International Annals of Science
ISSN: 2456-7132
Volume 14, Issue 1, pp. 10-20, 2024
DOI: https://doi.org/10.21467/ias.14.1.10-20

RESEARCH ARTICLE

The Herbicidal Potential of Euphorbia Species for Managing Common Weeds in the Mount Cameroon Region: A Promising Alternative to Synthetic Herbicides

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Received: 25 March 2024 / Revised: 08 September 2024 / Accepted: 13 November 2024 / Published: 07 December 2024

ABSTRACT

The utilization of botanicals in weed management is of great significance due to their biodegradability and eco-friendly nature. This research evaluates the herbicidal potentials of seven Euphorbia species (*Euphorbia hirta, Euphorbia maculata, Euphorbia heterophylla, Euphorbia prostrata, Euphorbia pulcherrima, Euphorbia trigona, and Euphorbia milii*) in the Mount Cameroon Region.The assessment of the herbicidal potential of the most promising Euphorbia species was conducted using the Sandwich method along with germination bioassay techniques. Qualitative phytochemical screening techniques were employed to investigate the herbicidal properties of *E. heterophylla* through the identification of classes of secondary metabolites. Additional analysis of Faculty of Agriculture and Veterinary Medicine, University of Buea, Cameroon, P.O Box 63, Cameroon. The crude extracts from *Euphorbia heterophylla* regarding the germination and development of five weed species revealed inhibiting effects on all the weeds examined. The results showed that *Euphorbia heterophylla* exhibited inhibitory effects on *Lactuca sativa* seeds, with a maximum inhibition of 85.75% at higher concentrations, indicating dose-dependent trends. *Bidens pilosa* had the highest herbicidal suppression rate of 83.91%, while *Mimosa invisa* exhibited the least suppression at 61.10%. Thirteen (13) chromatographic fractions of *Euphorbia heterophylla* were tested for their influence on the germination and growth of *Bidens pilosa* seeds. The findings showed that fractions F4, F6, F7, and F13 demonstrated total inhibition of the weed (100% suppression). Phytochemical analysis of *E. heterophylla* identified the presence of flavonoids, phenolic compounds, alkaloids, tannins, steroids, saponins, and cardiac glycosides. *Euphorbia heterophylla,* had a strong allelopathic potential, making it viable candidate for the isolation and development of bioactive compounds. These compounds could be utilized as bioherbicides and thereby contributing to sustainable weed management in crop production.

Keywords: Bioherbicide, Botanicals, *Euphorbia* species.

1 Introduction

For the past 20 years, agriculture in both developed and developing nations has primarily relied on synthetic herbicides, such as glyphosphates, which has caused the emergence glyphosate-resistant weeds [1]. Chemical herbicides have been shown to be an effective method of controlling weeds for many years [2], [3]. However, there is a growing concern regarding the development of non-target site mechanisms of resistance to herbicides [4], [5]. It has been reported that there are about 530 unique cases of herbicide resistant weeds globally and this occurred in 272 species [6]. Weeds have developed resistance to 168 distinct herbicides and 21 of the known sites of action of herbicides [6]. [7], identified nine weed species in the Southwest Region of Cameroon that are resistant to pesticides often used in banana plantations. There is an urgent need for novel systems with alternative modes of action to assist combat the emergence of herbicide resistance [8]. For future global food security and sustainability, controlling these weeds will be extremely difficult [9]. Severe weed infestations have been linked worldwide to enormous agricultural losses [10], [11]. Herbicidal qualities of several plant groupings, such as lichens, algae, annuals, and perennial weeds, are well documented [12]. Scientists are starting to investigate the use of allelopathic plants or their allelochemicals as an occasional alternative for weed control, as opposed to exclusively depending on synthetic herbicides. This has caused the development of herbicides based on natural products derived from allelopathic plants to get more attention [13]. There is an increasing need to minimize synthetic herbicide use by incorporating bio-herbicides in order to promote environmental sustainability and decrease reliance on them. Of the various approaches available, allelopathy has surfaced as a sustainable and holistic strategy, employing methods like crop rotation, cover cropping, intercropping, and the application of powders or extracts obtained from natural sources to efficiently manage weed control. A well-known paradigm in crop protection is using the bioactive potential of medicinal plants to mitigate the 20–30% yield loss brought on by weeds. Since plant-derived compounds are less harmful to humans, easily biodegradable, cost-effective, and target-specific, they provide an environmentally friendly weed control alternative to synthetic herbicides [14].

