutes to check the cutaneous infection before acclimatization. After that, fish were acclimatized for at least 10-15 days to the laboratory environment at 25-28 °C, 7.16 pH, and were fed twice daily with available fish food. However, feeding was stopped 24 hours prior to the start of the experiment.

2.4.2. Preparation of stock solution of toxicant

Imidacloprid (1-6-chloro-3-pyridyl methyl-N-nitro-imidazolidin-2-ylidene-amine) 17.8% SL is a commercial pesticide made by Pioneer Pesticides Pvt. Ltd. in Chandigarh, India, and was purchased from a local pesticide store in the Jhansi district of India. The stock solution of imidacloprid was prepared by diluting its 1 mL in 10 mL distilled water.

2.4.3. Acute toxicity bioassay

The median lethal concentration (LC_{50}) of imidacloprid for Zebrafish was determined through exploratory and definitive bioassays. In the exploratory phase, two preliminary concentrations 0.1 mL/L and 0.5 mL/L representing expected lower and higher toxicity thresholds, were selected and administered to two separate glass aquaria, each containing 10 fish, to observe mortality rates ranging from 0% to 100% over a 96 h period. Based on these results, a definitive test was conducted using six graded concentrations of imidacloprid: 0.20, 0.25, 0.30, 0.35, 0.40, and 0.45 mL/L. Ten fish were exposed to each concentration, and mortality was recorded at 24, 48, 72, and 96 h post-exposure. Dead individuals were promptly removed from the aquaria to maintain water quality and reduce potential confounding factors.

2.4.4. Probit analysis of LC_{50}

To statistically determine the LC_{50} at 96 h using probit analysis, the concentrations of imidacloprid taken in the definitive test were transformed into log concentrations, % mortalities after the 96 h exposure period, first into the correct percentage, and then into their empirical probit value using Finney's table [13]. Following that, using the log concentrations and the probit values, a graph was plotted. And with the help of the regression line on the graph, the LC_{50} of imidacloprid after the 96 h exposure period was determined. The correct percentage for 0% and 100% mortality were calculated by the following formulas [14]-

$$For\ 0\%\ mortality = 100\left(0.25/n\right)$$
$$For\ 100\%\ mortality = 100\left(n - 0.25/n\right)$$

Where, n = Number of fish

The standard error of LC_{50} was calculated by using the given formula [14]-

$$Standard\ error\ (SE)\ of\ LC_{50} = \frac{\log\ LC_{84} - \log\ LC_{16}}{\sqrt{2n}}$$

Where, n = Number of fish

The values of log LC_{84} and log LC_{16} values were calculated using their respective probit values, which approximately equate to 6 and 4 probits, respectively. Following the calculation, the antilog values were the SE of LC_{50}.

Table 1. Amount and % yield of crude extracts of MQ leaves

Extracts	Amount (g)	% Yield (W/W)
Ethyl acetate	1.54	2.05%
Acetone	0.85	1.13%
Ethanol	1.62	2.16%
Methanol	1.06	1.41%
Distilled water	10.93	14.57%

2.4.5. Preparation of leaf extract of MQ for in vivo study

For the preparation of aqueous leaf extract of MQ plant (MQE), twenty grams of dried and grounded leaves of the MQ plant were mixed with 200 mL of hot (98 °C) distilled water and left for 24 h. After that, leaf extract was filtered with Whatman filter paper and made up to 200 mL by adding distilled water and filtrate (100 mg/mL) was stored for later use.

2.4.6. Evaluation of effective concentrations of MQE after 96 h exposure period

To evaluate the effective concentration of MQE extract in mitigating the toxicity of imidacloprid at its 96 h LC_{50} level, a total of 60 fish were randomly allocated into six experimental groups, each comprising 10 individuals. The groups were exposed to MQE extract at concentrations of 0.25, 0.5, 1, 2, 4, and 8 mL/L, respectively, in combination with the 96 h LC_{50} dose of imidacloprid. The LC_{50} dose of imidacloprid alone served as the toxic control. Fish mortality was monitored across all treatment groups, and the concentration of MQE at which no mortality was observed was identified as the effective protective concentration.

