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Evaluation of Allelopathic Potentials from Medicinal Plant Species in Phnom Kulen National Park, Cambodia by the Sandwich Method

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Abstract: Phnom Kulen National Park, in north-western Cambodia, has huge richness in biodiversity and medicinal value. One hundred and ninety-five (195) medicinal plant species were collected from the national park to examine allelopathic potentials by using the sandwich method, a specific bioassay for the evaluation of leachates from plants. The study found 58 out of 195 medicinal plant species showed significant inhibitory effects on lettuce radicle elongation as evaluated by standard deviation variance based on the normal distribution. Three species including *Iris pallida* (4% of control), *Parabarium micranthum* (7.5% of control), and *Peliosanthes teta* (8.2% of control) showed strong inhibition of lettuce radicle elongation less than 10% of the control. The results presented could present as a benchmark for isolation and identification of allelochemicals among medicinal plants used in Cambodia.

Keywords: allelopathy; allelochemicals; leachates; sandwich method

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1. Introduction

Plant species in the natural diversity have been used by humans to treat numerous diseases worldwide. The various modes of medicinal plant use associated with traditional knowledge were found in different ways in different regions [1]. Hundreds of species have been used for curing various diseases such as fever, malaria, cough, flu, asthma, colds, chest diseases, skin itch, acne, headache, jaundice, nausea, ulcer, tumours, typhus, stomach pain, heart attack, chills, inflammation, herpes, hepatitis, swelling, and among others. [2]. Over the last three decades, no less than 80% of people worldwide relied on medicinal plants for primary healthcare and other factors [3]. Medicinal plants are a significant source of bioactive substances in the development of most drugs [4,5]. In the natural ecology, bioactive phytochemical constituents include alkaloids, tannins, flavonoids and some other phenolic compounds present in medicinal plants that produce a definite physiological action effect either on humans, animals, and other plants [6]. Interestingly, a wide range of these secondary metabolites was reported to have strong relativity in allelopathic activity [7]. Some bioactive compounds contained in medicinal plants including ferulic, coumaric, vanillic, caffeic and chlorogenic acids in medicinal plants were found to possess plant growth inhibitory effect [8,9]. The term allelopathy was introduced by Molisch in 1937, referring to a phenomenon observed in many plants that influence the physiological process of neighbouring plants and or organisms, interacting through secondary metabolites [10,11]. In this process, chemicals—called allelochemicals—are released from plants that impose allelopathic influences (stimulatory or inhibition) into the environment through volatilization, leaching, root exudation and decomposition of plant residues in soil [12]. Allelopathic substances from either specialized or varying amounts of different plant organs are consisted in a vast array of seemingly disconnected structures and possess different modes of action which are mostly interpreted in ecology as a defence against other plants, pests, or diseases [13,14]. Allelochemicals can also stimulate or inhibit the germination, growth, and development of plants [15,16]. The incorporation of allelopathic substances released from plant residues was introduced to reduce the use of synthetic herbicides which were reported to harmful to human health and to cause environmental deterioration [17–19]. Consequently, allelopathic potentials of medicinal plant species were suggested as a practical option for sustainable weed management [20–22]. A previous study linked the allelopathic potential of medicinal plants to the medicinal values (relative frequency of citation, fidelity level, and use values) of plants [23]. Research have focused much attention on the search for novel natural plant products to promote sustainable agriculture. This study, therefore, focused on medicinal plants in Phnom Kulen National Park, a region known for its cultural and medicinal value, in north-western Cambodia. The national park named from a lychee tree species (*Litchi chinensis*), elevated up to 500 m and covering 37,373 ha, was expected to have around 1500 plant species. However, only 500 species were currently recorded in taxonomy among 775 known plant species [24]. It is also believed that the medicinal value from this area is likely different from other regions in Cambodia, and it is home to 389 medicinal plant species associated with traditional knowledge that has been elucidated by the School for Field Studies in 2017 [25,26]. One hundred and ninety-five medicinal plant species belonging to 81 different families were collected from the national park to evaluate allelopathic potentials by using the sandwich method.

2. Materials and Methods

2.1. Material

The parts used of the medicinal plant species were collected and dried up (oven oven-dried at 60 °C for 3 hours) at the target area before being transferred for testing at the Laboratory of Department of International Environment and Agriculture, Tokyo University of Agriculture and Technology, Japan. The various plant parts collected for this study were leaves, stems, barks, bulbs, rhizomes, tubers, roots, flowers and fruits. Lettuce *(Lactuca sativa L.)* was selected as a test plant material in the bioassay due to its reliability in germination and its susceptibility to inhibitory and stimulatory chemicals [27].

