



Article Determination of the Allelopathic Potential of Cambodia's Medicinal Plants Using the Dish Pack Method

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Abstract: Plants produce several chemically diverse bioactive substances that may influence the growth and development of other organisms when released into the environment in a phenomenon called allelopathy. Several of these allelopathic species also have reported medicinal properties. In this study, the potential allelopathic effects of more than a hundred medicinal plants from Cambodia were tested using the dish pack method. The dish pack bioassay method specifically targets volatile allelochemicals. Twenty-five species were found to have significant inhibitory effects on lettuce radicle growth. Eleven different plant families, including Iridaceae (2), Apocynaceae (2), Poaceae (2), Sapindaceae, Araceae, Combretaceae, Orchidaceae, Clusiaceae, Zingiberaceae, Rutaceae and Asparagaceae had the plant species with high inhibitory effects. *Allophyllus serulatus* had the highest growth inhibitory effect on lettuce radicles more than 60%, followed by *Alocasia macrorrhiza*, *Iris pallida*, *Terminalia triptera*, *Wrightia tomentosa*, *Cymbidium aloifolium*, *Garcinia villersiana* and *Kaempferia parviflora*. The candidate species were subjected to further studies to identify the volatile allelochemicals in the volatile constituents.

Keywords: allelopathy; allelochemicals; volatile compounds; dish pack method

1. Introduction

Biodiversity refers to the variety and variability of life on Earth and plays a vital role in ecological functions. The integrative use of plant biodiversity is one approach to improve food security and sustainable agriculture. Species combinations, such as multi-cropping, inter-cropping, alley farming, rotations, and cover cropping, also have positive effects on crop productivity and yield stability [1]. Interaction among plant species may include the production and release of bioactive substances that directly or indirectly influence the growth and development of other organisms in a phenomenon known as allelopathy [2]. The definition was later revised to mean any process involving the secondary metabolites produced by plants, microorganisms, viruses and fungi that influence the growth and development of agricultural and biological systems (excluding animals), including positive and negative effects [3]. The secondary metabolites associated with allelopathy released into the environment through volatilization, leaching, root exudation and the decomposition of plant residues in soil are called allelochemicals. These allelochemicals are found in different parts of various plants, such as leaf, root, rhizome, stem, flower, pollen, fruit



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). and seed [4,5]. Allelopathy may play an important role in the biological invasion process in natural ecology. Some plants are not dominant competitors in their natural habitat, yet show strong succession when introduced to new areas [6]. Generally, allelopathy is accepted as a significant ecological factor in determining the structure and composition of plant communities [7].

Despite recent advances in the development of agrochemicals for pest control in modern agriculture, crop yields experience average losses of 35% worldwide. This is mainly due to pests, pathogens and weeds [8]. Weeds are particularly destructive: approximately 30 to 50% of producing crops are destroyed if weeds are not controlled in Asia and other continents [9–11]. More than 240 weeds have been found to have allelopathic effects on surrounding plants, whether on the same species (autotoxicity) or on other crops and weed species [12]. However, scientists in many different habitats around the world have demonstrated agrochemical pest control. Numerous allelopathic effects from plant species have been reported. For example, 84 out of 245 plant species in the Sino-Japan floristic region have been shown to cause significant inhibitory activity; of these, 10 species showed the strongest effects [13]. The evaluation of the allelopathic potential of 83 Iranian medicinal plants found more than 80% root growth suppression of lettuce by Peganum harmala, Berberis vulgaris, Artemisia aucheri and Ferulago angulate [14]. The evaluation of allelopathic potential in medicinal plant species used in Ghana found that 75 out of 183 medicinal plant species caused a significant inhibition of lettuce radicle growth through leaf leachates [15]. Identified and isolated bioactive compounds (allelochemicals) from plants are therefore important sources for alternative sustainable and eco-friendly weed control strategies [16], especially given that organic products have increased in popularity over the last decade [17]. The secondary metabolites present in medicinal plants are thought to have relatively strong allelopathic activity. Moreover, analyzing medicinal plants to find new natural compounds is easier than analyzing other plants [2,18–20]. Some bioactive substances, including ferulic, coumaric, vanillic, caffeic and chlorogenic acids in medicinal plants have been found to inhibit plant growth [21,22]. By using the sandwich method, the previous study identified more than fifty medicinal plants with allelopathic potentials through leachates [23]. This study, therefore, collected different parts of some medicinal plants from northwestern Cambodia to examine allelopathic effects using the dish pack method under laboratory conditions.

