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Phytotoxicity studies of *Canarium zeylanicum* Blume on lettuce and radish

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ABSTRACT

We did a preliminary investigation of the allelopathic activity of 60 invasive and medicinal plants in Sri Lanka on *Lactuca sativa* (lettuce) and *Raphanus sativus* (radish). Out of 60 plants, 14 significantly reduced lettuce seedling lengths than distilled water, and 55 plants reduced radish seedling growth. In consideration of all the parameters tested, *Cardiospermum halicacabum* and *Canarium zeylanicum* Blume showed the highest phytotoxicity. *C. zeylanicum* (family Burseraceae), an endemic plant in Sri Lanka was selected for the isolation of phytotoxic compounds. The whole tree has a distinct fragrance bears edible seeds and has potential health benefits. This plant is used for medicinal purposes and its bark contains 3-hydroxy-12-methoxy-8,11,13-podocarpatrien-3-one and acetyl aleuritolic acid. This is the first report of the aforementioned two compounds from *C. zeylanicum*. These compounds exhibited phytotoxicity against lettuce.

Keywords: Acetyl aleuritolic acid, allelopathy, *Canarium zeylanicum*, *Cardiospermum halicacabum*, *Lactuca sativa*, lettuce, radish, *Raphanus sativus*, 13-hydroxy-12-methoxy-8, 11,13-podocarpatrien-3-one, seed germination bioassay.

INTRODUCTION

Weeds infestation results in yield losses on agricultural lands. Currently, synthetic herbicides are used to control weeds. Their over use have led to development of herbicide-resistant weeds, which are harmful to both the environment and human health (2,3,8). Owing to these concerns, globally, researches are being done to reduce the use of synthetic herbicides and to use the naturally available plant products or environmentally friendly. In this regard, plant allelopathy has been suggested as an alternative option for achieving long-term weed control (20,23,25).

C. zeylanicum family Burseraceae (Figure 1), is grown in the wet zone of Sri Lanka. The whole tree has a distinct fragrance, bears edible seeds and has potential health benefits. The resins from the bark are used in fumigation, seed oil for cooking and oil lamps for household lighting (1,11). It has been investigated that the seed oil is rich in essential fatty acids (C18:3 and C18:2), p-hydroxybenzoic acid, ellagic acid and 3,4-dihydroxybenzoic acid. Further, seed oil and seed hull contained total phenol contents of 66 ± 6 mg/kg and 2014 ± 14 mg/kg respectively. The antioxidant activity of seed hull and oil is equivalent to artificial antioxidant, butylated hydroxytoluene (21). Furthermore, methanol extract from leaves and seeds showed significant larvicidal activity (LC_{50}<10.0 mg/L) against late 3rd instar larvae of *Culex quinquefasciatus* (19). However, terpenes [taraxerol, 3β-hydroxy-12-en-1l-one, canaric acid, 3β-hydroxyolean-12-en-11-one, elemene, olean-12-en-3,11-dione, a-pinene, urs-12-en-3, ll-dione, terpineol, α and β-amyrin, elemol, α and β-amyrenone, carvone limonene, sitosterol, α and β-hellandrene] were identified from timber and bark (1).

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Figure 1. *Canarium zeylanicum* Blume (Source: https://www.floraofsrilanka.com/species/1280)

The present study is a preliminary screening of the phytotoxic plants since a more detailed investigation that includes a greenhouse and/or field studies should be required to reproduce the above results in the natural environment. Thereafter, plant extracts or mulch could be used in integrated weed management in sustainable agriculture. These plant extracts might contain phytotoxic compounds. Therefore, chemical investigation should be carried out for the identification of the respective phytotoxic compounds. As *C. halicacabum*, *P. scutellaria*, *D. volubilis* and *B. nigra* are highly abundant plants in Sri Lanka, there is a potential for using these plant extracts or in crude form as mulch in weed management of agricultural fields. The limitation of the present study is that applications of the mulching materials are not commercially viable; however, phytotoxic mulching materials could be used for weed management in small-scale agricultural systems, organic farming and home gardens. Nevertheless, there are several constraints for employing plant mulching materials due to the extensive fieldwork involving huge quantities of plant mulching materials, which is frequently unaffordable in terms of the cost of operation.

Allelopathy is a natural approach that could be used to control weeds. It is defined as harmful or beneficial impacts on one microorganism or plant upon another via the production and release of allelochemicals into the surroundings. It is a known that plants influence other plants by releasing chemicals into the environment for thousands of years. This concept was practiced by the ancient Romans and Greeks as early as 64 AD. The ancient Greek Theophrastus suggested the harmful effects of peas on other plants in ca. 300 B.C. (9). The term allelopathy was introduced to describe the plant-plant interactions (16,27) and allelopathy has been proposed as an alternate weed control method for sustainable agriculture (10,27). These chemicals are released into the environment through leaching, root exudation, volatilization and plant biomass decomposition (8,27). Allelochemicals, also known as defensive secondary metabolites, are associated with allelopathic interactions, can have negative consequences for autotoxicity and heterotoxicity. These allelochemicals comprise an array of chemical groups, where phenols, terpenoids and nitrogen-containing compounds are the most prominent (20). Allelochemicals have a broad spectrum of modes of action with apparent impacts on target plants, such as inhibited seed germination and

