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Prospecting bioassays and enzyme inhibitory activities of *Alysicarpus vaginalis, Biophytum reinwardtii, Mikania cordata, and Plumeria obtusa*

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Abstract

Natural products have long been recognized as vital in medicine, agriculture, cosmetics, and food industry. A variety of structurally numerous new chemical compounds can be extracted from crude extracts of microorganisms, plants, or animals. *Alysicarpus vaginalis* (AV), *Biophytum reinwardtii* (BR), *Mikania cordata* (MC) and *Plumeria obtusa* (PO) are plants used in traditional medicine in Sri Lanka. This study was designed to determine the bioactivities of the whole plants of AV and BR, leaves of MC, and flowers of PO. The plant parts were collected from the central province of Sri Lanka, and processed by washing, air drying, and finally powdering. Extracts were obtained using *n*-hexane, ethyl acetate (EtOAc), and methanol (MeOH) and they were subjected to testing antifungal activity against *Cladosporium cladosporioides* (CC), cytotoxicity against brine shrimp, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging antioxidant activity, phytotoxic activity against lettuce seeds, α-amylase inhibitory activity, and lipase enzyme inhibitory activity. The EtOAc extract of MC (MCE) showed inhibitory properties against root and shoot elongation of lettuce seeds. EtOAc extract of PO (POE) and MeOH extract of PO (POM) showed considerable toxicity, and hexane extract of MC caused lethal effects in brine shrimp (LD_{50}: 1.54 mg L^{-1}). The POM displayed an inhibition zone around a spot of a separated compound from PO on a TLC plate against CC. The MeOH extract of *B. reinwardtii* (BRM) exhibited the highest antioxidant activity (IC_{50}: 43.7 mg L^{-1}) compared to ascorbic acid (IC_{50}: 2.21 mg L^{-1}). The MeOH extracts of BR, AV, and PO showed α-amylase enzyme inhibitory activity, where POM (IC_{50}: 582 mg L^{-1}), BRM (IC_{50}: 743.4 mg/L^{-1}), and MeOH extract of *A. vaginalis* (AVM) (IC_{50}: 1015.9 mg L^{-1}) showed inhibitory activity. The EtOAc extract of *A. vaginalis* (AVE) (IC_{50}: 332 mg L^{-1}) exhibited lipase enzyme inhibition. Since positive results were obtained for almost all the assays, it is worthwhile directing future studies towards the isolation of the responsible bioactive pure compounds in the plant extract.

Keywords: α-amylase, antifungal, antioxidant, cytotoxicity, lipase, phytotoxicity

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Introduction

Natural products are chemical compounds derived from living organisms such as plants, microorganisms, and animals. These compounds play crucial roles in various biological processes and have been widely utilized in pharmaceuticals, agriculture, and other industries. Natural products originate from diverse biological sources, including plants, fungi, bacteria, and marine organisms. Among different sources of natural products, plants have been a significant source of bioactive compounds due to their high content of secondary metabolites, which include alkaloids, flavonoids, terpenoids, and polyphenols. These compounds exhibit a broad spectrum of pharmacological properties, including antimicrobial, antifungal, antioxidant, and enzyme inhibitory activities.

Extraction of natural products is a crucial step in isolating bioactive compounds. Several extraction methods are used, including maceration, Soxhlet extraction, and solvent extraction. The choice of the extraction method depends on factors such as the nature of the plant material and the type of bioactive compounds targeted. The solvents commonly used include methanol, ethanol, chloroform, and water. The efficiency of extraction is evaluated through phytochemical screening and bioassay-guided fractionation.

The present study focuses on four plant species known for their medicinal and bioactive properties: *Alysicarpus vaginalis* (AV), which is traditionally used for treating various ailments due to its antimicrobial and antioxidant properties, *Biophytum reinwardtii* (BR) which is known for its anti-inflammatory and wound healing properties, *Mikania cordata* (MC) which used in traditional medicine for treating infections and inflammatory conditions, and *Plumeria obtusa* (PO) which is recognized for its antifungal and antibacterial activities in medicine. Rich in bioactive compounds, AV shows strong medicinal potential with low toxicity, supporting further pharmaceutical research (MR et al., 2018).

Studies on the phytochemistry of *Biophyum sensitivum* have revealed that it contains bioactive compounds like amentoflavone, flavonoids, and essential oils, exhibiting strong antioxidant, hypoglycemic, and anti-angiogenic effects (Bucar et al., 1998). It also enhances insulin secretion and inhibits tumor-induced angiogenesis, supporting therapeutic potential (Puri, 2001). The *MC*, a member of the Asteraceae family, contains bioactive compounds like polyphenols, flavonoids, and alkaloids (Rahman et al., 2020), exhibiting antibacterial, anti-inflammatory, anti-diarrheal, antipyretic (Strobel et al., 2020), and glucose-regulating properties (Jayatilake & Munasinghe, 2020), with low toxicity potential. *Plumeria* species possess purgative, diuretic, anticancer (Wong et al., 2011), antibacterial, and cytotoxic properties, with *PO* showing the strongest antimicrobial effects (Ali et al., 2014). Its bioactive compounds include amyrins, iridoids, plumieride, and benzyl salicylate (Ali et al., 2014).

The primary objectives of this research were to extract bioactive compounds from selected medicinal plants and evaluate their biological activities, including antifungal, antioxidant, and metabolic enzyme–modulating effects. Additionally, the study aims to assess the toxicity of these extracts using various bioassays to determine their safety and potential applications. By achieving these objectives, this research seeks to contribute to the broader understanding

of the medicinal potential of these plants, paving the way for their possible use in pharmaceutical and therapeutic developments.

Methodology

Materials and Instruments

The solvents used for extraction and chromatography were re-distilled in the laboratory before use. Herbs used in the study were ground into fine powder using a heavy-duty grinder. Extraction utilized a sonicator (Rocker Ultrasonic Cleaner, Soner 220H), and solvents were removed with a rotary evaporator (Heidolph Laborota 4000, BIOBASE CCA-420 Chiller). A vacuum oven (Heraeus Vacutherm VT 6025) ensured complete solvent removal. Weighing was performed with an analytical balance (KERN ABS 120-4N), and extracts were dissolved using a vortex mixer (Stuart® SA8). The pH was measured with a pH meter (EUTECH pH 700). Potato dextrose agar (PDA) (Dextrose-Avonchem, Oxon, UK; Agar-Meron, India) served as fungal culture media, sterilized in an autoclave (HIRAYAMA HV-110). Subculturing occurred in a laminar flow cabinet (NUAIRE NU-425-300E). TLC was performed using silica gel plates (Merck 1.05554.0007, 60F254) and analyzed under UV (Chromato-Vue CC-60). Optical density readings were obtained via a microplate reader.

