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 serratus* had the highest growth inhibitory effect on lettuce radicles more than 60%, followed by *Alcosia 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...
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 × 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.

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\text{Inhibitory} = 100 - \left( \frac{\text{Average length of treatment radicle/hypocotyl}}{\text{Average length of control radicle/hypocotyl}} \right) \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*, *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*, *Afxelia xylocarpa*, *Zingiber purpureum*, *Careya sphaerica*, *Congea tomentosa*, *Pseuderanthemum latifolium* and *Ventilago cristata*.
| Scientific Name                  | Plant Families | Part Used | Inhibition Activity (%) | Criteria |
|---------------------------------|----------------|-----------|-------------------------|----------|
|                                 |                |           |                         |          |
| **Allophyllus sserulatus** Radlk | Sapindaceae    | Leaf      | 68.6                    |          |
| **Alocasia macrorrhiza** (L.) G.Don | Araceae        | Tuber     | 23.4                    |          |
| **Iris pallida** Lam.           | Iridaceae      | Rhizome   | 22.1                    |          |
| **Terminalia tripiera** Stapf    | Combretaceae   | Stem      | 21.3                    |          |
| **Wrightia tomentosa** Roem-Schult | Apocynaceae    | Stem      | 21.1                    |          |
| **Garcinia villersiana** Pierre | Orchidaceae    | Leaf      | 20.4                    |          |
| **Cymbidium aloifolium** (Linn.) Swartz. | Orchidaceae | Leaf   | 20.4                    |          |
| **Eupatorium odoratum** (L.) R.M.King & H.Rob. | Asteraceae | Leaf | 5.1                    |          |
| **Eulthenone bulbosa** (Mill.) Urb. | Iridaceae      | Flower    | 18.7                    |          |
| **Pelosantha tetra** Andrew     | Asparagusaceae | Leaf      | 17.4                    |          |
| **Willughbeia edulis** Roxb.    | Apocynaceae    | Stem      | 17.2                    |          |
| **Eleusine indica** (L.) Gaertn | Poaceae        | Leaf      | 17.1                    |          |
| **Spatholobus parviflorus** Kuntz. | Fabaceae      | Leaf      | 16.4                    |          |
| **Asplenium vidale**            | Asplenaceae    | Stem      | 15.7                    |          |
| **Drynaria quercifolia** (L.) Sm | Lamiaceae      | Leaf      | 15.3                    |          |
| **Croton oblongifolius**        | Euphorbiaceae  | Leaf      | 15.2                    |          |
| **Zingiber officinale**         | Zingiberaceae  | Leaf      | 14.8                    |          |
| **Eleuthine bulbosa** (Mill.) Urb. | Iridaceae      | Leaf      | 14.6                    |          |
| **Croton lachnocarpus**         | Euphorbiaceae  | Leaf      | 14.6                    |          |
| **Suregada multiflora**         | Myristicaceae  | Stem      | 13.7                    |          |
| **Gomphrena celosioides**       | Amaranthaceae  | Flower    | 13.4                    |          |
| **Zizyphus cambodiana**         | Rhamnaceae     | Stem      | 13.3                    |          |
| **Artocarpus rigidus**          | Malvaceae      | Bark      | 12.7                    |          |
| **Kalanchoe Integra**           | Crassulaceae   | Stem      | 12.4                    |          |
| **Phyllanthus niruri**          | Euphorbiaceae  | Leaf      | 12.3                    |          |
| **Lettuce**                     | **Criteria**   |           |                         |          |
|                                 |                |           | Average at 41 mm        | Average for Whole Wells |
| R                               | H              | R         | H                        |          |
| 68.6                            | 67.3           | 63.1      | 63.1                     |