There are over 2000 species in the genus Euphorbia, which are widely found in both tropical and temperate regions [15]. These plants are easily recognized by their distinctive flower head (cynthum) and the toxic milky latex they release when harmed. There are 28 species of Euphorbia known from the Cameroon Guineo-Congolian rainforest, nine of which are listed on the Mount Cameroon Region's checklist [16]. There have been reports of chemical interactions between Euphorbia and other plant species resulting in the inhibition of their growth. Plant cells' form and structure are influenced by bioactive substances also known as allelochemicals [11]. These compounds interact with recipient plants and create a negative effect on their growth and development [11]. Aqueous extracts from a variety of temperate Euphorbia species have been shown in numerous studies to inhibit the growth of specific plants. Furthermore, the root extract of *Euphorbia heterophylla* has been shown to greatly hinder the germination and seedling development of *Sorghum bicolor* and *Lactuca sativa* [17], [18]. Comparable results have been noted regarding the suppression of germination and seedling growth in *Amaranthus spinosus*, *Zea mays*, *Bromus tectorum*, *Bidens pilosa, Melilotus indica*, and *Triticum aestivum* when treated with the aqueous extract of *Euphorbia hypericifolia* [19].This research focuses on the need for the discovery of new weed management technologies to overcome the limitations of synthetic herbicides, and it serves as a valuable foundation for the development of bioherbicides.The study therefore documents the herbicidal potentials of plants of *Euphorbia* species within the Mount Cameroon Region.

2 Material and Methods

2.1 Assessment of some *Euphorbia* species for allelopathic potentials using the Sandwich method

The study was conducted in the Biological Science laboratory at the Faculty of Science, University of Buea.The leaves of seven *Euphorbia* species, *Euphorbia hirta*, *Euphorbia maculata*, *Euphorbia heterophylla*, *Euphorbia prostrata, Euphorbia pulcherrima, Euphorbia trigona* and *Euphorbia milii* were collected from different localities of the Mount Cameroon Region at almost the same altitudes. The above-ground sections of the plant species were placed in labeled polyethylene bags and transported to the Life Sciences Laboratory at the University of Buea. The plant samples were rinsed with running tap water to eliminate any debris. Afterwards, the plants were placed in envelopes and dried in an oven at 60 °C for 48 hours. Agar powder (Nacalai Tesque Kyoto, Japan), which gels at a temperature of 30-31 °C, was utilized as the medium. For the sandwich technique, a 0.75% (w/v) agar solution was prepared.The solution was autoclaved at 115 ºC for 15 min and cooled to 45 ºC in a water bath. A six-well plate (10 cm^2 area per each dish) (Nalge Nunc Int,l., Denmark) was used for each of the seven *Euphorbia* species. 10 mg of dried leaves of the *Euphorbia* species were placed in three wells, while the other three wells had 50 mg of dried leaves of the various *Euphorbia* species. Two aliquots of 5 ml agar were added to each well to form two layers (the second layer acted as a buffer zone between the sample and the lettuce seeds) on the dried leaves. Five equal spaced lettuce seeds (*Lactuca sativa* L) were placed on top of the agar in each well and each plate was sealed with plastic tape. Control wells were filled with agar only and plated with lettuce seeds to serve as the control. The plates were incubated in a dark Cupboard for 3 days at 25 ^{0}C. The root and shoot lengths of germinated lettuce seeds were measured for each species. The percentage of inhibition in the root and shoot lengths of the seedlings treated with leachates was assessed by comparing them to the control seedlings, as detailed by the [20] as shown in equation (1).

Inhibition percentage = [1-(treatment/control)] x 100………………….. (1)

2.2 Extraction of *Euphorbia heterophylla* leaves and preparation of crude extract

In terms of inhibitory activity on the growth of the root and shoot of Lettuce seeds, *Euphorbia heterophylla* was the most potent plant amongst the seven species of *Euphorbia* screened. Fresh leaves of *Euphorbia heterophylla* were collected and air dried in the University of Buea, Life Sciences laboratory. The dried leaves were pulverized using a mill equipped with a 2 mm sieve to produce a fine powder.One kg of the powdered *Euphorbia heterophylla* leaf was macerated three times in a 1:1 methanol and methylene chloride (5000 mL of methanol and 5000 mL of methylene chloride). The mixture was filtered through Whatman filter paper No. 1, and the resulting filtrate was concentrated under reduced pressure using rotary evaporation (BUCHI Rotavapor R-200, Switzerland) at a temperature range of 73-81°C. Residual solvents were removed by air drying at room temperature (23 – 25^{o}C). The final residue yielded 200 g of crude extract of *Euphorbia heterophylla.* 30g of the crude extract was used in the germination bioassay and the rest stored at 4^{o}C for further use.

