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

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.

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\text{Elongation} = \left( \frac{\text{Average length of treatment radicle/hypocotyl}}{\text{Average length of control radicle/hypocotyl}} \right) \times 100
\]
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 Parabarrium 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 R | 10 mg H | 50 mg R | 50 mg H | Criteria |
|----------------------------------|----------------|-----------|---------|---------|---------|---------|----------|
| Iris pallida Lam                 | Iridaceae      | Rhizome   | 4.0     | 3.1     | 0       | ***     |
| Parabarium micranthum (A.DC.)    | Apocynaceae    | Leaf      | 7.5     | 5.9     | 3.2     | *****   |
| Pelosanthes teta Andrew          | Asparagaceae   | Leaf      | 8.2     | 7.2     | 19.7    | *****   |
| Crinum latifolium L              | Amaryllidaceae | Bulb      | 21.3    | 13.0    | 13.0    | ****    |
| Sureaua multiflora Baill         | Euphorbiaceae  | Stem      | 21.3    | 35.5    | 12.4    | ****    |
| Eruvatilis microphylla Kerr      | Apocynaceae    | Leaf      | 22.4    | 46.6    | 10.3    | ****    |
| Allophylus serrulatus Radlk      | Sapindaceae    | Leaf      | 23.3    | 17.5    | 12.8    | ****    |
| Eupatorium odoratum (L.) R.M.King & H.Rob | Asteraceae | Leaf      | 24.1    | 30.5    | 11.5    | ****    |
| Stephania rotunda Linn           | Menispermaceae | Tuber     | 28.7    | 24.6    | 10.0    | ***     |
| Cicelya barbata Miers            | Menispermaceae | Leaf      | 31.4    | 44.7    | 14.4    | ***     |
| Jasminum nobile C.B.Clarke       | Oleaceae       | Stem      | 31.7    | 89.1    | 24.4    | ***     |
| Kaempferia galanga Linn          | Zingiberaceae  | Bulb      | 32.1    | 34.1    | 21.6    | ***     |
| Holarhena curtisii King & Gamble | Apocynaceae    | Leaf      | 32.7    | 85.4    | 25.6    | ***     |
| Mimosa pudica Linn               | Fabaceae       | Leaf      | 32.8    | 76.4    | 21.1    | ***     |
| Eletherine bulbosa (Mill.) Urb    | Iridaceae      | Flower    | 34.5    | 28.5    | 19.1    | ***     |
| Cleistanthus tomentosus Hance    | Euphorbiaceae  | Stem      | 36.3    | 30.5    | 10.3    | ***     |
| Sindora siamensis Teysm          | Fabaceae       | Bark      | 37.5    | 27.0    | 12.2    | ***     |
| Cassia siame Lam                 | Fabaceae       | Leaf      | 38.0    | 86.0    | 29.0    | ***     |
| Phyllanthus amarus Schum. cl Thonn | Phyllanthaceae | Stem     | 38.6    | 56.0    | 13.2    | ***     |
| Spirolebias cambodiannum Baill   | Apocynaceae    | Stem      | 38.8    | 64.2    | 25.2    | ***     |
| Terminalia cordisca Pierre       | Combretaceae   | Bark      | 39.4    | 71.9    | 14.1    | ***     |
| Adina cordifolia Hok. F          | Rubiaceae      | Stem      | 39.7    | 35.5    | 9.40    | ***     |
| Croton oblongifolius Roxb        | Euphorbiaceae  | Leaf      | 41.0    | 43.3    | 21.6    | ***     |
| Carallia brachiata (Lour.) Merr  | Rhizophoraceae | Bark      | 42.6    | 72.3    | 26.5    | ***     |
| Euphorbia hirta Linn             | Euphorbiaceae  | Leaf      | 43.3    | 83.8    | 21.2    | ***     |
