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Bioactivity evaluations from stem bark extract fractions of *Myrica esculenta* Buch. -Ham.ex D. Don

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Abstract

The chemical constituents of *Myrica esculenta* (a plant distributed across Nepal, India, China, and Pakistan) are known for their potent antioxidant properties and reported pharmacological effects. The study used methanol extract to assess antioxidant activity (DPPH assay), anthelmintic effects (standard; albendazole and experimental organism; *Eisenia fetida*), anti-inflammatory properties (human red blood cell membrane stabilization), and cytotoxicity (brine shrimp lethality bioassay). The study revealed 39.06% yield with methanol and 2.01% with hexane. Phenolic and flavonoid content were measured at 96.43 mg GAE/g and 14.54 mg QE/g, respectively. Methanolic extract exhibited IC_{50} of 60.89 µg/mL and LC_{5o} of 160.5298 µg/mL. It also demonstrated anti-inflammatory activity, notably achieving a 37.8% membrane stabilizing action at 500 µg/mL. Anthelmintic potential was observed at different concentrations (50 mg/mL, 75 mg/mL, and 100 mg/mL), leading to paralysis and death of worms with corresponding times reported. Brine Shrimp Lethality Bioassay revealed concentration-dependent effects. Methanolic extract of *M. esculenta* displayed robust antioxidant and anthelmintic activities. The extract was identified as bioactive, holding promise as a source for plant-derived antitumor compounds. However, further investigation is essential to validate these findings.

KEYWORDS
anthelmintic activity, antimicrobial activity, brine shrimp lethality, DPPH radical scavenging activity, *M. esculenta*, phytochemical screening

1 | INTRODUCTION

Medicinal plants are essential to many indigenous medicinal systems, serving as vital components in the treatment of a wide range of ailments. Furthermore, a significant portion of Western medicines contain plant-based components, highlighting the widespread use of botanicals in healthcare. According to data from the World Health Organization, around 80% of people worldwide receive their main medical treatment through traditional medicine, underscoring the important role that plant-based traditional medicine systems play in healthcare. The antioxidant capabilities of restorative plants have been investigated. According to earlier studies, the high concentrations of phenolic components, including flavonoids, phenolic acids, vitamins C and E, and other carotenoids, are largely responsible for

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the antioxidant benefits of several botanicals (Koirala et al., 2019; Sawadogo et al., 2006). Natural antioxidants such as these work well in preventing the negative effects of oxidative stress, which is caused by too many free radicals (Betteridge, 2000). Different endogenous antioxidant molecules found in our bodies, such as glutathione peroxidase, catalase, and superoxide dismutase, can counteract free radicals and guarantee that our cells are working properly (Kurutas, 2015). Dietary antioxidants, however, might be necessary since endogenous antioxidants might not be sufficient to maintain optimal cellular functions in the presence of increased oxidative stress. Thus, it would be wise to investigate plant species that are used by many ethnic groups in order to harness the amazing bioresources and produce new bioactive phytopharmaceuticals in a sustainable manner (Panthi et al., 2020). Furthermore, a wealth of antibacterial compounds can be found in therapeutic plants. Many anti-microbials have been rendered ineffective by microorganisms (Kabra et al., 2019). Many disorders, including anemia, chronic diarrhea, liver illnesses, fever, ulcers, and inflammation, have historically been treated with it (Sood & Shri, 2018). Mahavisagarbha taila, bala taila, katphaladi churna, kass-har churna, katphala taila, katphala kwatha, and bala taila are among the ayurvedic formulations that most frequently contain the bark of *M. esculenta* (Figure 1) as an essential ingredient for the treatment of a variety of ailments, including rheumatoid arthritis, diarrhea, dysentery, headache, and menorrhagia (Kabra et al., 2019). With its wide range of applications in toxicity testing, such as initial evaluation of plant extracts, detection of fungal toxins, assessment of heavy metals, cyanobacteria toxins, pesticides, and cytotoxicity testing of dental materials, the Brine Shrimp Lethality Bioassay (BSLA) is useful in identifying potentially cytotoxic principles in plant extracts (Meyer et al., 1982). Many research projects have made extensive use of this assay, including testing for heavy metals, cyanobacteria toxins, pesticides, and cytotoxicity of dental materials. It has also been used extensively in the identification of fungal toxins and preliminary toxicity assessment of plant extracts. A strong correlation has been demonstrated by numerous investigations between the anticancer effects of cytotoxic medicines and this simple, affordable, laboratory-based assay (Carballo et al., 2002). As a preliminary screening technique, BSLA may prove beneficial in the discovery and combination of anticancer medicines. As the body's natural reaction to irritation and infection is brought on by outside chemicals, inflammation is essential for defending the body from damage (Bacchi et al., 2012). Research has revealed a number of naturally occurring substances that may be used as a novel therapeutic strategy for the treatment of inflammatory illnesses. These compounds have demonstrated potential anti-inflammatory properties both in vitro and in vivo (Maione et al., 2016). *Myrica esculenta* is one of the most commonly used plants that possess high medicinal value. *M. esculenta* is a small moderate-sized tree, which is 3–15 m in height (Panthari et al., 2013). It belongs to the family Myricaceae (Shankhwar et al., 2019). *Myrica esculenta*, commonly known as Kaphal in Nepal, stands out as one of the most frequently used plants with high medicinal value. This small to moderate-sized tree is distributed in the foothills of Nepal, India, China, and Pakistan, thriving in nitrogen-depleted soils, mixed forests, agricultural, and marginal lands (Kurutas, 2015).



