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Dehydrocostus lactone: A natural herbicide against the Invasive Weed *Parthenium hysterophorus*

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ABSTRACT: *Parthenium hysterophorus*, commonly known as Santa-Maria weed, is a notorious invasive species globally. It is toxic to crop germination and growth, leading to a nearly 25% reduction in crop yields in the least developed countries. This weed has significant socioeconomic impacts on human health, soil fertility, biodiversity, and agricultural productivity in Ethiopia. Currently, synthetic chemicals are widely employed to manage this weed. However, their frequent use poses numerous risks to human health and the environment. Herbicides like Roundup® are non-selective and affect all plant types. Conversely, allelopathic plants and their derivatives are emerging as promising sources for natural herbicide development. In this study, the root of *Echinops kebericho* was selected for its rich history of diverse biological activities against Santa-Maria weed. The crude ethanol extract of *E. kebericho* root completely inhibited the seed germination of Santa-Maria weed at a concentration of 0.05 mg/ml *in vitro*. Following a bioassay-guided protocol, the crude ethanol extract was further partitioned using hexane, $CHCl_3$, and MeOH, and screened against the weed. The hexane and $CHCl_3$ extracts exhibited 100% inhibition of Santa-Maria weed seed germination. Dehydrocostus lactone, isolated as an active compound from the nonpolar fractions, achieved 100% inhibition of the weed's germination and growth.

Keywords: Dehydrocostus lactone, *Parthenium hysterophorus*, phytotoxicity.

INTRODUCTION

Ethiopia's economy is predominantly driven by agriculture, which accounts for 40% of the nation's GDP (Asresie, 2015). Despite crops being the primary products, productivity has been declining over time due to parasitic weeds, insect pests, and diseases. Yield loss assessments indicate a 25% annual reduction due to weeds in the least developed countries (Gharde and Singh 2018).

Santa-Maria (*Parthenium hysterophorus* L.) is a highly destructive and aggressive annual herbaceous weed known for its allelopathic effects on other plants (Tamado *et al*., 2002). It spreads rapidly, infiltrating various habitats, and has a robust capability for reproduction and dispersal (Abdulkerim-Ute & Legesse 2016). This weed now covers extensive areas of eastern, central, and northern Ethiopia (Tamado & Milberg 2000). Santa-Maria weed significantly impacts crop yield, human health, and animal health (Gnanavel, 2013; Roy & Shaik 2013). Consequently, it has numerous socio-economic impacts on human life, competing with and suppressing crop growth due to its allelopathic nature (Niguse and Belachew 2016).

Previous phytochemical studies reveal that Santa-Maria weed is rich in sesquiterpene lactones and various phenolic acids, which contribute to its harmful effects on other crops and animals (Paudel, 2010; Satao & Shinde 2014). Effective control of this weed is essential to ensure food security for the Ethiopian population. Traditional control methods, such as tillage and hand weeding, have proven unsuccessful (Niguse and Belachew 2016). The use of synthetic chemicals alone has also failed to mitigate the weed's rapid spread. Therefore, alternative safe and effective control methods should be implemented to enhance crop productivity.

Organic herbicides offer a promising solution for controlling parasitic weeds. Using plants and their products as alternative weed control tools is considered the next generation of herbicides, especially in organic

agriculture. Natural products are less toxic to beneficial microorganisms, environmentally friendly, and more biodegradable than synthetic compounds (Bhadoria, 2011; Macias *et al.,* 2003). Chemicals can be released from one plant to another through leaching, root exudation, and residual decomposition (Qasem, 2012).