2.2. Sandwich Method

The sandwich method was introduced as a very useful tool for large scale allelopathic activity screening of plant leachates [28]. Multi-dish plastic plates were used as shown in Figure 1. Agar without plant material was set up as the untreated control. After lettuce seeding in each well, the multi-dish plastic plates were sealed with plastic tape, marked with a corresponding label and kept in an incubator (NTS Model MI-25S) at 25°C for three days. With three replication treatments, the germination percentage of the lettuce seedlings were measured and recorded including the mean of radicle and hypocotyl growth.

2.3. Statistical 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. And the means, standard deviation (SD), and SD variance (SDV) were also evaluated.

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

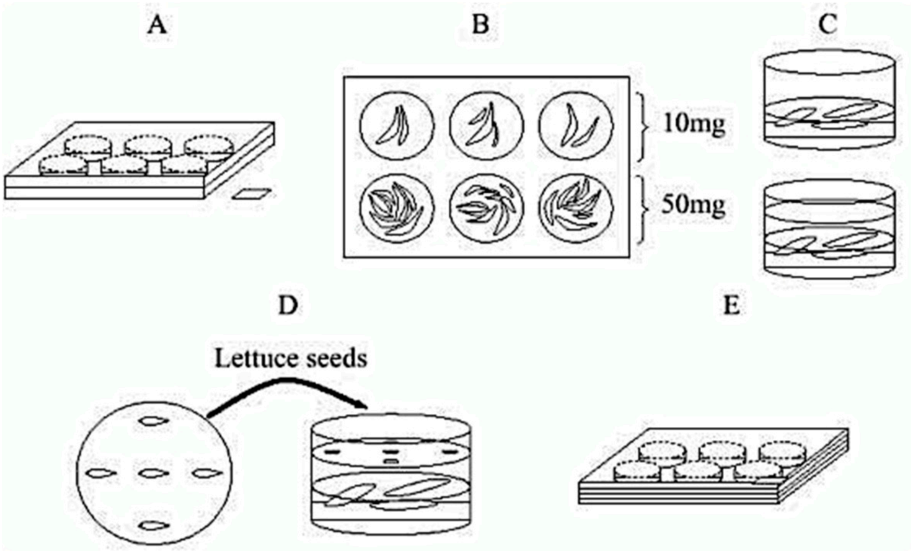

Figure 1. Sandwich method: (**A**) six-well multi-dish plastic plate; (**B**) 10 or 50 mg dried leaves placed in each well of the multi-dish plate; (**C**) addition of 5 mL plus 5 mL agar in two layers on the dried leaves; (**D**) five lettuce seeds vertically placed; (**E**) covered with plastic tape and appropriately labelled the multi-dish for incubation in dark conditions.

3. Results

The elongation percentages of radicle and hypocotyl of lettuce seedlings were affected by leachates from 195 medicinal plant species in the sandwich bioassay (Table 1). In this study, the radicle elongation percentages of lettuce seedlings were in the range of 4.0% to 132.5% and 3.1% to 119.7% for 10 mg and 50 mg, respectively. In both the 10 mg and 50 mg treatments, the lettuce radicle elongations were inhibited more than hypocotyl elongations. Concerning the 10 mg oven oven-dried treatment, we observed that only 58 species showed significant inhibition on lettuce radicle growth as evaluated by using standard deviation variance (SDV). The radicle growth elongation of >90% occurred in 64 species, 70–90% in 61 species, 50–70% in 36 species, 30–50% in 25 species, and 4–30% in 9 species. The six families with highest species number in all examined medicinal plants were Rubiaceae (13 species), Fabaceae (12 species), Euphorbiaceae (12 species), Apocynaceae (10 species), Moraceae (7 species) and Zingiberaceae (7 species). Our study found that 34 species from different plant families showed less than 50% of radicle elongation percentage. However, only three species from different families such as Iridaceae, Apocynaceae and Asparagaceae had lettuce radicle elongation growth less than 10%. The species with the strongest inhibition on lettuce radicle elongation was *Iris pallida* (4% of control), followed by *Parabarium micranthum* (7.5% of control), *Peliosanthes teta* (8.2% of control), *Crinum latifolium* (21.3% of control), *Suregada multiflora* (21.3% of control), *Ervatamia microphylla* (22.4% of control), *Allophyllus serrulatus* (23.3% of control) and *Eupatorium odoratum* (24.1% of control). Nonetheless, the phytochemicals that linked to phytotoxicity and the inhibitory activities of these top inhibiting medicinal plants might contain compounds or some unknown chemical constituents.