Extraction of the Plant Materials

The four plants were collected from Mahawaththa (7°17'34.4"N 80°40'41.2"E), Menikhinna (7°19'06.1"N 80°41'53.8"E), Bogaskumburegammedda (7°19'17.1"N 80°39'44.7"E). After washing and air-drying, they were powdered and sequentially extracted using n-hexane, ethyl acetate, and methanol via sonication. The extracts were evaporated and vacuum-dried to remove residual solvents. The abbreviations used to identify each extract are given in Table 1.

Table 1: Abbreviation used to identify the sequential extracts.

Crude extract	Abbreviation
EtOAc extract of *Alysicarpus vaginalis*	AVE
Hexane extract of Alysicarpus vaginalis	AVH
MeOH extract of *Alysicarpus vaginalis*	AVM
EtOAc extract of *Biophytum reinwardtii*	BRE
Hexane extract of Biophytum reinwardtii	BRH
MeOH extract of *Biophytum reinwardtii*	BRM
EtOAc extract of *Mikania cordata*	MCE
Hexane extract of Mikania cordata	MCH
MeOH extract of *Mikania cordata*	MCM
EtOAc extract of *Plumeria obtusa*	POE
Hexane extract of Plumeria obtusa	POH
MeOH extract of *Plumeria obtusa*	POM

Antifungal Activity Assay

The antifungal activity of the extracts was visualized with TLC-bioautography on Czapek Dox Broth, where visible zones of inhibition indicate the presence of active antifungal agents. The TLC plates were spotted with initial plant extracts and developed in an appropriate solvent system. The plates were then sprayed with a suspension of CC spores and incubated under humid conditions for five days. The presence of antifungal compounds was indicated by clear inhibition zones on the TLC plate, contrasting with the fungal growth areas. Benlate was used as the positive control (Homans & Fuchs, 1970).

Antioxidant Activity Assay

Antioxidant potential to prevent diseases caused by oxidative stress was measured using the DPPH radical scavenging assay, a widely accepted method that reflects the ability of the extract to donate hydrogen and neutralize free radicals (Kumarappan et al., 2012). A dilution series of each crude extract, ranging from 31.25 to 1000 mg/L, was prepared using methanol. Triplicates were carried out for each concentration. DPPH solution (0.3 mM) was freshly prepared and was mixed with different concentrations of the plant extract, and the mixture [DPPH (0.3 mM, 60 µL] with sample (150 µL) was incubated in the dark for 30 minutes.

The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The decrease in absorbance indicates the scavenging ability of the extract, which was compared to a standard antioxidant ascorbic acid. DPPH (0.3 mM, 60 µL), and methanol (150 µL) without the test sample were used as the control whereas methanol (210 µL) was used as the control blank. Test sample (150 µL) with methanol (60 µL) was used as the sample blank. Ascorbic acid was used as positive control. The percentage inhibition was calculated, and median inhibitory concentration (IC_{50}) values were determined (Tepe et al., 2007).

Brine Shrimp Lethality Assay

Cytotoxicity was assessed using the brine shrimp lethality assay, a simple and cost-effective test that often correlates with antitumor and broader toxic effects in higher organisms (Ullah et al., 2013). Brine shrimp (*Artemia salina*) eggs were hatched in artificial seawater under continuous aeration. A dilution series of each crude extract ranging from 1000 to 31.25 mg/L was prepared using artificial seawater. The nauplii (larvae) were then exposed to different concentrations of plant extracts in a 24 well-plate.

After 24 hours of incubation, the number of surviving shrimps was counted. Triplicates were carried out for each concentration of samples. Artificial seawater was used as the negative control and atropine was served as the positive control of the assay. The median lethal concentration (LC_{50}) was calculated using probit analysis. A lower LC_{50} value indicates higher toxicity, making this assay a useful preliminary screening method for potential cytotoxic agents (Krishnaraju, 2005).

Phytotoxicity Assay

Phytotoxic effects on plant growth were evaluated by observing germination of *Lactuca sativa* seeds to predict agricultural implications such as herbicidal or growth-promoting properties. Seeds were sterilized and placed in petri dishes containing filter paper. Crude extracts were dissolved in distilled water. A dilution series of the crude extracts ranging from 1000 to 31.25 mg/L were prepared. 6 well plates were used for the assay. Different concentrations of plant extracts were applied, and the seeds were incubated under controlled conditions. After 5 days, germination percentage, root length, and shoot length were recorded. Triplicates were carried out for each sample. Distilled water was used as the negative control and abscisic acid was used as the positive control for the assay. The phytotoxicity of the extracts was determined by comparing growth parameters with untreated control seeds (Baratelli et al., 2012).

α-Amylase Inhibitory Assay

The extracts were tested for their ability to inhibit α-amylase, which slows carbohydrate digestion and supports diabetes management. A starch solution was used as the substrate, and α-amylase was mixed with plant extracts at different concentrations. Phosphate buffer (pH 6.9), pancreatic α- amylase enzyme (Sigma- Aldrich, EC 3.2.1.1) (25 units/ mL), starch solution (1% w/v) were used along with sample series and DNSA. Acarbose (1 mg/mL) was used as the positive control. After incubation, the reaction was stopped by adding a color-developing reagent, and the absorbance was measured at 540 nm using a spectrophotometer. The level of inhibition was calculated, with acarbose used as a standard inhibitor. The IC_{50} value was determined, indicating the concentration of extract required to inhibit 50% of enzyme activity (Nickavar et al., 2008).

Lipase Enzyme Inhibitory Assay

Inhibition of lipase impedes fat digestion and is relevant for obesity control. Pancreatic lipase enzyme was dissolved in phosphate-buffered saline (PBS, pH 7.4), while p-nitrophenyl butyrate (pNPB) was dissolved in acetonitrile as the substrate. Crude extracts were diluted (8000–250 mg/L) in PBS and incubated with pancreatic lipase in a 96-well plate at 37 °C. After adding pNPB, absorbance was measured at 400 nm. Orlistat (1 mg/mL) was used as positive control, and inhibition percentages were calculated to determine IC_{50} values (Choi et al., 2003).

Statistical Analysis

All samples underwent triplicate chemical analysis, with results expressed as mean ± standard deviation. Microsoft Excel 2021 was used for basic statistical reporting, while SPSS (Ver 26) performed probit analysis for IC_{50} values. Data analysis included two-way ANOVA with Tukey's test at a 95% confidence level ($p \leq 0.05$). Pearson's correlation method assessed relationships between variables.

Results

Yields of the crude extracts

Table 2.0 gives the percentage yield of each extract from the selected plants. When considering all the plant extracts, the highest yield was recorded in the MeOH extract of BR and the lowest yield was recorded in AV.

Table 2: Percentage yield (w/w %) of the extracts

Plant	Extract		
	Hexane	**EtOAc**	**MeOH**
Alysicarpus vaginalis (whole plant)	0.78	1.48	4.00
Biophytum reinwardtii (whole plant)	1.36	1.46	6.92
Mikania cordata (leaves)	2.32	3.50	2.92
Plumeria obtusa (flowers)	3.90	2.34	5.00

Antifungal activity assay

Antifungal activity of all crude extracts was tested according to the TLC bioautography method using the common plant pathogen, *Cladosporium cladosporioides*.