2.5. Statistical analysis

The results of total phenolic content and total antioxidant content are expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was performed for statistical analysis, followed by a Tukey *post hoc* test for multiple comparisons. Pearson correlation analysis was conducted to evaluate the relationship between total phenolic and antioxidant content. Lethal concentration results are represented as LC_{50} ± standard error (SE). The Kruskal–Wallis test was used to assess the effect of different concentrations of MQE extract on fish mortality after 96 hours of exposure. All statistical analyses were performed using IBM SPSS Statistics software (Version 29.0.2.0, Armonk, NY, USA) and the result values were considered statistically significant at $P<0.05$. Additionally, all standard graphical representations were generated using Microsoft Excel 2021.

3. RESULTS

3.1. In vitro phytochemical study of MQ leaves

3.1.1. Yield of crude extracts

Extraction of MQ leaves was carried out by using non-polar to polar solvents such as ether, ethyl acetate, acetone, ethanol, methanol, and distilled water. After the extraction and evaporation process, the obtained amounts of various crude extracts are given in Table 1, from which it can be seen that the aqueous extract had the highest yield.

3.1.2. Preliminary phytochemical test

Preliminary phytochemical tests on each of the extracts of MQ leaves were carried out in order to detect either the presence or absence of alkaloids, flavonoids, phenolic compounds, tannins, carbohydrates, saponins, glycosides, and anthraquinones. The results for the preliminary phytochemical analysis of MQ leaf extracts are shown in Table 2.

3.1.3. Total phenolic content

The results of the total phenolic contents of the various extracts of MQ leaves are represented as mg of gallic acid equivalent per gram of each crude extract (mg GE/g) and are shown in Table 3. The following linear regression equation, which was derived from the gallic acid standard curve, was used to calculate these values.

$$y = 0.0068x + 0.0654, R^2 = 0.994$$

Where y is absorbance and x is gallic acid concentration in µg/mL

Table 2 Phytochemical components detected in the various extracts of MQ leaves

Phytochemicals	Phytochemical test	EAE	AE	EE	ME	DWE
Alkaloids	Dragendroff's test	+	+	+	-	+
Flavonoids	Shinoda test	+	-	+	+	-
Phenolic and Tannins	Ferric chloride test	+	+	+	+	+
Carbohydrate	Fehling's test	-	-	+	+	+
Saponins	Foam test	-	-	+	+	+
Glycosides	Keller-Killiani Test	-	-	+	+	+
Anthraquinones	Borntrager's test	-	-	-	-	-

Key: EAE = Ethyl acetate extract; AE = Acetone extract; EE = Ethanol extract; ME = Methanol extract; DWE = Distilled water extract; (+) = Presence of constitute; (-) = Absence of constitute.

Table 3 Total phenolic and antioxidant content of various extracts of MQ leaves

Crude extracts	Total phenolic content (mg GE/g)	Total antioxidant content (mg AAE/g)
Ethyl acetate	15.873±2.25[a]	58.954±1.99[a]
Acetone	33.03±2.94[c]	146.31±3.45[c]
Ethanol	25.186±1.70[b]	89.988±3.98[b]
Methanol	22.735±1.47[b]	61.253±5.27[a]
Distilled water	31.069±0.85[c]	65.851±7.18[a]

Each value represents mean ± standard deviation of three replicates, different letters (a-c) in the same column were significant at $P<0.05$

3.1.4. Total antioxidant content

The results of the total antioxidant contents of the various extracts of MQ leaves are shown in Table 3. These values were calculated using the following linear regression equation, which was derived from the ascorbic acid standard curve, and are given as mg of ascorbic acid equivalent per gram of each crude extract (mg AAE/g).