| 23.4                            | –5.22          | 20.5      | –5.62                     |
| 22.1                            | 6.71           | 14.3      | 7.34                      |
| 21.3                            | 9.22           | 13.7      | –1.82                     |
| 21.1                            | 9              | 15.9      | 2.12                      |
| 20.4                            | 14.2           | 10.7      | 12.4                      |
| 20.2                            | 8.11           | 8.72      | 4.12                      |
| 19.8                            | –4.72          | –0.92     | –4.53                     |
| 18.7                            | 2.42           | 16.7      | 5.62                      |
| 17.4                            | 13.4           | –0.54     | 6.32                      |
| 17.2                            | 9.2            | 8.92      | 1.83                      |
| 17.1                            | 13.9           | 3.12      | 11.6                      |
| 16.4                            | 6.92           | 10.9      | 0.81                      |
| 15.7                            | 4.73           | 9.14      | 7.32                      |
| 15.3                            | 5.92           | 9.12      | –5.91                     |
| 15.2                            | 7.6            | 9.32      | 6.63                      |
| 14.8                            | 17.4           | 8.41      | 11.2                      |
| 13.9                            | 19.5           | 4.62      | 11.6                      |
| 12.7                            | –3.12          | 13.4      | 3.12                      |
| 12.4                            | 21.8           | 0         | 12.1                      |
| 12.3                            | 1.4            | 2.33      | –3.74                     |
| 12.3                            | 0              | 17.6      | 1.82                      |
| 11.5                            | –28.4          | 5.07      | –19.1                     |
| 11.4                            | –5.21          | 19.1      | 4.31                      |
| 11.4                            | 57.1           | –0.92     | 41.6                      |
| 11.3                            | 8.8            | 5.61      | 1.82                      |
| 11.1                            | 12.2           | 5.12      | 8.93                      |
| 11                             | 11.5           | 6.2       | 10.9                      |
| 10.1                            | 2.7            | 9.12      | 4.71                      |
| 9.8                            | 15.1           | –1.93     | 7.74                      |
| 9.42                           | 6.94           | 4.12      | 0                         |
| 9.23                           | –4.43          | 7.04      | –2.21                     |
| 8.94                           | –0.94          | 9         | 2.24                      |
| 8.21                           | 2.72           | 6.74      | –2.91                     |
| 8.14                           | 7.31           | –1.43     | 2.72                      |
| 8.04                           | 2.33           | 2.91      | –0.82                     |
| 8.02                           | –0.22          | 0.52      | 3.51                      |
| 8                             | –1.64          | 9.61      | –1.83                     |
| 7.26                           | 14.1           | 2.12      | 3.91                      |
| 7.23                           | –2.6           | –4.04     | –3.3                      |
| 7.14                           | –7.12          | 5.32      | –4.5                      |
| 7.13                           | 7.16           | 3.21      | 10.2                      |
| 7.1                            | 6.93           | 3.32      | 6.71                      |
| 7                             | 4.74           | 7         | 4.12                      |