| Bracca javanica (Linn) Merr      | Simaroubaceae  | Stem      | 43.8    | 21.8    | 68.6    | ***     |
| Courouzia guianensis Aubert      | Lecythidaceae  | Flower    | 43.9    | 45.3    | 19.6    | ***     |
| Dialium cochincheninse Pierre    | Fabaceae       | Bark      | 43.9    | 67.2    | 14.2    | ***     |
| Cyperus rotundus Linn            | Cyperaceae     | Leaf      | 44.8    | 106     | 11.5    | ***     |
| Dracaena angustifolia Roxb       | Asparagaceae   | Leaf      | 45.0    | 95.3    | 31.7    | ***     |
| Hymenocardia punctata Wall. ex Lindl | Euphorbiaceae | Stem     | 46.4    | 58.9    | 69.3    | ***     |
| Melaleuca leucadendra L          | Myrtaceae      | Leaf      | 46.6    | 74.5    | 22.3    | ***     |
| Diospyros decandra Lour          | Ebenaceae      | Bark      | 47.3    | 77.7    | 31.2    | ***     |
| Dillenia pentagyna Roxb          | Dilleniaceae   | Stem      | 49.5    | 58.1    | 13.1    | ***     |
| Ficus pumila L                   | Moraceae       | Leaf      | 50.2    | 69.9    | 18.1    | ***     |
| Diospyros nitida Merr            | Ebenaceae      | Leaf      | 50.3    | 39.1    | 15.6    | ***     |
| Rhodomyrtus tomentosa (Ait) Hassk | Myrtaceae      | Leaf      | 50.4    | 80.2    | 24.3    | ***     |
| Streptacaulon juventus Merr      | Apocynaceae    | Stem      | 50.7    | 84.4    | 27.8    | *       |
| Kaempferia parviflor Wall. ex Baker | Zingiberaceae | Bulb     | 50.8    | 108     | 120     | *       |
| Acacia harmandiana (Pierre) Gagnep | Fabaceae      | Bark      | 51.6    | 70.9    | 84.5    | *       |
| Derrick scandens (Roxb.) Benth   | Fabaceae       | Stem      | 51.6    | 36.9    | 20.1    | *       |
| Peltophorum dasycladhich (Misq.) Kurz | Caesalpinioideae | Bark   | 52.3    | 85.2    | 77.8    | *       |
| Tetracera schdens (L.) Merr      | Dilleniaceae   | Leaf      | 52.7    | 111     | 46.1    | *       |
| Harrisonia perforata Merr        | Rutaceae       | Bark      | 53.4    | 87.9    | 91.5    | *       |
| Spatholobus parviflorous Kunz    | Fabaceae       | Stem      | 54.2    | 93.2    | 111     | *       |
| Lagerstroemia floribunda Jack    | Lythraceae     | Bark      | 57.3    | 47.7    | 109     | *       |
| Scoparia dulcis L                | Plantaginaceae | Stem     | 57.6    | 107     | 95.0    | *       |
| Ampelocissus matrinii Planche    | Vitaceae       | Stem      | 58.8    | 59.5    | 118     | *       |
| Macaranga triloba (Blume) Muell.Arg | Euphorbiaceae | Stem   | 59.2    | 72.1    | 107     | *       |
| Acalypha boehmerioides Misq.     | Euphorbiaceae  | Leaf      | 60.0    | 106     | 41.5    | *       |
| Pteridium aquinimum (L) Kuhn     | Dennstaedtiaceae | Bark | 60.3    | 71.7    | 107     | *       |
| Coptosapelta flavescens North    | Rubiaceae      | Stem      | 60.7    | 125     | 73.9    | *       |
| Nepenthes kampotiana Lecomte     | Nepenthaceae   | Flower    | 60.9    | 114     | 123     | *       |
| Plumbago zeylanica L             | Plumbaginaceae | Stem      | 61.0    | 103     | 26.1    | *       |
| Mesua ferrea L                   | Calophyllaceae | Leaf      | 61.1    | 69.8    | 95.5    | *       |
| Scindapsus officinalis (Roxb.) Schott | Araceae   | Stem      | 61.1    | 70.3    | 80.7    | *       |