2. Materials and Methods

2.1. Material

All the collected medicinal plant samples were oven-dried at 60 °C for 3 h at target areas and transferred to the Laboratory of the Department of International Environment and Agriculture, Tokyo University of Agriculture and Technology, Japan to test their allelopathic activities. Lettuce (*Lactuca sativa* L.) was used as a test plant material in bioassay due to its reliability in germination and its susceptibility to inhibitory and stimulatory chemicals [24].

2.2. Dish Pack Method

The dish pack method was adopted for the analysis of volatile allelochemicals of plant species. Most importantly, it allowed us to obtain very quick results, as shown in Figure 1 [25]. Therefore, it was applied to screen all collected medicinal plant species with possibly volatile substances that could influence (promote or inhibit) the growth of lettuce. Multi-well plastic dishes with six wells (36 mm \times 18 mm each) were used in this experiment. The distances from the centre of the source well (where the plant sample was placed) to the centre of other wells were 41, 58, 82 and 92 mm. The source well was filled with 200 mg of oven-dried plant materials. Filter papers were laid in the other wells, then 0.75 mL of distilled water was added to each well that contained filter paper. The control treatment did not contain any plant sample in the source well. Seven lettuce seeds were placed on the filter paper in each well. The multi-well dishes were tightly sealed using cellophane tape to avoid desiccation and the loss of volatile compounds. To exclude

light, aluminium foil was wrapped around the dishes and placed in an incubator (NTS Model MI-25S) at 25 °C for three days. With three replications, the radicle and hypocotyl lengths of lettuces were measured and recorded; they were then compared to the lettuce in the control during analysis. The degree of inhibition was estimated by the relationship between lettuce seedling growth inhibition and its distance from the source well.



Figure 1. Dish pack method, multi-well plastic plate filled with plant sample and lettuce seeds in each well to test plant allelopathy through volatile substances.

2.3. Statistic Analysis

The treatment tested was arranged in a complete randomized design with three replicates. Statistical analysis of the experimental data was conducted with Microsoft Excel 2010. The means, standard deviations (SDs), and SD variances (SDVs) were also evaluated.

$$Inhibitory = 100 - \frac{(Average \ length \ of \ treatment \ radicle/hypocotyl)}{(Average \ length \ of \ control \ radicle/hypocotyl)} \times 100.$$

3. Results

The inhibition effects on the radicle and hypocotyl of lettuce seedlings from 195 medicinal plants using the dish pack bioassay method are shown in Table 1. The allelopathic effects of the collected medicinal plants were presented either as the promotion or inhibition of lettuce growth on the radicle and hypocotyl, which ranged from -19.2% to 68.6% and -30.2% to 67.3%, respectively. The negative value for the lettuce radicle growth indicates the stimulatory effects compared to the control. The study found several strong candidate species: 25 species from different plant families showed a significant inhibition of lettuce radicle growth among the tested plants. These species came from 11 different families, including Iridaceae (two), Apocynaceae (two), Poaceae (two), Sapindaceae, Araceae, Combretaceae, Orchidaceae, Clusiaceae, Zingiberaceae, Rutaceae and Asparagaceae. However, only Allophyllus serrulatus inhibited more than 60% on lettuce radicle growth among the tested plants. Radicle growth inhibition in the range of 20–30% occurred in seven species: Alocasia macrorrhiza, Iris pallida, Terminalia triptera, Wrightia tomentosa, Garcinia villersiana, Cymbidium aloifolium and Kaempferia parviflora. Ten further species, Harrisonia perforate, Eleutherine bulbosa, Imperata cylindrica, Peliosanthes teta, Willughbeia edulis, Eleusine indica, Spatholobus parviflorous, Asplenium nidus, Drynaria quercifolia and Croton oblongifolius demonstrated lettuce radicle inhibitory effects of between 15 and 20%. The lowest effects on lettuce radicle growth were Kaempferia galanga, Afzelia xylocarpa, Zingiber purpureum, Careya sphaerica, Congea tomentosa, Pseuderanthemum latifolium and Ventilago cristata.