2.3 Germination bioassay using crude extract of *Euphorbia heterophylla*

Euphorbia heterophylla was further assayed (Germination test) on some weed species reported to be dominant in agricultural production systems in the Southwest Region of Cameroon [21]. Seeds of *Bidens pilosa*, *Chromolaena odorata*, *Amaranthus spinosus*, *Ageratum coniziodes* and *Mimosa invisa* were collected in their natural habitat in Buea when the seeds reached maturity. Seeds removed from the seed heads were separated from unwanted appendages by winnowing and kept in seed bags at ambient temperature. Preliminary germination tests using the blotter method were performed on the acceptor species to ascertain the viability of these weed seeds.

For the germination assay, 300 mL of stock solutions was prepared by dissolving 30 g crude extract of *Euphorbia heterophylla* using distilled water. Five treatments were prepared from the crude extract: T1 as the control (0%), T2 at 25%, T3 at 50%, T4 at 75%, and T5 at 100%. [22]-[24]. Weeds seeds were disinfected with hydrogen peroxide (H_2O_2) for five minutes and raised with sterile distilled water. Five (5) mL of the extract of *Euphorbia heterophylla* was poured with the help of a 10 ml syringe into 75 petri dishes underlain with Whatman filter paper No.1. Fifteen (15) petri dishes were lined with Whatman filter paper, and 10 mL of distilled water was added to each, serving as the control.The Petri dishes were plated with 10 weed seeds from each species per treatment. Three replicates were used for each of the five treatments. The petri dishes were then placed in an incubator at room temperature (25 ^{0}C) for 21 days. Germination was considered to have taken place when the radicle extended beyond the seed coat by at least 1 mm. The lengths of the roots and shoots of all the germinated weed seeds were measured in centimeters using a printable graph paper with 10 squares.The inhibitory percentage of roots and shoots lengths of all the seedlings in petri dishes were calculated as described by [20].

2.4 Column chromatography (CC) of *Euphorbia heterophylla*

Further experiments were conducted to narrow down to the active fractions responsible for the inhibitory property of *Euphorbia heterophylla* using Column and Thin Layer Chromatographs. 90g crude extract was fractionated using column chromatography (CC) on a silica gel and eluded with gradients of ethyl acetate (EtoAc) in hexane of increasing polarity. 90g of silica gel was weighed and dissolved in fresh hexane (in a beaker) while stirring vigorously. The mixture was poured into the column while tapping the sides of the column with both hands (the tap was left open). When the solvent head was about 2cm from the silica gel, the material consisting of 90g of silica gel and 90g of crude extract of *Euphorbia heterophylla* was poured into the column through the filter paper. Cotton wool was inserted into the column using a spatula while ensuring that the cotton wool was in contact with the silica gel. Fresh hexane was added (including recovered hexane used to wash the column) to the column. 200 ml fractions were collected at 5% increasing polarity and evaporated in a rota-vapour (73-81 ^{0}C). This gave a total of 201 fractions collected as shown in Table 1. The 201 subfractions collected were regrouped on the basis of TLC analysis to major factions. Five ml of Methylene chloride (mobile phase) was added to the fractions using a pipette, aliquots spotted on TLC plates (stationary phase) so that a migration was visible. Grouping of fractions was done based on observation using UV lamp. Active and fluorescent compounds were group together into 13 fractions (F_1-F_{13}) as shown in Table 1.

Table 1: *Chromatographic profile of crude extract of Euphorbia heterophylla collected from the Mount Cameroon Region*

Eluent system HEX: EA	Fractions	Combined fractions	New fractions/weight (g)	Information from TLC profile (without or under UV and or/exposed to iodine)
100: 0	1-11	2	F1, 0.18	Pure white solid
		3-6	F2, 2.17	Mixture of compound with mostly non polar compounds
		7-12	F3, 1.91	Mixture of compounds with mostly non polar and slightly polar compounds
95: 5	12-22	13-21	F4, 6.79	Mixture of compounds with different polarity
90: 10	23-33	22-29	F5, 9.416	
85: 15	34-44	30-78	F6, 1.16	
80: 20	45-55			
75: 25	56-66			
70: 30	67-77			
65: 35	78-88			
60: 40	89-99	79-91	F7, 3.67	
55: 45	100-111	92-101	F8, 1.148	
50: 50	112-122	102-115	F9, 1.318	
45: 55	123-133	116-145	F10, 4,21	Mixture of compounds with mostly polar compounds
40: 60	134-144			
35: 65	145-155	146-189	F11, 0.31	
30: 70	156-166			
25: 75	167-177			
20: 80	178-188			
50% CH_2Cl_2: 50% MeOH	189-198	190-194	F12, 22.68	
100% MeOH	199-201	195-201	F13, 2.068	