| Scientific Name          | Plant Families | Part Used | 10 mg R | 10 mg H | 50 mg R | 50 mg H | Criteria |
|-------------------------|----------------|-----------|---------|---------|---------|---------|----------|
| Moringa oleifera Lamk  | Moringaceae    | Bark      | 62.5    | 112     | 13.9    | 61.9    | *        |
| Pandanus tectorius Parkinon ex Du Roi | Pandanaceae | Leaf | 63.0 | 122 | 28.3 | 87.1 | *        |
| Dillenia orthata Wall. ex Hook.f | Dilleniaceae | Bark | 63.3 | 100 | 35.6 | 90.6 |      |
| Alpinia conchigera Grulf | Zingiberaceae | Bark | 63.7 | 117 | 45.3 | 117 |      |
| Oroxyllum indicum (Linn.) Kurz | Bignoniaceae | Bark | 64.7 | 120 | 41.4 | 132 |      |
| Careya sphaerica Roxb | Lecythidaceae  | Leaf | 67.4 | 138 | 35.1 | 129 |      |
| Blumea balsamifera DC | Asteraceae     | Bark | 68.7 | 120 | 49.8 | 129 |      |
| Croton lachnocarpus Benth. | Euphorbiaceae | Leaf | 69.1 | 126 | 75.2 | 115 |      |
| Eleusine indica (L) Gaertn | Poaceae      | Leaf | 69.2 | 126 | 55.3 | 115 |      |
| Aquilaria crassna Pierr | Thymeleaceae   | Root | 69.7 | 126 | 44.9 | 115 |      |
| Drynaria quercifolia (L.) J Sm | Polygodaceae | Leaf | 69.9 | 133 | 44.9 | 115 |      |
| Lagerstroemia calyculata Kurz | Lythraceae | Leaf | 70.2 | 126 | 75.2 | 115 |      |
| Ergothroxyllum cambodiamum Pierre | Erythroxylaceae | Stem | 70.6 | 126 | 44.9 | 115 |      |
| Cnestis pulala (Lour.) Merr | Connaraceae   | Leaf | 70.9 | 133 | 44.9 | 115 |      |
| Capparis micranthta DC | Capparaceae    | Stem | 70.7 | 126 | 75.2 | 115 |      |
| Glycosmis pentaphylla (Retz) Correa | Rutaceae | Stem | 70.5 | 133 | 44.9 | 115 |      |
| Ventilago cristata Pierre | Rhamnaceae    | Stem | 70.7 | 126 | 75.2 | 115 |      |
| Dioscorea rugosa desert | Solanaeaceae  | Stem | 70.9 | 133 | 44.9 | 115 |      |
| Solanum touroum Swartz | Solanaeaceae  | Stem | 71.2 | 126 | 75.2 | 115 |      |
| Hoya diversifolia Blume | Asclepiadaceae | Leaf | 72.1 | 126 | 44.9 | 115 |      |
| Bauhinia bassacensis Pierre | Fabaceae      | Stem | 72.6 | 133 | 44.9 | 115 |      |
| Garcinia villersiana Pierre | Clusiaceae    | Stem | 72.6 | 133 | 44.9 | 115 |      |
| Polyalthia everta (Pierre) Finet et Gagnep | Annonaceae | Stem | 72.9 | 126 | 75.2 | 115 |      |
| Gardenia philastri Pierre-ex-Pit | Rubiaceae | Stem | 73.4 | 133 | 44.9 | 115 |      |
| Schlechteria olerosa (Lour.) Oken | Sapindaceae | Stem | 78.0 | 126 | 75.2 | 115 |      |
| Entada phaseoloides Merr | Fabaceae      | Stem | 78.0 | 126 | 75.2 | 115 |      |
| Calamus rudement Lour | Arecaceae     | Stem | 78.0 | 126 | 75.2 | 115 |      |
| Tilia corda triandra Diels | Menispermaeae | Stem | 78.0 | 126 | 75.2 | 115 |      |
| Alstonia scholaris R-Br | Apocynaceae   | Bark | 78.0 | 126 | 75.2 | 115 |      |
| Congea tomentosa Roxb | Lamiaceae     | Stem | 78.0 | 126 | 75.2 | 115 |      |
| Gnetum montanum Markgr | Gnetaceae     | Stem | 78.0 | 126 | 75.2 | 115 |      |
| Andrographis paniculata (Burm.f.) | Acanthaceae | Stem | 78.0 | 126 | 75.2 | 115 |      |
| Anacardium occidentale Linn | Anacardiaceae | Stem | 78.0 | 126 | 75.2 | 115 |      |
| Imperata cylindrica Beav | Poaceae       | Stem | 78.0 | 126 | 75.2 | 115 |      |