| 6.7                            | 8.4            | 6.21      | 0                         |
| 6.62                           | 11.6           | 4.43      | 4.61                      |
| 6.32                           | 1.8            | 3.13      | –1.82                     |
| 5.84                           | 8.54           | 11.4      | 0.81                      |
| 5.82                           | –9.42          | 4.63      | –6.32                     |
| 5.62                           | –3.61          | –3.71     | –11                       |
| 5.48                           | 8.61           | 0.52      | 2.73                      |
| 5.44                           | 7.32           | 1.21      | 3.52                      |
| 5.33                           | –8.73          | 7.21      | –7.64                     |
| 5.17                           | –1.14          | 3.12      | 2.21                      |
| 5.1                            | 20              | 4.72      | 12.2                      |

**Table 1.** The radicle and hypocotyl inhibition percentages of lettuce seedlings grown using the dish pack method.
| Scientific Name | Plant Families | Part Used | Inhibition Activity (%) | Average at 41 mm | Average for Whole Wells |
|----------------|----------------|-----------|-------------------------|-----------------|-------------------------|
|                |                |           | Criteria                | R H             | R H                     |
| Dipterocarpus tuberculatus Roxb. | Dipterocarpaceae | Stem | 4.88 | −5.72 | 3.91 | −1.82 |
| Licuala spinosa Wurmb. | Areceae | Root | 4.84 | −10.2 | 4.23 | −15.2 |
| Nepenthis kampotiana Lecomte | Nepenthaceae | Flower | 4.83 | −10.6 | 3.12 | −11.1 |
| Smilax ovalifolia Roxb. | Smilacaceae | Stem | 4.17 | −7.72 | −4.51 | −0.92 |
| Cnestis palata (Lour.) Merr. | Combretaceae | Leaf | 4.67 | −7.72 | 4.12 | −11.2 |
| Similas chinasa 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 | −0.2 | 7.82 |
| Lagerstroemia calycistyla Kurz. | Lythraceae | Bark | 4 | 3.81 | 7.24 | 6.34 |
| Symphytum polyanthum (Wight) Walp. | Myrsinaceae | Stem | 3.9 | 5.64 | −4.93 | −0.91 |
| Streblus asper Lour. | Moraceae | Stem | 3.81 | −6.72 | 4 | −2.23 |
| Tinospora crispa (Linn) Miers ex. Hook. | Menispermacae | Stem | 3.77 | −3.13 | −30.9 | 0 |
| Anacapillus chinensis (Lam.) | Rubiaceae | Bark | 3.46 | −1.53 | 7.61 | −0.72 |
| Borassus flabellifera Linn | Areceae | Root | 3.42 | −7.31 | 7.21 | −2.81 |
| Cassia alata L. | Fabaceae | Stem | 3.28 | −9.2 | −6.32 | −0.91 |
| Mangifera duperreana Pierre | Anacardiaceae | Bark | 3.23 | −2.12 | 11.6 | 10.3 |
| Tetracera scordens (L.) Merr. | Dilleniaceae | Leaf | 2.83 | 4.34 | 3.62 | 5.91 |
| Lygodium flexuosum (L.) SW. | Lygodiaceae | Leaf | 2.62 | −4.52 | 2 | −4.12 |
| Blumea balsamifera DC. | Asteraceae | Stem | 2.41 | 19.6 | 7.52 | 14.7 |
| Diospyros decandra Lour. | Ebenaceae | Bark | 2.37 | −4.1 | 10.3 | 3.91 |
| Bauhinia baccassensis Pierre | Fabaceae | Stem | 2.12 | 10.7 | 9.73 | 6.42 |
| Clerodendrum schmidtii B.Clarke | Lamiaceae | Stem | 1.97 | 7.74 | −4.11 | −11.3 |
| Elaeocarpus stipularis Blume | Elaeocarpaceae | Stem | 1.92 | −16.7 | 4.62 | −11.4 |
| Memecylon laeveilum Blume | Melastomataceae | Stem | 1.72 | 3.42 | 0.41 | 6.43 |
| Illigera rhodantha Hance. | Hernandiaceae | Stem | 1.58 | −7.62 | 2 | −6.73 |
| Phyllanthus amarus Schum. et Thonn. | Phyllanthaceae | Stem | 1.53 | 1.12 | 12.2 | 4.91 |
| Ficus hispida L. | Moraceae | Stem | 1.26 | 8.81 | −0.21 | 9 |
| Ancistrocladus tectorius (Lour.) Merr. | Ancistrocladaceae | Stem | 1.18 | 15.1 | −0.54 | 10.2 |
| Morinda 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 depauperatum (Miq.) Kurz | Fabaceae | Bark | 1.12 | 11.7 | −9.12 | 7.31 |
| Prismonatris tetrandra (Roxb.) K.Schum | Rubiaceae | Stem | 1.1 | −4.91 | 7.9 | −8.12 |
| Dipterocarpus obtusifolius (Teissm.-ex-Miq) | Dipterocarpaceae | Stem | 0.82 | 4.52 | −3.21 | −0.93 |
| Macaranga trifida (Blume) Muell.Arg. | Euphorbiaceae | Stem | 0.76 | 0.23 | 4.12 | −1.81 |