FIGURE 1 Leaf and dried bark of *Myrica esculenta* used in this study.

2 | MATERIALS AND METHODS

Chemicals: Gallic acid (GA), ascorbic acid (AA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and quercetin were procured from Hi-Media Lab. FC reagent and aluminum chloride (AlCl3) were procured from Thermo Fisher Scientific India Pvt. Ltd. Reference standards diclofenac and albendazole were procured from Lomus Pharmaceuticals Pvt. Ltd. All other chemicals were of standard analytical grade.

Plant materials: The bark of *M. esculenta* was collected from Rampur-9, Palpa, Nepal (1100–1350 m) (collection period: September 2022). The plant sample was authenticated by National Herbarium and Botanical Laboratories (NHBL) Voucher number: 273.

Preparation of extracts: Fresh barks were washed altogether by running tap water to remove the soil or dust particles and then cut into small pieces with the help of a stainless-steel knife. Then barks were dried in shade under normal room temperature for about 15–20 days. After drying, barks were then processed to a fine powder using an electric grinder. Methanol was used as a polar solvent and n-hexane as a nonpolar solvent to extract the powdered plant material. For this, a predetermined amount of the powdered sample was used. The Soxhlet apparatus was used to extract the material from n-hexane and methanol while maintaining the solvent's boiling point. Using a rotary evaporator, the extract was concentrated, and it was allowed to dry at temperatures below 40°C. Dried extract was the subject of various investigations.

2.1 | Determination of total phenolic content

The TPC of the extract was estimated by Folin–Ciocalteau reagent as the method described by (Modi et al., 2021; Singleton & Rossi, 1965). Briefly, 1 mL of methanolic extract (1 mg/mL) was mixed with Folin–

Ciocalteu's reagent (5 mL, 1:10 v/v diluted with distilled water) and aqueous sodium carbonate (4 mL, 7%) solution. Next, the mixture was agitated and allowed to incubate for 30 min at 40°C in a water bath before the absorbance was measured at 760 nm using a UV-visible spectrophotometer. The TPC value was determined from a calibration curve prepared with a series of GA standards (10–200 μg/mL), and the total polyphenolic compound concentration in the extracts was expressed as milligrams of GA equivalent per gram of dry weight (mg GAE/g) of the extract.

2.2 | Determination of total flavonoid content

The total flavonoid content was determined as per Ghafar et al. (2017). To a 10 mL volumetric flask containing 4 mL of distilled water, an aliquot of 1 mL of different extracts in methanol was added. Initially, 0.3 mL 5% sodium nitrite was added to the flask, and then 0.3 mL of 10% AICI3 was added to the flask after 5 min. To this mixture, 2 mL of 1 M sodium hydroxide was added exactly at 6 min. Immediately, the entire volume of the mixture was increased to 10 mL by adding 2.4 mL of distilled water and thoroughly mixing it. The absorbance of the pink-colored mixture was measured at 510 nm, while a blank containing all the reagents but no extracts was used as a reference. The total flavonoid concentration (TFC) value of the extract was calculated using a calibration curve constructed with a range of quercetin standards (10–100 μg/mL), and expressed in milligrams of quercetin equivalents (QE) per gram of extract (mg QE/g).