White spot-on Figure 1B indicates the presence of antifungal compounds in POM flower. No antifungal properties were observed from any other extract (Figures 2 and 3).

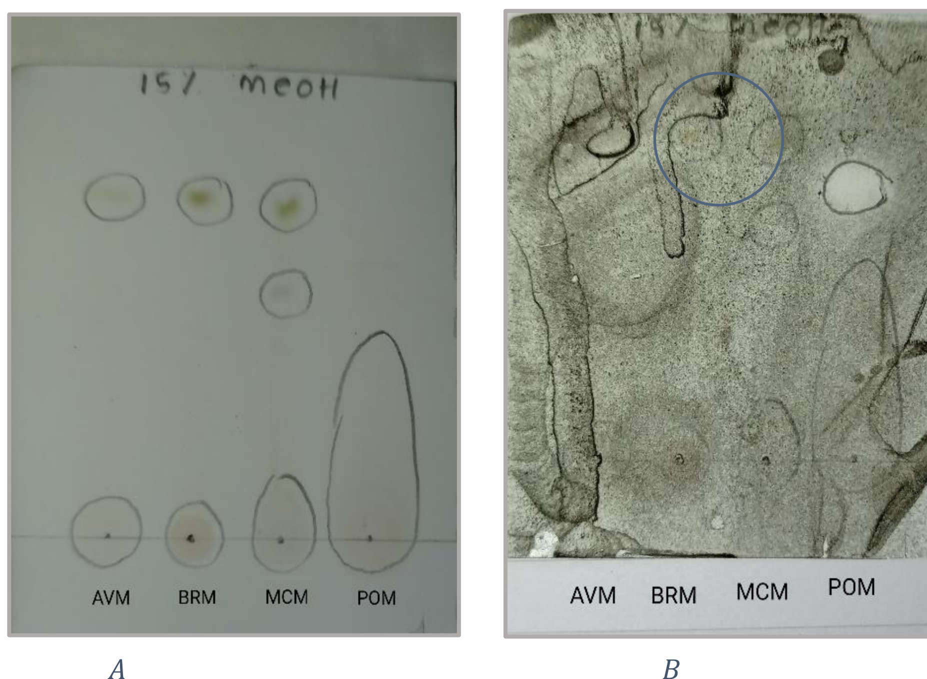

Figure 1. TLC plate with spots of extracts of plants A: under UV 254nm B: The ***Cladosporium cladosporioides*** sprayed TLC plate after3 days of incubation, solvent system – 15% MeOH / $CHCl_3$.AVM – The MeOH extract of AV, BRM – The MeOH extract of BR, MCM – The MeOH extract of MC, POM – MeOH extract of PO.

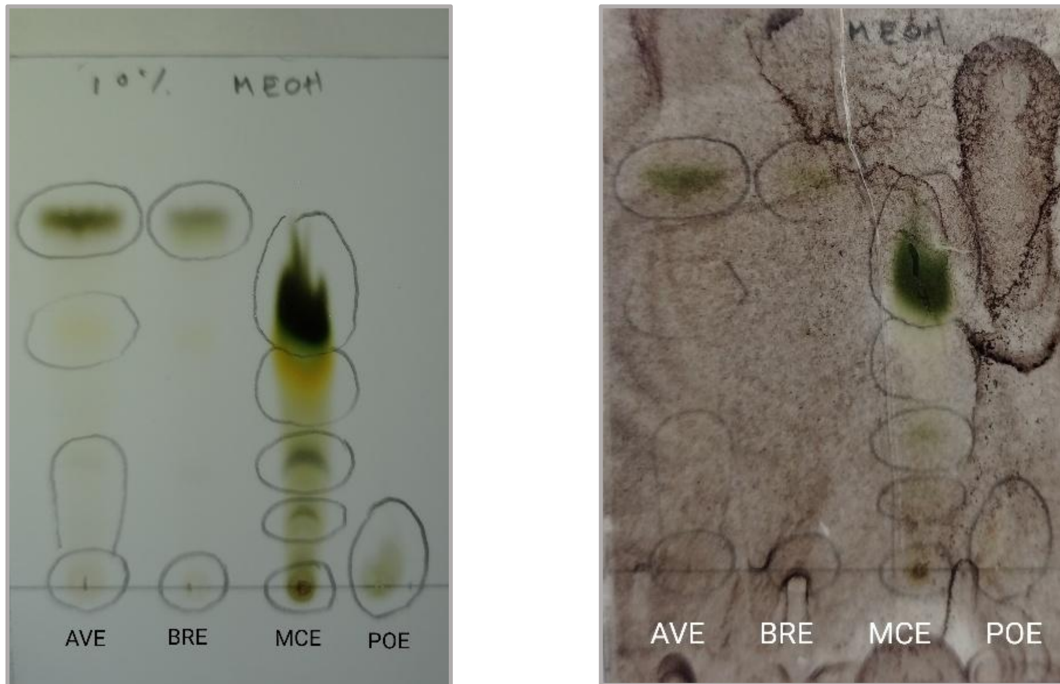

Figure 2. TLC plate with spots of extracts of plants A: under UV 254nm B: The ***Cladosporium cladosporioides*** sprayed TLC plate after3 days of incubation; solvent system – 10% MeOH / $CHCl_3$. **AVE – The EtOAc extract of AV, BRE – The EtOAc extract of BR**, **MCE – The EtOAc extract of MC, POE – The EtOAc extract of PO**.