HEX (Hexane), EA (Ethylacetate), MEOH (Methanol), CC (Column chromatography), TLC (Thin layer chromatography

2.5 Germination bioassay of *Euphorbia heterophylla* fractions on *Bidens pilosa*

Only *Bidens pilosa* out of five weeds in the previous germination bioassay was used for the germination bioassay of fractions. Though there was a significant effect of *Euphorbia heterophylla* extracts on the other weeds seed, *Bidens pilosa* growth was highly inhibited by the extract at all four concentrations reason why it was chosen for the germination bioassay of fractions. The 13 fractions of *Euphorbia heterophylla* were assayed against *Bidens pilosa*. The same procedure as described in the case of germination bioassay using crude extract was exploited. 10 ml solutions were prepared by diluting a stock solution of dried fractions of F1-F13 made of 1 ml methanol plus distilled water. In each treatment, four petri dishes were fitted with Whatman filter paper No.1 and plated with 10 seeds of *Biden Pilosa*. Four treatments T1, control (0 %), T2 (100 %), T3 (50 %), and T4 (25 %) were made from dried fractions of F1-F13 following [22]-[24] with some modifications and wetted with 5mL of solutions diluted from the stock (T1, 5ml of distilled water, T2, 5mL of stock, T3, 2.5mL of stock and 2.5mL of distilled water and T4, 0.5ml stock and 4.5mL of distilled water.The petri dishes were placed in a dark cabinet at room temperature for a duration of 21 days. Germination was considered to have occurred when the radicle extended beyond the seed coat by a minimum of 1 mm.

2.6 Preparation of crude extracts of *Euphorbia heterophylla* for Qualitative phytochemical screening

After the evaluation of the herbicidal properties of the eight *Euphorbia* species, *Euphorbia heterophylla* was shortlisted and screened for its phytoconstituents. Leaf material of *Euphorbia heterophylla* was thoroughly washed several times with tap water to remove dust and air dried for five minutes to drain the water. The leaf was placed in an envelope and oven dried at 60 ^{0}C for 72 hours. The dried materials were crushed using a hand mill and then sifted through a 2 mm sieve to obtain a fine powder. Three hundred grams (300g) of the powdered leaves of *Euphorbia heterophylla* were sequentially soaked in three liters of hexane, chloroform, acetone, and methanol, respectively, for two days with each solvent. The mixture was filtered through Whatman filter paper No. 1, and the resulting filtrate was concentrated under reduced pressure using a rotary evaporator (BUCHI Rotavapor R-200, Switzerland) at temperatures between 45-60 °C for hexane. A minimal amount of acetone was utilized to reclaim the solvent. Any remaining solvent was eliminated by air drying at room temperature (23-25 °C), and the extract was weighed and kept at 4 °C for future use. A portion of the crude extract was set aside for phytochemical analyses.

2.7 Phytochemical Tests

Phytochemical analysis of the powdered dried leaves of Euphorbia heterophylla was conducted following extraction with four solvents (hexane, methanol, acetone, and chloroform) to identify secondary plant metabolites, including saponins, flavonoids, steroids, tannins, alkaloids, and cardiac glycosides, based on [25] with some modifications. To detect saponins, 2 mg of the crude extract was dissolved in 3 ml of hot distilled water in a test tube. The mixture was then vigorously shaken for one minute, and the presence of saponins was confirmed by the formation of persistent foam measuring approximately 1-2 cm. Flavonoids were identified by dissolving 2 mg of the crude extract in methanol, followed by the addition of 2 ml of concentrated hydrochloric acid. A spatula of magnesium turnings was included, and the mixture was observed for effervescence. The appearance of a brick-red color indicated the presence of flavonoids.