2.3 | Antioxidant activity of the extract

The antioxidant activity of plant extract was carried out to check the ability of the extract to kill or scavenge the free radicals, which are responsible for the oxidation reaction (Hossen et al., 2022; Peron et al., 2023).

Procedure: The methodology for antioxidant activity was adapted from Hossen et al. (2022). Reference samples of AA and sample solutions of plant extract were prepared in methanol at different concentrations (50,100, 200, and 1000 μg/mL) using minimum DMSO to dissolve the extracts. 0.1 mM solution of DPPH was prepared in methanol and 4 mL of this solution was added to 1 mL of each concentration of sample extracts and AA solutions. The solution was left in darkness for 30 min. Similarly, as a control, 4 mL of 0.1 mM DPPH was combined with 1 mL of methanol. The absorbance was measured at 517 nm after 30 min. The ability to scavenge the DPPH radical was determined using the following formula:

$$\% \text{ Scavenging} = [(A_0 - A_1)/A_0] \times 100$$

where,

A_0 = absorbance of control (DPPH solution and methanol)

A_1 = absorbance of test or reference sample

The percentage scavenging was then plotted against concentration and regression equation was obtained from which IC_{50} (micromolar concentration required to inhibit DPPH radical formation by 50%) values were calculated for each plant extract. The higher the antioxidant activity, the lower the value of IC_{50} value.

2.4 | Anti-inflammatory activity of the extract

Anti-inflammatory activity was performed in vitro by human RBC membrane stabilization technique according to Shinde et al. (1999), with a small modification. The blood was drawn from a human volunteer with good health who had not taken any NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment. The same amount of blood and sterilized Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl) were combined. This mixture was then transferred into centrifuge tubes, which were centrifuged at 3000 rpm for 10 min and washed three times with an equal volume of normal saline. The blood volume was measured and then mixed with normal saline to create a 10% v/v suspension (Shinde et al., 1999). Following this, plant extracts at various concentrations were prepared. To each concentration, 1 mL of phosphate buffer (0.15 M, pH 7.2), 2 mL of hyposaline (0.36), and 0.5 mL of HRBC suspension were added. Diclofenac sodium was used as a reference drug. After a 30-min incubation at 37°C, each assay mixture was centrifuged for 20 min at 3000 rpm. The same procedure was applied to the sample, with 2 ml of distilled water replacing hyposaline in the control solution. The hemoglobin concentration of the supernatant liquid, after decantation, was determined using a spectrophotometer set to 560.

$$\text{Membrane stabilization} = 100 - [\text{OD of the test}/\text{ OD of the control}] \times 100$$

2.5 | Anthelmintic activity of the extract

Eisenia fetida, an adult earthworm, was used to test the anthelmintic activity. It shares morphological and physiological similarities with the intestinal roundworm parasite of human beings. Five groups, each consisting of around six earthworms, were released into 50 mL of the intended formulation. Each group was treated with one of the following: Vehicle (5% DMSO in normal saline), albendazole (10 mg/mL), and extract (50 mg/mL, 25 mg/mL, and 12.5 mg/mL) in normal saline containing 5% DMSO. First, the paralysis time and then the death time (DT) were observed. When the worms failed to resurrect in regular saline, it was claimed that paralysis had occurred. When the worms lost their ability to move and then the body color gradually faded away, it was considered that they were dead. The anthelmintic activity was performed as per Jain and Jain (2010) with slight modification (Jain & Jain, 2010).