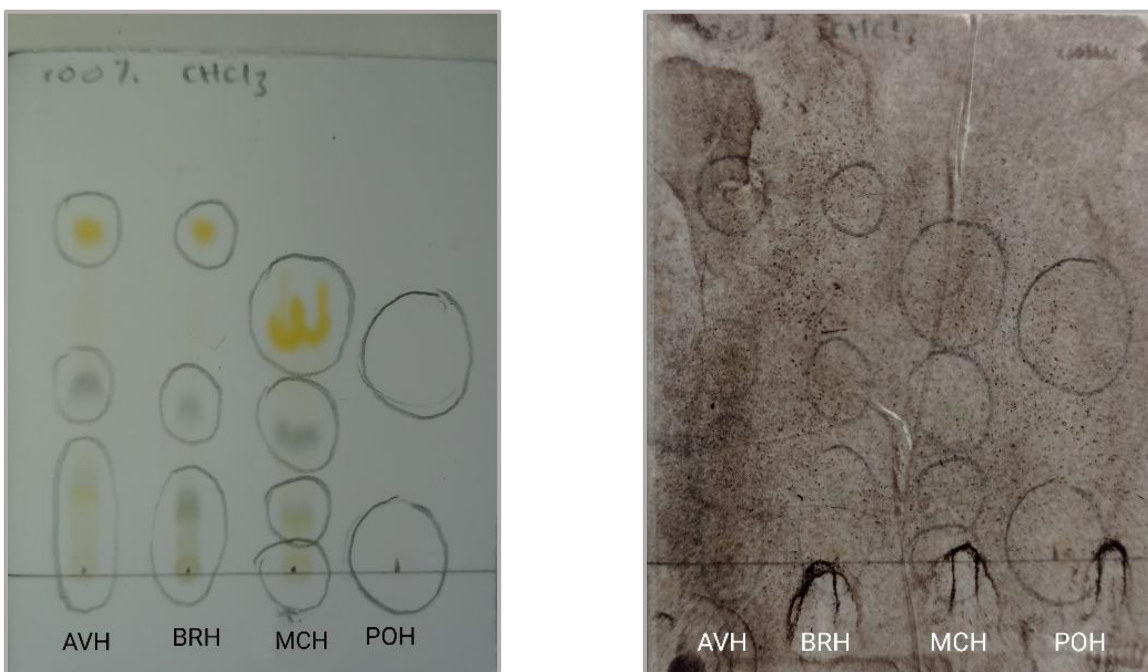

Figure 3. TLC plate with spots of extracts of plants A: under UV 254nm B: The ***Cladosporium cladosporioides*** sprayed TLC plate after3 days of incubation; solvent system – 100% $CHCl_3$. **AVH – The hexane extract of AV**, **BRH – The hexane EtOAc of BR**, **MCH – The hexane extract of MC**, **POH – The hexane of PO**.

Antioxidant activity

Antioxidant activity was tested quantitatively using DPPH free radical scavenging method for all the crude extracts. IC_{50} values of ascorbic acid and BHA were 2.21 mg/L and 5.20 mg/L respectively (Figure 4).

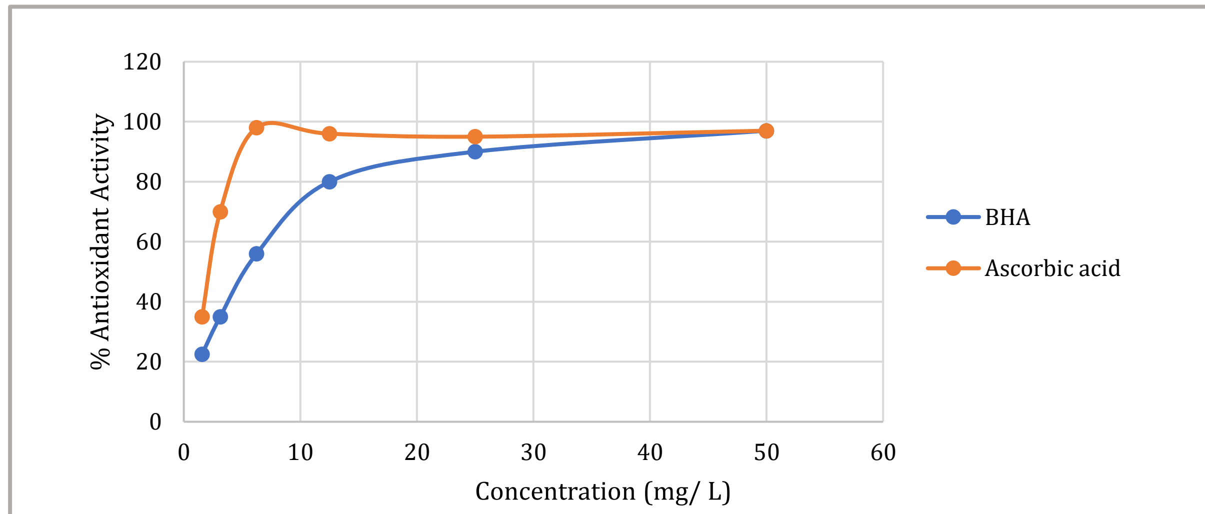

Figure 4. The percentage radical scavenging activity of BHA and ascorbic acid (n = 3)

The Percentage Radical Scavenging Activity of BHA and Ascorbic Acid Standards increased and reached a plateau, indicating that the DPPH radical scavenging activity of the standards reaches saturation at higher concentrations. The IC_{50} value is inversely proportional to antioxidant activity, and therefore, high IC_{50} values correspond to samples having a low antioxidant capacity.

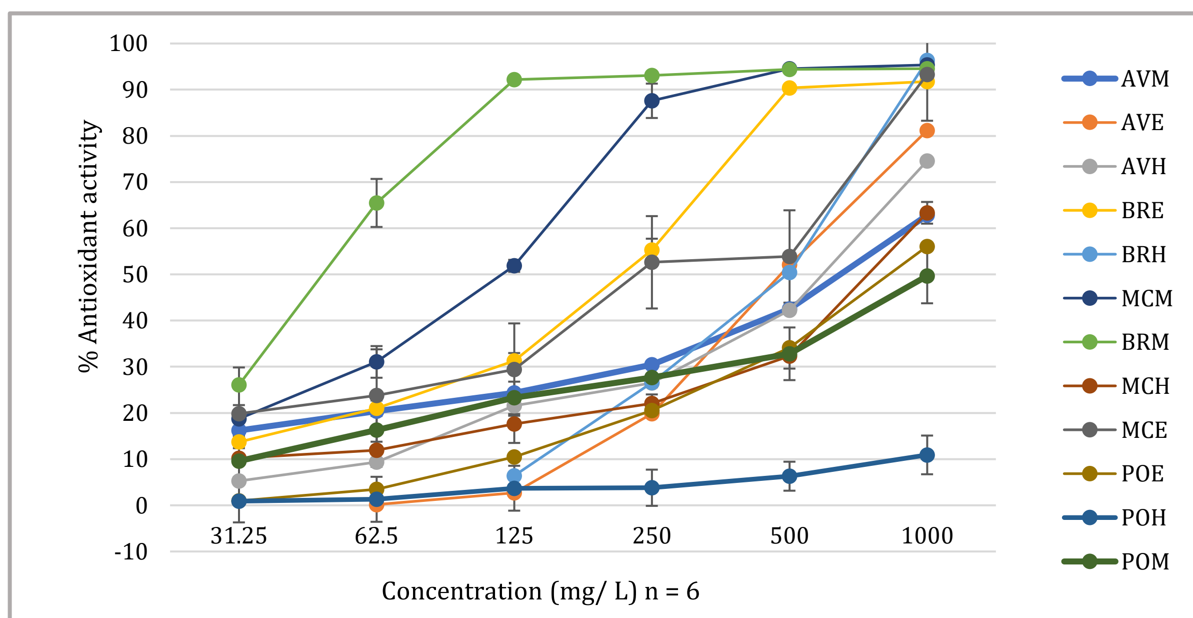

Figure 5. The percentage radical scavenging activity of all the crude extracts

The percentage radical scavenging activity of the extracts of all the plants increased with concentration (31.25–1000 mg/L) (Figure 5). In AV, the IC_{50} values ranged from 493 to 665 mg/L, indicating moderate antioxidant activity. Significant differences ($p \leq 0.05$) in absorbance were observed across solvent extracts. Correlation analysis showed a strong negative correlation ($r = -0.64$) between absorbance and IC_{50}, meaning higher absorbance corresponds to lower IC_{50} values.