Echinops kebericho (Asteraceae) is a plant endemic to Ethiopia. It is a shrub that grows up to 1.2 meters with stocky roots. The root is widely used to treat various diseases and is a well-known endemic medicinal plant in Ethiopia (Manahlie & Feyissa 2014). The root smoke is used to treat ailments such as headaches, diarrhea, malaria, hemorrhoids, and typhus, and it serves as a fumigant to prevent airborne diseases and repel mosquitoes and snakes (Manahlie & Feyissa 2014; Tadesse & Mesfin 2010; Toma *et al.,* 2015). Studies on the genus Echinops have shown a range of biological activities, including antibacterial, antifungal, and anti-inflammatory properties (Ameya *et al.,* 2016). The crude polar extract of *E. kebericho* roots (3.12 to 25 µg/ml) has demonstrated significant antimicrobial activities against Staphylococcus aureus, Candida albicans, and Aspergillus flavus (Ameya *et al.,* 2016). Other studies indicate its antimalarial, anti-diarrheal, and anti-spasmodic properties (Karunamoorthi *et al.,* 2008; Toma *et al.*, 2015). Phytochemical research has isolated various compounds from Echinops species, including sesquiterpene lactones, polyacetylenes, and acetylenic thiophenes (Abegaz *et al.,* 1991). Specifically, *E. kebericho* is rich in sesquiterpenes, with Dehydrocostus lactone being a major component. Other identified compounds include caryophyllene epoxide, costunolide, santamarin, reynosin, and various thiophene derivatives (Abegaz *et al*., 1991).

The volatile fractions of *E. kebericho* roots are rich in essential oils. The main constituent in the hydrodistilled essential oil is eudesm-7(11)-en-4-ol, followed by caryophyllene oxide and T-cadinol. SPME analysis has detected high amounts of β-cubebene, β-patchoulene, longifolene, and cyperene (Abegaz *et al.,* 1991).

In another study, Dehydrocostus lactone, obtained from nonpolar extracts of Saussurea lappa root, was found to inhibit the growth of crabgrass, maize, and soybean by more than 85%, and black nightshade by 40% (Cho *et al.,* 2010). The primary aim of this study was to investigate the phytotoxic potential of *E. kebericho* against the invasive Santa-Maria weed.