	Plant Families	Part Used					
Scientific Name			Average at 41 mm		Average for Whole Wells		Criteria
			R	Н	R	Н	-
Allophyllus serrulatus Radlk	Sapindaceae	Leaf	68.6	67.3	63.1	63.1	***
Alocasia macrorrhiza (L.) G.Don	Araceae	Tuber	23.4	-5.22	20.5	-5.62	***
<i>Iris pallida</i> Lam.	Iridaceae	Rhizome	22.1	6.71	14.3	7.34	***
<i>Terminalia triptera</i> Stap f	Combretaceae	Stem	21.3	9.22	13.7	-1.82	**
Wrightia tomentosa Roem-Schult	Apocynaceae	Stem	21.1	9	15.9	2.12	**
Garcinia villersiana Pierre	Clusiaceae	Stem	20.4	1.32	16.2	2.11	**
Cymbidium aloifolium (Linn) Swartz.	Orchidaceae	Leaf	20.4	14.2	10.7	12.4	**
Kaempferia parviflora Wall. ex Baker	Zingiberaceae	Rhizome	20.2	8.11	8.72	4.12	**
Harrisonia perforata Merr.	Rutaceae	Bark	19.8	-4.72	-0.92	-4.53	**
Eleutherine bulbosa (Mill.) Urb.	Iridaceae	Flower	18.7	2.42	16.7	5.62	**
Imperata cylindrica Beauv	Poaceae	Leaf	18.2	15.2	12.4	9.91	**
Peliosanthes teta Andrew	Asparagaceae	Leaf	17.4	13.4	-0.54	6.32	**
Willughbeia edulis Roxb.	Apocynaceae	Stem	17.2	9.2	8.92	1.83	**
Eleusine indica (L) Gaertn	Poaceae	Leaf	17	1.34	13.9	7.32	**
Spatholobus parviflorous Kuntz.	Fabaceae	Stem	16.4	6.92	10.9	0.81	*
Asplenium nidus L.	Aspleniaceae	Leaf	15.7	4.77	9.14	7.42	*
Drynaria quercifolia (L.) J Sm	Polypodiaceae	Leaf	15.3	-5.92	9.12	-5.91	*
Croton oblongifolius Roxb.	Euphorbiaceae	Leaf	15.2	7.6	9.32	6.63	*
Kaempferia galanga Linn.	Zingiberaceae	Rhizome	14.8	17.4	8.41	11.2	*
Afzelia xylocarpa (Kurz) Craib.	Fabaceae	Bark	14.6	-8.9	7.72	-5.31	*
Zingiber purpureum Roscoe.	Zingiberaceae	Rhizome	13.9	19.5	4.62	11.6	*
Careya sphaerica Roxb.	Lecythidaceae	Bark	12.7	-3.12	13.4	3.12	*
Congea tomentosa Roxb.	Lamiaceae	Stem	12.4	21.8	0	12.1	*
Pseuderanthemum latifolium (Vahl) B. Hansen	Acanthaceae	Leat	12.3	1.4	2.33	-3.74	*
Ventilago cristata Pierre	Rhamnaceae	Stem	12.3	0	17.6	1.82	*
Sterculia foetida Linn	Sterculiaceae	Stem	11.5	-28.4	5.07	-19.1	
Croton lachnocarpus Benth.	Euphorbiaceae	Leat	11.4	-5.21	19.1	4.31	
Zingiber ottensii Valeton	Zingiberaceae	Rhizome	11.4	57.1	-0.92	41.6	
Ervatamia microphylla Kerr.	Apocynaceae	Leaf	11.3	8.8	5.61	1.82	
Vitex pubescens Vahl.	Lamiaceae	Stem	11.1	12.2	5.12	8.93	
Morinda tomentosa Koth	Rubiaceae	Stem	11	11.5	6.2	10.9	
