

Artículo Original / Original Article

**Antileishmanial, insecticidal, anti-leukemic, phytotoxicity and anti-pancreatic lipase effects of ethanolic extracts of *Moringa peregrina* Seed**[Efectos antileishmaniales, insecticidas, anti-leucémicos, fitotóxicos y anti-lipasa pancreática de extractos etanólicos de semillas de *Moringa peregrina*]Shaymaa Fadhel Abbas Albaayit<sup>1</sup>, Rukesh Maharjan<sup>2</sup>, Rasedee Abdullah<sup>3</sup>,  
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<https://doi.org/10.37360/blacpma.25.24.2.16>**Abstract:** In this study, the ethanolic extracts of *Moringa peregrina* seeds (MPSE) were evaluated for their antiparasitic, insecticidal, herbicidal, anti-leukemic, and anti-pancreatic lipase activities. The MPSE showed moderate antileishmanial activity against *Leishmania major* with an IC<sub>50</sub> of 71.7 ± 0.46 µg/mL, compared to 10 ± 0.05 µg/mL and 4 ± 0.05 µg/mL for pentamidine and amphotericin B, respectively. The extract demonstrated moderate insecticidal activity with 41% mortality in *Rhyzopertha dominica* and 15.7% in *Tribolium castaneum*. MPSE exhibited potent herbicidal activity against *Lemna minor* at 1000 µg/mL. Additionally, MPSE inhibited the proliferation of leukemia K562 cells with an IC<sub>50</sub> of 25 µg/mL and porcine pancreatic lipase with an IC<sub>50</sub> of 83.7 ± 0.57 µg/mL. These findings suggest that MPSE may be beneficial as an antiparasitic, insecticidal, herbicidal, anti-leukemic, and anti-pancreatic lipase agent.**Keywords:** Anti-parasitic; Insecticidal; Herbicidal; Leukemic K562 cell line; Pancreatic lipase**Resumen:** En este estudio, se evaluaron los extractos etanólicos de semillas de *Moringa peregrina* (MPSE) por sus actividades antiparasitarias, insecticidas, herbicidas, anti-leucémicas y anti-lipasa pancreática. Los MPSE mostraron una actividad antileishmanial moderada contra *Leishmania major* con un IC<sub>50</sub> de 71.7 ± 0.46 µg/mL, en comparación con 10 ± 0.05 µg/mL y 4 ± 0.05 µg/mL para pentamidina y anfotericina B, respectivamente. El extracto demostró una actividad insecticida moderada con un 41% de mortalidad en *Rhyzopertha dominica* y un 15.7% en *Tribolium castaneum*. Los MPSE exhibieron una potente actividad herbicida contra *Lemna minor* a 1000 µg/mL. Además, los MPSE inhibieron la proliferación de células de leucemia K562 con un IC<sub>50</sub> de 25 µg/mL y la lipasa pancreática porcina con un IC<sub>50</sub> de 83.7 ± 0.57 µg/mL. Estos hallazgos sugieren que los MPSE pueden ser beneficiosos como agentes antiparasitarios, insecticidas, herbicidas, anti-leucémicos y anti-lipasa pancreática.**Palabras clave:** Extracto etanólico de semillas de *Moringa peregrina*; Antiparasitario; Insecticida; Herbicida; Lipasa pancreática

## INTRODUCTION

The incidence of leishmaniasis, a vector-borne disease, is widespread across 98 countries globally, leading to an annual surge of between 700,000 and 1 million new cases, while an estimated 350 million people worldwide face the risk of infection (Kareem *et al.*, 2016; Jewely & Zuhair, 2022). Current treatment options for leishmaniasis involve the use of glucantime, pentostam, and pentamidine, with Amphotericin B drug being recommended for the cutaneous form of the infection. However, these treatment regimens are now at risk of developing resistance to the emerging forms of leishmania. Consequently, there is a growing necessity to increase their dosage to achieve the desired therapeutic effectiveness, which has, in turn, contributed to a rising incidence of kidney and liver disorders (Bekhit *et al.*, 2018). Hence there is an urgent demand for the discovery of effective compounds, whether natural or synthetic, with minimal side effects for treating this infection. Medicinal plants hold significant promise as a source of anti-leishmanial compounds (Abdullah & Alqaisi, 2022).