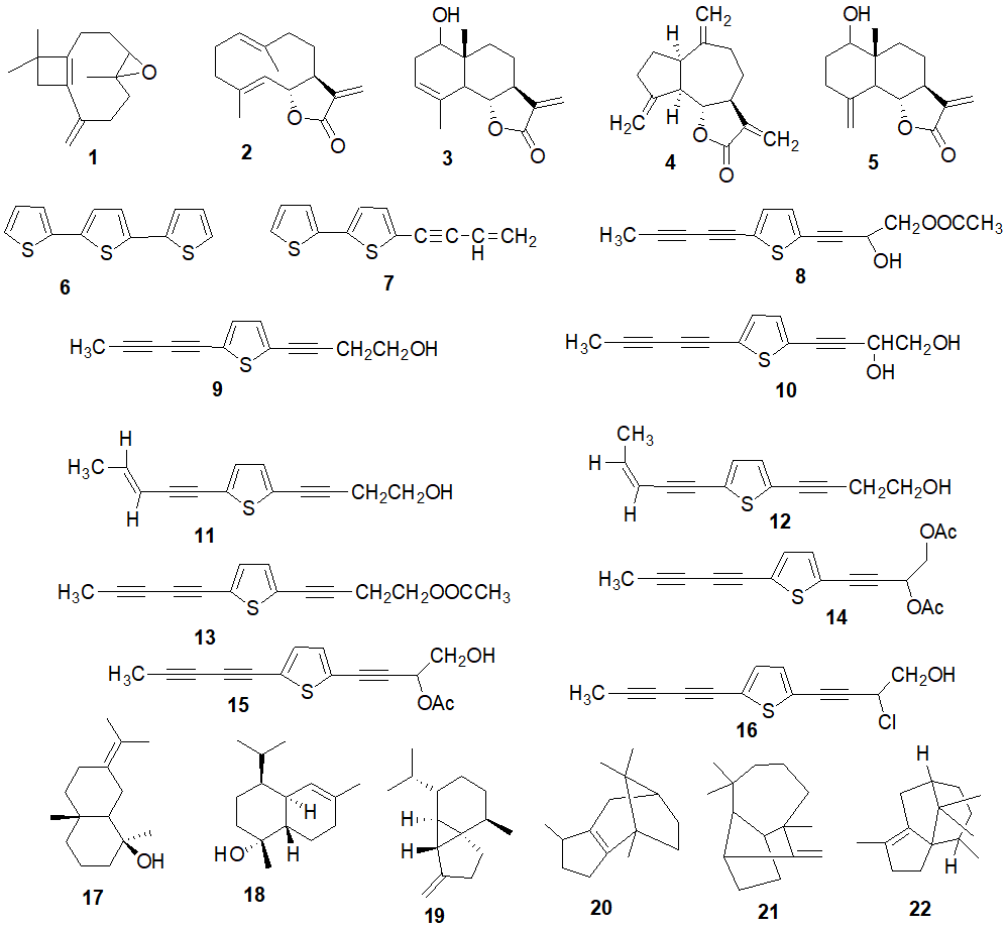

Fig. 1. Secondary metabolites isolated from *E. kebericho* by different scholars.

MATERIAL AND METHODS

A. Materials used for purification and spectroscopic analysis

Two sizes of column chromatography, medium and small, were employed for separation. Thin-layer chromatography (TLC) was carried out on aluminum-coated plates. Melting points were noted as uncorrected, with detection using UV light at 254 and 366 nm, and spray reagents vanillin-H_2SO_4. Infrared (IR) data was measured using Perkin Elmer 1600 and Pye Unicam Infrared spectrophotometers. Both 1H and ^{13}C NMR spectra were recorded in $CDCl_3$, using the chloroform solvent peaks at δ_H 7.2 and δ_C 77.2 as internal references. Chemical shift values are reported in δ (ppm) units. The NMR data was acquired on a Bruker UltrashieldTM 400 spectrometer at 400 MHz for 1H and 100 MHz for 13C, with TMS and solvents as internal standards.

B. Extraction of plants for bioassay test

The semi-dried root of *E. kebericho*, purchased from a local market in Addis Ababa, Ethiopia (Gizachew, 2019), was left to dry at room temperature for over a month. Once fully dried, it was ground into a fine powder using a grinder. One hundred grams of this powder were soaked in 500 mL of ethanol and placed on a shaker for six hours. Afterward, the mixture was filtered and concentrated using a rotary evaporator at 40°C, yielding 12 grams (12%) of semi-solid crude extract.

For the bioassay test, 100 mg of the ethanol extract was dissolved in 100 mL of a 5% acetone-in-water solution to create a 1 mg/mL test solution. The remaining crude extract was partitioned with chloroform (CHCl3), and the resulting 3 grams of CHCl3 extract were subjected to silica gel column chromatography (70 g). Using hexane and a hexane:ethyl acetate (EtOAc) solvent system as the eluent, 12 fractions were collected. These fractions were then combined and reduced to five subfractions based on their TLC profile. Left overnight, a white crystal precipitated from subfraction 1, which was identified as compound 4 (480 mg).

C. Santa-Maria fever few weed seed preparation for bioassay

The seeds of the Santa-Maria weed were air-dried and hand-threshed. Only viable seeds were chosen and then surface-sterilized by shaking them in a 1% Sodium hypochlorite (NaOCl) solution for five minutes. To prevent any fungal contamination during the screening test, the seeds were rinsed with distilled water for three minutes right before use.

D. Phytotoxicity screening test procedure (in vitro)

The phytotoxicity test was carried out at the Ambo Agricultural Research Center (AARC) in Ethiopia. We prepared Petri dishes lined with filter paper and sowed ten pre-washed and disinfected test seeds in each dish. To create the test solution, we dissolved the extract in acetone and water to achieve a concentration of 1 mg/ml and added it to the Petri dishes until the filter paper was fully moistened. The dishes were then covered and placed in a greenhouse with temperatures ranging from 23-30°C.