reduced seedling growth. The primary changes include interfering with cell membrane permeability, inhibition of cell elongation and division, respiration, enzymatic activities photosynthesis, etc. (20). Moreover, mixtures of allelochemicals produce the allelopathic effects in field conditions (20). There are diverse range of phytotoxic constituents in plants. The allelochemicals are present in every organ of the plant and they frequently indicate selectivity, similar to synthetic herbicides. Since the majority of these chemical compounds are phytotoxic, they could be used as herbicides or as templates for novel herbicide families (27). Therefore, unidentified phytotoxic compounds might be used as potential growth inhibitors and as novel herbicides (13). Allelopathy can be used to manage weeds through strategies including cover crops and their residues, crop rotations and intercropping, or by using phytotoxic chemicals as natural herbicides (8,27). It is reported that some plants are incorporated in paddy soil after harvest by indigenous farmers in Southeast Asia. For example, a leguminous plant, *Stylosanthes guianensis*, commonly used in Southeast Asia as livestock feed, mulch, cover crop, and to improve soil fertility, most significantly increases rice yield (13). Sri Lanka has a diverse flora, of them about 3800 higher plant species, approximately 28 % are endemic (18). The comprehensive allelopathic studies of the plant wealth in Sri Lanka could be beneficial to develop sustainable and economical natural herbicides. In our previous investigation on search for allelopathic compounds from Sri Lankan flora, a phytotoxic compound, mikanolide was identified from an invasive plant, *Mikania scandens* (17).

Various combinations or formulations of natural substances, primarily derived from plants, are marketed as crop protection agents for utilization in organic farming (18,20). Natural herbicides are harmless from an environmental toxicology perspective and have an extremely short half-life (16,26). Bio-herbicides are generally referred to as naturally occurring substances that are either secondary metabolites of organisms to control target weed populations without harming the environment (16,20). Plant-based bio-herbicides are becoming more and more popular as a means of controlling weed resistance and reducing their negative effects on the environment, owing to the numerous advantages provided: most of them are water-soluble, have an environmentally friendly chemical structure, possess novel molecular targets in weeds and have the acceptance of the public (20). This study aimed to identify the phytotoxicity of invasive and medicinal plants of Sri Lanka and identify their phytotoxic compounds. These phytotoxic plants could be utilized as an alternative option for integrated weed management. Furthermore, the introduction of new phytotoxic compounds from natural sources is imperative to develop eco-friendly herbicides.

MATERIAL AND METHODS

Materials

Lettuce (RAPIDO 344) and radish (big Ball Radish, Quality seeds, Nongwoo Bio Co. Ltd, Korea) seeds were purchased from Kandy, Sri Lanka. Solvents, n-hexane, dichloromethane, ethyl acetate, methanol, sulfuric acid and glacial acetic acid were purchased from Fisher Scientific, UK. Anisaldehyde and abscisic acid were purchased from Sigma Aldrich, Germany.

Preparation of plant extracts

Plant materials were collected in from Kandy Sri Lanka (Elevation: 500 m, Latitude: 7.290572º, Longitude: 80.633728º, Annual Rainfall: 198.12 mm, Maximum Temperature: 30 °C, Minimum Temperature: 26 °C.). Plants were identified by comparing the voucher specimens in the Royal Botanical Garden, Sri Lanka. All the voucher specimens were kept in NIFS (National Institute of Fundamental Studies, Sri Lanka). *C. zeylanicum* leaves (200 g) and bark (2 kg) were collected. Fresh plant material was cleaned and ground in distilled water using a grinder. The water extract was freeze-dried (Freeze-dryer, EYELA FD-1, Fisher Scientific) (17).

Lettuce (*Lactuca sativa*) and radish (*Raphanus sativus*) germination bioassays

Seeds of radish and lettuce were treated with NaOCl (5 %) for 10 min and washed with autoclaved water. Five viable seeds were sown per Petri plate covered with filter paper (Whatman No. 4.) containing freeze-dried plant extract soluble in water (5 mL, 1000 ppm). Abscisic acid (10 ppm) and water were used as positive and negative controls, respectively. Treatments were replicated 4-times in complete randomised design. Petri dishes were placed for 5- days at 25 ° C in dark in incubator. Germinated seeds were counted and root and shoot lengths of seedlings were measured after 5-days (7,22).

Radish Parameters

(i). Rate of germination

The number of radish seed germinated were monitored for 5- days. The germination rate was determined as under (22).

$$\text{Seed Vigour} = \frac{\sum \text{ Daily counts of number of seeds germinated}}{\text{Number of days}}$$

(ii). Fresh/Dry weight

Radish seedlings fresh weight was recorded immediately after sampling and Dry weight after 5-days dried using paper towels (6).