Pest infestations significantly impact crop yield and the quality of vegetables, fruits, and grains, making them a major concern, especially in grain storage and the maintenance of high-quality products (Ismail *et al.*, 2012). In crop farming, pesticides, especially insecticides, are widely employed to enhance production, minimize losses, and ensure product quality (Damalas, 2009). However, the presence of pesticide residues in food products can lead to a range of short- to long-term health issues in humans (Daqer *et al.*, 2018).

Numerous plants are known to contain phytochemicals with potential pesticidal properties. Some of these include *Madhuca indica*, *Sapindus mukorossi*, *Curcuma longa*, *Pongamia glabra*, *Eupatorium adenophorum*, *Tagetes erecta*, *Rheum emodi*, and *Anethum sowa* (Walia *et al.*, 2017). One such plant is *Moringa peregrina* (Forssk) Fiori, whose products boast a diverse array of biological activities, making them promising candidates for drug development (Elbatran *et al.*, 2005; Albaayit *et al.*, 2019). *M. peregrina* leaves, and seeds have traditionally been used to treat various ailments, including wounds, gastrointestinal disorders, cytotoxic, urease inhibition, immunomodulating properties (Qaralleh *et al.*, 2015; Albaayit & Ozaslan,

2019; Albaayit & Maharjan, 2024). Several phytochemicals in *M. peregrina* seeds, such as phenolics, isothiocyanate, tocopherols, triterpenoids, and oleic acid, are known for their immunomodulatory effects.

Herbicides play a crucial role in crop farming, but many of them can be toxic if not handled with care. Natural products with herbicidal properties are considered safer for both humans and animals. These natural herbicides, or phytotoxins, target various molecular components, including membrane structures, lipid components, gene expression, microstructures, and the plant cell cycle (Dayan & Duke, 2014). *Moringa oleifera* has been reported to possess herbicidal activity against *Econocloa colonum* grassy weed (El-Rokiek *et al.*, 2022) and *Euphorbia heterophylla* (Oluwafemi, 2013).

Leukemia ranks as the second leading cause of cancer-related deaths in children (Du *et al.*, 2022), with its incidence on the rise. The search for leukemia therapeutics, especially from plants with minimal side effects, is ongoing. Many anticancer compounds are derived from plants (Albaayit *et al.*, 2021; Maher *et al.*, 2021; Albaayit & Mohammed, 2023). Notably, the anticancer drug homoharringtonine is derived from the evergreen tree *Cephalotus harringtonia* (Luo *et al.*, 2004). The isothiocyanate, with anticancer properties has been identified in the *M. peregrina* seeds. *Moringa oleifera* has shown promising antileukemic effects (Wu *et al.*, 2021).

Obesity, characterized by the excessive accumulation of body fat due to high-energy intake and low energy expenditure, has become a global problem affecting approximately 1.9 billion people (Haththotuwa *et al.*, 2020). It is linked to various health issues, including metabolic, endocrine, and cardiovascular disorders (Piché *et al.*, 2020). Pancreatic lipase inhibitors are suggested as effective treatments for obesity, preventing the absorption of dietary triglycerides and reducing fat deposition in the body (Abdulaziz & Khazaal, 2015). Many natural products are known for their anti-pancreatic lipase activity and help in the regulation of obesity (Jaradat *et al.*, 2017; Albaayit, 2021).

*Moringa* plant species are rich in pharmacologically active components with a wide range of therapeutic effects including immunomodulation, antidiabetic, antioxidant,

antimicrobial, and anti-cancer effects (Senthilkumar *et al.*, 2018; Albaayit, 2024). However, the potential of *M. peregrina* seed extracts as antiparasitic, insecticidal, anti-leukemic, and anti-pancreatic lipase agents remains to be explored and determined.

## METHODOLOGY

### Preparation of plant

Crude *M. peregrina* seed extract (MPSE) was obtained as reported earlier by Albaayit *et al.*, 2019.

### Phytochemical identification of the ethanolic extract of *Moringa peregrina* seed

To find the important phytochemical compounds from MPSE, it was subjected to LC-MS/MS analysis by following the protocol described by Mayakrishnan *et al.* (2013) and analysed in AB Sciex 3200 QTRAP.