BRM had the strongest antioxidant activity (IC_{50} = 43.70 mg/L), followed by ethyl acetate (IC_{50} = 169.70 mg/L) and hexane (IC_{50} = 500.00 mg/L). Absorbance values differed significantly ($p \leq 0.05$) across solvents and concentrations. Correlation analysis showed a strong negative correlation ($r = -0.70$) between absorbance and concentration and a weak positive correlation ($r = 0.47$) between absorbance and IC_{50}.

The MeOH extract of *Mikania cordata* (MCM) had the highest antioxidant activity (IC_{50} = 97.4 mg/L), followed by EtOAc (IC_{50} = 222.7 mg/L) and hexane (IC_{50} = 870.4 mg/L). A significant difference ($p \leq 0.05$) was noted in absorbance at different concentrations. A strong negative correlation ($r = -0.75$) was observed between absorbance and concentration, while a weak positive correlation ($r = 0.27$) was found between absorbance and IC_{50}.

POM and POE exhibited moderate antioxidant activity (IC_{50} = 1304.20 mg/L and 832.70 mg/L, respectively). Hexane extract showed negligible antioxidant activity (IC_{50} > 2000.00 mg/L). No significant differences were observed between solvent extracts at $P \geq 0.05$. A strong negative correlation ($r = -0.75$) was found between absorbance and concentration, while a weak negative correlation ($r = -0.29$) was found between absorbance and IC_{50}.

The MeOH extracts consistently demonstrated the highest antioxidant activity across all four plant species. MC and BR extracts had stronger antioxidant activities than AV and PO. Environmental factors and extraction methods may significantly influence results, as seen in variations with previous studies.

Brine shrimp lethality assay

Atropine was used as the positive control. LD_{50} value of Atropine was 25 mg/ L. According to (Moshi et al., 2010), the brine shrimp results were interpreted as follows: LC_{50} < 1.0 µg/ mL – highly toxic; LC_{50} –1.0-10.0 µg/ mL – toxic; LC_{50} 10.0-30.0 µg/ mL – moderately toxic; LC_{50} > 30 < 100 µg/ mL as non–toxic.

The lethality of the plant extracts on brine shrimp indicated the presence of potent cytotoxic components in these plants (Figure 6). According to the findings, MCH had the lowest LC_{50} value (1.54 mg/L) while all the plant extracts had the LC_{50} value greater than 100 µg/mL.

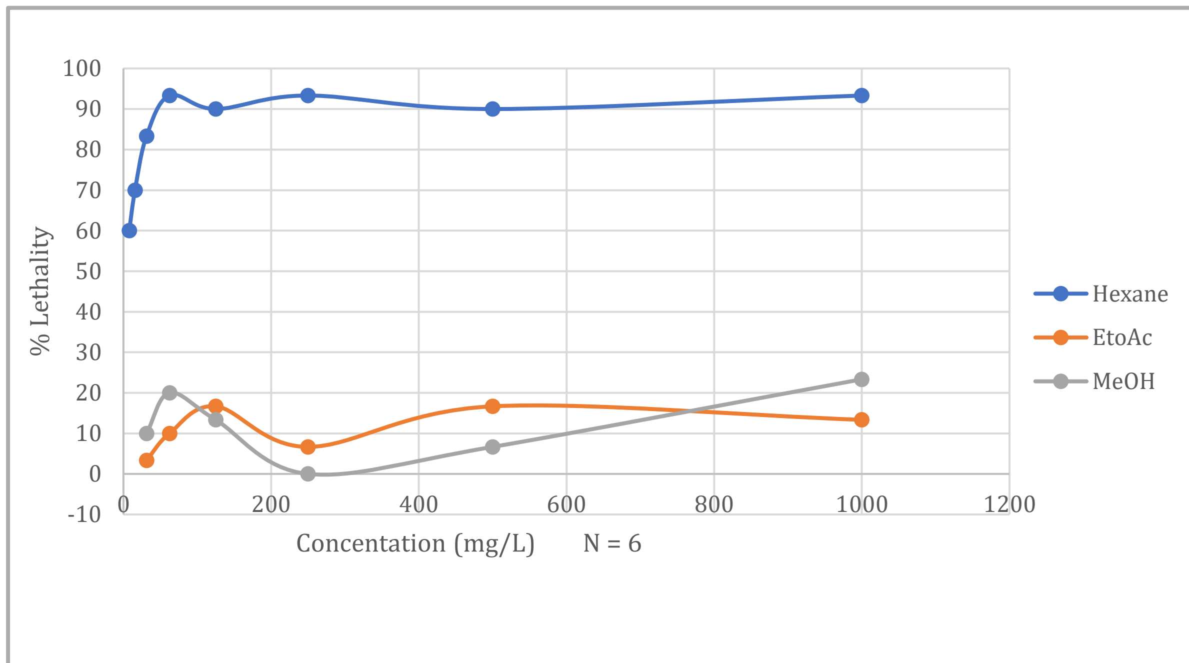

Figure 6. The percentage lethality of brine shrimp in crude extracts of MC.