(iii). Total seedling dry weight

The dry weight of radish seedlings was measured after 9-days and incubated for 4 h at 65 ˚C. The inhibition (%) was determined by following equation (6).

$$\text{Inhibition (\%)} = [(\text{control-extracts})/\text{control}] \times 100$$

Lettuce seed germination assay (water-insoluble compounds and extracts)

Test compounds, 3-hydroxy-12-methoxy-8, 11,13-podocarpatrien-3-one and acetyl aleuritolic acid were dissolved in $CHCl_3$/MeOH added to filter paper (Whatman No 4) and kept overnight in vacuum oven. After that, Tween 20 (5 mL, 0.05% (*v/v*)) was added to it and 5 seeds were sown in each petri plate. Tween 20 (0.05% (*v/v*)) was utilized as a negative control. Seed germination was monitored for 5 days (12).

Isolation of compounds

Acidic anisaldehyde was used to visualize TLC spots following the identification of TLC plots using a UV lamp. Silica gel (Merck Art. 7734, Germany) and Sephadex LH-20

(Fluka, Germany), thin-layer chromatography (Merck, 60F254, Germany) were used. NMR (400 MHz for ^{1}H NMR (Nuclear Magnetic Resonance), HMQC (Heteronuclear Multiple Quantum Correlation), HMBC (Heteronuclear Multiple Bonded Correlation) and 100 MHz for ^{13}C NMR) spectra were recorded on a Varian Mercury (400 MHz) spectroscopy (TMS as internal standard).

C. zeylanicum (stem bark 2 kg) collected in Kandy, Sri Lanka was cleaned and powdered. Its successive extractions were done using cold hexane (n), dichloromethane, EtOAc (ethyl acetate) and methanol using a horizontal shaker (VWR, Germany). Weights of extracts were 8.1 g (0.35 %), 10.2 g (0.40 %), 11.6 g (0.44 %) and 6.8 g (0.24 %) respectively. Activity-guided fractionations were performed for each extract and the extract which showed the strong phytotoxicity was selected for the chemical investigation. Column chromatography was done to purify the compounds from dichloromethane extract (10.2 g). These column chromatographic fractions were further purified using size exclusion chromatography, gravity column and preparative thin layer chromatography. Two compounds, 13-hydroxy-12-methoxy-8, 11, 13-podocarpatrien-3-one **(1)** (20 mg, (5) and acetyl aleuritolic acid **(2)** (100 mg, (15) were found.

13-hydroxy-12-methoxy-8, 11, 13-podocarpatrien-3-one (1)

White needles (Hexane/CH_2Cl_2) (20 mg, 0.0008 %) m. p. 136-138 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H: 6.81 (1H, s, H-14), 6.52 (1H, s, H-11), 5.46 (1H, s, OH), 3.84 (3H, s, OMe), 2.85 (2H, m, H-7), 2.63 (2H, m, H-2), 2.38 (2H, m, H-1), 1.86 (1H, m, H-5), 1.74 (2H, m, H-6), 1.26 (3H, s, Me-17), 1.15 (3H, s, Me-16), 1.12 (3H, s, Me-15). **^{13}C NMR** (100 MHz, $CDCl_3$) δ_C: 217.6 (C-3), 145.0 (C-12), 144.1 (C-13), 140.4 (C-9), 126.6 (C-8), 111.5 (C-14), 110.8 (C-11), 56.1 (OMe), 50.9 (C-5), 47.5 (C-4), 37.9 (C-1), 37.2 (C-10), 34.9 (C-2), 30.8 (C-7), 27.1 (C-16), 24.7 (C-17), 21.3 (C-15), 20.6 (C-6).

Acetyl aleuritolic acid (2)

White needles (Hexane/CH_2Cl_2) (100 mg, 0.004 %) m. p. 288-290 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H: 5.51 (1H, m, H-15), 4.45 (1H, m, H-3), 2.38 (2H, dd, H-16), 2.23 (1H, m, H-18), 2.11 (3H, s, -$COCH_3$), 1.62 (2H, m, H-2), 1.61 (2H, m, H-1, 22), 1.60 (2H, m, H-21), 1.42 (1H, m, H-9), 1.31 (2H, m, H-19), 1.22 (2H, m, H-7), 1.15 (2H, m, H-12), 1.02 (2H, m, H-11), 0.94 (6H, s, H-26, 27), 0.93 (3H, s, H-29, 24), 0.92 (2H, m, H-6), 0.91(3H, s, H-30), 0.90 (1H, m, H-5), 0.87 (3H, s, H-23), 0.84 (3H, s, H-25). **^{13}C NMR** (100 MHz, $CDCl_3$) δ_C: 184.7 (C-28), 171.2 (-O-$COCH_3$), 160.7 (C-14), 117.1 (C-15), 81.1 (C-3), 55.7 (C-5), 51.7 (C-17), 49.2 (C-9), 41.5 (C-18), 40.9 (C-19), 39.2 (C-4), 38.1 (C-8), 37.9 (C-10), 37.6 (C-1), 37.5 (C-13), 35.5 (C-12), 33.5 (C-21), 32.0 (C-26, 29), 31.5 (C-16), 30.9 (C-22), 29.9 (C-7), 29.5 (C-20), 28.8 (C-30), 28.1 (C-23), 23.7 (C-2), 22.7 (C-27), 21.5 (-O-$COCH_3$), 18.9 (C-6), 17.5 (C-11), 16.8 (C-24), 15.8 (C-25).