### Anti-leishmania activity

The half maximal inhibitory concentration (IC<sub>50</sub>) for the promastigotes of *Leishmania. major* was determined following the protocol of Okokon *et al.* (2013). The MPSE were serially diluted with RPMI 1640 medium to a total volume of 100 µL to concentrations ranging from 6.25 to 100 µg/mL. The log phase concentrations of *L. major* were prepared in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum, and 100 µL of parasite culture was aliquoted to each well containing 100 µL of MPSE to a final concentration of 1 × 10<sup>6</sup> cells/mL. Non-treatment controls received medium without extract while the positive controls were received 20 µL of amphotericin B (Hebei, China) and 20 µL of pentamidine (Hebei, China), the

commercial anti-leishmanial compounds. The plate was incubated at 21 to 22°C for 72 h. 20 µL of each dilution was placed in the Neubauer chambers and examined under the binocular microscopy (Optica, 500 series), to determine parasite viability.

### Herbicidal activity

Inorganic E-medium essential for the growth of plant was prepared by mixing appropriate inorganic constituents with distilled water, pH 7.0 to 7.2 and maintained by adding KOH pellets before storing at 4°C (30). A 20 mg/mL stock MPSE solution in ethanol was prepared. 10, 100, and 1000 µL of the stock MPSE solution were dispensed to the small conical flasks in triplicates and left overnight to evaporate to dry. 20 mL of working E-medium solution was added to all flasks such that the final concentration would be 10, 100, and 1000 µg/mL. 20 fronds of green, healthy, and paired *Lemna minor* were added to the flasks, which was sealed with parafilm. Small holes were punched with a needle into the parafilm to allow for air passage. The nontreatment control did not receive the extract and served as the positive growth control. Paraquat (Sinon USA, Inc), a standard plant growth inhibitor at its IC<sub>50</sub> value of 0.02 µg/mL was used. These flasks were incubated in a growth cabinet at 27°C, under 8500-9000 lux light intensity and 54 ± 6% relative humidity. After 7 days of incubation, the flasks were removed and the number of dead and live fronds in each flask was determined. The percentage inhibition of *L. minor* was determined using following formula.

$$\% \text{ Inhibition} = 100 - \frac{\text{Number of live fronds in test sample}}{\text{Total number of live fronds in untreated control}} \times 100$$

### Insecticidal activity

*Tribolium castaneum*, and *Rhyzopertha dominica* were used to determine the insecticidal activity of the MPSE using the filter paper method described by Ismail *et al.* (2012). 1 mL MPSE stock solution (30 mg/mL) in absolute ethanol was loaded on the filter paper (9 cm) and left overnight to evaporate to dry. 10 healthy insects were placed on dried extract-

infiltrated filter paper in a petri plate and incubated in a growth chamber at 27°C under 50% relative humidity for 24 h. The drug control was similarly treated with filter paper impregnated with 1mL of Permethrin. In untreated control, absolute ethanol (1 mL) was treated with filter paper. The next day, the percentage insect mortality in each plate was calculated using the following formula.

$$\text{Mortality (\%)} = 100 - \frac{\text{Number Of Live Insect In Test Sample}}{\text{Total Number Of Live Insects In Untreated Control}} \times 100$$

### MTT assay of MPSE-treated human leukemia, K562, cells

The human leukemia cancer, K562, cell line was obtained from the Cell Culture Biobank (PCMD, ICCBS). The cancer cells were cultured and passaged using DMEM medium according to the method described by Albaayit *et al.* (2021). The cytotoxic effect of the MPSE on the K562 cells was determined using the MTT assay.  $5 \times 10^5$  K562 cells were placed in each well in a 96-well plate and incubated at 37°C, under 5% CO<sub>2</sub> for 24 h, before treating with MPSE at concentration ranging from 6.25 to 100 µg/mL. The

nontreatment control cells received medium only. After 48 h, 20 µL of 5 mg/mL MTT reagent was added to each and the plate incubated at 37°C for another 3 to 4 h. The purple formazan crystals formed were dissolved by adding 100 µL DMSO and the absorbance was measured at 570 nm using an ELISA plate reader (Multiskan<sup>GO</sup>, Thermo Scientific). In this assay, doxorubicin, a well-known antineoplastic drug at IC<sub>50</sub> value of 0.501 µg/mL was used as a standard drug. The percentage of cytotoxicity of MPSE was determined by using the following formula.