| Sterculia lycophora Hance | Sterculiaceae | Stem | 78.0 | 126 | 75.2 | 115 |      |
| Melodorum fruticosium Lour | Annonaceae    | Stem | 78.0 | 126 | 75.2 | 115 |      |
| Physalis angulata L | Solanaeaceae  | Stem | 78.0 | 126 | 75.2 | 115 |      |
| Afcelia xylorcarpa (Kurz) Craib | Fabaceae | Bark | 78.0 | 126 | 75.2 | 115 |      |
| Licuyla spinosa Wurbm | Arecaceae     | Stem | 78.0 | 126 | 75.2 | 115 |      |
| Diospyros venosa Wall | Ebenaceae     | Stem | 81.4 | 133 | 44.9 | 115 |      |
| Illigeria rhodanta Hance | Hermiadaceae  | Stem | 81.4 | 133 | 44.9 | 115 |      |
| Asplenum nidus L | Aspleniaceae  | Leaf | 81.4 | 133 | 44.9 | 115 |      |
| Shorea roxburghii G Don | Dipterocarpaceae | Bark | 81.4 | 133 | 44.9 | 115 |      |
| Mallotus paniculatus (Lam.) Mull.Arg | Euphorbiaceae | Stem | 81.4 | 133 | 44.9 | 115 |      |
| Gymnophyto seseoides Mart | Amananthaceae | Flower | 81.4 | 133 | 44.9 | 115 |      |
| Lithl chinensis Sonn | Sapindaceae   | Stem | 81.4 | 133 | 44.9 | 115 |      |
| Elaeocarpus stipularis Plume | Elaeocarpaceae | Stem | 81.4 | 133 | 44.9 | 115 |      |
| Lea rubra Bl | Vitaceae      | Stem | 81.4 | 133 | 44.9 | 115 |      |
| Streblus asper Lour | Moraceae      | Stem | 81.4 | 133 | 44.9 | 115 |      |
| Kalanchoe Integra Kuntze | Crassulaceae  | Stem | 81.4 | 133 | 44.9 | 115 |      |
| Anthocephalus chinensis (Lam.) | Rubiaceae | Bark | 81.4 | 133 | 44.9 | 115 |      |
| Microcos paniculata L | Malvaceae     | Stem | 81.4 | 133 | 44.9 | 115 |      |
| Manilkara hexandra (Rosxb.) Dubard | Sapotaceae | Leaf | 81.4 | 133 | 44.9 | 115 |      |
| Uvaria rufa Blume | Annonaceae    | Stem | 81.4 | 133 | 44.9 | 115 |      |
| Pristatomeris tetrandra (Rosxb.) K.Schum | Rubiaceae | Stem | 81.4 | 133 | 44.9 | 115 |      |
| Memecylon laevigatum Blume | Melastomataceae | Stem | 81.4 | 133 | 44.9 | 115 |      |
| Anomum xanthioides Wall. | Zingiberaceae | Stem | 81.4 | 133 | 44.9 | 115 |      |
| Scientific Name          | Plant Families       | Part Used | 10 mg | 50 mg | Criteria |
|-------------------------|----------------------|-----------|-------|-------|----------|
| *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* | Leguminosae      | Stem      | 87.4  | 101   | 80.1     | 117     |
| *Cassia alata L*        | Linderniaceae        | Stem      | 87.5  | 135   | 69.2     | 117     |
| *Lindernia crustacea (L.) F.Muell* | Linderniaceae   | Stem      | 87.5  | 135   | 69.2     | 117     |
| *Parameria laevisata (Juss.) Moldenke* | Apocynaceae     | Bark      | 87.7  | 123   | 58.0     | 127     |
| *Albizia lebek (L.) Benth* | Mimosaceae          | Stem      | 87.9  | 108   | 42.2     | 84.3     |
| *Lygodium latifolia*    | Lygodiaceae         | Stem      | 90.3  | 126   | 54.8     | 90.5     |
| *Hydnophytum formicarium* | Rubiaceae         | Tuber     | 88.8  | 118   | 77.6     | 128     |
| *Scleria terrestris*    | Euphorbiaceae       | Stem      | 91.0  | 104   | 74.4     | 94.8     |
| *Broussonetia papyrifera (L.) L. Her. ex Vent* | Urticaceae     | Stem      | 89.2  | 114   | 55.8     | 123     |
| *Colona auriculata (Desv.) Craib* | Moraceae        | Stem      | 89.6  | 117   | 80.3     | 117     |