Statistical analysis

The statistical analysis was done by using MINITAB (version 11.12). One-way analysis of variance proceeded by Turkey's test for pair-wise mean comparisons and Dunnett's comparisons were carried out. The mean values were separated using the least significant difference at a probability level of 0.05.

RESULTS AND DISCUSSION

Lettuce seed germination bioassay

In lettuce seed germination bioassays, only germination (%) radicle and hypocotyl lengths of lettuce seedlings were recorded. A radish seed germination bioassay was used to determine germination rate, fresh and dry weight inhibition, as well as seedling length. Sixty plants used in this study are given in Table 1.

Table 1. Plants used for preliminary phytotoxicity screening

Invasive plants	**Medicinal plants**	
Alternanthera sessilis	*Myristica fragrans*	*Murraya koenigii*
Argyreia populifolia	*Solanum nigrum*	*Morinda citrifolia*
Centrosema vainas	*Tagetes erecta*	*Anisomeles indica*
Clidemia hirta	*Coccinia grandis*	*Elaeocarpus serratus*
Croton laccifer	*Careya arborea*	*Alstonia macrophylla*
Hevea brasiliensis	*Mentha viridis*	*Michelia champaca*
Impatiens balsamina	*Cardiospermum halicacabum*	*Adenanthera pavonina*
Ipomoea aquatica	*Trema orientalis*	*Lasia spinosa-Rhizome*
Ipomoea cairica	*Sauropus androgynus*	*C. Zeylanicum*
Lantana camara	*Sida rhombifolia*	*Coriandrum sativum*
Manihot esculenta	*Calamus rotang*	*Languas galanga*
Mikania scandens	*Cassia auriculata*	*Amaranthus viridis*
Mimosa pudica	*Dregea volubilis*	*Eugenia caryophyllata*
Panicum maximum	*Costus speciosus*	*Colocasia esculenta*
Polyscias scutellaria	*Passiflora edulis*	*Alstonia scholaris*
Sphagneticola trilobata	*Solanum indicum*	*Garcinia mangostana*
Tithonia diversifolia	*Centella asiatica*	*Sesbania grandiflora*
Vernonia cinerea	*Lasia spinosa*	*Carum petroselinum*
	Bacopa monnieri	Lactuca sativa
	Basella alba	*Acalypha indica*
	Brassica nigra	*Aerva lanata*

Table 2. Effects of plant extracts on *Lactuca sativa* and *Raphanus sativus*

Plant extract	***Lactuca sativa***		***Raphanus sativus***	
	Germination (%)	**Radicle length (mm)±SE**	**Radicle length (mm)±SE**	**Dry weight inhibition (%)**
Acalypha indica (L.)	70	33±2.10	186±2.16*	42.70±3.65
Adenanthera pavonina (L.).	90	31.9±2.68	142.25±4.38*	36.97±5.21
Aerva lanata (L.)	70	11.9±1.02*	90.25±6.84*	47.91±5.25
Alstonia scholaris (L.)	80	29.0±2.43	132.75±3.68*	32.36±3.98
Alstonia macrophylla (L.)	90	35.1±1.51	130.75±3.78*	39.58±4.23
Alternanthera sessilis (L.)	90	28.6+ 1.67	150.25±1.70*	8.33±1.25
Amaranthus viridis (L.)	65	19.5±2.44*	110.5±3.02*	47.39±10.26
Anisomeles indica (L.)	80	37.6±3.26	194.75±8.65	32.81±6.22
Argyria populifolia (L.)	90	30.3±2.20	157.25±4.57*	43.22±7.86
Asparagus officinalis (L.)	85	25.8±1.53	168.25±4.56*	47.91±8.05
Baccopa monierri (L.)	77.5	34.6±3.86	140.75±6.18*	56.25±5.68
Basella alba (L.)	70	24.3±2.16*	135±6.21*	47.91±8.67