For steroid testing, 2 mg of the crude extract was dissolved in 0.5 mL of dichloromethane to create a dilute solution. Then, 0.5 mL of acetic anhydride was added along with three drops of concentrated sulfuric acid. The emergence of a blue-green ring at the interface indicated a positive reaction, confirming the presence of steroids. To test for tannins, 1 mg of the crude extract was dissolved in a tube containing 20 mL of boiling distilled water and boiled for one hour. Afterward, a few drops of ferric chloride were added and allowed to sit for color development. The appearance of a blue-black coloration indicated the presence of tannins. In detecting alkaloids, 2 mg of the crude extract was dissolved in dichloromethane and spotted onto a thin-layer chromatography plate, which was developed in 20% hexane in ethyl acetate. The presence of alkaloids in the developed chromatogram was identified by spraying with freshly prepared Dragendorf's reagent in a fume hood. A positive reaction was indicated by an orange or darker spot against a yellow background, confirming the presence of alkaloids in the plant extract.

Cardiac glycosides were detected by dissolving 2 mg of the crude extract in dichloromethane to create a dilute solution. Then, 2 mL of glacial acetic acid and one drop of ferric chloride were added, followed by 1 mL of concentrated sulfuric acid. The appearance of violet and brownish rings at the interface, along with a greenish ring in the acetic acid layer, indicated the presence of cardiac glycosides. Lastly, phenolics were identified by dissolving 2 mg of the crude extract in 1 mL of the plant extract, to which one drop of 5% $FeCl_3$ (w/v) was added. The formation of a greenish precipitate indicated the presence of phenolics.

2.8 Data analysis

For the sandwich method, the collected data were analyzed statistically by using the Independent Sample t-Test to compare the means of all the plants inhibition pattern of 10 and 50 mg for both roots and shoots of lettuce seeds. To investigate whether the concentration applied in the germination bioassay could influence the growth of roots and shoots of the examined weed seeds, an analysis of variance (ANOVA) was conducted using SPSS 22.0 (SPSS, Inc., USA) software. Significant mean values were distinguished using Duncan's Multiple Range Test (DMRT) at a significance level of $P < 0.05$.

3 Results

3.1 The Herbicidal potentials of some *Euphorbia* species on lettuce (*Lactuca sativa*) by sandwich method

Both root and shoot elongations of lettuce seedlings were affected by the leaf leachates of different donor plant species ranging from almost complete inhibition of root at higher leaf concentrations to stimulation of shoot growth at the lower dosages. The applied plant extracts inhibited the root length more than that of the shoot of the test plant, though they did not show significant difference between them. When the lettuce seedlings were grown with 10 mg and 50 mg dry leaves of selected *Euphorbia species*,the greatest inhibition of root growth was observed with the leachate from *Euphorbia heterophylla* at both 10 mg and 50 mg concentrations, showing -10.89% and 85.74% inhibition for roots, and -29.39% and 68.54% for shoots, respectively (Table 2).

Table 2. *Percentage inhibition on the germination of seeds of lettuce (Lactuca sativa) as influenced by leaves of eight Euphorbia species in the Mount Cameroon Region*

Plant species	Percentage root inhibition (%)		Percentage shoot inhibition (%)	
	Treatment (10 mg)	Treatment (50 mg)	Treatment (10 mg)	Treatment(50mg)
Euphorbia prostrata	-23.27	48.92	32.06	27.24
Euphorbia maculata	6.23	56.83	-16.41	33.41
Euphorbia hirta	-22.52	73.38	-34.95	49.46
Euphorbia trigona	-2.92	73.52	-36.03	37.89
Euphorbia milii	40.13	58.20	19.23	50.10
E. pulcherrima	23.36	75.26	-15.59	52.52
E. heterophylla	-10.89	85.74	-29.37	68.54
P<0.05	0.031		0.105	

The influence of leaf leachate from *Euphorbia heterophylla* and *Euphorbia pulcherrima* on the root length of lettuce seedlings demonstrated notable differences when compared to other species at $P < 0.05$.Percentage inhibition of the growth of lettuce seeds by *Euphorbia* species decreased in the order; *Euphorbia heterophylla* > *Euphorbia pulcherrima* > *Euphorbia hirta* > *Euphorbia trigona* > *Euphorbia milii* > *Euphorbia maculata* > *Euphorbia prostrata.* There was growth promotion for both roots and shoots at 10 mg leachate of *Euphorbia hirta* (-22.52 % and -34.95 %) respectively and *Euphorbia heterophylla* (-10.89 % and -29.37 %). The lowest activity was recorded for exudates released by the leaf of *Euphorbia prostrata* with the minimum suppression of -23 % of the root elongation (Table 2).