Table 1. The radicle and hypocotyl elongation percentages of lettuce seedlings grown containing oven-dried plant materials tested using the sandwich method.

Scientific Name	Plant Families	Part Used	10 mg		50 mg		Criteria
			R	H	R	H	
Iris pallida Lam	Iridaceae	Rhizome	4.0	7.1	3.1	0	*****
Parabarium micranthum (A.DC.) Pierre	Apocynaceae	Leaf	7.5	16.8	5.9	3.2	*****
Peliosanthes teta Andrew	Asparagaceae	Leaf	8.2	38.9	7.20	19.7	*****
Crinum latifolium L	Amaryllidaceae	Bulb	21.3	65.5	5.50	13.0	****
Suregada multiflora Baill	Euphorbiaceae	Stem	21.3	57.7	12.4	35.5	****
Ervatamia microphylla Kerr	Apocynaceae	Leaf	22.4	104	10.3	46.6	****
Allophyllus serrulatus Radlk	Sapindaceae	Leaf	23.3	22.5	12.8	17.5	****
Eupatorium odoratum (L.) R.M.King & H.Rob	Asteraceae	Leaf	24.1	77.5	11.5	35.0	****
Stephania rotunda Linn	Menispermaceae	Tuber	28.7	46.2	10.0	24.6	***
Cyclea barbata Miers	Menispermaceae	Leaf	31.4	94.1	14.4	44.7	***
Jasminum nobile C.B.Clarke	Oleaceae	Stem	31.7	83.2	24.4	89.1	***
Kaempferia galanga Linn	Zingiberaceae	Bulb	32.1	59.3	21.6	34.1	***
Holarrhena curtisii King & Gamble	Apocynaceae	Leaf	32.7	95.1	27.6	85.4	***
Mimosa pudica Linn	Fabaceae	Leaf	32.8	91.9	21.1	76.4	***
Eleutherine bulbosa (Mill.) Urb	Iridaceae	Flower	34.5	56.9	19.1	28.5	***
Cleistanthus tomentosus Hance	Euphorbiaceae	Stem	36.3	90.5	10.3	30.5	***
Sindora siamensis Teysm	Fabaceae	Bark	37.5	70.0	12.2	27.0	***
Cassia siamea Lam	Fabaceae	Leaf	38.0	90.0	29.0	86.0	**
Phyllanthus amarus Schum.ct Thonn	Phyllanthaceae	Stem	38.6	115	13.2	56.0	**
Spirolobium cambodianum Baill	Apocynaceae	Stem	38.8	88.5	25.2	64.2	**
Terminalia corticosa Pierre	Combretaceae	Bark	39.4	69.5	14.1	71.9	**
Adina cordifolia Hok. F	Rubiaceae	Stem	39.7	68.0	9.40	35.5	**
Croton oblongifolius Roxb	Euphorbiaceae	Leaf	41.0	107	21.6	44.3	**
Carallia brachiata (Lour.) Merr	Rhizophoraceae	Bark	42.6	76.9	26.5	72.3	**
Euphorbia hirta Linn	Euphorbiaceae	Leaf	43.3	104	21.2	83.8	**
Brucea javanica (Linn) Merr	Simaroubaceae	Stem	43.8	68.6	10.8	21.8	**