Brassica nigra (L.)	72.5	23.0±1.93*	134±3.21*	80.21±3.65*
Calamus totang (L.)-Rhizome	95	26.7±2.28	162.75±8.26*	32.29±8.56
Canarium Zeylanicum (L.)-Bark	75	15.1±1.12*	91.75±6.07*	50±7.32
Cardiospermum halicacabum	25	7.70±1.75*	104.75±2.87*	86.25±1.32
Carum petroselinum (L.)	75	25.0±0.69*	98.25±4.71*	33.33±6.32
Cassia auriculata (L.)	87.5	28.6±3.97	139.75±8.26*	40.62±6.48
Centella asiatica (L.)	80	30.1±2.00	113±10.29*	50.52±5.45
Centrocema vainas (L.)	85	34.3±2.80	154.25±5.05*	44.79±1.56
Clidemia hirta (L.)	80	27.1±3.04	129.25±6.39*	31.25±5.26
Coccinia grandis (L.)	77.5	25.4±2.01	116±10.61*	36.23±1.35
Colocasia esculenta (L.)	85	32.9±1.72	123.5±4.43*	28.12±3.25
Coriandrum sativum (L.)	87.5	26.1±2.14	170.25±7.84*	26.04±7.36
Costus speciosus (L.)	85	29.8±1.02	66.75±12.47*	40.32±3.21
Croton lacciferus (L.)	80	25.1±2.44	73.5±3.87*	47.5±4.25
Dregea volubilis (L.)	85	22.3±1.95*	91.5±7.59*	89.58±6.21*
Elaeocarpus serratus (L.)	85	34.9±4.77	112.02±3.21*	29.36±3.99
Eugenia caryophyllata (L.)	90	37.5±3.51	120.75±6.5*	46.35±7.21
Garcinia mangostana (L.)	95	40.9±1.77	113.89±3.61*	28.12±3.25
Hevea brasiliensis (L.)	100	34.4±1.81	111.02±10.20*	23.02±3.22
Impatience balsamina (L.)	80	33.5±3.69	199±8.60	52.08±5.64
Ipomoea aquatica (L.)	70	34.5±2.74	112±1.63*	55.20±2.35
Ipomoea carica (L.)	80	24.2±2.16*	107.75±2.21*	56.77±4.35
Lactuca sativa (L.)	85	34.8±0.77	163.75±4.64*	30.20±3.24
Languas galanga (L.)-Leaves	80	39.2±2.90	149.5±32.05*	36.45±2.36
Lantana camara (L.)	95	20.0±1.91*	93.75±11.75*	55.20±4.25
Lasia spinosa (L.)	85	36.0±1.87	133±3.82*	29.16±3.21
Lasia spinosa (L.)-*Rhizome*	80	26.4±0.76	91.5±1.29*	39.58±4.32
Manihot esculenta (L.)	80	30.2±1.17	149±6.81*	36.45±4.36
Mentha viridis (L.)	80	30.1±1.78	121.25±8.84*	35.41±1.39
Mikania scandens (L.)	85	19.4±2.33*	112.5±9.14*	41.14±5.36
Michelia champaca (L.)	85	41.1±1.40	101±1.82*	51.51±4.36
Mimosa pudica (L.)	85	26.4±0.76	127.5±10.40*	46.35±2.69
Morinda citrifolia (L.)	90	32.0±3.67	197.75±51.11	29.16±5.36
Murraya koenigii (L.)	82.5	33.1±1.41	114±3.16*	35.41±5.36
Panicum maximum (L.)	80	31.5±1.30	169.75±12.14*	40.62±2.32
Passiflora edulis (L.)	82.5	27.2±2.97	182.25±15.84	51.56±1.39
Polyscias scutellaria (L.)	72.5	10.6±1.29*	63.75±3.58*	83.12±4.36*
Sauropus androgynus (L.)	77.5	26.9±0.90	106.5±5.06*	43.75±6.47
Sesbania grandiflora (L.)	52.5	15.2±2.93*	60.25±3.30*	43.75±2.14
Sida rhombifolia (L.)	85	29.7±2.63	105±9.12*	52.32±4.32
Sphagneticola trilobata (L.)	75	26.3±1.63	198.25±5.31	37.21±4.35
Solanum nigrum (L.)	90	27.6±2.56	124±3.21*	41.14±5.36
Tagete erecta (L.)	90	28.7±1.59	151.5±1.29*	37.5±1.25
Tithonia diversifolia (L.)	85	18.0±1.10*	105.75±3.5*	47.91±2.15
Trema orientalis (L.)	90	32.5±2.33	90.25±14.24*	33.85±3.21
Vernonia cinera (L.)	87.5	33.5±1.37	166.25±7.36*	39.06±2.36
Wedelia trilobata (L.)	70	33.5±2.57	137.75±4.64	35.48±4.32
Distilled Water	90	38.6±0.96	198.75±3.25	
Positive Control (Abscisic acid 10 ppm)	0			

* Plants showed a significant difference compared to distilled water.

Applied plant extracts affected the radicle and hypocotyl lengths of lettuce seedlings. Out of 60-plant extracts, 14-plant extracts (Table 2) significantly reduced the radicle length of seedlings than distilled water. Among them, extracts of *C. zeylanicum*, *P. scutellaria*, *C.halicacabum* and *A. lanata* were most inhibitory to seedling growth (Table 2). *C. halicacabum* and *C. zeylanicum* caused highest reduction (75 %) in seedling length.