Hoya diversifolia Blume	Asclepiadaceae	Leaf	10.1	2.7	9.12	4.71	
<i>Uvaria rufa</i> Blume	Annonaceae	Stem	9.8	15.1	-1.93	7.74	
Scoparia dulcis L	Plantaginaceae	Stem	9.42	6.94	4.12	0	
Polyaltnia evecta (Pierre) Finet et Gagnep.	Annonaceae	Stem	9.23	-4.43	7.04	-2.21	
Litchi chinensis Sonn	Sapindaceae	Bark	9.04	-7.71	2.82	-5.91	
Artocurpus rigiuus biume	Moraceae	Dark	8.71	6.72	5.22	0.93	
Kalanchoe Integra Kuntze.	Crassulaceae	Stem	8.7	-0.94	9	2.24	
Hymenocurata punctata Wall. ex Linal.	Euphorbiaceae	Stem	8.4Z	1.15	3.07	4.12	
Zizypnus camboatana Pierre	Rhamhaceae	Stem	8.33 8.31	3.63	4.62	-2.21	
Copiosuperiu judescens Korin.	Calmana	Stem	0.21	2.72	0.74	-2.91	
Connu integerrima (Lour) Merr.	Zingiharagaaa	Stem	8.14 8.04	7.31	-1.43	2.72	
Curcumu uromuticu Salisb.	Eabaaaaa	Paul	0.04 8.02	2.55	2.91	-0.62	
Sunaada multiflora Boill	Fabaceae	Stom	0.02 8	-0.22	0.52	1.82	
Diagnumas sugaras Mall	Euphorbiaceae	Stem	7 26	-1.04	9.01	-1.65	
Diospyros venosu vvali.	Cristaceae	Stem	7.20	14.1	2.12	2.91	
Knowa alabularia Warb	Muristicaceae	Stem	7.23	-2.0	-4.04	-3.3	
Sterkania rotunda Linn	Monispormassa	Tubor	7.14	-7.12	3.52	-4.5 10.2	
Microsco naniculata I	Malvassas	Storm	7.13	6.02	2.22	10.2	
Costus anaciosus (Koonig) LE Smith	Costaceae	Reat	7.1	0.93	5.52	0.71	
Amomum xanthioidae Wall	Zingiharagaaa	Storm	67	4.74	6 21	4.12	
Amomum xuninoues wan.	Bignoniaceae	Bark	6.62	0.4	0.21	4.61	
Doudran naroracilis (Bourd) Pidadala	Pubiaceae	Storm	6.32	11.0	2.12	1.01	
Rombar caiba I	Malyaceae	Bark	6.32	7 75	20.1	-1.82	
Donar grandis Ridlov	Popcopo	Stom	5.94	11.2	20.1	6.81	
Carallia brachiata (Lour) Merr	Rhizophoraceae	Bark	5.86	4 54	8 54	11 1	
Pouzolzia zeulanica (I.) Ronn	Urticaceae	Stem	5.80	-942	4 61	_6 32	
Comphrena celosioides Mart	Amaranthaceae	Flower	5.62	_3.42	-3.71	11	
Crinum Jatifalium I	Amaryllidaceae	Bulb	5.02	-0.01 8.61	-3.71	2 73	
Struchnos voallichiana Stoud Ex DC	Loganiaceae	Stom	5.40	7 22	_1 21	2.73	
Melastoma zillooum I	Molastomatacoac	Stom	5.33	_8 73	-1.41 7.01	_7.64	
Irora chinensis I am	Rubiaceae	Loof	5.33	-0.75 -114	2.12	-7.0 4 2.21	
Eunatorium odoratum (I) R M King & H Pob	Asteraceae	Leat	5.17	-1.14 20	4 72	12.21	
ENDATION THE CONTRACT OF A DESCRIPTION O	maicrattat	LCAI	J.1	<u> 4</u> 0	7./4	14.4	