$$y = 0.0029x - 0.0343, R^2 = 0.991$$

Where y is absorbance and x is ascorbic acid concentration in µg/mL

3.2. In vivo effect of MQ extract against imidacloprid

3.2.1. Acute toxicity bioassay

The results of the exploratory test and the definitive test are given in Table 4. During the assessment of acute toxicity of imidacloprid after different exposure period, the exploratory test showed 100% mortality of fish at 0.50 mL/L concentration after 48 h and no mortality at 0.10 mL/L concentration up to 96 h. The definitive test after 96 h exposure showed 0% mortality at 0.20 mL/L, 10% at 0.25 mL/L, 30% at 0.30 mL/L, 50% at 0.35 mL/L, 80% at 0.40 mL/L, and 100% at 0.45 mL/L concentration. The statistically accurate LC_{50} of imidacloprid at the 96 h exposure period was calculated from the regression line on the graph in Figure 1 (Table 5). The resultant value of LC_{50} for imidacloprid at 96 h was 0.324±0.032 mL/L (LC_{50}±SE) with 95% confidence upper limit 0.356 mL/L and lower limit 0.292 mL/L

3.2.2. Effective concentration of MQE after 96 h exposure period

The effectiveness of MQE against the imidacloprid-induced mortality of fish is shown in Figure 2. When the 96 h LC_{50} of imidacloprid (0.324 mL/L) was administered in combination with 0.25 mL/L of MQE, 50% death happened. The mortality rate reduced as MQE concentrations increased, and no death occurred when a 4 mL/L concentration of MQE was combined with the LC_{50} of imidacloprid. On further increasing the concentration of MQE to 8 mL/L along with the LC_{50} of imidacloprid, the mortality rate was also increasing. Therefore, the effective concentration of MQE against the LC_{50} of imidacloprid after the 96 h exposure period was found to be 4 mL/L.

The Kruskal–Wallis test was conducted to evaluate the effect of different concentrations of MQE extract on fish mortality at 96 h exposure period. The analysis yielded no statistically significant difference in mortality across treatment groups ($H \approx 4.71$, $p \approx 0.45$), suggesting that elevated concentrations of the extract did not provide substantially varied mortality outcomes within the examined range.

Table 4 Exploratory test and definitive test for LC50 determination

S.No.	Conc. of imidacloprid (mL/L)	No of fish	24 h		48 h		72 h		96 h	
			M	M%	M	M%	M	M%	M	M%
Exploratory Test										
1	0.10	10	0	0%	0	0%	0	0%	0	0%
2	0.50	10	9	90%	1	100%	-	-	-	-
Definitive Test										
1	0.20	10	0	0%	0	0%	0	0%	0	0%
2	0.25	10	0	0%	0	0%	0	0%	1	10%
3	0.30	10	0	0%	0	0%	2	20%	1	30%
4	0.35	10	0	0%	1	10%	3	40%	1	50%
5	0.40	10	2	20%	3	50%	2	70%	1	80%
6	0.45	10	5	50%	2	70%	2	90%	1	100%

Key: M = number of mortalities; M% = percentage mortality relative to the number of fish; (-) indicates no data recorded due to complete mortality before the given time point.