Radish seed germination bioassay

Considering the bioassay results of radish seed germination (according to Dunnett's comparison), out of 60-plants, 55-plants except *M. citrifolia*, *Impatiens balsamina*, *A. indica*, *P. edulis and S. trilobata* exhibited a significant reduction in radish seedling growth at 1000 ppm concentration than distilled water (Table 2). In radish seed germination bioassay, one-way ANOVA and Dunnett's test were used to identify significant differences in the seed germination rate, dry weight and fresh and dry weight inhibition after exposure to different plant extracts. Out of 60-plants, 4-plants (*D. volubilis*, *P. scutellaria*, *C. halicacabum* and *B. nigra*) significantly reduced the fresh weight of radish seedlings. While *S. rhombifolia* significantly reduced the dry weight of radish seedlings compared to distilled water. *B. sativa*, *B. alba*, *I. carica*, *D. volubilis*, *P. scutellaria*, *B. sativa and C. halicacabum* showed significant ($P \leq 0.05$) dry weight inhibition in radish. *C. halicacabum* was most inhibitory to dry weight (67 % reduction), while *P. scutellaria*, *D. volubilis and B. nigra* showed 62.5 % dry weight inhibition. The dry-weight inhibition of other plant extracts ranged from 21 % to 58.3 %. In radish germination bioassay, *C. speciosus*, *L. galanga* and A. *sessilis* reduced seed germination than distilled water.

Isolation of compounds from *C. zeylanicum*

The bark of *C. zeylanicum* was successively extracted with organic solvents. Two compounds, compound **1** (20 mg) and compound **2** (100 mg) were isolated from the phytotoxic fraction of dichloromethane extract using chromatographic techniques (Figure 2). Structure elucidation of compounds was done using spectroscopic analysis.

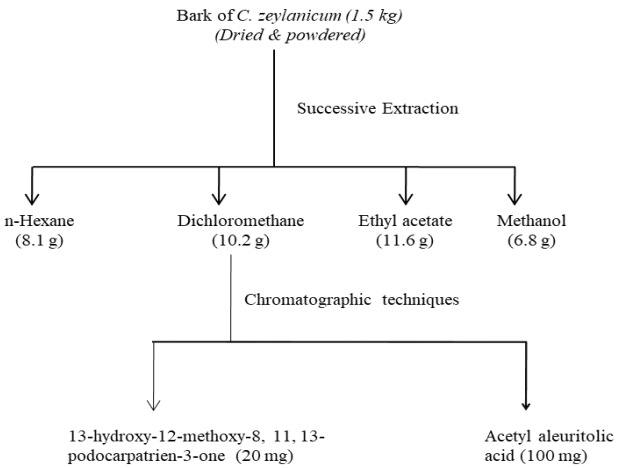


Figure 2. Schematic diagram for solation of compounds

In this study, 60 plants were selected for the initial screening of their allelopathic activity using field observations viz., less naturally occurring weed density than other nearby plant used as green manure, plants used by local farmers for management of pests and weed for invasive plants, plants resistant to decomposition, plants resistant to pest attacks. In our study, indicator plants, including radish and lettuce, were used as they were susceptible to allelochemicals at very low concentrations, cheap and easy to handle in the laboratory (13). The phytotoxicity of these plants was assessed using germination bioassays of radish and lettuce. Out of sixty plant extracts, 14 exhibited a significant reduction in seedling lengths compared to distilled water; this may be due to the presence of allelopathic active constituents. Hence, observations of this study suggest that the aforementioned plants could be utilized as sources of natural herbicides in the future after a detailed evaluation of their allelopathic activity using bioassays and greenhouse experiments followed by field trials.

In consideration of radish seed germination bioassay, according to the results of Dunnett's comparison, out of 60 plants, 55 plants except *M. citrifolia, Impatiens balsamina, A. indica, P. edulis and S. trilobata* were exhibited the significant reduction in radish seedlings growth compared to distilled water. Therefore, it is necessary to re-investigate radish germination bioassay using lower concentrations of plant extracts. The reduction of the fresh weight, dry weight inhibition and rate of germination of radish might be due to the presence of phytotoxic compounds in the plants of D. *volubilis, P. scutellaria, C. halicacabum and B. nigra.*

In the present study, activity guided fractionation was carried out for the extracts of *C. zeylanicum.* Dichloromethane extract of *C. zeylanicum* exhibited the highest phytotoxicity. Further chemical investigation of dichloromethane extract, two compounds was isolated. The major compound **(1)** was isolated as a white powder and recrystallized with methanol and dichloromethane to give white needles of melting point (M.P.) 136-138° C. The ^{1}H NMR spectrum of compound **1** showed 1H singlets at δ_H 6.52 and 6.81 and they could be assigned for two aromatic protons. In the ^{1}H NMR spectrum, methyl groups (3 Nos.) appeared as singlets at δ_H 1.12, 1.15 and 1.26 and one methoxy group appeared at δ_H 3.84 as a singlet. The ^{13}C NMR spectrum gave evidence for 18 carbons in the compound. Analysis of the ^{1}H and ^{13}C NMR, HMQC spectral data provided evidence that compound possessed seven quaternary carbons, one methoxy carbon at δ_C 56.1 and three methyl carbons at δ_C 21.3, 24.1 and 27.1. The chemical shift of carbon at δ_C 217.6 confirmed a carbonyl carbon in the molecule. Further analysis on HMQC and HMBC spectral data elucidated that five of the non-protonated carbon atoms showed at δ_C 145.0, 143.6, 126.6 and 140.4 and two protonated carbon atoms at δ_C 110.8 and 111.5 to be aromatic. Compound **1** was identified as 13-hydroxy-12-methoxy-8, 11, 13-podocarpatrien-3-one by comprehensive spectroscopy analysis of ^{1}H, ^{13}C NMR, HMQC and HMBC as well as the comparison with the literature (5) (Table 3 and Figure 3).