$$\% \text{ cytotoxicity/inhibition} = 100 - \frac{\text{O.D of treated well} - \text{O.D of media control}}{\text{O.D of untreated control} - \text{O.D of media control}} \times 100.$$

### Porcine pancreatic lipase inhibition

The lipase inhibition assay was carried out spectrophotometrically in 96-well plate using Tris-HCl buffer as described by Ong *et al.* (2016). The reaction mixture contained various concentrations of MPSE (0.2 mg/mL), porcine pancreatic lipase (PPL) (200 units/mL) and p-nitrophenyl butyrate substrate

solution (10 mM in acetonitrile). After incubation at 37°C for 5 min, the absorbance of the released PPL was measured at 410 nm ELISA plate reader (Spectra Max M2, Molecular Devices, CA, USA). Mean ± SD had been used to analysis data from which IC<sub>50</sub> values were calculated.

$$\text{Enzyme Inhibition (\%)} = 100 - \frac{\text{O.D of enzyme with inhibitor}}{\text{O.D of enzyme without inhibitor}} \times 100$$

### Statistical Analysis

One way analysis of variance (ANOVA) and Tukey's posttest were used to determine the difference in IC<sub>50</sub> values of various concentrations of MPSE. The Graphpad Prism software version 5.0 was used to determine the significant differences among means at  $\alpha = 0.05$ .

## RESULTS

### LCMS/MS screening

MPSE has high contents of flavanoids, caffeoyl glucose, Apigenin 6 C glucoside, and methyl 2-[cyclohex-2-en-1-yl(hydroxy)methyl]-3-hydroxy-4-(2-hydroxyethyl)-3-methyl-5-oxoprolinate (Figure No. 1).

### Anti-leishmaniasis activity

In order to evaluate the anti-leishmaniasis activity, MPSE was incubated at various concentrations with promastigotes of *L. major*. Standard drugs, amphotericin B and pentamidine were also used due

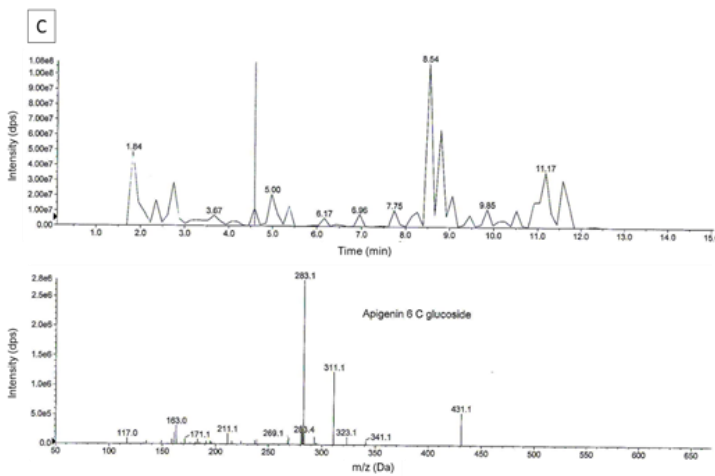
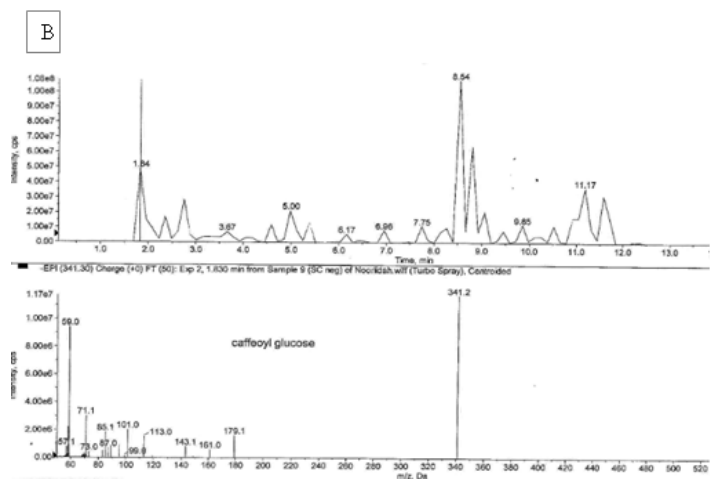
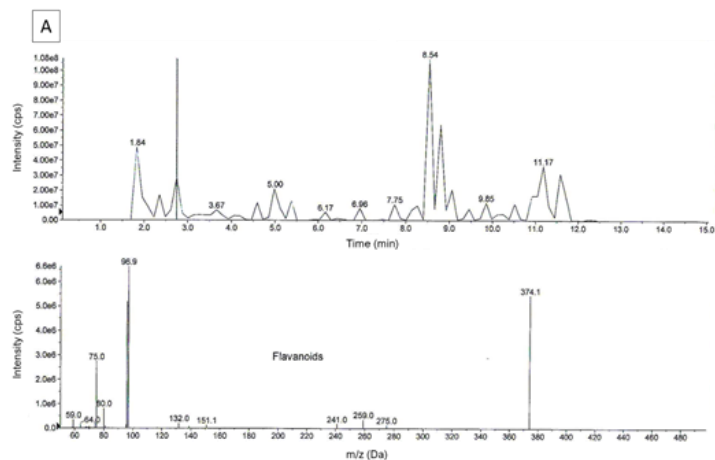
to their different mode of action. The IC<sub>50</sub> of the MPSE was found to be  $71.7 \pm 0.46$  µg/mL, which was much higher than standard anti-leishmania drugs, pentamidine and amphotericin B with their IC<sub>50</sub> values at  $10 \pm 0.05$  and  $4 \pm 0.05$  µg/mL, respectively.