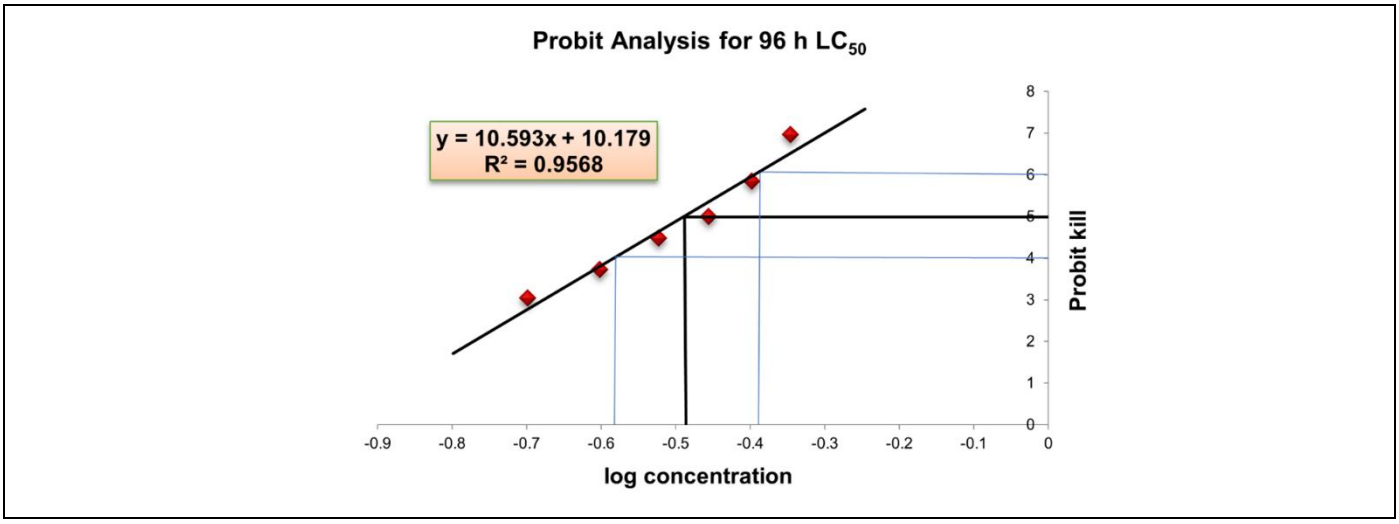

Figure 1. Plot of log concentrations versus probit kill after 96 h exposure of imidacloprid

4. DISCUSSION

Medicinal plants are sources of many biologically active phytochemicals, also known as secondary metabolites, and their screening plays a very essential role in assessing the therapeutic properties of medicinal plants. In this study, the preliminary phytochemical screening of ethyl extract, acetone, ethanol, methanol, and distilled water extract of the leaves of *Merremia quinquefolia* (L.) Hallier f. (MQ) revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, carbohydrates, saponins, and glycosides. However, anthraquinones were absent in all the extracts. Alkaloids were present in all the extracts except methanol. Flavonoids were only present in ethyl acetate, ethanol, and methanol extracts. All of the extracts were found to be rich in phenolic compounds and tannins. Furthermore, the only extracts containing carbohydrates, saponins, and glycosides were ethanol, methanol, and distilled water. The occurrence of all these phytochemicals may indicate the therapeutic role of MQ leaves in combating several diseases, as a study found that consuming a diet rich in plant foods with significant phytochemicals is associated with a lower chance of developing chronic diseases like cancer, diabetes, obesity, and neurological and cardiovascular disorders [15]. Alkaloids, phenolic compounds, and flavonoids are the important classes of phytochemicals that contribute to various medicinal properties such as antioxidant, anti-allergic, anti-angiogenic, anticancer, anti-inflammatory, and antimicrobial activities [16–18]. Among the five investigated solvents, ethanol yielded the most diverse range of phytochemicals, indicating its potential as the primary solvent for extracting bioactive components from MQ leaves. Its broad-ranging solubility, because to its intermediate polarity, effectively dissolves both polar and semi-polar molecules. These results of the phytochemical screening not only identify the ethanol extract as the most interesting fraction for future pharmacological or bioactivity studies, but also provide a solid framework for solvent selection in phytopharmacological research. To the best of our knowledge, this is the first report on the phytochemical screening of the various extracts of MQ leaves. In addition to the qualitative analysis of phytochemicals, quantitative analysis of phenolics and antioxidants provides important insights into the MQ leaf extracts.

The present study indicated that the results of total phenolic content in the various extracts of MQ leaves varied from 15.873±1.83 to 33.03±2.4 mg GE/g. Among the five extracts, acetone extract was found to contain the significantly highest amount of phenolic content, followed by aqueous, ethanol, methanol, and ethyl acetate extract. This variation may be attributed to the tendency of different phenolic components to be

Table 5 Probit analysis of LC_{50} of imidacloprid after 96 h exposure period

Conc. of imidacloprid mL/L	log Conc.	No. of fish	% Mortality	Correct %	Probit value	Obtained linear equation	LC_{50} ± SE mL/L
0.20	-0.699	10	0%	2.5%	3.04	y = 10.593x + 10.179	0.324 ± 0.032
0.25	-0.602	10	10%	10%	3.72		
0.30	-0.523	10	30%	30%	4.48		
0.35	-0.456	10	50%	50%	5		
0.40	-0.398	10	80%	80%	5.84		
0.45	-0.346	10	100%	97.5%	6.96		