Every day for 15 days, we moistened the filter paper by adding the test solution. The experiment was conducted in triplicate using a Completely Randomized Design. As controls, we used 5% acetone in water (negative control) and a standard commercial herbicide, Roundup® (positive control). We observed the seed germination daily and counted the number of germinated seeds after fifteen days. Germination inhibition was calculated using the formula GI% = [1-(Gt/Gc)] × 10% (Kumar, 2015), where Gt is the number of germinated seeds in the treatment group, and Gc is the number in the control group.

E. Greenhouse experiment procedures

Following the standard protocol by Belz and Hurle (2016), we conducted our greenhouse experiment. We collected red soil, sand, and compost from the Ambo area and mixed them in a 2:1:1 ratio. This soil mixture was then surface-sterilized at Ambo University. We prepared equal-sized plastic pots, each with an upper diameter of 20 cm and a depth of 18 cm, and filled them with 3 kg of the sterilized soil mixture. The pots were watered until moistened and placed in a greenhouse with temperatures ranging from 23-30°C.

Initially, we sowed 10 viable Santa-Maria weed seeds in each pot and watered them daily for 15 days. After this period, we thinned the seedlings to 3 uniform ones per pot. Using the foliar spray bioassay technique, we sprayed the extract solution on the seedlings for 15 days, applying an average of 5 mL per spray (2 drops per leaf) as described by Javaid *et al.* (2013). The treatments were arranged in a Completely Randomized Block Design with three replications. We used 5% acetone in water as the negative control and Roundup® as the positive control.

Two weeks after treatment, we carefully uprooted the seedlings from both control and treatment groups, washed their roots with water, and allowed them to dry separately. We recorded the dry biomass of the seedlings before and after treatment. The percentage growth inhibition was calculated using the standard formula provided by Inderjit *et al.* (2008).

$$GI\% = \frac{[1 - \text{Average dry biomass difference of treatment}]}{\text{Average dry biomass difference of control}} \times 100$$

RESULTS AND DISCUSSION

A. Bio-assay results (in vitro)

Our in vitro bio-assay revealed that the ethanol (EtOH) extract of *E. kebericho* root exhibited potent phytotoxic activity, completely inhibiting (100%) the germination of Santa-Maria weed seeds at a concentration of 1 mg/mL. We then tested lower concentrations of 0.02, 0.05, 0.1, and 0.2 mg/mL to explore their phytotoxic effects.

At the lowest concentration of 0.02 mg/mL, the inhibition was minimal, with less than 20% seed germination inhibition. However, at concentrations of 0.05, 0.1, and 0.2 mg/mL, the EtOH extract of *E. kebericho* consistently demonstrated strong phytotoxic activity, achieving 100% germination inhibition (Fig. 2).

Table 1: Impact of Ethanol Extract Concentration of *E. kebericho* on Phytotoxicity against Santa-Maria Weed.

Species name	Activity at different concentration (mg/mL)						
	Local name	parts	0.02	0.05	0.1	0.2	100
E. kebericho	Kebericho	Ap	17	100	100	100	100
Round up®	+ve control	Rt	27	100	100	100	100
5%acetone in H_2O	-ve control	Lf	0	0	0	0	0

The ethanol extract from the root of *E. kebericho* showed impressive phytotoxic activity, achieving 100% growth inhibition (GI) at just 0.05 mg/mL. Therefore, this concentration was established as the minimum required for growth inhibition in our study. Remarkably, this extract consistently demonstrated similar phytotoxic effects over various time intervals and was used as a benchmark when screening other botanical extracts.