Table 3. **^{1}H** and **^{13}C** NMR and HMBC data of 13-hydroxy-12-methoxy-8, 11, 13-podocarpatrien-3-one in $CDCl_3$

^{1}H (δ)	**^{13}C (δ)**	**HMBC ^{1}H-^{13}C (δ)**	**H/C Assignment**
1.12 (3H, s)	21.3	217.6, 47.5, 27,1	15-Me
1.15 (3H, s)	27.1	217.6, 50.9, 21.3	16-Me
1.26 (3H, s)	24.1	140.4, 50.9	17-Me
1.74 (2H, m)	20.6	50.9, 37.2	6-CH_2
1.86 (1H, m)	50.9	50.9, 47.5, 21.3, 24.1, 27.1,37.2	5-CH
2.38 (1H, m), 1.90 (1H, m)	37.9	217.6, 50.9, 24.1, 37.2	1-CH_2
2.63 (2H, m)	34.9	217.6, 50.9, 37.2	2-CH_2
2.85 (2H, m)	30.8	110.8, 126.6, 140.4, 50.9, 21.3	7-CH_2
3.84 (3H, s)	56.1	145.0, 56.1	-OMe
5.46 (1H, s)		144.1, 111.5	-OH
6.52 (1H, s)	110.8	140.4, 144.1	11-CH
6.81 (1H, s)	111.5	145.0, 126.6, 37.2	14-CH

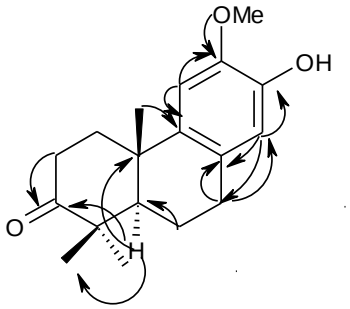
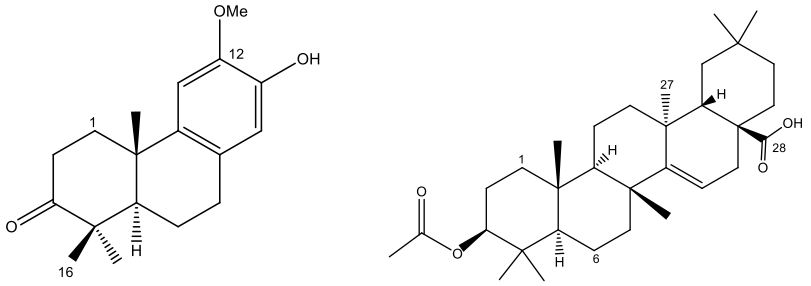


Figure 3. HMBC (Heteronuclear Multiple Bonded Correlation) of compound **1**

Compound **2** was isolated as a white powder and recrystallized with methanol and dichloromethane to give white needles of melting point (M.P.) 288-290°C. The ^{13}C NMR spectrum depicted 32 carbons in the compound. The chemical shift of carbon at δ_C 81.1 suggested that it may be attached to an acetate group. The proton under the above acetyl group appeared at δ_H 4.45. ^{1}H NMR spectrum showed one-olephinic protons appeared at δ_H 5.52 and eight methyl groups at δ_H 0.93, 0.84, 0.94, 0.87 and 0.91 as singlets. Further, a highly shielded methyl group appeared as a singlet at δ_H 2.11 for an acetyl methyl. The chemical shift of carbons at δ_C 184.7 and 171.2 suggested the indication of acid and ester groups respectively. Further, HMQC and HMBC correlation studies (see Table 4) and the direct comparison of literature data revealed the structure of the compound as 3*β*-OAc, 28-COOH and acetyl aleuritolic acid (14).

It is reported that diterpenoid compound **(1),** podocarpane-type diterpenes rarely occur in nature had been previously isolated from *Taiwania cryptomerioides* (5). Also, compound **2** was reported from *Maprounea guianensis*, the plant that is well known to produce triterpenes with cytotoxic activity (14). Further, it is stated that compound **2** showed anticancer properties, significantly inhibiting tumor cell growth. The scratch assay showed that compound **2** (Figure 4) was an efficient migration inhibitor in autophagy induction (24).