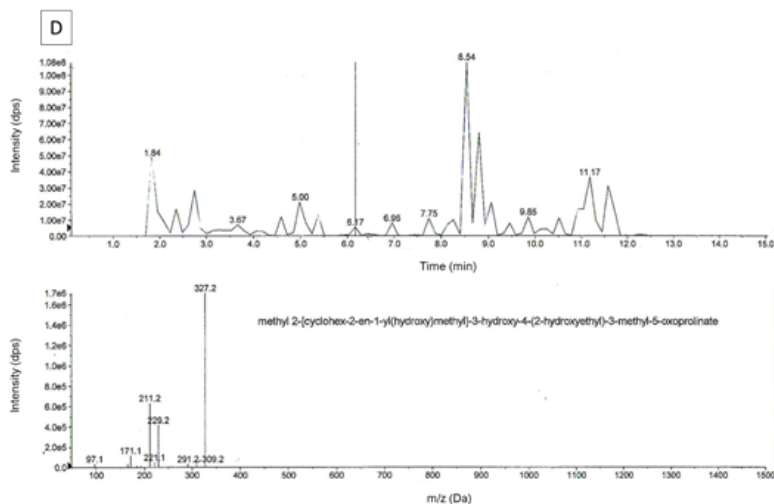
### Insecticidal activity

The MPSE at concentration of 30 mg/mL showed moderate insecticidal activities against *R. dominica* with  $41.0 \pm 1.0\%$  mortality. However, it showed low lethality against *Tribolium castaneum* with 15% mortality. Permethrin, a standard drug showed 100% lethality against both insects.

### Herbicidal activity

MPSE showed an IC<sub>50</sub> of  $241.1 \pm 0.46$  µg/mL on the *L. minor* fronds. At 1000 µg/mL, MPSE was 100% phytotoxic to the *L. minor* fronds. The phytotoxic effect of MPSE was much lower at lower concentrations of 100 and 10 µg/mL. The IC<sub>50</sub> of paraquat was 0.02 µg/mL.