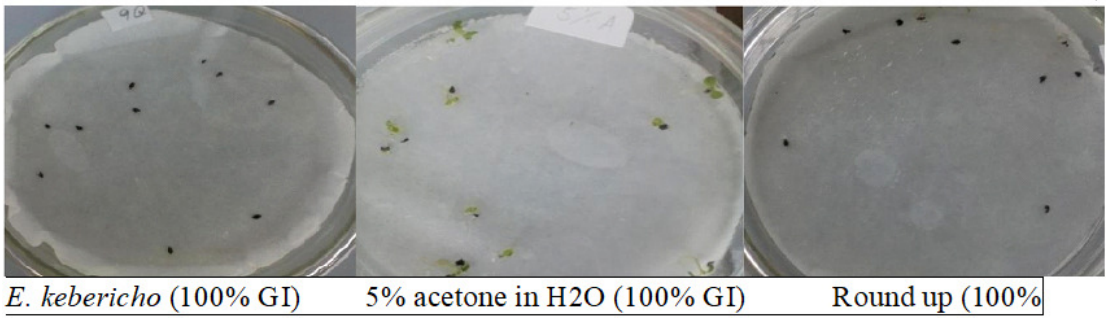

Fig. 2. Phytotoxicity results of *E. kebericho* at 0.05 mg/mL.

B. In vivo bioassay results of the crude ethanol extracts of E. kebericho root

The ethanol extract from the root of *E. kebericho* was tested for herbicidal activity in a greenhouse setting at a concentration of 1 mg/mL and showed an impressive average activity of 90% over various time periods. The $CHCl_3$-soluble part of this ethanol extract was even more potent, suppressing the growth of 15-day-old seedlings by 92%.

Table 2: *In vivo* bioassay results of EtOH extract of *E. kebericho* root.

	Average dry biomass of seedlings before treatment (mg)				Average dry biomass of seedling after treatment (mg)				Mass difference	
	T1	T2	T3	M1	T1	T2	T3	M2	M2-M1	%GI
E. kebericho	81	60	69	70	352	140	310	267	197.5	92
Roundup®	81	60	69	70	141	120	129	130	60	97
5% acetone	81	60	69	70	2515	2500	2620	2545	2475	0

The hexane, $CHCl_3$, and MeOH portions of the crude ethanol extract were tested against Santa-Maria weed. The hexane and $CHCl_3$ portions proved to be more effective than the methanol portion. At a concentration of 0.05 mg/mL in vitro, both the hexane and $CHCl_3$ portions completely inhibited Santa-Maria weed seed germination (100%). In comparison, the methanol portion showed lower activity, inhibiting 60% of seed germination.

In a greenhouse study, the $CHCl_3$ fraction suppressed Santa-Maria weed seedling growth by 92%. For reference, the commercial herbicide Roundup® used as

a positive control also achieved 100% seed germination inhibition at the same concentration as the botanical extracts.

C. Bioassay guided isolation of a compound from E. kebericho

Using lab and greenhouse bioassay results, we applied the $CHCl_3$ extract of *E. kebericho* root to silica gel column chromatography. This process led to the isolation of Dehydrocostus lactone (compound 4). The characterization of this compound was performed using various spectroscopic methods, as described below.

D. Interpretation of compound 4

We obtained this compound as white crystals (480 mg, 16%) from the CHCl3 soluble portion of the ethanol extract of *E. kebericho* root, using hexane as the eluent. The compound melts at 55-56°C. TLC analysis, using a hexane:EtOAc (4:1) solvent system and vanillin as a spraying agent, revealed a single blue spot (Rf 0.5).

The ^{1}H-NMR spectrum ($CDCl_3$) of compound **4** displayed signals at δ 3.90 (1H, *t, J* = 9.2 Hz), indicating an oxygenated methine proton. Exocyclic double bonds were confirmed by the signals at δ 6.12 (1H, *d*) and 5.43 (1H, *d*), δ5.19 (1H, *s*) and δ4.99 (1H, *s*) and δ4.83 (1H, *s*), and δ4.74 (1H, *s*). A multiplet signal appeared at δ 2.85 was attributed to three different methine protons. The methylene protons that appeared at δ 6.12 and 5.43 (each 1H) are coupled each other. The remaining exocyclic protons are observed as each a singlet indicating they are not coupled with to each other.