| Taphonaeflua triloba (Blume) Scop. | Euphorbiaceae | Stem | 0.65 | −2.83 | 4.31 | −6.42 |
| Scindapsus officinalis (Roxb.) Schott | Araceae | Stem | 0.54 | 4.81 | −2.12 | 1.31 |
| Erythroxylum camaldulensis Pierre | Erythroxylaceae | Bark | 0.54 | −2.54 | −1.63 | 0.52 |
| Spirulobium camaldulensis Baill. | Apocynaceae | Stem | 0.52 | −1.22 | | |
| Caesalpinia sappan Linn. | Fabaceae | Bark | 0.37 | 16.3 | −0.52 | 9.73 |
| Melastoma normale (Kuntze) Merr. | Melastomataceae | Stem | 0.33 | 2.32 | 1 | 11.9 |
| Heliotropium indicum L. | Boraginaceae | Leaf | 0.22 | −9.44 | −2.81 | −8.82 |
| Erycoucha 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 |
| Bauhinia sappan Linn. | Fabaceae | Stem | 0.1 | −9.42 | −7.2 | −8.21 |
| Scheffera elliptica (Blume) Harms. | Araliaceae | Stem | 0.22 | 5.35 | 1.61 | 4.52 |
| Manilkara hirsuta (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 |
| C neutronia guianensis Aubert | Lecythidaceae | Flower | 0.64 | −7.12 | −3.9 | 1.4 |
| Cleistanthos tonentosus Hance | Euphorbiaceae | Stem | 1.45 | −8.05 | 0.92 | −4.12 |
| Albizia lebbeck (L.) Benth. | Mimosaceae | Stem | 1.57 | 13.4 | −5.81 | 13.6 |
| F hologynous emblica L. | Ficus sygitis Vahl. | Moraceae | 1.94 | −15.5 | −0.52 | −6.31 |
| Alpinia officinarum (Roxb.) | Fabaceae | Stem | 2 | −4.13 | 8.62 | −0.91 |
| Pandanus capensis Marc | Pandanaeae | Root | 2.33 | −13.4 | 8 | −15.3 |
| Streptocarpus jaunettensis Merr. | Apocynaceae | Stem | 2.37 | 3.31 | 0.22 | 3.83 |
| Dioscorea bulbifera L. | Dicocereae | Tuber | 2.52 | 0.44 | −1.41 | −2.52 |
| Scientific Name | Plant Families | Part Used | Inhibition Activity (%) | Criteria |
|-----------------|----------------|----------|--------------------------|-----------|
|                 |                |          | Average at 41 mm | Average for Whole Wells | R | H | R | H |
| 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 olesa (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 | −3.1 | 0 | 5.31 | 2.11 |
| Glycosmis pentaphylla (Retz) Correa | Rutaceae | Stem | −3.24 | −7.32 | −2.53 | −3.81 |
| Acalypha boehmerioides Miq. | Euphorbiaceae | Leaf | −3.41 | −10.2 | −6.12 | −11.4 |
| Lagerstroemia floribunda Jack. | Lythraceae | Bark | −3.57 | −5.4 | −4.31 | −4.9 |
| Micromelum falcatum (Lour.) Tanak. | Rutaceae | Stem | −3.58 | −10.4 | −6.33 | −10.4 |
| Ficus benjamina L. | Moraceae | Stem | −4.1 | −5 | 3.7 | 6.12 |
| Hydrophyllum formicarium Jack | Rubiaceae | Tuber | −4.12 | 9.53 | −6.04 | 6.6 |
| Capparis micrantha DC. | Capparaceae | Stem | −4.21 | 8.34 | 0.23 | 6.92 |
| Terminalia cirtostica Pierre. | Combretaceae | Bark | −4.24 | 2.83 | −11.8 | −3.72 |
| Pteridium aquilum (L) Kuhn. | 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 Gagnep | Annonaceae | Stem | −4.51 | 1.2 | −1 | 3.31 |
| Parinari anamensis Hance | Chrysobalanaceae | Bark | −5.3 | −1.64 | 0 | −0.92 |
| Gardenia philastri Pierre-ex-Pit. | Rubiaceae | Stem | −5.35 | −1.44 | 7.23 | 0 |
| Parameria laevigata (Juss.) Moldenke | Apocynaceae | Bark | −5.41 | −4.82 | −1.72 | −6.5 |
| Alstonia scholaris R-Br | Apocynaceae | Bark | −5.51 | 3.37 | −5.91 | 0.9 |
| Tiliacora triandra Diels | Menispermaceae | Stem | −5.52 | −3.83 | −3.81 | −3.34 |
| Dracaena angustifolia Roxb. | Asparagaceae | Leaf | −5.53 | 2.85 | −6.74 | −1.06 |
| Holarøhena curtisii King &Gamble | Apocynaceae | Leaf | −5.53 | −1.44 | 7.23 | 0 |
| Parabarium micranthum (A.D.C.) Pierre | Apocynaceae | Leaf | −5.53 | −4.82 | −1.72 | −6.51 |
| Dialium cochinchinense Pierre | Fabaceae | Bark | −5.71 | 13.3 | −7.72 | 5.43 |
| Jasminum nobile C.B.Clarke | Oleaceae | Stem | −5.78 | −14.3 | −2.91 | −2.7 |
| Melaleuca cajuputi Powell | Myrtaceae | Leaf | −5.79 | −4.84 | 1.31 | 0.32 |
| Hymenodictyon excisum (Roxb) w. | Rubiaceae | Leaf | −5.8 | −7.11 | 3.11 | −2.81 |
| Derris elliptica (Wall) Benth. | Fabaceae | Stem | −6.21 | 7.72 | −1.62 | 5.9 |
| Lea rubra Bl. | Vitaceae | Stem | −6.23 | −8.32 | −2.11 | −13.8 |
| Rhodomyrtus tomentosa (Ait) Hassk | Myrtaceae | Bark | −6.28 | 14.2 | −6.08 | 6.31 |
| Brucia javanica (Linn) Merr. | Simaroubaceae | Bark | −6.31 | −8.83 | −4.81 | −10.1 |