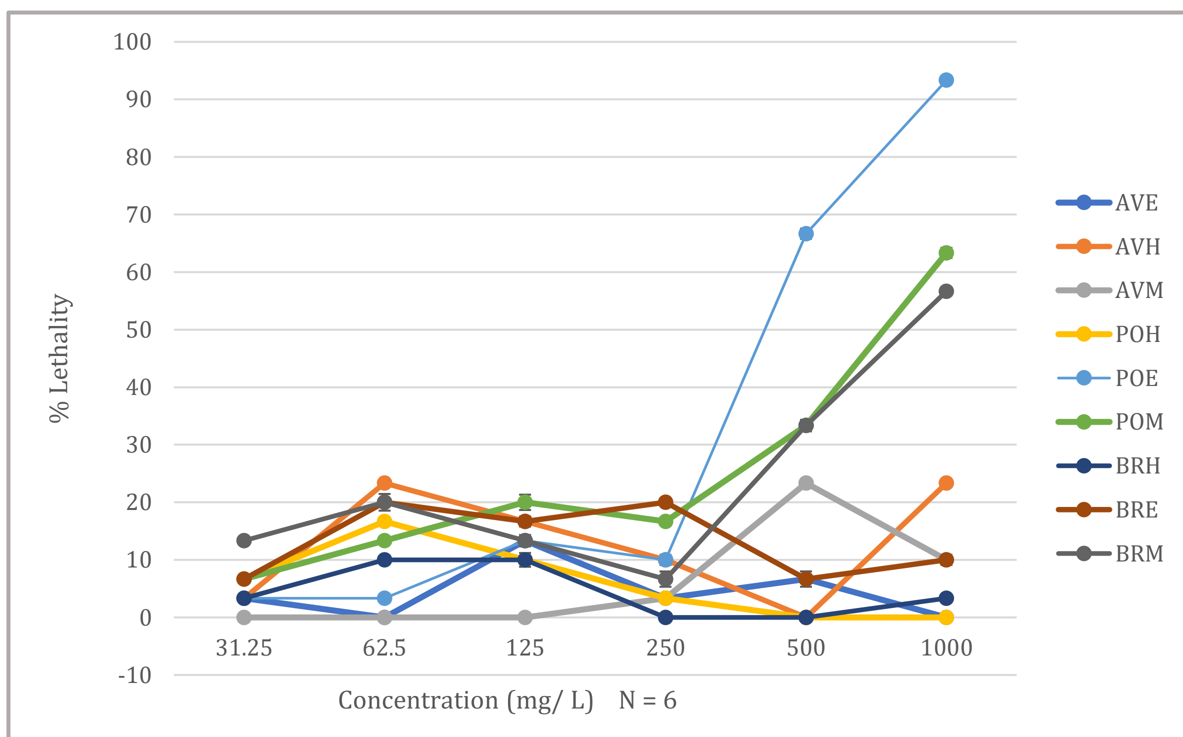

Figure 7. The percentage lethality of brine shrimp in crude extracts of AV, BR, PO.

Since 100% lethality was obtained for 31.25 mg/L concentration of MCH sample, the procedure was repeated using 7.81 mg/L as the lowest concentration and determined the LD_{50} as 1.54 mg/L. According to the findings, hexane extract with an LC_{50} of 1.54 mg/ mL is toxic. The toxicity of hexane extract of MC differ significantly ($P < 0.05$) with MeOH and EtOAc extracts based on the Tukey test (Figure 6).

ANOVA indicated that there were no significant differences in the lethality as inferred from absorbance readings with respect to solvent type or its concentration in AV and BR extracts ($p \geq 0.05$) (Figure 7).

Extracts that have LC_{50} values greater than 100 µg/ mL can be considered non-toxic (Moshi et al., 2010). Therefore, all extracts can be considered non-toxic since the % lethality of all extracts were less than 50 even up to 250 µg/ mL ..

Phytotoxic activity against lettuce seeds

Abscisic acid (positive control) showed 100% shoot and root inhibition at 5 mg/L (Figure 8). IC_{50} values of root and shoot inhibition of abscisic acid were at 0.99 mg/L and 1.11 mg/L respectively.

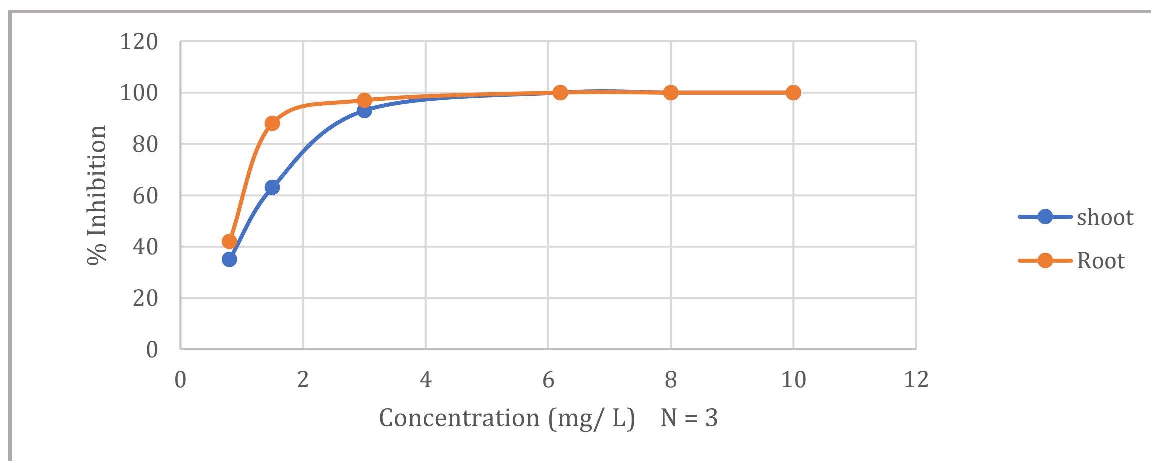

Figure 8. The percentage lettuce shoots and root elongation inhibition by abscisic acid

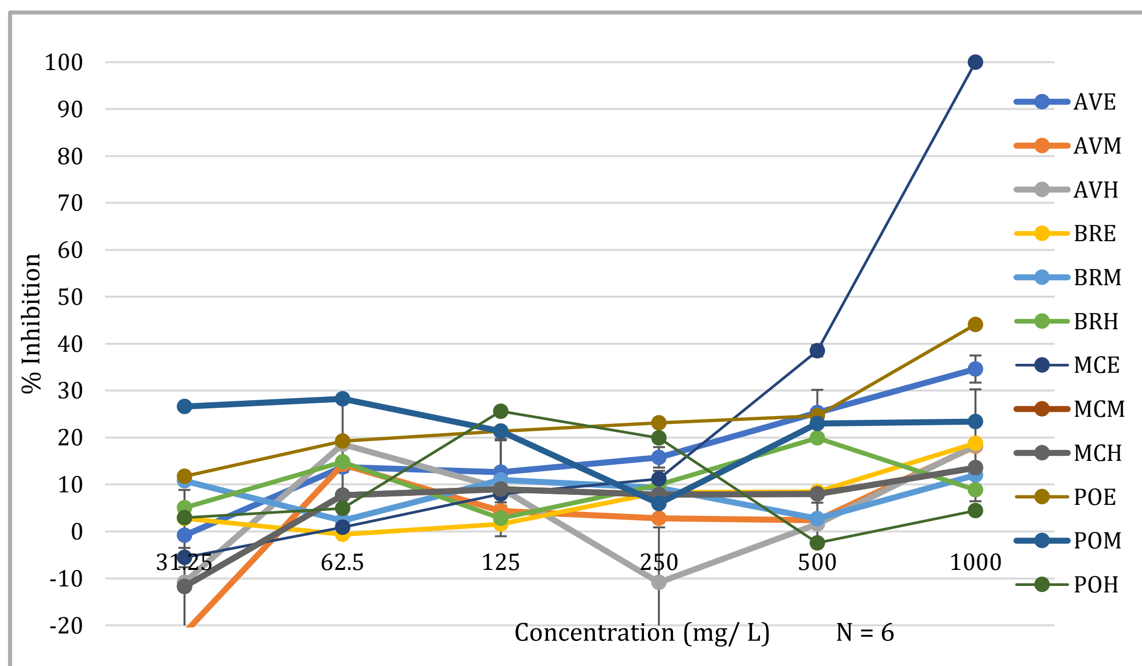

Figure 9 The percentage lettuce shoot elongation inhibition by the crude extracts