Table 1. The radicle and hypocotyl inhibition percentages of lettuce seedlings grown using the dish pack method.

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	Plant Families	Part Used					
Scientific Name			Average at 41 mm		Average for Whole Wells		_ Criteria
			R	Н	R	Н	-
Dipterocarpus tuberculatus Roxb	Dipterocarpaceae	Stem	4.88	-5.72	3.91	-1.82	
Licuala spinosa Wurmb	Arecaceae	Root	4.84	-30.2	0.23	-15.2	
Nepenthes kampotiana Lecomte	Nepenthaceae	Flower	4.83	-10.6	3.12	-11.1	
Smilax ovalifolia Roxb.	Smilacaceae	Stem	4.8	14.8	-0.42	3.64	
Cnestis palala (Lour.) Merr.	Connaraceae	Leaf	4.67	7.72	-4.51	-0.92	
Smilax china L.	Smilacaceae	Stem	4.65	13.1	1.62	11.2	
Dillenia pentagyna Roxb	Dilleniaceae	Stem	4.58	-12.7	1.21	-7.62	
Gonocaryum lobianum (Miers) Kurz	Icacinaceae	Stem	4.55	-13.8	2.23	-6.74	
Physalis angulata L.	Solanaceae	Root	4.52	0.82	2.71	7.42	
Irvingia malayana Olive. Ex Benn.	Irvingiaceae	Bark	4.42	-4.62	6.12	0.4	
Dioscorea hispida Dennst.	Dioscoreaceae	Tuber	4.16	8.44	0	7.82	
Lagerstroemia calyculata Kurz.	Lythraceae	Bark	4	3.81	7.24	6.34	
Syzygium polyanthum (Wight) Walp.	Myrtaceae	Bark	3.9	5.64	-4.93	-0.91	
Streblus asper Lour.	Moraceae	Stem	3.81	-6.72	4	-2.23	
<i>Tinospora crispa</i> (Linn) Miers ex Hook.	Menispermaceae	Stem	3.77	-3.13	-30.9	0	
Anthocephalus chinensis (Lam.)	Kubiaceae	Bark	3.46	-1.53	7.61	-0.72	
Borassus flabellifera Linn	Arecaceae	Root	3.42	-7.31	7.21	-2.81	
Cassia alata L.	Fabaceae	Stem	3.28	-9.2	-6.32	-0.91	
Totracora condena (L.) Morr	Dilloniaceae	Dark	3.23	-2.12	11.0	10.3 E 01	
Lucedium Genuers (L.) Merr.	Dilleniaceae	Lear	2.83	4.34	3.62	5.91	
Lygoutum flexuosum (L.) Svv.	Lygodiaceae	Lear	2.62	-4.52	∠ 7 52	-4.12	
Biumea daisumifera DC.	Asteraceae	Lear	2.41	19.6	10.2	14.3	
Diospyros uccunuru Lour Paukinia haccacencie Pierro	Ebenaceae	Storm	2.37	-4.1 10.7	0.72	5.91	
Claradandrum cehmidtii C B Clarko	Lamiacoao	Stem	2.12	7.74	9.73	0.42	
Elaocarnus stimularis Blumo	Flagocarpaceae	Stom	1.97	167	-4.11	-11.3	
Mamaculon laggigalum Blumo	Molastomatacoao	Stom	1.92	3 42	4.02	6.43	
Illigera rhodantha Hance	Hernandiaceae	Stem	1.72	_7.62	_2	-6.73	
Phullanthus amarus Schum et Thonn	Phyllanthaceae	Stem	1.50	1 12	12.2	4 91	
Ficus hispida L	Moraceae	Stem	1.00	8.81	-0.21	9	
Ancistrocladus tectorius (Lour.) Merr.	Ancistrocladaceae	Stem	1.18	15.1	-0.54	10.2	