Couroupia guianensis Aubert	Lecythidaceae	Flower	43.9	83.8	19.6	45.3	**
Dialium cochinchinense Pierre	Fabaceae	Bark	43.9	101	14.2	67.2	**
Cyperus rotundus Linn	Cyperaceae	Leaf	44.8	115	22.8	106	**
Dracaena angustifolia Roxb	Asparagaceae	Leaf	45.0	106	31.7	95.3	**
Hymenocardia punctata Wall. ex Lindl	Euphorbiaceae	Stem	46.4	69.6	31.3	58.9	**
Melaleuca leucadendra L	Myrtaceae	Leaf	46.6	91.0	22.3	74.5	**
Diospyros decandra Lour	Ebenaceae	Bark	47.3	96.9	31.2	77.7	**
Dillenia pentagyna Roxb	Dilleniaceae	Stem	49.5	91.1	13.1	58.1	**
Ficus pumila L	Moraceae	Leaf	50.2	110	18.1	69.9	**
Diospyros nitida Merr	Ebenaceae	Stem	50.3	95.5	15.6	39.1	**
Rhodomyrtus tomentosa (Ait) Hassk	Myrtaceae	Leaf	50.4	79.3	24.2	80.2	**
Streptocaulon juventas Merr	Apocynaceae	Stem	50.7	97.0	27.8	84.4	*
Kaempferia parviflora Wall. ex Baker	Zingiberaceae	Bulb	50.8	120	33.6	108	*
Acacia harmandiana (Pierre) Gagnep	Fabaceae	Bark	51.6	84.5	31.6	70.9	*
Derris scandens (Roxb.) Benth	Fabaceae	Stem	51.6	80.8	20.1	36.9	*
Peltophorum dasyrhachis (Miq.) Kurz	Caesalpinioideae	Bark	52.3	77.8	24.7	85.2	*
Tetracera scendens (L.) Merr	Dilleniaceae	Leaf	52.7	114	46.1	111	*
Harrisonia perforata Merr	Rutaceae	Bark	53.4	91.5	36.2	87.9	*
Spatholobus parviflorous Kuntz	Fabaceae	Stem	54.2	111	69.3	93.2	*
Lagerstroemia floribunda Jack	Lythraceae	Bark	57.3	109	8.60	47.7	*
Scoparia dulcis L	Plantaginaceae	Stem	57.6	95.0	92.2	107	*
Ampelocissus matinii Planch	Vitaceae	Stem	58.8	118	16.7	59.5	*
Macaranga triloba (Blume) Muell.Arg	Euphorbiaceae	Stem	59.2	107	39.5	72.1	*
Acalypha boehmerioides Miq	Euphorbiaceae	Leaf	60.0	149	41.5	106	*
Pteridium aquilinum (L) Kuhm	Dennstaedtiaceae	Leaf	60.3	107	17.0	71.7	*
Coptosapelta flavescens Korth	Rubiaceae	Stem	60.7	73.9	64.2	125	*
Nepenthes kampotiana Lecomte	Nepenthaceae	Flower	60.9	120	43.9	114	*
Plumbago zeylanica L	Plumbaginaceae	Stem	61.0	130	26.1	103	*
Mesua ferrea L	Calophyllaceae	Leaf	61.1	95.5	22.3	69.8	*
Scindapsus officinalis (Roxb.) Schott	Araceae	Stem	61.1	80.7	8.60	70.3	*