2.6 | Brine shrimp lethality bioassay

The cytotoxic activity of the plant was assessed using Brine Shrimp Lethality Bioassay method where 6 graded doses (viz 800, 400, 200, 100, 50, and 25 µg/mL) were used. As test organisms, brine shrimp (*Artemia salina* Leach) nauplii were employed. Eggs were incubated for 48 h in artificial sea salt with a steady supply of oxygen to hatch. Then, 10 fully grown nauplii were employed in the study. In addition to being a solvent, DMSO served as a negative control. In this instance, vincristine sulfate served as the reference standard. After 24 h, the survivors were tallied. The numbers of survivors were counted after 24 h. If, during several seconds of observation, no internal or exterior movement was seen in the larvae, they were considered to be dead. No food was given to the larvae. To ensure that mortality observed in the bioassay could be attributed to bioactive compounds and not starvation, we compared the dead larvae in each treatment with dead larvae in the control (Meyer et al., 1982).

The lethal concentration of the test sample was calculated using the probit analysis method described by Finney as a measure of the toxicity of the plant extract.

$$\text{Mortality} = (\text{No. of Dead Larvae}) / (\text{Total no. of Larvae}) \times 100\%$$

2.7 | Statistical analysis

A triplicate analysis of each sample was carried out. All findings presented are means (±SEM) of at least three independent experiments.

3 | RESULTS

The percentage yield of methanolic extract was found to be 39.06% and that of hexane extract was 2.01%.

3.1 | Total Phenolic Content

Using the Folin–Ciocalteu method, the extract's TPC was calculated and represented as GA equivalents (GAE), in accordance with Singleton and Rossi's (1965) methodology. Using standards for GA, a calibration curve was created to determine the extract's phenolic content.

The regression equation of the calibration curve is given as $y = 0.0098x + 0.0017$, with an R^2 value of 0.9988. This equation allows for the conversion of absorbance values obtained at different concentrations of GA to corresponding concentrations. The high R^2 value suggests a strong correlation between the concentration of GA and the absorbance values, indicating the reliability of the calibration curve.

The TPC value of the sample, expressed in terms of GA equivalents, is reported as 96.43 mg GAE/g. This value represents the TPC present in the analyzed extract. A higher TPC value generally implies a greater concentration of phenolic compounds, which are often associated with antioxidant and health-promoting properties. The Folin–Ciocalteu method, coupled with the calibration curve, provides a quantitative measure of the phenolic content in the extract under investigation (Table 1).

3.2 | Total flavonoid content

Using an AlCl3 colorimetric assay, the extracts' TFC was calculated; the results are expressed in terms of QE. A calibration curve made with quercetin standards was used to determine the amount of flavonoids present in the extract.

The regression equation of the calibration curve is given as $y = 0.0044x + 0.002$, with an R^2 value of 0.9946. This equation allows for the conversion of absorbance values obtained at different concentrations of quercetin to corresponding concentrations. The high R^2 value indicates a strong correlation between the concentration of quercetin and the absorbance values, suggesting the reliability of the calibration curve.

The TFC value of the sample, expressed in terms of QE, is reported as 14.54 mg QE/g. This value represents the total flavonoid content present in the extract. Flavonoids are a group of polyphenolic compounds known for their antioxidant properties and potential health benefits. The colorimetric assay with AlCl3, along with the calibration curve (Table 2) provides a quantitative measure of the flavonoid content in the extract under investigation.

TABLE 1 Absorbance value for Gallic acid measured for calibration curve.

Concentration (µg/mL)	Absorbance at 760 nm
10	0.11
25	0.25
50	0.45
100	1.01
200	1.947
Sample (1000 µg/mL)	0.945

TABLE 2 Absorbance value for quercetin measured for calibration curve.

Concentration (µg/mL)	Absorbance at 510 nm
10	0.04
25	0.11
50	0.23
75	0.35
100	0.43
Sample (1000 µg/mL)	0.064

3.3 | Antioxidant activity

IC_{50} value of the extract was calculated using a regression equation derived from plotting the percentage scavenging effect against the concentrations of the extract (Table 3). A lower IC_{50} value indicates higher scavenging abilities. The IC_{50} of the methanolic extract was determined to be 60.89 µg/mL. In comparison to the standard, that, the extract exhibits slightly lower activity, as a lower IC_{50} value indicates higher scavenging activity (Figure 2).