Moringa oleifera Lamk	Moringaceae	Bark	1.16	-2.92	-1.21	-0.83	
Melodorum fruticosum Lour	Annonaceae	Stem	1.15	-1.65	-0.32	2.7	
Peltophorum dasurhachis (Mig.) Kurz	Fabaceae	Bark	1.12	11.7	-9.12	7.31	
Prismatomeris tetrandra (Roxb.) K.Schum	Rubiaceae	Stem	1.1	-4.91	7.9	-8.12	
Dipterocarpus obtusifolius Teijsmex-Mig	Dipterocarpaceae	Stem	0.82	4.52	-3.21	-0.93	
Macaranga triloba (Blume) Muell.Arg.	Euphorbiaceae	Stem	0.76	0.23	4.12	-1.81	
Typhonium trilobatum Schott	Araceae	Stem	0.65	-2.83	-4.31	-6.42	
Scindapsus officinalis (Roxb.) Schott	Araceae	Stem	0.54	4.81	-2.12	1.31	
Erythroxylum cambodianum Pierre	Erythroxylaceae	Stem	0.54	-2.54	-1.63	0.52	
Spirolobium cambodianum Baill.	Apocynaceae	Stem	0.52	-1.22	1	-5.24	
, Caesalpinia sappan Linn.	Fabaceae	Bark	0.37	16.3	-0.52	9.73	
Melastoma mormale (Kuntze) Merr.	Melastomataceae	Stem	0.33	2.32	1	11.9	
Heliotropium indicum L.	Boraginaceae	Leaf	0.22	-9.44	-2.81	-8.82	
Eurycoma longifolia Jack	Simaroubaceae	Bark	0.17	4.51	-2.72	3.31	
Shorea roxburgii G Don	Dipterocarpaceae	Bark	0.15	3.21	-0.71	1.82	
Plumbago zeylanica L.	Plumbaginaceae	Stem	0	2.84	-2.24	3.22	
Rauwenhoffia siamensis Scheff	Annonaceae	Stem	-0.1	-9.42	-7.2	-8.21	
Scheffera elliptaca (Blume) Harms.	Araliaceae	Stem	-0.22	5.35	1.61	4.52	
Manilkara hexandra (Roxb.) Dubard	Sapotaceae	Leaf	-0.25	0.2	-1.72	2.1	
Senna siamea Lam	Fabaceae	Leaf	-0.27	-18.1	-2.71	-14.2	
Fagraea fragrans Roxb.	Loganiaceae	Stem	-0.63	14.4	3.31	10.5	
Dracaena loureiri (Gagnep.)	Asparagaceae	Bark	-0.65	12.5	1.21	11.1	
Couroupita guianensis Aubert	Lecythidaceae	Flower	-1.42	-7.12	-3.9	1.4	
Cleistanthus tomentosus Hance	Euphorbiaceae	Stem	-1.45	-8.05	0.92	-4.12	
Albizia lebbek (L.) Benth.	Mimosaceae	Stem	-1.57	13.4	-5.81	13.6	
Fhyllanthus emblica L.	Euphorbiaceae	Stem	-1.63	-9.12	-0.52	-8.53	
Alpinia conchigera Grulf	Zingiberaceae	Leaf	-1.72	-0.2	4.14	0.91	
Ficus sagitta Vahl.	Moraceae	Leaf	-1.94	-15.5	-0.52	-6.31	
Derris scandens (Roxb.) Benth.	Fabaceae	Stem	-2	-4.13	8.62	-0.91	
Andrographis paniculata (Burm.f.)	Acanthaceae	Leaf	-2.14	1.1	9.9	-1.31	
Pandanus capusii Marc	Pandanaceae	Root	-2.33	-13.4	-8	-15.3	
Streptocaulon juventas Merr.	Apocynaceae	Stem	-2.37	3.31	0.22	3.83	
Dioscorea bulbifera L.	Discoreaceae	luber	-2.52	0.44	-1.41	-2.52	

 Table 1. Cont.