3.2 Evaluation of the herbicidal potentials of 1:1 methylene chloride/methanolic extract of *Euphorbia heterophylla* on early seedling growth of five weed species by germination bioassay technique.

The findings regarding the percentage of inhibition of the weed seeds at various extract concentrations of *Euphorbia heterophylla* revealed significant differences (P < 0.05) compared to the control, as presented in Table 3 and Table 4. Maximum inhibition was observed in *Bidens pilosa* root (83.91 %) and shoot (70.07 %) among weed species while minimum suppression of growth was observed in *Mimosa invisa* (61.0 %) root and shoot (64.92 %). The root length of almost all test species except for *Mimosa invisa* was more sensitive to the extracts at all concentration with potencies increasing with increased in extract concentration. The degree of percentage inhibition in different weeds species was in the order. *Bidens pilosa* > *Chromolaena odorata* > *Amaranthus spinosus* > *Ageratum coniziodes* > *Mimosa invisa* (Table 3 and Table 4)

Table 3. *The herbicidal effect of different concentrations of the crude extract of Euphorbia heterophylla on percentage inhibition of roots of different weeds.*

Treatments (Concentrations, %)	Percentage inhibition of roots of weed species				
	Bidens pilosa	*Mimosa invisa*	*Amaranthus spinosus*	*Chromolaena odorata*	*Ageratum coniziodes*
25	46.58[c]	33.29[c]	42.75[c]	49.34[c]	30.40[c]
50	59.35[cb]	40.45[cb]	52.43[cb]	54.74[cb]	41.55[cb]
75	74.76[ab]	52.84[ab]	60.28[ab]	64.53[ab]	64.85[ab]
100	83.91[a]	61.0[a]	68.37[a]	73.55[a]	70.43[a]

Values sharing the same letter within a column are not significantly different (P ≤ 0.05) according to the results obtained from Duncan's Multiple Range Test (DMRT).

Table 4: *The herbicidal effects of different concentrations of the crude extract from Euphorbia heterophylla on the percentage inhibition of shoots of different weed species.*

Treatments Concentrations, %)	Percentage inhibition of shoots of weed species				
	B. pilosa	*M. invisa*	*A. spinosus*	*C. odorata*	*A. coniziodes*
25	34.36[d]	27.59[c]	26.01[c]	16.84[c]	31.66[c]
50	42.37[cd]	40.07[bc]	49.38[bc]	43.48[b]	43.38[b]
75	63.85[ab]	60.08[a]	60.16[ab]	68.94[a]	59.72[ab]
100	70.07[a]	64.92[a]	74.85[a]	69.17[a]	65.91a

Values sharing the same letter within a column are not significantly different (P ≤ 0.05) according to the results obtained from Duncan's Multiple Range Test (DMRT).

3.3 Herbicidal effect of chromatographic fractions of *Euphorbia heterophylla* on percentage inhibition of roots and shoots of *Bidens pilosa*

The bioassay guided fractions of methanol/methylene extract of *Euphorbia heterophylla* has strong herbicidal property against the root and shoot of *Bidens pilosa* (83.91 % and 70.07 % respectively). The highest herbicidal suppression of *Bidens pilosa* root and shoot lengths was caused by fractions F4, F6, F7 and F13 with complete inhibitions of 100 % (Table 5 and Table 6). The lowest inhibition percentage of root length was recorded for fraction F1 (63.71 %) while Fraction F2 recorded the lowest shoot length of 42.88 %.

Table 5: *The herbicidal effect of different concentrations of chromatographic fractions of Euphorbia heterophylla on percentage inhibition of roots of Bidens pilosa in relations to weed*

Fractions (F) of *E. heterophylla*	Percentage inhibition of roots of *Bidens pilosa* in various treatments		
	0.5 %	2.5 %	5 %
F1	63.71[b]	85.06[a]	92.22[a]
F2	71.16[b]	82.84[a]	92.22[a]
F3	82.07[a]	84.64[a]	93.12[a]
F4	100.00[a]	100.00[a]	100.00[a]
F5	90.29[a]	95.58[a]	95.62[a]
F6	100.00[a]	100.00[a]	100.00[a]
F7	100.00[a]	100.00[a]	100.00[a]
F8	86.24[a]	95.05[a]	100.00[a]
F9	91.24[a]	94.47[a]	100.00[a]
F10	88.64[a]	92.57[a]	99.99[a]
F11	96.667[a]	97.73[a]	100.00[a]
F12	95.62[a]	96.61[a]	96.61[a]
F13	100.00[a]	100.00[a]	100.00[a]

Values sharing the same letter within a column are not significantly different ($P \leq 0.05$) according to the results obtained from Duncan's Multiple Range Test (DMRT).