Table 1. *Cont.*

Scientific Name	Plant Families	Part Used	10 mg		50 mg		Criteria
			R	H	R	H	
Moringa oleifera Lamk	Moringaceae	Bark	62.5	112	13.9	61.9	*
Pandanus tectorius Parkinson ex Du Roi	Pandanaceae	Leaf	63.0	122	28.3	87.1	*
Dillenia ovata Wall. ex Hook.f	Dilleniaceae	Bark	63.3	100	35.6	90.6	
Alpinia conchigera Grulf	Zingiberaceae	Leaf	63.7	117	45.3	117	
Oroxylum indicum (Linn.) Kurz	Bignoniaceae	Bark	64.7	120	41.4	132	
Careya sphaerica Roxb	Lecythidaceae	Bark	65.3	119	41.1	136	
Blumea balsamifera DC	Asteraceae	Leaf	65.9	110	42.3	102	
Croton lachnocarpus Benth.	Euphorbiaceae	Leaf	66.3	112	31.9	96.9	
Eleusine indica (L) Gaertn	Poaceae	Leaf	67.4	138	35.1	129	
Aquilaria crassna Pierr	Thymeleaceae	Root	67.5	134	60.4	127	
Drynaria quercifolia (L.) J Sm	Polypodiaceae	Leaf	68.7	120	49.8	129	
Lagerstroemia calyculata Kurz	Lythraceae	Bark	68.7	108	53.7	61.9	
Erythroxylum cambodianum Pierre	Erythroxylaceae	Stem	69.7	84.7	67.3	113	
Cnestis palala (Lour.) Merr	Connaraceae	Leaf	69.8	103	44.9	103	
Capparis micracantha DC	Capparaceae	Stem	70.6	98.2	42.2	93.1	
Glycosmis pentaphylla (Retz) Correa	Rutaceae	Stem	70.7	137	44.4	113	
Ventilago cristata Pierre	Rhamnaceae	Stem	70.7	124	32.8	102	
Dioscorea hispida Dennst	Dioscoreaceae	Tuber	71.0	104	39.0	114	
Solanum torvum Swartz	Solanaceae	Stem	71.4	104	65.4	113	
Hoya diversifolia Blume	Asclepiadaceae	Leaf	72.1	116	46.8	105	
Bauhinia bassacensis Pierre	Fabaceae	Stem	72.6	118	46.9	110	
Garcinia villersiana Pierre	Clusiaceae	Stem	72.6	101	44.5	86.9	
Polyalthia evecta (Pierre) Finet et Gagnep	Annonaceae	Stem	72.9	125	24.9	72.3	
Gardenia philastrei Pierre-ex-Pit	Rubiaceae	Stem	73.6	125	24.3	92.6	
Schleicheria oleosa (Lour.) Oken	Sapindaceae	Stem	74.0	103	31.5	94.2	
Entada phaseoloides Merr	Fabaceae	Fruit	75.0	103	46.9	80.2	
Calamus rudentum Lour	Arecaceae	Stem	75.2	124	53.5	102	
Tiliacora triandra Diels	Menispermaceae	Stem	75.2	114	28.1	75.0	
Alstonia scholaris R-Br	Apocynaceae	Bark	76.2	93.2	84.1	110	
Congea tomentosa Roxb	Lamiaceae	Stem	76.3	120	43.0	90.5	
Gnetum montanum Markgr	Gnetaceae	Stem	76.5	118	24.1	64.1	
Andrographis paniculata (Burm.f.)	Acanthaceae	Leaf	77.1	136	44.9	75.2	
Anacardium occidentale Linn	Anacardiaceae	Bark	77.8	99	14.8	55.0	
Imperata cylindrica Beauv	Poaceae	Leaf	78.2	91.9	69.1	99.1	
Sterculia lychnophora Hance	Sterculiaceae	Stem	78.8	125	49.4	93.7	
Melodorum fruticosum Lour	Annonaceae	Stem	79.1	131	52.9	109	
Physalis angulata L	Solanaceae	Root	79.2	126	55.3	115	
Afzelia xylocarpa (Kurz) Craib	Fabaceae	Bark	79.3	141	82.6	125	
Licuala spinosa Wurmb	Arecaceae	Root	79.4	129	60.6	147	
Diospyros venosa Wall	Ebenaceae	Stem	79.6	126	44.2	109	
Illigera rhodantha Hance	Hernandiaceae	Stem	80.2	131	43.9	99.2	
Asplenium nidus L	Aspleniaceae	Leaf	80.8	120	64.6	106	
Shorea roxburgii G Don	Dipterocarpaceae	Bark	81.4	93.2	39.4	81.0	
Mallotus paniculatus (Lam.) Mull.Arg	Euphorbiaceae	Stem	81.7	120	22.2	64.4	
Gomphrena celosioides Mart	Amaranthaceae	Flower	82.0	135	45.8	122	
Litchi chinensis Sonn	Sapindaceae	Bark	82.1	103	14.8	78.8	
Elaeocarpus stipularis Blume	Elaeocarpaceae	Stem	83.0	120	37.1	92.1	
Leea rubra Bl	Vitaceae	Stem	83.8	118	32.0	109	
Streblus asper Lour	Moraceae	Stem	83.9	149	52.1	126	
Kalanchoe Integra Kuntze	Crassulaceae	Stem	84.0	186	49.8	166	
Anthocephalus chinensis (Lam.)	Rubiaceae	Bark	84.2	95.2	92.8	118	
Microcos paniculata L	Malvaceae	Stem	84.4	103	43.2	95.8	
Manilkara hexandra (Roxb.) Dubard	Sapotaceae	Leaf	85.1	105	58.9	98.1	
Uvaria rufa Blume	Annonaceae	Stem	86.1	120	56.2	84.5	
Prismatomeris tetrandra (Roxb.) K.Schum	Rubiaceae	Stem	86.3	110	73.2	112	
Memecylon laevigalum Blume	Melastomataceae	Stem	86.4	123	56.3	114	
Amomum xanthioides Wall.	Zingiberaceae	Stem	87.0	161	58.1	139	