| Mimosia pudica Linn. | Fabaceae | Leaf | −6.56 | −5.64 | 0.72 | −0.37 |
| Legydonium conform C. Chr. | Lygodiaeaceae | Leaf | −7.4 | 5.42 | 1.41 | 8.6 |
| Adina cordifolia Hok. F | Rubiaceae | Stem | −7.5 | 2.81 | −10.7 | 0 |
| Aquilaria crassa Kerr. | Thymelaeaceae | Root | −7.52 | −5.62 | −0.92 | −2.71 |
| Ficus pumila L. | Moraceae | Leaf | −7.55 | 12.2 | −5.72 | 7.71 |
| Scleria terrestres (L) Fassett | Cyperaceae | Leaf | −7.72 | −25.6 | −2.61 | −15.7 |
| Calamus radentum Loure. | Arecales | Leaf | −7.82 | −12.1 | −0.8 | −4.04 |
| Neorea nesis safari (Rosh)Merr. | Rubiaceae | Bark | −7.9 | 8.93 | −4.3 | −2.51 |
| Broussonetia papyrifera (L) L’Hérit ex Vent. | Urticaceae | Stem | −7.9 | −1 | −10.1 | −3.2 |
| Diospyros nitida Merr. | Ebenaceae | Stem | −8 | 0.92 | −3 | 3.81 |
| Ziziphus oenoplia Mill | Rhamnaceae | Bark | −8.12 | −8.42 | −9.08 | −5.62 |
| Cyclea barbata Miers | Menispermaceae | Bark | −8.41 | −5.95 | −9.11 | −3.63 |
| Dillenia ootia Wall. ex Hook.f. | Dilleniaceae | Bark | −8.44 | −6.14 | −9.8 | −9.43 |
| Homonoia riparia Loure. | 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 cambodioida Pirrol ex Pit | Rubiaceae | Bark | −9.77 | −0.62 | −10.6 | −6.83 |
| Pandanus tectorius Parkinson ex Du Roi | Pandanaceae | Leaf | −10 | −8.34 | −7.05 | −10.1 |
| Cyperus rotundus Linn. | Cyperaceae | Leaf | −10.3 | −19.2 | −6.91 | −15.7 |
| Aganosoma marginata G. Don | Apocynaceae | Stem | −10.7 | −0.92 | 15.2 | 2.61 |
| Mesua ferrea L | Calophyllaceae | Leaf | −10.7 | 12.5 | −10.2 | 5.92 |
| Lindernia crustacae (L) F.Muell | Linderniaceae | Stem | −10.9 | 5.63 | −1.44 | 7.82 |
| Zanthoxylum rheta DC. | Rutaceae | Bark | −11.4 | 9.56 | −11.4 | −7.11 |
| Walsara 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 |
| Amygdalocissus matini Planch | Vitaceae | Stem | −13 | 0.52 | −9.91 | −0.92 |
| Euphorbia bitha 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 |

**Criteria:**
- **R:** Number of reactions.
- **H:** Number of herbal extracts.

**Table 1. Cont.**
Table 1. Cont.

| Scientific Name       | Plant Families | Part Used | Inhibition Activity (%) | Criteria |
|-----------------------|----------------|-----------|-------------------------|----------|
|                       |                |           | Average at 41 mm        | R H      |
|                       |                |           | Average for Whole Wells | R H      |
| *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     | ++       |
| *Ficus hirta* Vahl var roxburghii (Misq). | Moraceae | Stem      | –16                     | –1.72    |
|                       |                |           | –12.8                   | –1.33    | ++       |
| *Sterculia lycoperschophora* Hance | Sterculiaceae | Stem      | –19.2                   | –10.2    |
|                       |                |           | –12.1                   | –0.42    | +++      |

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 *Schlechteria 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-[beta]-D-glucopyranoside, rutin and apigenin-4-O-[beta]-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, antiuretic, 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, ascobic 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-[beta]-irone, [alpha]-irone, trans-2,6-[gamma]-irone, [beta]-isometilionone; benzophenone and other dominant terpenes, including 4-isobutylphenone, benzophenone, hexahydrofarnesyl acetone, neoartadien and squelane 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 harrisitone A–E, haperforine A, haperforine E, 12-desacetylhap-erforine A, haperforine C2, haperforine F, haperforine G, Foritin, harrisonol A, pucerin-7-methyl ether, O-methyl-lloptaeroxylin, perforatic acid, eugenin, saikochromone A, greveichromenol and perforamone A–D [43]; β-sitosterol, obacunone, herteropeucenin-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 haperforatin, 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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