3.4 | Anti-inflammatory activity

At various concentrations, the extract demonstrated efficacy in suppressing hemolysis generated by hypotonicity. These provide evidence that its anti-inflammatory activity is due to the mechanism of membrane stabilization. Absorbance of extract for anti-inflammatory test is given in Table 4. The extract prepared using methanol exhibited the strongest membrane stabilizing effect, reaching 37.8% at a concentration of 500 µg/mL (Figure 3a; Table 5). The extract showed its effectiveness in preventing the hemolysis caused by hypotonicity at various concentrations. This evidence supports the notion that membrane stabilization is the underlying mechanism responsible for its anti-inflammatory effect. The EC_{50} value of the methanolic extract was determined to be 571.24 mg/mL.

TABLE 3 Percentage scavenging effect of the extract and ascorbic acid.

Concentration (µg/mL)	% RSA of Extract	% RSA of Ascorbic acid
10	16.7	4.67
50	43.5	58.8
100	75.1	93.224

3.5 | Anthelmintic activity of the extract

In this experiment, adult earthworms (*Einesia foetida*) were employed to evaluate the effectiveness of the plant extract as an anthelmintic. Different concentrations (50, 75, and 100 mg/mL) of the extract were studied, and paralysis and DT were reported as 28.19, 20.2, 10.6, 75.66, 57.96, and 51.85 min, respectively (Table 6). The outcomes demonstrated a higher level of activity at a concentration of 100 µg/mL when testing against Einesia foetida. The time it took for the earthworms to become paralyzed and eventually die is visually represented in the graph (Figure 3b).

3.6 | Brine shrimp lethality bioassay

LC_{50} values for various concentrations were calculated. The LC50 value of the plant extract was determined to be 160.5298 µg/mL. This indicates that at a concentration of 160.5298 µg/mL of the plant extract, it is projected that 50% of the target organisms or cells will experience negative effects or even death. In contrast to the anticancer drug, vincristine sulfate, exhibited an LC50 value of 1.707 µg/mL (Table 7). The lower LC50 value for vincristine sulfate indicates that it is more toxic to the target cells, which is a positive characteristic for an anticancer drug. Conversely, the plant extract,

TABLE 4 Absorbance of the extract for anti-inflammatory test.

Concentration (µg/mL)	Absorbance of extract
100	0.775 ± 0.0017
200	0.73 ± 0.0003
300	0.635 ± 0.001
500	0.54 ± 0.001

Note: Values are in Mean ± SEM ($n = 3$).

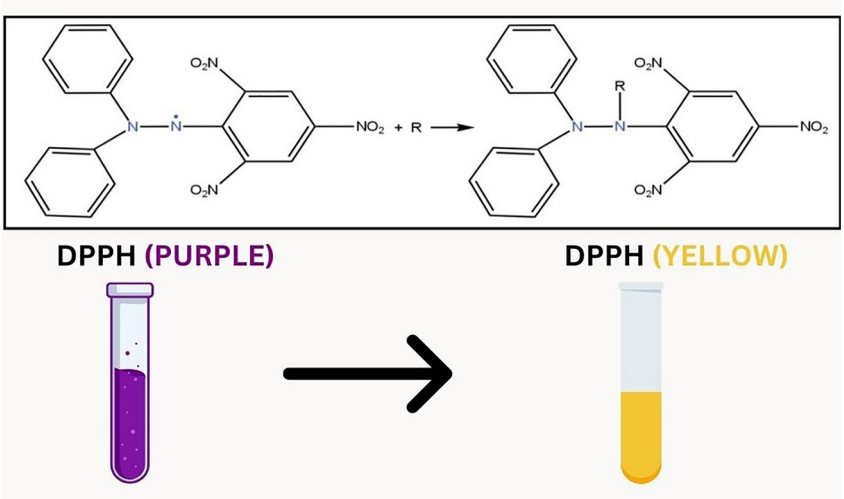


FIGURE 2 Mechanism of DPPH scavenging.