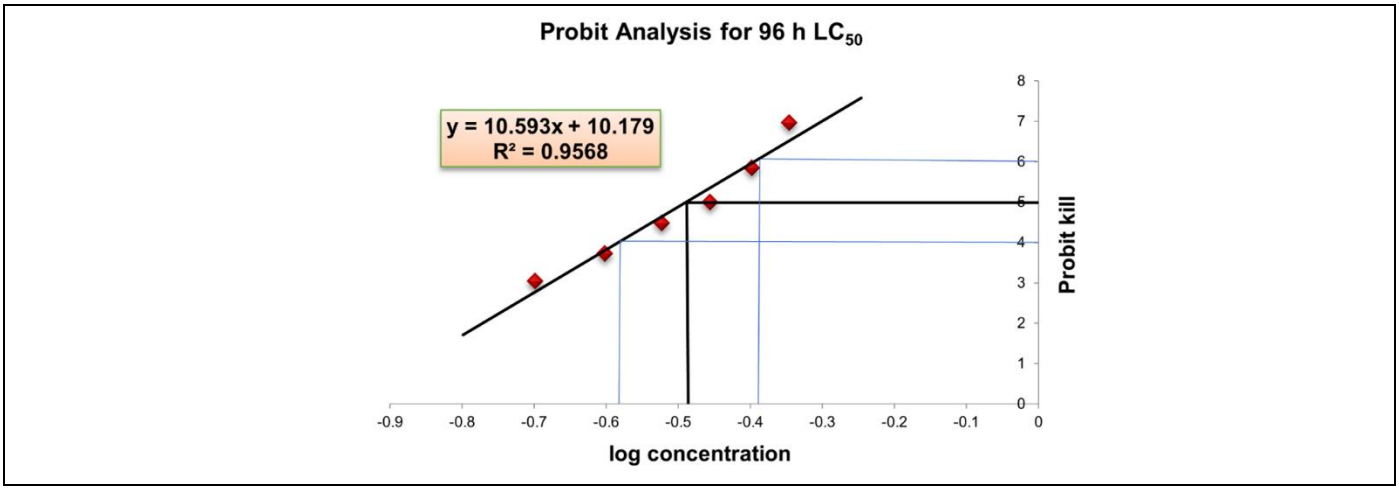

Figure 2. Assessment of effective concentration of MQE against 96 h LC_{50} of imidacloprid

extracted with solvents having different polarities. In fact, a similar tendency of different extracting solvents in altering the phenolic and antioxidant content of raw vegetables was observed by Sulaiman *et al.* [19].Additionally, Trabelsi *et al*. reported on the extraction of phenolics, including antioxidants, using various organic solvents and found that extracts obtained using higher polar solvents were more effective than those obtained using lower polar solvents [20]. Also, the results of total antioxidant content in the various extracts of MQ leaves varied from 58.954±1.63 to 146.31±2.82 mg AAE/g. Among the five extracts, acetone extract was found to contain the significantly highest amount of antioxidant content, followed by ethanol, aqueous, methanol, and ethyl acetate extract. These results showed that all the extracts have a considerable amount of antioxidant content, which supports the traditional use of MQ leaves. The highest antioxidant content in the acetone extract may be due to its high phenolic content. To examine the correlation between total phenolic and antioxidant content, a Pearson correlation analysis was performed on extracts prepared with five different solvents. The analysis revealed a moderate positive correlation between total phenolic and antioxidant content ($r = 0.665$, $p = 0.220$). Although this correlation was not statistically significant at the 0.05 level, possibly due to the small sample size, it does indicate a tendency for higher phenolic contents to contribute to greater antioxidant levels. Similar moderate correlations between total phenolic and antioxidant content have been reported in several studies. For instance, Saeed *et al.* observed a significant but moderate correlation in a study on *Torilis leptophylla* plant extracts [21]. Similarly, methanolic extracts from 12 different traditional medicinal plants of India yielded moderate correlations [22]. Dohre *et al.* also established a positive correlation *between* total phenolic and the antioxidant content of *Sansevieria trifasciata* and different fruit extracts [23]. These results support the notion that, although phenolic content plays a major role in antioxidant capacity, other phytochemicals and possible synergistic interactions also probably have a big impact.