Table 1. *Cont.*

Scientific Name	Plant Families	Part Used	10 mg		50 mg		Criteria
			R	H	R	H	
Tinospora crispa (Linn) Miers ex Hook	Menispermaceae	Stem	87.0	134	45.0	112	
Morinda tomentosa Roth	Rubiaceae	Stem	87.1	119	50.6	73.0	
Ficus sagitta Vahl	Moraceae	Leaf	87.4	159	74.9	150	
Psydrax pergracilis (Bourd.) Ridsdale	Rubiaceae	Stem	87.4	101	80.1	117	
Cassia alata L	Leguminosae	Stem	87.5	123	48.4	95.7	
Lindernia crustacea (L.) F.Muell	Linderniaceae	Stem	87.5	135	69.2	117	
Parameria laevigata (Juss.) Moldenke	Apocynaceae	Bark	87.7	123	58.0	127	
Albizia lebbek (L.) Benth	Mimosaceae	Stem	87.9	108	42.2	84.3	
Lygodium conforme C. Chr	Lygodiaceae	Leaf	88.0	109	66.3	94.6	
Zingiber purpureum. Roscoe	Zingiberaceae	Tuber	88.4	97.6	48.1	53.7	
Fhyllanthus emblica L	Euphorbiaceae	Stem	88.6	126	59.8	130	
Hydnophytum formicarium Jack	Rubiaceae	Tuber	88.8	118	77.6	128	
Scleria terrestris (L.) Fassett	Cyperaceae	Leaf	89.0	166	50.2	161	
Broussonetia papyrifera (L.) L'Hér. ex Vent	Urticaceae	Stem	89.2	114	55.8	123	
Colona auriculata (Desv.) Craib	Tiliaceae	Stem	89.6	112	38.7	96.7	
Micromelum falcatum (Lour.) Tanak	Rutaceae	Stem	89.8	117	80.3	117	
Typhonium trilobatum Schott	Araceae	Stem	89.8	127	44.8	123	
Madhuca butyrospermoides A.Chev	Sapotaceae	Bark	90.0	110	29.0	86.0	
Cananga latifolia Finet et Gagnep	Annonaceae	Stem	90.3	126	54.8	90.5	
Gonocaryum lobianum (Miers) Kurz	Icacinaceae	Stem	90.3	110	71.5	109	
Sterculia foetida Linn	Sterculiaceae	Stem	90.3	131	59.4	133	
Wrightia tomentosa Roem-Schult	Apocynaceae	Stem	90.5	138	74.3	120	
Zanthoxylum rhetsa DC.	Rutaceae	Bark	90.7	162	65.5	130	
Smilax china L	Smilacaceae	Stem	91.0	104	74.4	94.8	
Parinari anamensis Hance	Chrysobalanaceae	Bark	91.3	129	59.8	119	
Strychnos wallichiana Steud. Ex DC	Loganiaceae	Stem	93.1	100	54.7	90.5	
Borassus flabellifera Linn	Arecaceae	Root	93.2	122	79.0	132	
Donax grandis Ridley	Poaceae	Stem	93.6	136	71.3	124	
Lxora chinensis Lam	Rubiaceae	Leaf	93.6	147	83.8	130	
Ochna integerrima (Lour) Merr	Ochnaceae	Stem	94.3	133	62.8	112	
Vitex pubescens Vahl	Lamiaceae	Stem	94.3	118	87.2	104	
Artocarpus rigidus Blume	Moraceae	Bark	95.0	116	87.5	126	
Costus speciosus (Koenig) J.E.Smith	Costaceae	Root	95.0	149	78.6	147	
Phyllanthus reticulatus Poir	Euphorbiaceae	Stem	95.4	113	71.4	105	
Melastoma mormale (Kuntze) Merr	Melastomataceae	Stem	95.6	102	68.0	107	
Derris elliptica (Wall.) Benth	Fabaceae	Stem	96.6	118	55.1	94.8	
Elephantopus scaber L	Asteraceae	Leaf	96.8	155	67.5	109	
Knema globularia Warb	Myristicaceae	Stem	96.8	110	64.6	114	
Sida rhombifolia L	Malvaceae	Root	96.8	155	75.8	162	
Heliotropium indicum L	Boraginaceae	Leaf	97.2	150	79.9	154	
Ancistrocladus tectorius (Lour.) Merr	Ancistrocladaceae	Stem	97.6	115	27.5	69.0	
Curcuma aromatica Salisb.	Zingiberaceae	Leaf	97.8	119	81.3	121	
Salacia chinensis Linn	Celastraceae	Stem	97.9	106	69.3	102	
Lygodium flexuosum (L.) SW	Lygodiaceae	Leaf	98.6	141	76.5	116	
Scheffera elliptaca (Blume) Harms.	Araliaceae	Stem	98.9	154	61.0	124	
Zizyphus oeniplia Mill	Rhamnaceae	Stem	98.9	122	42.9	96.0	
Cymbidium aloifolium (Linn) Swartz	Orchidaceae	Leaf	99.2	112	61.8	129	
Fagraea fragrans Roxb	Loganiaceae	Stem	99.2	98.2	107	110	
Hymenodictyon excelsum (Roxb) w	Rubiaceae	Leaf	99.3	129	77.0	132	
Mussaenda cambodiana Pirrl ex Pit	Rubiaceae	Stem	99.6	140	82.6	125	
Smilax ovalifolia Roxb	Smilacaceae	Stem	100	125	53.7	101	
Ficus hirta Vahl var roxburghii (Miq)	Moraceae	Stem	100	141	75.5	127	
Caesalpinia sappan Linn	Fabaceae	Bark	101	91.8	106	87.0	
Clerodendrum schmidtii C.B.Clarke	Lamiaceae	Stem	101	130	97.6	132	
Zizyphus cambodiana Pierre	Rhamnaceae	Stem	102	130	91.1	140	
Pouzolzia zeylanica (L) Benn	Urticaceae	Stem	102	120	76.6	132	
Aganosma marginata G. Don	Apocynaceae	Stem	102	114	94.1	116	
Eurycoma longifolia Jack	Simaroubaceae	Bark	102	91.7	54.9	53.9	