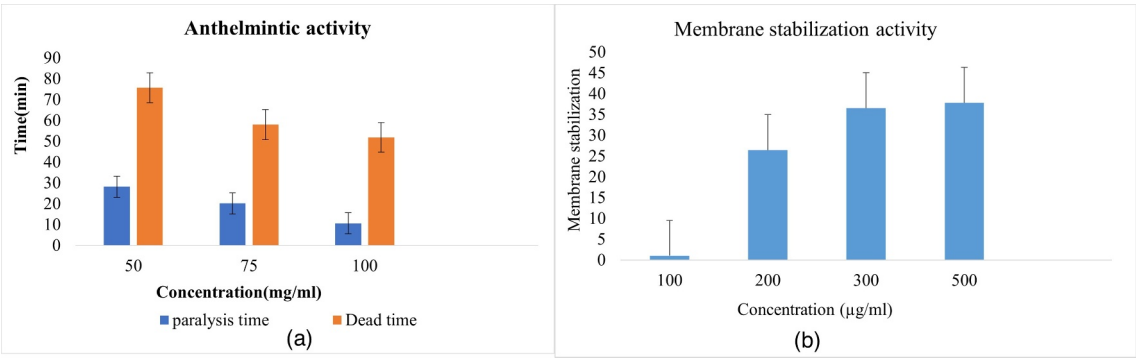


FIGURE 3 (a) Membrane stabilizing activity of the extract; (b) anthelmintic activity of the extract.

TABLE 5 % hemolysis and protection of methanolic extract.

	Methanolic extract	
Concentration (µg/mL)	% hemolysis	Membrane stabilization
100	98.97	1.03
200	73.56	26.44
300	63.5	36.5
500	62.2	37.8

TABLE 6 Anthelmintic activity of extracts and albendazole.

	Methanolic extract (minutes)	
Concentration (mg/mL)	Paralysis time	Dead time
50	28.19 ± 1.1702	75.66 ± 2.3333
75	20.2 ± 3.06159	57.96 ± 1.3159
100	10.6 ± 0.702	51.85 ± 1.0275

although still showing cytotoxic effects (as indicated by its LC50 value), may be regarded as less potent than vincristine sulfate.

4 | DISCUSSION

In comparison to hexane, the extractive value was greater in methanol. This confirms that the mass of the polar component compound is in greater proportion than nonpolar. The methanol extraction value was higher than 19.02%, as shown in the previous article by Shrestha Shrimita, Bhandari Sudip, Aryal Babita et al. (Shresta et al., 2021).

There is a growing interest in phenolic compounds because of their nutritional significance and a variety of bioactivities, such as antioxidant, antiaging, anti-inflammatory, and antiproliferative qualities. Phenolic compounds are a class of chemical compounds that are abundant in the natural world. It has been shown that methanol extracts polyphenolic compounds far more effectively. Given that polar solvents could dissolve higher amounts of phenols than nonpolar solvents, the results demonstrated that polar solvents were superior at extracting phenols. In contrast to the earlier research, which determined a TPC of 91.8 mg GAE/g, the TPC value of the sample was slightly elevated, reaching 96.43 mg GAE/g (Patel et al., 2017). Nonetheless, the phenolic content in *M. esculenta* can be influenced by factors such as the region it is grown in, the time of year it is harvested, and when it is collected. Among the phenolics that are most frequently discovered in different plant tissues are flavonoids. Flavonoids may be able to prevent cancer, according to studies on the subject. According to Peng et al. (2017), quercetin is thought to be the prototype for a naturally occurring chemical with chemo-preventive qualities. A modified AlCl3 coulometric technique was employed in this investigation to calculate the QE of the total flavonoids present in various organic crude plant extracts. The result showed the TFC value of the *M. esculenta* 14.54 mg QE/g, which is higher than as depicted in a previous study by Shrestha Shrimita, et al., that is, 6.84 mg QE/g. This variation in the results may be attributed to differences in location or the time of data collection (Shresta et al., 2021).

The antioxidant activity was determined by the antioxidant compound's ability to diminish the DPPH radical, as evidenced by a drop in absorbance at 517 nm until the reaction reached a plateau. IC_{50} of methanolic extract was found to be 60.89 µg/mL. In comparison to standard AA extract, the lower IC_{50} value indicates higher scavenging activity. In a previous study conducted by Prashant Kumar and his team in 2017, they found that the IC50 value for the methanolic extract was approximately 60 ± 1.15 µg/mL (Kumar et al., 2017).