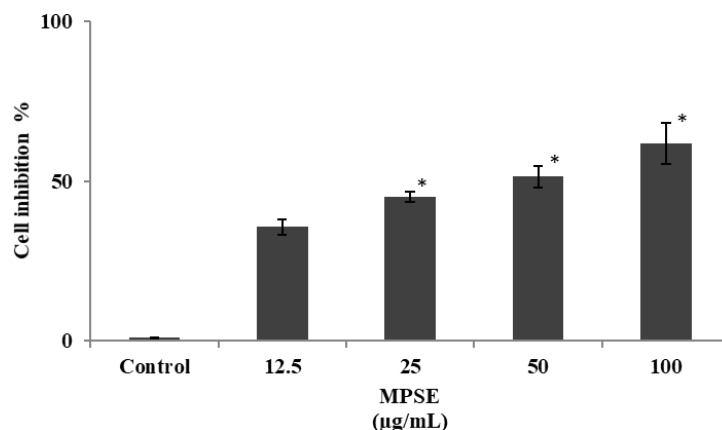
**Figure No. 1**

LCMS/MS characterizations of ethanol *M. peregrina* seed extract showing its high contents of (A) flavanoids, (B) caffeoyl glucose, (C) Apigenin 6 C glucoside, and (D) methyl 2-[cyclohex-2-en-1-yl(hydroxy)methyl]-3-hydroxy-4-(2-hydroxyethyl)-3-methyl-5-oxoprolinate

**Effects of *M. peregrina* seed extract on leukemia, K562, cells**

MPSE inhibited *in vitro* proliferation of leukemia K562 cells. The  $IC_{50}$  of MPSE on the K562 cells was 25  $\mu\text{g/mL}$ . With increase in concentration, there was

slight increase in cytotoxicity. At 100  $\mu\text{g/mL}$ , MPSE caused 60-65% inhibition on the proliferation of the cancer cells. To increase its cytotoxic effect, concentration has to be increased above 100  $\mu\text{g/mL}$  (Figure No. 2).



**Figure No. 2**

Effect of *M. peregrina* seed extract (MPSE) on the proliferation of the human leukemia, K562, cells, determined via the MTT assay. The cells were treated with MPSE for 48 h

**Pancreatic lipase inhibition assay**

The MPSE enzyme inhibition activity was determined on Porcine Pancreatic lipase. The  $IC_{50}$  value of MPSE was found at  $83.7 \pm 0.57 \mu\text{g/mL}$  and produced  $63.10 \pm 0.30\%$  inhibition in pancreatic

lipase activity. This enzyme is responsible for fat absorption, therefore, inhibition of pancreatic lipase will reduce fat absorption and help in the regulation of obesity.

**Table No. 1**  
**Insecticidal activity of *M. peregrina* seed extract (MPSE)**

Insect	Lethality (%)	
	MPSE	Permethrin
<i>Rhyzopertha dominica</i>	41.0 ± 1.0	100
<i>Tribolium castaneum</i>	15.7 ± 5.7	100

**Note: Ethanol as negative control has no insecticidal activity**

## Discussion

*M. peregrina* plant from Moringaceae family is the second most studied plant after *Moringa oleifera*. These plant parts are widely used by various societies for the treatment of diabetes, hypertension, liver disease, gastrointestinal disorders, antimicrobial, anti-inflammatory, and cancers. Many phytochemical and biological data are available on the *M. oleifera* seeds. However, only limited research had been done on the seeds of *M. peregrina*. Therefore, our present study focused to explore the new biological activities of *M. peregrina* seeds.

*M. oleifera* flower, root, and leaves had been reported for its antileishmanial activity against promastigotes of *Leishmania donovani* (Kaur et al., 2014; Singh et al., 2015). The *M. oleifera* leaf extract in combination with amphotericin B showed a synergistic antileishmanial effect against the promastigotes of *L. major* (Hammi et al., 2020). In spite of the antileishmanial effect of the extract of this *Moringa* sp., the antiparasitic effect of the MPSE was not well explored. In our study, MPSE showed moderate anti-leishmanial activity and its IC<sub>50</sub> value was higher than amphotericin B and pentamidine. Abdalla et al. (2022), reported the main polyphenolic compounds in the *M. peregrina* seeds as gallic acid, chlorogenic acid, cinnamic acid, catechin, caffeic acid and rutin. Among these phenolic compounds, gallic acid and catechin had been reported for its antileishmanial activity against *L. donovani*, *L. amazonensis* (Ribeiro et al., 2015; Sharquie et al., 2016; Dutra et al., 2019; Keshav et al., 2021). The seeds of *M. peregrina* are rich in phenolic compounds, which could be main contributors to the anti-parasitic effect of MPSE.