The ^{13}C-NMR spectrum of compound **4,** along with DEPT 135, showed seven methylene signals (at δ30.2, 30.9, 32.6, 36.3, 109.4, 112.5 and 120.1), four methine (at δ45.0, 47.5, 51.9 and 85.3), and four quaternary (at δ151.3, 149.2, 139.6 and 170.1). The ^{1}H and ^{13}C-NMR data of compound **4** were compared with the literature values of Dehydrocostus lactone (DHCL) and found to be in close agreement (Table 3) (Cho *et al.*, 2010).

Table 3: Comparison of NMR spectral data ($CDCl_3$) of compound 4 with literature values of DHCL.

Experimental data of compound 4			Literature report of DHCL (Cho *et al.*, 2010)		
	^{13}C-NMR ($CDCl_3$)	^{1}H-NMR of Cpd4	^{13}C-NMR of DHCL		^{1}H-NMR of DHCL
1	47.5	2.85 (1H, *m*)	47.5		2.9 (1H, *m*)
2	32.6	1.87 (2H, *m*)	32.5		1.9 (2H, *m*)
3	30.2	2.47 (2H, *m*)	30.2	2.5	(2H, *m*)
4	151.3		150.9		
5	51.9	2.85 (1H, *m*)	51.9		2.8 (1H, *m*)
6	85.3	3.90 (1H, *t,* 9.2)	85.1		3.9 (1H, *t, 9.3*)
7	45.0	2.85 (1H, *m*)	45.0		2.9 (1H, *m*)
8	30.9	2.20 (1H, *m*)	30.9	2.24	(1H, *m*)
		1.37 (1H, *m*)		1.46	(1H, *m*)
9	36.3	2.44 (1H, *m*)	36.2	2.52	(1H, *m*)
		2.10 (1H, *m*)			2.27 (1H, *m*)
10	149.2		148.8		
11	139.6		139.5		
12	170.1		170.0		
13	120.1	6.12 (1H, *d,* 3.6)	119.9	6.21	(1H, *d,* 3.4)
		5.43 (1H, *d*, 2.8)		5.49	(1H, *d,* 2.9)
14	112.5	5.19 (1H, *s*)	112.4	5.27	(1H, *d,* 2.2)
		4.99 (1H, *s*)		5.07	(1H, *d,* 2.2)
15	109.5	4.83 (1H, *s*) & 4.74 (1H, *s*)	109.4	4.90	(1H, *s*), 4.82 (1H,*s*)

Chemical shifts are reported in parts per million ($CDCI_3$,), *J* values are in Hertz.

The spectroscopic data of compound 4 closely matched the literature for Dehydrocostus lactone. Therefore, we propose that this compound is indeed Dehydrocostus lactone, previously isolated from *E. kebericho* and known for various biological activities, including antibacterial, antifungal, insecticidal, antidiarrheal, and ex-vivo spasmolytic effects (Abegaz *et al.,* 1991).

We studied the seed germination inhibition effect of Dehydrocostus lactone and found it completely inhibited Santa-Maria weed seed germination at a concentration of 0.05 mg/mL (Table 4). Further greenhouse experiments demonstrated its potent herbicidal activity, showing 92% phytotoxicity against the target weed, comparable to the standard herbicide Roundup®. The growth of 15-day-old Santa-Maria weed seedlings was inhibited at a concentration of 1 mg/mL. This herbicidal property of Dehydrocostus lactone has been reported previously (Cho *et al.*, 2010).

Fig. 3. *In vivo* phytotoxic activity of DHCL at green house against Santa-Maria weed.