Table 1. *Cont.*

Scientific Name	Plant Families	Part Used	10 mg		50 mg		Criteria
			R	H	R	H	
Gnetum latifolium Blume	Gnetaceae	Stem	102	116	73.0	125	
Homonoia riparia Lour	Euphorbiaceae	Bark	103	138	59.0	104	
Syzygium polyanthum (Wight) Walp	Myrtaceae	Bark	103	116	14.2	57.2	
Rauwenhoffia siamensis Scheff	Annonaceae	Stem	103	122	100	135	
Mangnifera duperreana Pierre	Anacardiaceae	Bark	105	137	64.2	118	
Randia tomentosa Bl	Rubiaceae	Stem	107	114	83.5	112	
Bombax ceiba L	Malvaceae	Bark	107	142	98.8	159	
Ficus benjamina L	Moraceae	Stem	107	136	62.6	121	
Ficus hispida L	Moraceae	Stem	107	143	77.6	110	
Dipterocarpus tuberculatus Roxb	Dipterocarpaceae	Stem	108	142	31.3	90.7	
Millingtonia hortensis Linn	Bignoniaceae	Stem	110	138	74.3	112	
Irvingia malayana Olive. Ex Benn	Irvingiaceae	Bark	110	129	19.1	125	
Dipterocarpus obtusifolius Teijsm.-ex-Miq	Dipterocarpaceae	Stem	111	128	55.1	95.5	
Neonauclea sessilifolia (Roxb.)Merr	Rubiaceae	Bark	112	137	48.5	86.5	
Dracaena lourieri (Gagnep.)	Asparagaceae	Bark	112	104	97.5	127	
Alocasia macrorrhiza (L.) G.Don	Araceae	Bulb	114	140	51.9	104	
Terminalia triptera Stap f	Combretaceae	Stem	114	130	19.1	53.2	
Pseuderanthemum latifolium (Vahl) B. Hansen	Acanthaceae	Leaf	114	149	89.0	142	
Willughbeia edulis Roxb	Apocynaceae	Stem	115	119	43.2	84.4	
Dioscorea bulbifera L	Discoreaceae	Tuber	115	150	103	145	
Walsura villosa Wall. Ex Hiern	Meliaceae	Bark	116	121	184	141	
Pandanus capusii. Marc	Pandanaceae	Root	117	138	79.0	142	
Melastoma villosum L	Melastomataceae	Stem	119	137	92.2	120	
Zingiber ottensii Valeton	Zingiberaceae	Tuber	133	97.4	120	71.1	

Note: Criteria indicates stronger inhibitory activity of test sample on the radicle elongation of lettuce by standard deviation variance (SDV) where: * = M-0.5(SD), ** = M-1.0(SD), *** = M-1.5(SD), **** = M-2.0(SD), and ***** = M-2.5(SD). Species with more * indicates increasing inhibitory activity. M: mean of radicle elongation, SD: standard deviation of radicle length. R: radicle, H: hypocotyl, %: percentage of control growth. Values close to 0% indicate strong inhibitory activity in that plant species.