(**I**) (**II**)

Figure 4. Structures of 13-hydroxy-12-methoxy-8,11,13-podocarpatrien-3-one (**I**) and acetyl aleuritolic acid (**II**)

Table 4. ^{1}H and ^{13}C NMR and HMBC data of acetyl aleuritolic acid in $CDCl_3$

^{1}H (δ)	**^{13}C (δ)**	**HMBC ^{1}H-^{13}C (δ)**	**H/C Assignment**
1.61	37.6	23.7, 81.1, 39.2, 55.7, 37.9, 16.8	1
1.62	23.7	37.6, 81.1	2
4.45	81.1	171.2, 28.123.7,39.2	3
-	39.2	-	4
0.90	55.7	37.9,18.9, 29.9	5
0.92	18.9	55.7, 29.9, 37.9	6
1.22	29.9	38.1, 18.9, 49.2	7
-	38.1	-	8
1.42	49.2	17.5, 37.9, 16.8	9
-	37.9	-	10
1.02	17.5	49.2, 35.5	11
1.15	35.5	26.4, 37.5, 17.5	12
-	37.5	-	13
-	160.7	-	14
5.51	117.1	37.5, 31.5	15
2.38	31.5	117.1, 160.7, 51.7, 41.5, 184.7	16
-	51.7	-	17
2.23	41.5	51.7	18
1.31	40.9	33.5	19
-	29.5	-	20
1.60	33.5	30.9, 184.7	21
1.61	30.9	22.6,29.5	22
0.87	28.1	15.8, 81.1	23
0.93	16.8	28.1, 55.7	24
0.84	15.8	55.7, 38.1	25
0.94	32.0	160.7, 117.1	26
0.94	22.7	160.135.5	27
-	184.7	-	28
0.93	32.0	33.5	29
0.91	28.8	29.5, 33.5	30
-	171.2	-	-O-CO
2.11	21.5	171.2	-O-$COCH_3$

A bioassay for lettuce seed germination was conducted to investigate the phytotoxic ity of the two chemical compounds. At 1000 ppm, these two chemicals entirely inhibited the germination of lettuce seeds, indicating the highest level of phytotoxicity. A bioassay on the germination of lettuce seeds was conducted using a dilution series of each component ranging from 100 to 1000 ppm to ascertain the minimal inhibitory concentration. The minimum inhibitory concentration values of compounds **1** and **2** were 600 ppm and 500 ppm respectively (Table 5).

Table 5. Minimum inhibitory concentration of the compounds isolated from *C. zeylanicum* on the lettuce seed germination bioassay

Compound	**Minimum Inhibitory Concentration (ppm)**
13-hydroxy-12-methoxy-8, 11, 13-podocarpatrien-3-one (**1**)	600 ppm
Acetyl aleuritolic acid (**2**)	500 ppm

The minimum inhibitory concentration value of the positive control, abscisic acid was less than 0.001 ppm. Therefore, these two compounds have exhibited weak phytotoxicity compared with the positive control. The limitation of this study is that the fractions used to isolate these two compounds showed the highest phytotoxicity and isolated compounds showed weak phytotoxic activity compared to the positive control. This may be due to the synergistic effect of plant extracts. It is reported that promoters of the natural product mixtures used for medicinal purposes frequently claim that these crude mixtures are more effective compared to purified constituents due to beneficial "synergistic" effects (4). As plant extract was comprised of several compounds, we were able to isolate and identify two compounds with the resources available. Unidentified compounds might have higher phytotoxicity compared to the isolated ones. Therefore, it is necessary to re-investigate the active extracts to identify the compound/s with higher activity and their synergistic effect. Also, the crude formulation of this plant could be used for integrated weed management in small-scale agricultural fields.

In this study, *D. volubilis, P. scutellaria, C. halicacabum, C. zeylanicum* and *B. nigra* exhibited remarkable phytotoxicity for the laboratory bioassays. However, further greenhouse experiments and field trials should be carried out to obtain a comprehensive understanding of how these mulching plants behave in the natural environment. It is stated that phytotoxic secondary metabolites act on an array of overlooked herbicide target site (20). New herbicide modes of action are extremely important due to the occurrence of herbicidal-resistant weeds and the need to explore new market niches (20, 28). Therefore, these plant extracts might contain phytotoxic compounds and further chemical investigation should be required for the identification of the respective phytotoxic compounds.

CONCLUSIONS

Allelopathic activity of 60 invasive and medicinal plants were stdied on *Lactuca sativa* (lettuce) and *Raphanus sativus* (radish). Out of 60 plants, 14 significantly reduced lettuce seedling lengths than distilled water. Fifty-five plants except *M. citrifolia, I. balsamina, A. indica, P. edulis, and S. trilobata* reduced radish seedling growth at 1000 ppm concentration over distilled water. *D. volubilis, P. scutellaria, C. halicacabum,*

C. zeylanicum, and *B. nigra* showed remarkable phytotoxic activity against all the parameters examined: fresh weight and dry weight inhibition, rate of germination, and percentage dry weight inhibition. Two weak phytotoxic compounds, 13-hydroxy-12-methoxy-8, 11,13-podocarpatrien-3-one, and acetyl aleuritolic acid, have been identified from C. zeylanicum, followed by activity-guided fractionation, indicating a possibility of missing the active ingredients during the fractionation process. Therefore, it is necessary to re-investigate the active extracts to identify the compounds with higher activity.

ETHICAL STATEMENT

This is to inform you that in this study, we have not been involved in any animal and human studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. All authors agree to publish it.

DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

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