F1 (combination 2 of fractions of E. heterophylla), F2 (combinations 3-6 fractions of E. heterophylla), F3 (combinations 7-12 of fractions of E. heterophylla) F4 (combinations 13-21 of fractions of E. heterophylla) F5 (combinations 22-29 fractions of E. heterophylla), F6 (combination 30-78 fractions of E. heterophylla), F7 (combination 79-91 fractions of E. heterophylla) F8 (combination 92-101 fractions of E. heterophylla), F9 (combination 102-115 fractions of E. heterophylla), F10 (combination 116-145 fractions of E. heterophylla), F11 (combination 146-189 fractions of E. heterophylla), F12 (combination 190-198 fractions of E. heterophylla) and F13 (combination 199-201 fractions of E. heterophylla).

Table 6: *The herbicidal effect of different concentrations of fractions of Euphorbia heterophylla on percentage inhibition of shoot of Bidens pilosa*

Fractions	percentage inhibition of shoots of *Bidens pilosa in various treatments*		
	0.5%	2.5%	5%
F1	62.76[b]	81.77[a]	87.94[a]
F2	42.88[c]	77.57[b]	86.97[a]
F3	79.23[ba]	92.71[a]	100.00[a]
F4	100.00[a]	100.00[a]	100.00[a]
F5	88.94[a]	91.58[a]	93.54[a]
F6	91.44[a]	100.00[a]	100.00[a]
F7	100.00[a]	100.00[a]	100.00[a]
F8	75.56[b]	87.93[a]	100.00[a]
F9	88.06[a]	89.86[a]	100.00[a]
F10	80.62[a]	86.31[a]	96.02[a]
F11	92.64[a]	94.85[a]	100.00[a]
F12	94.22[a]	95.93[a]	100.00[a]
F'13	100.00[a]	100.00[a]	100.00[a]

Values sharing the same letter within a column are not significantly different ($P \leq 0.05$) according to the results obtained from Duncan's Multiple Range Test (DMRT).

F1 (combination 2 of fractions of E. heterophylla), F2 (combinations 3-6 fractions of E. heterophylla), F3 (combinations 7-12 of fractions of E. heterophylla) F4 (combinations 13-21 of fractions of E. heterophylla) F5 (combinations 22-29 fractions of E. heterophylla), F6 (combination 30-78 fractions of E. heterophylla), F7 (combination 79-91 fractions of E. heterophylla) F8 (combination 92-101 fractions of E. heterophylla), F9 (combination 102-115 fractions of E. heterophylla), F10 (combination 116-145 fractions of E. heterophylla), F11 (combination 146-189 fractions of E. heterophylla), F12 (combination 190-198 fractions of E. heterophylla) and F13 (combination 199-201 fractions of E. heterophylla).

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3.4 Phytochemical Constituents of *Euphorbia hetrophylla*

The phytochemicals including steroids, phenolics, alkaloids, cardiac glycosides, flavonoids, tannins, and saponins were found in varying amounts in *Euphorbia heterophylla*, as illustrated in Table 7. All the extracts of the different solvents, except chloroform and acetone showed positive results for the presence of saponins.

Table 7: *Phytochemical screening of Euphorbia heterophylla in the Mount Cameroon Region*

Solvent	Phytochemical constituents						
	Steroid	Flavonoid	Alkaloid	Cardiac glycosides	Phenolic	Tannins	Saponins
Methanol	+	++	+++	+++	++	+	++
Acetone	++	++	++	++	+	+++	-
Chloroform	+++	+	+	+++	++	-	-
Hexane	++	++	+	+	+	++	+

Legend: (+) present, (-) absent, (++) moderate concentration, (+++) High concentration

4 Discussion

The sandwich method utilizes Euphorbia species to inhibit root and shoot growth in *Lactuca sativa* seedlings. Leaf leachate dry weight affects growth, with higher amounts of 50mg inhibiting root and shoot, while lower amounts of 10mg stimulate shoot growth. The high herbicidal suppressive attribute of *Euphorbia heterophylla* on the root and shoot lengths of lettuce seeds was dose dependent with hormesis/attraction observed at low dosage and inhibition/repellent exhibited at high concentrations. Similar results were obtained by [26, 27]. Hormesis is a unique aspect of allelopathy, which is the biochemical interaction between different plants through the release of chemicals called allelochemicals that promote growth. The donor plants inhibited the root length more than that of the shoot at either 10 mg or 50 mg of dried leaves, which is in line with the finding of [24]. The observed inhibition could be attributed to the allelochemicals present in the examined plants, which are compounds that can affect the growth and development of neighboring plants by suppressing their germination or growth processes. However, the specific effects can vary depending on plant species, concentration, composition of allelochemicals, and environmental factors.