Figure 8 confirms the inhibition of shoot and root elongation assay with abscisic acid as the positive control. The MCE exhibited the lowest IC_{50} of 498.70 mg/ L, among all the plant extracts while the IC_{50} values of all the other extracts (AVE, AVH, AVM, BRE, BRH, BRM, MCH, MCM, POE, *POH and POM)* could not be calculated due to low inhibitory activities. For AV, shoot inhibition showed significant differences ($P \leq 0.05$) in inhibitory activity based on both the solvent and concentration. Hexane extract differed significantly from EtOAc and MeOH extracts, and the 31.25 mg/L concentration differed from 62.50, 125.00, 500.00, and 1000 mg/L. In contrast, there were no significant differences ($P \geq 0.05$) in inhibition of shoot elongation by BR, MC, and PO plant extracts based on solvent or concentration (Figure 9).

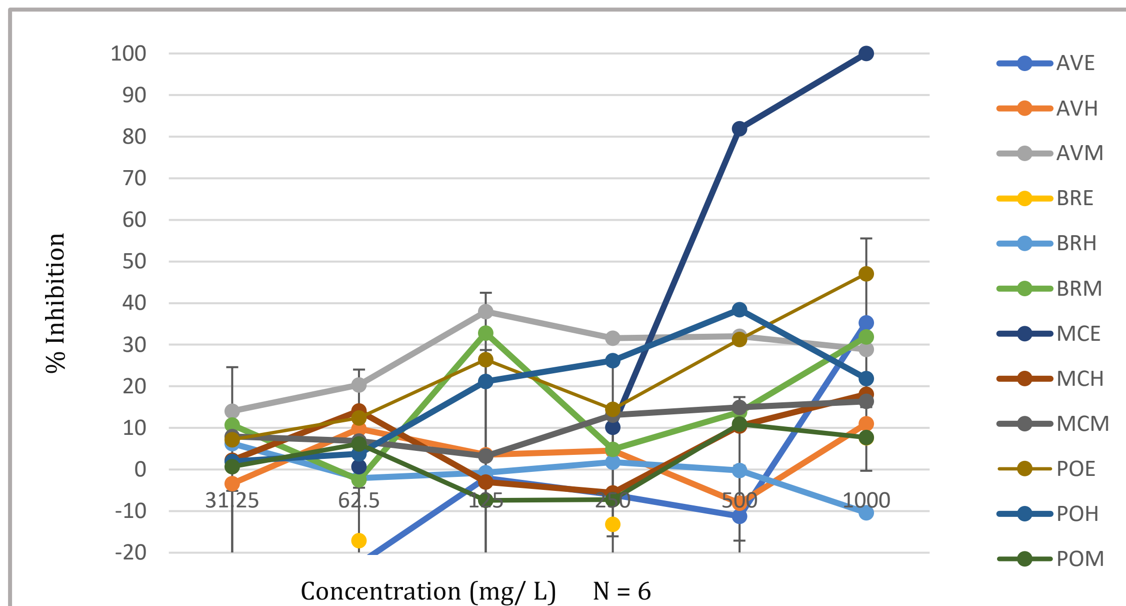

Figure 10. The percentage lettuce root elongation inhibition by the crude extracts

MCE exhibited the lowest IC_{50} of 373.91 mg/ L for inhibition of root elongation, among all the plant extracts. None of the remaining extracts exceeded 50% inhibition of root elongation at the concentrations under investigation (Figure 10).

Significant differences ($P \leq 0.05$) were observed between solvents for inhibition of elongation of roots by AV extracts. MeOH extract differed from EtOAc and hexane. However, no significant differences were found between the inhibition of root elongation with increasing concentrations indicating low level of toxicity even up to the highest tested concentration. In BR, significant differences ($P \leq 0.05$) were also seen between solvents, with MeOH differing from EtOAc, while increasing concentrations of each extract showed no significant difference indicating low level of toxicity of extracts of BR even at the highest level of concentrations tested. In MC and PO plants, there were no significant differences ($P \geq 0.05$) in activity based on either solvent or concentration.

α- amylase inhibitory assay

The IC_{50} value of BRM and POM are 743.4 mg/L and 582.1 mg/L respectively, implying moderate α- amylase enzyme inhibitory activity (Table 3) while the inhibitory activity of AVE, AVH, BRE,

BRH, MCE, MCH, MCM, POE as well as POH on α-amylase enzyme was observed as weak and did not reach at least to the 50% of the enzyme inhibition level.

Table 3: α- amylase inhibitory activity of the plant extracts

Crude extract	IC_{50} value (mg/L)
AVM	1015.98 ± 0.02^{a}
BRM	743.43 ± 0.09^{b}
POM	585.05 ± 0.02^{c}

Values are expressed as mean ± S.D. Values with different superscript (a, b and c) down the column are significantly different ($P < 0.05$)

Lipase enzyme inhibitory assay

Percentage inhibition by Orlistat (1 mg/mL) used as the positive control is 98.8%. The IC_{50} value of lipase enzyme inhibitory activity of the AVE is 332 mg/L while BRM had an IC_{50} value of 905.35 mg/L. The IC_{50} values of all other plant extracts could not be calculated due to low inhibitory activity(Table 4).

The ANOVA results indicated that there were significant differences ($P \leq 0.05$) in the activity when comparing solvents in all four plants: AV, BR, MC, and PO. There was no significant difference ($P \geq 0.05$) in activity with increasing concentrations of extracts from AV, BR, MC, and PO due to low activity.

Discussion

This study examined the bioactivities and enzyme inhibition of, AV, BR, MC and PO. The crude extract yields were generally above 1%, except for the hexane extract of AV. Since bioassays required relatively small sample amounts, the low yield was not considered a significant issue.

MeOH extract of *Plumeria obtusa* flower indicates the presence of antifungal compounds. Previous studies have shown that the n-butanol fraction of *Alysicarpus vaginalis* (AV) exhibits strong activity against *Staphylococcus aureus* and *Bacillus subtilis*, while the acetone fraction is effective against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and fungi including *Aspergillus flavus*, *Aspergillus niger*, and *Candida albicans* (Tapadiya et al., 2017b). However, antifungal activity of AV against *Cladosporium cladosporioides* has not yet been reported. The acetone leaf extract of *Biophytum sensitivum*, a species in the same genus as *B. reinwardtii*, has been reported to exhibit significant antifungal activity, effectively inhibiting *Aspergillus fumigatus*, *Aspergillus niger*, *Cryptococcus neoformans*, and *Nocardia* sp. using the disc diffusion method (Vijayan et al., 2010b). Nevertheless, the antifungal potential of *B. reinwardtii* has not yet been explored. Antifungal activity of MC has not been reported. Although the antibacterial properties of PO have been reported, its antifungal activity against specific species has not yet been investigated.