4. Discussion

We observed that *Iris pallida* showed higher plant growth inhibitory activity (4% of control) than *Eleutherine bulbosa* (34% of control) on lettuce radicle elongation among the Iridaceae family. Irises contain up to 80 genera and 300 species that are distributed worldwide, but abundant and diversified in Southern Africa and Asia. Many of them are common ornamental plants [29]. The *Iris* species are rich sources of isoflavonoids and flavonoids [30]; and they are primarily used in traditional medicine [31–33]. Sweet iris (*Iris Pallida*) is a perennial herb native to the Dalmatian coast, Croatia [34]. Iridals (tritepenoids) from sweet iris were reported to prevent cancer formation and act as anti-plasmodial [35,36]. The content of irones extracted from iris rhizomes contain aromatic principles which mostly responsible for the characteristic scent, and also commercialize in many industries [37,38]. Additionally, many compounds were also reported from the leaf and rhizome of iris essential oil. The major compounds were fatty acids, alkanes, aromatic compounds, sesquiterpenes, and triterpenes [14]; however, its allelochemicals were yet to be reported. On the other hand, *Eleutherine bulbosa*, known as an exotic ornamental and medicinal plant, is native to South America. The underground bulbous part was reported to with a wide range of pharmacognostical and physicochemical properties [39]. Some bioactive compounds contained in ethyl acetate extract of bulbs *Eleutherine bulbosa* including phenolic compounds, flavonoids, quinones and saponins were also reported [40].

The extract of the bulbs of *Eleutherine bulbosa* was reported to have strong activity in the direct bio-autography assay with phytopathogenic fungus *Cladosporium sphaerospermum* [41]. Four compounds were isolated from fungitoxic components including eleutherinone [8-methoxy-1-methyl-1,3dihydro-naphtho(2,3-c)furan-4,9-dione]; eleutherin [9-methoxy1(R),3(S)-dimethyl-3,4-dihydro-1H-benzo(g) isochromene-5,10-dione]; isoeleutherin

[9-methoxy-1(R),3(R)-dimethyl-3,4-dihydro-1H-benzo(g) isochromene-5,10-dione] and eleutherol [4-hy droxy-5-methoxy-3(R)-methyl-3H-naphtho (2,3-c)furan-1-one].

Parabarium micranthum showed the strongest inhibition activity (7.5% of control) among the other ten medicinal plants in Apocynaceae family. *Parabarium micranthum* known as a climbing shrub is native to China but widespread across in East and Southeast Asia and Himalayas. The branches of *P. micranthum* have inconspicuous lenticels and its leave-ovate elliptics are 5–8 cm long and 1.5–3 cm wide. Some part like bark and roots are used for the treatment of infantile paralysis, rheumatalgia, injury, and fractures [42]. Two phytochemical compounds were also identified including 2,2-dime thoxybutane and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one. The containing of catechol and quinic acid in this plant was contributed to extract in anti-aging activities [43].

Another interesting medicinal plant is *Peliosanthes teta* from Asparagaceae family. This plant also showed strong inhibitory activity (8.2% of control) in leachates treatment. *Peliosanthes teta* is a perennial herb with thick roots, short stem and blade-linear leaves. The solitary flower and bursting seed of this plant were shown during the early stage [43]. Although a monotypic genus of *Peliosanthes teta* ranging from India to China, it is well distributed in southeast Asia, particularly in wet evergreen forest [44]. The medicinal values such as earache treatment, energy tonic, circulation and postpartum care were also reported [45,46]. However, its allelochemicals have not yet been exploited.

5. Conclusions

This is the first comprehensive screening of medicinal plants used in Cambodia to evaluate their allelopathic effects. The results presented could serve as a benchmark to elucidate chemical involvement in allelopathy phenomenon. Such information could help researchers to develop new and potent bioactive compounds from natural products to enhance sustainable agriculture and effective use of biological functions. We hereby presented *Iris pallida* for the next study in the isolation and identification of allelochemicals.

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