Methanolic extract of *M. esculenta* was effective at preventing hypotonicity-induced hemolysis at various doses in the RBC membrane stabilization activity test. These findings suggest that the mechanism behind their anti-inflammatory effects is related to the stability of cell membranes. The extract prepared using methanol exhibited the strongest membrane stabilizing effect, reaching 37.8% at a concentration of 500 µg/mL. EC_{50} value of methanolic extract was found to be 571.24 mg/mL. Percentage membrane stabilization of methanolic extract was found to be lesser than as depicted in a former research article by Agnihotri Supriya et al. in the case of essential oil isolated from *M. esculenta*, the topical anti-inflammatory (85.3%) (Agnihotri et al., 2012).

TABLE 7 LC_{50} of plant extract and vincristine sulfate at different concentrations.

Test Sample	Concentration (µg/mL)	% mortality	LC50 (µg/mL)
Extract	50	20	160.5298
	100	36.66	
	200	56.6	
	400	73.33	
	800	93.33	
Vincristine sulfate	0.25	30	1.707
	0.5	0	
	1	50	
	5	90	
	10	100	

From the lowest concentration (50 µg/mL) to the highest concentration (800 µg/mL), the level of lethality exhibited by *M. esculenta* was found to be directly related to the extract concentration. This concentration-dependent increase in the percentage of brine shrimp nauplii mortality caused by *M. esculenta* may be evidence that the extract contains cytotoxic agents. LC_{50} value of methanolic extract was found to be greater than as depicted in a former research article by Gyawali Rajendra et al. (Gyawali et al., 2015). The brine shrimp assay's usefulness as a prescreening tool for anticancer drug development was proven by the National Cancer Institute (NCI, USA) by a strong association with the in vitro growth inhibition of human solid tumor cell lines (Anderson et al., 1991).

When three different methanolic extract concentrations (50, 75, and 100 mg/mL) were investigated, the paralysis and death times were found to be 28.19, 20.2, 10.6, 75.66, 57.96, and 51.85 min, respectively. The results against *Einesia foetida* demonstrated more pronounced effect at a concentration of 100 mg/mL. According to research conducted by Jain K. Vibhor and Jain Bindu, a 12.5 mg/mL concentration of an aqueous ethanolic extract caused the adult Indian earthworm *Pheritima posthuma* to die and become paralyzed after 20.11 and 41.25 min, respectively (Jain & Jain, 2010). The results could differ depending on the concentration and helminth species used.

5 | CONCLUSION

In conclusion, our study delves into the diverse therapeutic potential of *Myrica esculenta*, uncovering its remarkable antioxidant, anti-inflammatory, anthelmintic, and antitumor properties. The substantial levels of total phenolic and total flavonoid content in *M. esculenta*, surpassing established benchmarks, underscore its role as a functional plant with robust antioxidant capabilities.

Furthermore, our results indicate that *M. esculenta* may have promising anti-inflammatory and anthelmintic properties, making it a flexible option for treating parasite infections and inflammatory diseases. The methanolic extract's significant bioactivity against brine shrimp highlights its potential as a source of antitumor chemicals derived from plants.

While our study sheds light on the remarkable therapeutic attributes of *M. esculenta*, we acknowledge the need for further research to validate our findings and pinpoint the specific active constituents responsible for the observed effects on cancer cells, inflammation, and parasitic activity. This nuanced understanding is crucial for unlocking the full therapeutic potential of *M. esculenta* and harnessing its benefits for medical applications. The comprehensive analysis presented here contributes valuable insights to the existing body of knowledge on medicinal plants. Future studies should reveal new facets of *M. esculenta's* medicinal uses as we investigate the complex relationships inside the organism. In order to develop a more nuanced understanding of *M. esculenta's* many health-promoting qualities, our study encourages scientists to explore the plant's potential in greater detail.

AUTHOR CONTRIBUTIONS

Asmita Khanal: Investigation; methodology; writing—original draft. **Bechan Raut**: Supervision. **Dharma Prasad Khanal**: Conceptualization; project administration; validation. **Niranjan Koirala**: Data curation; project administration; supervision; validation; writing—review & editing

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

DATA AVAILABILITY STATEMENT

The datasets used during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This study was approved by the review committee of Manmohan Memorial Institute of Health Sciences Kathmandu, Nepal.

CONSENT FOR PUBLICATION

All the authors provided the consent for the publication in this journal.

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