The farm food production industry is plagued with excessive use of insecticides to control pest infestations and to prevent destruction of stored grains. The use of insecticides has resulted in the rise of drug resistance and also left over residue drugs

poses health issues. Over the last few decades, the search for plant products that could serve as insecticides had been intensive with numerous plant products showing potent insecticidal activities. Among the *Moringa* sp., *M. oleifera* seeds had been shown to exhibit insecticidal properties against *Sitophilus zeamais* (de Oliveira et al., 2020), and has been suggested for pest control management (Osman & Elsobki, 2019). In our study, the MPSE caused moderate mortality to *Rhyzopertha dominica* and less to *Tribolium castaneum*, an effect similar to that shown by the *M. oleifera* seed extract. Therefore, moringa species plant extract could be used against some limited insects like *Rhyzopertha dominica*. This MPSE need to be tested against other insects to broaden its insecticidal activity.

In sustainable agriculture, controlling the excessive weed growth is an important process. However, excessive use of chemicals had led to herbicidal resistance and environmental pollution. Therefore, researchers now searching for allelopathic plants by which weed growth could be managed (Al-Jobori & Ali, 2014). A recent review suggested that the herbicidal effect of natural products is due to the presence of phenolic compounds, flavonoids, terpenoids, saponins, phlobatannins, and alkaloid components (Mohammed & Najem, 2020; Mousavi et al., 2021). These phytochemicals are all present in the *M. peregrina* seed extract (Rouhi-Boroujeni et al., 2016; Senthilkumar et al., 2020). MPSE was shown to be moderate phytotoxic to the duckweed (*L. minor*) fronds. MPSE allelopathic effect was found to correlate with previously studies in which it showed allelopathic effect against *Hordeum vulgare* (Aytah, 2017).

*M. peregrina* seeds have been reported to show anticancer effects on the CACO-2, HeLa, AU565, MCF-7, L929, PC-3, and HepG2 cell lines (Elsayed et al., 2016; Abou-Hashem et al., 2019; Albaayit, 2020). However, the effect of MPSE on

leukemic cells was not explored. Earlier studies showed that the phenolic/flavonoid components of plant products, apigenin (Abdalla *et al.*, 2022) can induce apoptosis by activating the caspase-3 dependent apoptosis, by inhibiting PI3K/PKB pathways, by downregulating anti-apoptotic genes (Bcl-2, Bcl-xL), and upregulating caspase 3 (Zhou *et al.*, 2017). These components were present in MPSE and were reported for its anti-leukemia effect (Ruelade-Sousa *et al.*, 2010; Isoda *et al.*, 2014).

Obesity is an ongoing problem in the modern society and it is on the increase. There are many drugs that are being used to combat obesity and among these are dinitrophenol, amphetamine, phentermine. However, these drugs are associated with side effects ranging from diarrhea to hypertension and depression. Drugs and compounds to be used for the treatment for the condition should not only be efficacious but also safe. The pancreatic lipase is the target for the anti-obesity effect of candidate compounds because the enzyme plays a key role in the hydrolyzation of triacylglycerol to 2-monoacylglycerol and fatty acids and intestinal absorption of triacylglycerol, resulting fatty acid deposition in tissues (Al-Haidari *et al.*, 2020). Currently, among the *Moringa* sp., *M. oleifera* extracts showed anti-obesity effects (Redha *et al.*, 2021). In our study, MPSE was tested invitro for its pancreatic lipase inhibition activity and found to have

63% inhibition of this enzyme. As pancreatic lipase enzyme was inhibited therefore less amount of available pancreatic lipase will cause lower fat absorption from diets and help to regulate the obesity. There are multiple factors for obesity condition, but pancreatic lipase inhibition to some extent will help in reducing fat gain in the body. We propose that the flavonoid and phenolic compounds in MPSE are responsible for the porcine pancreatic lipase inhibitory activity, preventing intestinal absorption of lipolytic products and development of obesity.

This study is the preliminary screening of *M. peregrina* seed extract to find out its other biological properties which were not previously reported. There is no direct correlation between these studies in terms of antileishmanial, anti-leukemia, insecticidal or herbicidal activity. Instead, it gave new information that this extract has antileishmanial, anti-leukemia, herbicidal, and insecticidal properties. Thus, the isolated compounds from this plant could be tested for these studies and explore their useful biological properties for possible therapeutic treatment.

## CONCLUSION

The study showed that MPSE has several effects including antileishmanial, insecticidal, herbicidal, and anti-cancer activities. MPSE can also be used in obese people to reduce the fat absorption and limit the weight gain.

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