From the study, it can be concluded that *Euphorbia heterophylla* showed the highest allelopathic effect on the tested weeds. The extracts of *Euphorbia heterophylla* exhibited herbicidal potential on seed germination and seedling growth of some selected weeds in the bioassays indicating that extracts contained some inhibitory and stimulatory compounds that could prevent promote germination and reduced seedling growth. This aligns with the results of [28], which demonstrated that extracts from the leaves of the *Annona muricata* plant both inhibited and promoted the growth and development of the tested seedlings, depending on the concentration used. Growth inhibitions were dose dependent with higher dosage accounting for significant reduction in root and shoot lengths of all weed species. It was observed that the bioassay guided methanol/methylene chloride chromatographic fractions of *Euphorbia heterophylla* have strong herbicidal property against *Bidens pilosa.* The inhibition or stimulation of seedlings growth was mainly due to the presence of allelochemicals in different amounts. The allelochemical responsible for differential allelopathic behaviour of *Euphorbia heteropylla* under this study might be phenolics, tannins, alkaloids, saponins, cardiac glycosides and sterols as confirmed by phytochemical analysis of the presence of these metabolites in Euphorbia heterophylla.

Data from the phytochemical screening of leaves of *Euphorbia heterophylla* was analysed qualitatively. The Secondary metabolites steroids, flavonoids, phenolic, cardiac glycosides, tannins and saponins were present in different intensities. This result is in agreement with [29], who reported alkaloids, flavonoids, saponins, and tannins in *Euphorbia heterophylla* using a single solvent as extractant. These results implies that the identified secondary metabolites maybe responsible for the herbicidal effect observed on *Lactuca sativa* seeds and weed seeds of *Bidens pilosa, Chromolaena odorata, Amaranthus spinosus, Ageratum coniziodes,* and *Mimosa invisa.*

5 Conclusion

The study found that *Euphorbia heterophylla*, among the Euphorbia species in the Mount Cameroon Region, exhibited growth stimulatory effects at lower concentrations and the strongest weed killer potency as evidenced by the activities of fractions F4, F6, F7 and F13. This implies that this plant species has the potential to be further explored and utilized as an environmentally friendly bioherbicide for weed management. The stimulatory aspect of the allelopathy of *Euphorbia heterophylla*, could be exploited to enhanced crop productivity, reduced reliance on synthetic inputs, improved weed suppression, biodiversity promotion, and potential for allelopathic crop cultivars. This offers opportunities for sustainable agriculture, reduced chemical inputs through its use as biostimulant and as green manure, and enhanced biodiversity. The phytochemical screening identified six classes of secondary metabolites in *Euphorbia heterophylla*, including saponins, phenols, tannins, cardiac glycosides, flavonoids, or steroids. The presence of these metabolites suggests that they may contribute to the herbicidal properties of *Euphorbia heterophylla*. The extraction and characterization of these secondary metabolites from *Euphorbia heterophylla* may provide a foundational basis for the formulation of novel natural herbicides. Overall, these findings highlight the potential of *Euphorbia heterophylla* as a source of biostimulants and bioherbicides and the importance of studying and harnessing the herbicidal properties of specific plant species as sustainable alternatives for weed management in agricultural practices.

6 Declarations

6.1 Acknowledgments

The authors are grateful to the University of Buea for providing facilities used for this project.

6.2 Competing Interests

The authors declare that there is no conflict of interest.

6.3 Publisher's Note

AIJR remains neutral with regard to jurisdictional claims in published institutional affiliations.

How to Cite this Article:

Egbe *et al.*, "The Herbicidal Potential of Euphorbia Species for Managing Common Weeds in the Mount Cameroon Region: A Promising Alternative to Synthetic Herbicides ", *Int. Ann. Sci.*, vol. 14, no. 1, pp. 10–20, Dec. 2024. https://doi.org/10.21467/ias.14.1.10-20

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