Table 4: Bioassay results of Dehydrocostus lactone under greenhouse conditions

compound	source	number of germinated seeds					
		T1	T2	T3	mean	STDV	%GI
Dehydrocostus	*E.kebericho*	0	0	0	0	0	100
Roundup®		0	0	0	0	0	100
5% acetone		9	8	8	8.3	0.57	0.79

To investigate the seasonal variation of Dehydrocostus lactone, we conducted phytotoxicity tests every two months. The results showed consistent phytotoxic activity (90%) at the same concentration each time.

Another study reported that Dehydrocostus lactone caused 85% necrotic injury to crabgrass, maize, and soybean at a concentration of 4000 ppm. However, it only caused about 40% necrotic injury to black nightshade, indicating that Dehydrocostus lactone lacks gross morphological selectivity. Based on our experimental results and supporting literature, we conclude that Dehydrocostus lactone is not selective to Santa-Maria weed.

E. Selectivity study

Our study revealed that Dehydrocostus lactone did not inhibit the germination of field pea, chickpea, niger, linseed, maize, and wheat. However, it did inhibit the germination of sorghum seeds to a tolerable extent. In vitro tests showed that seed germination of tef, barley, sorghum, common bean, faba bean, niger, and linseed was highly inhibited.

A similar selectivity study was conducted under greenhouse conditions. The results showed that Dehydrocostus lactone did not negatively affect the growth of most test crops, except for minimal phytotoxicity on common bean, faba bean, maize, and wheat at an early stage after application. This preliminary result suggests that the compound lacks gross morphological selectivity. However, further intensive studies are needed to identify susceptible and resistant plants and reach concrete conclusions.

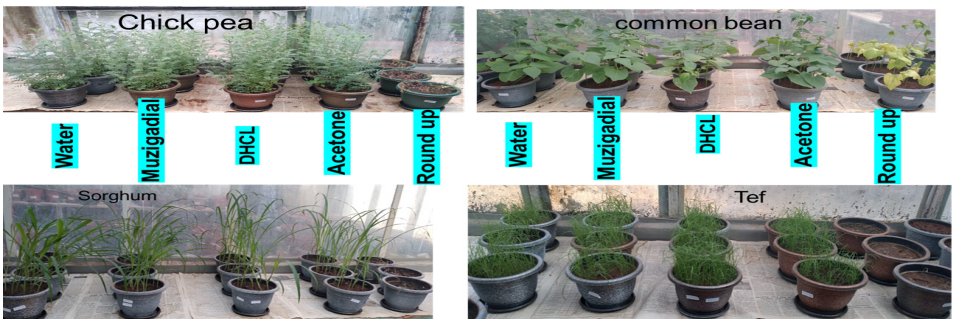

Fig. 4. Shows the selectivity study of DHCL on chick pea, common bean, sorghum and tef.

CONCLUSION

In conclusion, organic agriculture needs natural herbicides that are nontoxic, easily biodegradable, and environmentally friendly. This study investigated the herbicidal potential of *E. kebericho* against the allelopathic Santa-Maria weed. The in vitro results showed that the $CHCl_3$-soluble portion of the ethanol extracts from *E. kebericho* completely inhibited seed germination at a 0.05% concentration. When tested on 15-day-old seedlings under greenhouse conditions, only the $CHCl_3$ extract of *E. kebericho* effectively suppressed the growth of Santa-Maria weed seedlings by 92%.

Dehydrocostus lactone, isolated from the root of *E. kebericho,* was identified as the active principle against the target weed. It demonstrated comparable phytotoxic activity (92%) to the well-known herbicide Roundup®.

In our efforts to study its selectivity towards various cereal crops, including pea, chickpea, niger, linseed,

maize, wheat, tef, barley, sorghum, common bean, and faba bean, we concluded that Dehydrocostus lactone is selective to all these cereal crops. However, it showed minimal phytotoxicity on common bean, faba bean, maize, and wheat at an early stage after application. Therefore, Dehydrocostus lactone stands out as a promising natural herbicide candidate for managing *P. hysterophorus* weed.

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