Kongolo Kalemba et al., (2024), classifies extracts based on IC_{50} in DPPH/ABTS assays as IC_{50} < 10 μg/mL → *extremely strong,* 10–50 μg/mL → *strong,* 50–100 μg/mL → *moderate and* 250 μg/mL → *inactive/weak.* In the present study, all extracts of AV showed approximately similar radical scavenging activity values, with IC_{50} values ranging from 493–665 mg/L, indicating weak antioxidant activity. Previous studies, however, reported stronger activity, where MeOH and EtOAc extracts showed IC_{50} values of 34.70 mg/L and 12.49 mg/L, respectively (Shomudro et al., 2023) and methanol, chloroform, petroleum ether, and aqueous extracts showed IC_{50} values of 144.92, 243.64, 475.36, and 371.17 μg/mL, respectively (Arya & Mehta, 2017). These variations may be due to environmental factors, soil conditions, or differences in extraction methods such as solvent polarity, temperature, and time. For BR, the MeOH extract showed the strongest antioxidant activity with an IC_{50} value of 43.71 mg/L, while EtOAc and hexane extracts had IC_{50} values of 169.72 mg/L and 500 mg/L, respectively, indicating weak activity. Antioxidant activity of *Mikania micrantha* has been reported in earlier studies, but no data are available for MC. For PO, the EtOAc and MeOH extracts showed IC_{50} values of 832.67 and 1304.16 mg/L, indicating weak activity, while the hexane extract showed no antioxidant activity (IC_{50} > 500,000 mg/L). Previous reports on related genus, such as *Plumeria rubra*, confirm similar trends, with MeOH extract showing the highest activity (IC_{50} = 59.39 μg/mL) (Mohamed Isa et al., 2018).

According to (Moshi et al., 2010), brine shrimp lethality is classified as follows: LC_{50} < 1.0 μg/mL = highly toxic; 1.0–10.0 μg/mL = toxic; 10.0–30.0 μg/mL = moderately toxic; 30–100 μg/mL = non-toxic. The hexane, EtOAc, and MeOH extracts of AV with LC_{50} values above 100 μg/mL are considered non-toxic, while atropine is moderately toxic. Kurian & Josekumar (2016) reported LC_{50} values of 900.05 μg/mL and 754.35 μg/mL for the hexane and EtOAc extracts of AV, and no lethality up to 1000 μg/mL for the methanolic extract. However, Shomudro et al. (2023) found much lower LC_{50} values for the EtOAc and MeOH extracts (3.89 μg/mL and 8.71 μg/mL), indicating toxicity. These differences may be due to variations in experimental design, methods, or natural differences among plant populations. For BR, the hexane, EtOAc, and MeOH extracts had LC_{50} values above 100 μg/mL, suggesting low toxicity (Moshi et al., 2010). In contrast, Sreeshma S L & Nair (2014) reported IC_{50} values below 10 μg/mL for root, stem, leaf, and flower extracts, indicating toxicity. Such differences may occur because cytotoxic compounds in crude extracts may be present in low concentrations, inactive forms, or require specific combinations to be effective. Extraction methods and interfering compounds may also influence activity. For AV , Moshi et al. (2010), reported the hexane extract (LC_{50} 1.538 mg/mL) as toxic, while EtOAc and MeOH extracts were not toxic. Other studies on MC also showed varying LC_{50} values depending on extraction solvents (Khatun et al., 2021); (Sekendar Ali et al., 2011), with inconsistencies possibly caused by contamination or solvent impurities. For *Plumeria obtusa* (PO), LC_{50} values above 100 μg/mL (Moshi et al., 2010), indicate low toxicity, though Handayani & Chasani (2012)LC_{50} values vary with the solvents used. Some crude extracts did not dissolve well in water, and 5% DMSO was not used as a solvent because it caused 100% lethality in controls. This may have reduced the actual concentrations of the samples and affected results.

Finding seeds of *L. sativa* which meets the conditions of the protocol phytotoxic activity assay was challenging, since the seed germination may not exceed 90% or the seeds do not produce similar root or shoot lengths within the given period. Because they are readily available and produce results quickly, lettuce seeds are preferred over other types of seeds for the phytotoxicity assay. The LD_{50} values were 498.7 mg/L and 373.9 mg/L respectively. These observations are in accordance with some authors that states; the inhibition of root length treated by *Mikania*

micrantha was stronger than the shoot length, which was observed only when the concentration of *M. micrantha* extracts exceeded 200 mg/L (Ma et al., 2020). Although phytotoxicity was reported earlier in genus *Mikania,* there is no evidence of phytotoxicity in this particular species; MC*,* and this is the first time to report this. According to the results, POE has a slight phytotoxicity against the elongation of roots of lettuce seeds.

All other crude extracts did not show any considerable phytotoxicity against the elongation of shoots and roots of lettuce seeds. Phytotoxicity of POM is previously recorded and no other evidence is found about other extracts in this study. Many *in vitro* methods are available to screen plants for antidiabetic activity (Soumyanath, 2019).

MeOH extracts of AV, BR, and PO showed α- amylase enzyme inhibitory activity where IC_{50} values are 582, 743.4 mg/L and 1015.9 mg/L respectively. In Indian Ayurvedic medicine AV is renowned for its antidiabetic property. The mature leaves of BR and PO are given in the treatment for diabetes (Varadarajan et al., 2017;Wong et al., 2011b). Based on our findings, inhibition of the α-amylase enzyme might contribute to the antidiabetic properties of these plant extracts.

AVE (IC_{50} 332 mg L^{-1}) and BRE (905.4 mg L^{-1}) exhibited lipase enzyme inhibition compared to the other crude extracts. However, the crude extracts were only soluble in 5% DMSO. Therefore, the suspended particles may have interfered with the studies based on absorbance such as DPPH radical scavenging antioxidant assay, lipase enzyme inhibitory assay and α-amylase inhibitory assay. This limitation should be addressed in future studies by comparing the activity in a suitable solvent system. Furthermore, the chemicals responsible for each bioactivity were not isolated in this study. Therefore, further studies are warranted to delineate the compounds responsible for the observed bioactivities.

Conclusion

Extracts of both AV and BR showed α- amylase enzyme inhibitory activity, lipase enzyme inhibition and higher antioxidant activity. MC leaves showed a lethality against brine shrimp, inhibitory properties against root and shoot elongation of lettuce seeds and antioxidant activity. PO showed α- amylase enzyme inhibitory activity, lethality against brine shrimp, inhibition against CC and higher antioxidant activity. This study revealed that all four plants contain compounds with bioactive properties. Therefore, further studies are recommended to isolate the bioactive compounds.

Conflict of interest statement

The authors declare no conflict of interest.

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