Scientific Name	Plant Families	Part Used	Inhibition Activity (%)				
			Average	at 41 mm	Average for Whole Wells		- Criteria
			R	Н	R	Н	-
Gnetum latifolium Blume	Gnetaceae	Stem	-2.55	4.73	-7.41	-0.44	
Entada phaseoloides Merr.	Fabaceae	Fruit	-2.6	3.24	-4.51	-7.23	
Mallotus paniculatus (Lam.) Mull.Arg.	Euphorbiaceae	Stem	-2.64	-4.6	-7.92	-2.92	
Schleicheria oleosa (Lour.) Oken.	Sapindaceae	Stem	-2.8	0.5	-11.2	-1.91	
Elephantopus scaber L.	Asteraceae	Leaf	-3	-13.3	-4.12	-11.6	
Solanum torvum Swartz	Solanaceae	Stem	-3.11	0	5.31	2.11	
<i>Glycosmis pentaphylla</i> (Retz) Correa	Rutaceae	Stem	-3.24	-7.32	-2.53	-3.81	
Acalypha boehmerioides Mig.	Euphorbiaceae	Leaf	-3.41	-10.2	-6.12	-11.4	
Lagerstroemia floribunda Jack.	Lvthraceae	Bark	-3.57	-5.4	-4.31	-4.9	
Micromelum falcatum (Lour.) Tanak	Rutaceae	Stem	-3.58	-10.4	-6.33	-10.4	
Ficus beniamina L.	Moraceae	Stem	-4.1	-3	3.7	6.12	
Hydnophytum formicarium Jack	Rubiaceae	Tuber	-4.12	9.53	-6.04	6.6	
Capparis micracantha DC.	Capparaceae	Stem	-4.21	8.34	0.23	6.92	
Terminalia corticosa Pierre.	Combretaceae	Bark	-4.24	2.83	-11.8	-3.72	
Pteridium aauilinum (L) Kuhm.	Dennstaedtiaceae	Leaf	-4.45	2.62	-5.61	5.32	
Sida rhombifolia L.	Malvaceae	Root	-4.48	-12.9	-5.4	-7.81	
Cananga latifolia Finet et Gagnen	Annonaceae	Stem	-4 51	12	-1	3.31	
Parinari anamensis Hance	Chrysobalanaceae	Bark	-4.56	-1.64	0.2	-0.92	
Gardenia nhilastrei Pierre-ex-Pit	Rubiaceae	Stem	-4 72	-4.21	-6.91	-13.4	
Parameria laevigata (Juss.) Moldenke	Apocynaceae	Bark	-474	-0.72	-7.52	-3.21	
Alstonia scholaris R-Br	Apocynaceae	Bark	-51	3 37	-5.91	0.9	
Tiliacora triandra Diels	Menispermaceae	Stem	-5.15	-3.83	-3.81	-3.34	
Dracaena angustifolia Roxb	Asparagaceae	Leaf	-53	2 65	-6.74	-1.06	
Holarrhena curtisii King & Gamble	Apocynaceae	Leaf	-5 35	_1 44	-7.23	0	
Paraharium micranthum (A DC) Pierre	Apocynaceae	Leaf	-5.41	-4.82	-1.72	-6.51	
Dialium cochinchinanse Pierro	Fabaceae	Bark	-5 71	13.3	_7 72	5.43	
Jasminum nobile C B Clarke	Oleaceae	Stom	-5.78	_14.3	_2.91	_2 7	
Melaleuca cajunuti Powell	Murtaceae	Leaf	-5 79	_4.84	1 31	0.32	
Humenodictuon excelsum (Roxh) w	Rubiaceae	Leaf	-5.8	-711	3 11	-2.81	
Derris elliptica (Wall) Bopth	Fabaceae	Stom	6.21	772	1.62	-2.01	
Leeg ruhra Bl	Vitaceae	Stom	6.23	832	-1.02	13.8	
Rhodomurtus tomentosa (Ait) Hossk	Murtaceae	Loof	6.25	14.2	-2.11	6 31	
Pruga japanica (Lipp) Morr	Simaroubaceae	Stom	-0.20	8 22	-0.00	10.1	
Mimoca nudica Linn	Eabaceae	Loof	-0.31	-6.33	-4.81	-10.1	
Ivitnosu puuttu Liitti.	Ivgodiaceae	Leaf	-0.0	-5.04	0.72	=0.7	
Lygouium conforme C. Chr.	Bubiaceae	Lear	-7.4	3.4Z	1.41	0.0	
Autitu coratjotta FIOK. F	Theresele	Deet	-7.3	2.01	-10.7	0	
Aquitaria crassna Pierr.	Inymeleaceae	Koot	-7.52	-5.62	-0.92	-2.71	
Ficus pumila L.	Moraceae	Lear	-7.55	12.2	-5.72	1.71	
Coloriu terrestris (L.) Fassett	Cyperaceae	Lear	-7.72	-25.6	-2.61	-15.7	
Viennes rutentum Lour.	Dubiner	Deal	-7.62	-12.1	-0.8	-4.04	
Neonauciea sessilifolia (Roxb.)Merr.	Kubiaceae	Bark	-7.9	8.93	-4.3	-2.51	
Broussonetia papyrijera (L.) L'Her. ex vent.	Urticaceae	Stem	-7.9	-1.9	-10.1	-3.2	
Diospyros nitida Merr.	Ebenaceae	Stem	-8	0.92	-3	3.81	+
Zizypnus oentplui Mill	Knamnaceae	Stem	-8.12	-8.42	-9.08	-5.62	+
Cyclea barbata Miers	Menispermaceae	Lear	-8.41	-5.95	-9.11	-3.63	+
Dillenia ovata vvall. ex Hook.f.	Dilleniaceae	Bark	-8.44	-6.14	-9.8	-9.43	+
Homonoia riparia Lour.	Euphorbiaceae	Bark	-8.49	8.34	-6.32	2.31	+
Colona auriculata (Desv.) Craib	Tiliaceae	Stem	-9.71	-6.08	-11.5	-8.6	+
Mussaenda cambodiana Pirrl ex Pit	Rubiaceae	Stem	-9.77	-0.62	-10.6	-6.83	+
Pandanus tectorius Parkinson ex Du Roi	Pandanaceae	Leaf	-10	-8.34	-7.05	-10.1	+
<i>Cyperus rotundus</i> Linn.	Cyperaceae	Leat	-10.3	-19.2	-6.91	-15.7	+
Aganosma marginata G. Don	Apocynaceae	Stem	-10.7	-0.92	-15.2	2.61	+
Mesua ferrea L	Calophyllaceae	Leat	-10.7	12.5	-10.2	5.92	+
Lindernia crustacea (L.) F.Muell.	Linderniaceae	Stem	-10.9	5.63	-1.44	7.82	+
Zanthoxylum rhetsa DC.	Rutaceae	Bark	-11.4	9.56	-11.4	-7.11	+
Walsura villosa Wall. Ex Hiern.	Meliaceae	Bark	-12.4	-5.24	-9.71	-1.32	+
Acacia harmandiana (Pierre) Gagnep.	Fabaceae	Bark	-12.5	-13.1	-15.7	-4.1	++
Ampelocissus matinii Planch	Vitaceae	Stem	-13.1	0.52	-9.91	-0.92	++
Euphorbia hirta Linn.	Euphorbiaceae	Leaf	-13.3	-20.8	-13.5	-15.7	++
Madhuca butyrospermoides A.Chev.	Sapotaceae	Bark	-13.3	-2.1	-7.94	-1.44	++