The results of the in vivo study support the medical use of MQ leaves. Imidacloprid is a neonicotinoid pesticide. The toxicity of imidacloprid is reflected by its 96 h LC_{50} value, which is 0.324±0.032 mL/L. An almost similar finding of the LC_{50} value of imidacloprid (0.27 mL/L) for 96 h of exposure in Zebrafish has also been reported in previous studies [24]. Earlier in vivo research on imidacloprid-induced toxicity in zebrafish has demonstrated that high concentrations (0.3, 1.25, and 5 mg/mL) of imidacloprid after 7, 14, 21, and 28 days of exposure can induce oxidative stress by generating excessive amounts of ROS, as well as cause DNA damage in zebrafish, which worsens with prolonged exposure and duration [25].

In the present study, when fish were treated with the combination of 96 h LC_{50} of imidacloprid and varying concentrations of MQ leaf extract, mortality of fish started decreasing as the concentration of leaf extract increased, and no mortality occurred at the effective concentration (4 mL/L) of extract even though the fish were treated along with LC_{50} dose. Although mortality rates appeared to decline with increasing concentrations of the MQE extract, statistical analysis using the Kruskal–Wallis test ($H = 4.71$, $p = 0.45$) indicated that these differences were not statistically significant. The lack of significance may be attributed to the absence of repeated measurements, which limits statistical power. Further study with replicate groups and larger sample sizes is required to establish the reliability and significance of these findings. Despite the lack of statistical significance, the observed patterns, especially the complete absence of mortality at 4 mL/L, suggests a potentially protective effect of the MQE extract. On the basis of these findings, we can explain that the leaf extract of MQ mitigates the toxicity produced by imidacloprid in Zebrafish, which may be

due to the high antioxidant capacity of MQ leaf extract. The phytochemicals present in the extract, including flavonoids, tannins, and alkaloids, are known for their ability to reduce oxidative stress by scavenging ROS [26], thereby enhancing cellular defence mechanisms and preventing mortality.

However, although the concentration of 4 mL/L of the MQE extract was identified as the most effective concentration, in vivo results showed that concentrations above this level resulted in elevated fish mortality. This biphasic response may be explained by the dose-dependent nature of phytochemicals within the MQE extract. At moderate concentrations, these compounds exert antioxidant and protective effects, but at higher concentrations, these same compounds may exert pro-oxidant effects, disrupt cellular redox balance, or lead to cytotoxicity [27]. Furthermore, excessive concentration of MQE extract may also alter water quality parameters, such as decreasing dissolved oxygen levels, increasing organic load, or changing pH, all of which may be responsible for fish mortality [28]. These results emphasize the importance of careful dosage optimization when applying phytotherapeutic drugs in aquaculture to avoid unwanted toxicological consequences. Yadav *et al*. also conducted a study where antioxidant-rich moringa leaf extract was used to normalize the altered values of hepatic enzymes in imidacloprid-intoxicated Zebrafish [29]. Besides it, further studies on the liver and kidney markers of Zebrafish are needed to verify the effectiveness of MQ leaves against the toxicity induced by imidacloprid.

5. CONCLUSIONS

In the present study, phytochemical screening and total phenolic and antioxidant contents of *Merremia quinquefolia* (L.) Hallier f. leaves, extracted in five different solvents, are determined for the first time. This study is necessary to locate and isolate the phytochemicals exhibiting pharmacological action. Also, further characterization of the isolated compounds can be helpful for developing new drugs. Furthermore, the information obtained from in vitro and in vivo investigations would be useful for the medicinal use of leaves of the *Merremia quinquefolia* (L.) Hallier f. as an antioxidant to combat several chronic diseases and as a therapeutic alternative to overcome adverse effects caused by pesticides.

6. ACKNOWLEDGEMENTS

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Author contribution

All authors contributed to the study conception and design. Material preparation, animal maintenance, data collection, and analysis were performed by Kritika Verma. Data analysis and experimental supervision was done by Surabhi Yadav. Kaneez Zahra and Vikash Verma also actively participated by providing advice for result interpretation and contributing to the reviewing process. The first draft of the manuscript was written by Kritika Verma and all authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

The animal study protocol was reviewed and approved by the Institutional Animal Ethical Committee with approval no. BU/Pharm/IAEC/A/21/18 and was according to CPCSEA guidelines.

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