| *Tithonia diversifolia Schott* | Asteraceae      | Stem      | 89.7  | 100   | 54.7     | 90.5     |
| *Madhuca butyracea (A.Chev)* | Sapotaceae       | Bark      | 90.0  | 110   | 29.0     | 86.0     |
| *Cassia alata* | Leguminosae        | Stem      | 90.3  | 110   | 39.7     | 96.7     |
| *Lindernia crustacea*   | Euphorbiaceae       | Stem      | 89.2  | 114   | 55.8     | 123     |
| *Colona auriculata*     | Moraceae            | Stem      | 89.6  | 117   | 80.3     | 117     |
| *Madhuca butyracea*     | Sapotaceae          | Bark      | 90.0  | 110   | 29.0     | 86.0     |
| *Cassia alata*          | Leguminosae         | Stem      | 87.4  | 101   | 80.1     | 117     |
| *Ficus hirta* | Moraceae            | Stem      | 100   | 141   | 75.5     | 127     |
| *Caesalpinia sappan*    | Fabaceae            | Bark      | 100   | 141   | 75.5     | 127     |
| *Clidemia hirta*        | Fabaceae            | Bark      | 100   | 141   | 75.5     | 127     |
| *Zizyphus cambodiana*   | Rhamniaceae         | Stem      | 99.3  | 129   | 77.0     | 132     |
| *Smilax glabra*         | Euphorbiaceae       | Stem      | 97.2  | 150   | 79.9     | 154     |
| *Ancistrocladus tectorius (Lour.) Merr* | Rhamniaceae   | Stem      | 97.6  | 115   | 27.5     | 69.0     |
| *Curcuma aromatica*     | Zingiberaceae       | Stem      | 97.8  | 119   | 81.3     | 121     |
| *Salacia chinensis*     | Celastraceae        | Stem      | 97.9  | 106   | 69.3     | 102     |
| *Lygodium flexuosum*    | Rhamniaceae         | Leaf      | 98.6  | 141   | 76.5     | 116     |
| *Scheffera elliptica*   | Araliaceae          | Stem      | 98.9  | 154   | 61.0     | 124     |
| *Zizyphus oenoplia*     | Rhamniaceae         | Stem      | 98.9  | 122   | 42.9     | 96.0     |
| *Cymbidium aloifolium*  | Orchidaceae         | Stem      | 99.2  | 112   | 61.8     | 129     |
| *Fagraea fragrans*      | Loganiaceae         | Stem      | 99.2  | 98.2  | 107      | 110     |
| *Musanga cambodiensis*  | Rhamniaceae         | Stem      | 99.3  | 129   | 77.0     | 132     |
| *Smilax ovalifolia*     | Euphorbiaceae       | Stem      | 100   | 125   | 53.7     | 101     |
| *Ficus hirta* | Moraceae            | Stem      | 100   | 141   | 75.5     | 127     |
| *Caesalpinia sappan*    | Fabaceae            | Bark      | 100   | 141   | 75.5     | 127     |
| *Clidemia hirta*        | Fabaceae            | Bark      | 100   | 141   | 75.5     | 127     |
| *Zizyphus cambodiensis* | Rhamniaceae         | Stem      | 102   | 130   | 91.1     | 140     |
| *Pouzolzia zeifan*      | Rhamniaceae         | Stem      | 102   | 120   | 76.6     | 132     |
| *Aganosma marginata*    | Apocynaceae         | Stem      | 102   | 114   | 94.1     | 116     |
| *Eurycoma longifolia*   | Smaragdaceae        | Bark      | 102   | 91.7  | 54.9     | 53.9     |
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 antiplasmodial [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 have 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)fur-an-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-hydroxy-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-dimethoxybutane 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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