 Table 1. Cont.

					Criteria			
Scientific Name	Plant Families	Part Used	Average at 41 mm			Average for Whole Wells		
			R	Н	R	Н	-	
	Millingtonia hortensis Linn	Bignoniaceae	Stem	-13.4	-5.42	-9.71	2.23	++
	Phyllanthus reticulatus Poir	Euphorbiaceae	Stem	-14.1	-14.1	-10.5	-12.9	++
	Randia tomentosa Bl.	Rubiaceae	Stem	-15.4	-3.61	-12.4	1.41	++
	Anacardium occidentale Linn	Anacardiaceae	Bark	-15.9	-14.3	-10.1	-6.74	++
	Salacia chinensis Linn.	Celastraceae	Stem	-15.9	2.54	-12.8	6.72	++
	<i>Ficus hirta</i> Vahl var roxburghii (Miq).	Moraceae	Stem	-16	-1.72	-12.8	-1.33	++
	Sterculia lychnophora Hance	Sterculiaceae	Stem	-19.2	-10.2	-12.1	-0.42	+++

Table 1. Cont.

Note: Criteria (*), (**) and (***) refer to radicle elongation shorter than the mean value plus 1.0(SD), 1.5(SD) and 2(SD)—that is, SDV = 12, 17 and 22, respectively. + Criteria (+), (++) and (+++) refer to radicle elongation longer than the mean value minus 1.0(SD), 1.5(SD) and 2(SD)—that is, SDV = -8, -13 and -18, respectively.

4. Discussion

In the Sapindaceae plant family, *Allophylus serratus*, a large shrub found all over India, showed a stronger inhibition activity through volatile compounds than *Litchi chinensis* and *Schleicheria oleosa*. *Allophylus serratus* is used as an anti-inflammatory and carminative due to its strong pharmacological activity. This plant is also used to treat numerous medical conditions, such as elephantiasis, oedema and bone fractures, as well as several gastrointestinal disorders, including dyspepsia, anorexia and diarrhea [26]. Bioactive substances contained in *Allophylus serratus* include phenolic compounds, flavonoids, tanning substances, steroids, alkaloids and saponins were reported [27]. Other compounds isolated from *Allophylus serratus*, such as quercetin, pinitol, luteolin-7-O- β -D-glucopy-ranoside, rutin and apigenin-4-O- β -D-glucoside. However, only rutin showed an increase in osteoblast mineralization, as assessed by alizarin extraction; its use has been suggested for menopausal osteoporosis [28].

Another interesting species is *Alocasia macrorrhiza* (common name Elephant Ear Taro), a giant plant with distinctive leaves, which is mostly used for ornamental purposes and belongs to the Araceae family [29]. Elephant Ear Taro is a massive herb formed by a thick erect trunk in large plants and up to 4 m in height; its leaves are held erect with petioles (leaf stalks) that are up to 130 cm long [30]. It has antifungal, antidiuretic, laxative, antitubercular and antioxidant properties; it also features other compounds such as flavonoids, oxalic acid, cyanogenic glycosides, alocasin, cholesterol, amino acids, gallic acid, malic acid, ascorbic acid, succinic acid, glucose, fructose, sucrose and beta-lectins [31]. Additionally, 14 compounds have been isolated and identified from giant taro, including 5 new lignan amides, 1 new monoindole alkaloid and 8 known compounds [32].

Iris pallida from the Iridaceae family also showed potential inhibitory effects. *Iris* contains up to 80 genera and 300 species that are distributed worldwide; it is abundant and diversified in the regions of Southern Africa and Asia. Many of these species are common ornamental plants [33]. *Iris pallida*, known as the sweet iris, is a perennial herb native to the Dalmatian coast, Croatia; it is mostly cultivated for its essential oils and use in aromatherapy and traditional medicine [34,35]. The rhizomes of *Iris pallida* found to have strong allelopathic activity contain the isoflavones irigenin, iristectorigenin A, nigericin, nigricanin, irisflorentin, iriskumaonin methyl ether, irilone, iriflogenin and others [23,36–39]. In total, 16 and 26 volatile components were found from the essential oil of rhizomes and leaves, respectively. Dihydro- β -irone, α -irone, trans-2,6- γ -irone, β -isometilionone; benzophenone and other dominant terpenes, including 4-isobutylphenone, benzophenone, hexahydrofarnesyl acetone, neofitadien and squalene were also reported [40]. The bioactive substances, including irones in *iris* rhizomes could offer commercial potential in the form of iris essential oil [41].

In the Rutaceae family, *Harrisonia perforata* Merr, known as a prickly shrub, is native to China but widely distributed across Southeast Asia. This plant is nearly upright, growing up to 6 m tall. Several parts of this prickly shrub are gathered from the wild and used locally

as medicines to treat some diseases, such as dysentery and cholera, and to relieve itching. It is also reported that its root when dried contains antipyretic and anti-inflammatory properties that are used to deal with wound healing and diarrhea [42]. The leaves, fruits, branches and roots of *Harrisonia perforate* have been reported to contain several chromones, limonoids, triterpenoids and prenylated polyketides, including harrisotone A–E, haperforine A, haperforine E, 12-desacetylhap-erforine A, haperforine C2, haperforine F, haperforine G, Foritin, harrisonol A, peucenin-7-methyl ether, *O*-methyla-lloptaeroxylin, perforatic acid, eugenin, saikochromone A, greveichromenol and perforamone A–D [43]; β -sitosterol, obacunone, herteropeucenin-7-methyl ether, perforatic acid and harrisonin [44–47]; and harperforatin, harperfolide and harperamone [48].

5. Conclusions

This study presents a preliminary analysis of the potential volatile allelopathic effects of some medicinal plants in Cambodia. The revealed data could help future researchers to isolate and identify volatile allelochemicals to demonstrate bio-herbicides for sustainable weed control. *Allophyllus serrulatus*, which showed the highest inhibitory effect, was recommended for the further identification and characterization of allelopathic substances.

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