ALLELOPATHIC ACTIVITY OF SOME MEDICINAL PLANT EXTRACTS AND ALLELOPATHIC ACTIVITY GUIDED FRACTIONATION OF METHANOLIC LEAF EXTRACT OF *CASSIA ALATA*

M.H. Haroon[1] & J.M.R. Jayasekara[2]

Correspondence: haroonmh@seu.ac.lk

ABSTRACT

The current study investigated the allelopathic activity of twenty six medicinal plant extracts using Radish seed germination bioassay. The investigation result suggested that the methanolic leaf extract of *Dregea volubilis*, *Cassia alata* and *Azadirachta indica* have the highest allopathic activity against seed germination. As a continuation of our research work on allelopathic activity studies of some medicinal plant and seaweeds, methanolic leaf extract of *Cassia alata* was further investigated with the hope of discovering new eco-friendly natural herbicides. Methanolic leaf extract (25 g) of Cassia alata was chromatographed on a column of silica gel (50 g, Merk Kiselegel 60, 230-400 mesh ASTM) using n-hexane, ethyl acetate, methanol and water as eluents to give fifteen major fractions F-1 to F-15. Lettuce seed germination bioassay which is widely used in the detection of allelochemicals, throughout the world was carried out to examine the active fractions for seed germination inhibitory activity. Out of 15 fractions and crude tested, F-11 (80% -100% ethyl acetate in hexane), F-12 (0% - 10% methanol in ethyl acetate) and crude showed statistically potent seed germination inhibitory activities (0 % germination), this might be due to the allelochemicals present in the *Cassia alata*. F-9 (45% to 65% Ethyl acetate with hexane) has also shown significant inhibition activity with 12.5% germination.

Keywords: Medicinal plants, Allelopathic activity, *Cassia alata*, column chromatography, lettuce seed germination bioassay, radish seed germination bioassay.

1. Introduction

In nature, plants can inhibit or stimulate the growth and/or development of other surrounding plant species, through the various interactions and this phenomena is known as “Allelopathy”. The term Allelopathy first introduced by an Austrian scientist Hans Molisch in 1937 (Inderjit et al., 2005). It was derived from two Greek words “allelon” which means “to each other” and “pathos” which means “to suffer”. The term allelopathy was defined as “any process involving 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” by the international allelopathy society in 1996 (Nath et al., 2016). Secondary metabolites that are responsible for the allelopathic interactions are known as allelochemicals or allelotoxins and these chemicals can be presented in different concentrations in different parts of the plant including leaves, flowers, roots, fruits, seeds, rhizomes, pollens or stems. These allelochemicals can be released into the environment by root exudation, leaching dew and rain from the plant surface, secretion of volatile compound or

[1] Faculty of Applied Sciences, South Eastern University of Sri Lanka.
[2] Faculty of Applied Sciences, South Eastern University of Sri Lanka.

decaying plant litters (Devkota, 2013). The ability of an allelochemical to inhibit or delay the growth of plant and/or seed germination are defined as it's allelopathic potential and these effects carry out by disrupting various physiological processes such as photosynthesis, respiration, cell division, membrane permeability, ion uptake, water and hormonal balance and enzyme activity (Gniazdowska and Bogatek, 2005).

In the weed management of agricultural system, the application of chemical herbicides is an effective way. However long-term and large scale use of chemical herbicides have raised many environmental and health problems. And also induction of herbicide resistance property in weed plants have raised the need of introduction of new herbicides (Amini et al., 2016). The present study describe the allelopathic activity medicinal plants along with bioassay guided fractionation of methanolic leaf extract of *Cassia alata.*

2. Results and Discussion

Out of twenty seven crude MeOH extracts of medicinal plants, some extracts exhibited significant differences in their mean radical length, compared to the control, sterile distilled water (Table 1). However, *Dregea volubilis (0.00%), Cassia alata* (6.25%) and *Azadirachta indica (6.25 %)* showed the lowest germination values in the Radish seed germination while *Ricinus communis, Centella asiatica and Justicia adhatoda showed* a 12.5% germination value and appeared to possess the smallest average radical length of 0.12 ± 0.08 mm. Interestingly, *Plectranthus amboinicus, Aloe vera, Cynodon dactylon, Leucas aspera and Ocimum sanctum showed* evidence of seed germination stimulation activity. Even though, other extracts have not inhibited the radish seed germination at a significant level, reduced root lengths might increase the chance of desiccation in seedling before establishment and delay growth since water uptake is necessary for seedling growth.

Table 1: % germination and the average radicle length of seedlings under different treatments.

Name	Germination Percentage (%)	Root length (mm)
Plectranthus amboinicus	100	5.31 ± 0.82
Cassia siameae	81.25	*2.23* ± 0.68
Aloe vera	100	0.63 ± 0.14
Scoparia dulcis	93.75	3.57 ± 0.82
Cynodon dactylon	100	5.80 ± 0.55
Dregea volubilis	0	0.00 ± 0.00
Leucas aspera	100	4.88 ± 0.88
Spondias dulcis	56.25	1.89 ± 0.37
Murraya koenigii	56.25	1.36 ± 0.45
Solanum trilobatum	75	2.87 ± 1.00
Asparagus racemosus	12.5	0.70 ± 0.63 b
Coccinia grandis	31.25	0.36 ± 0.15 b
Ricinus communis	12.5	0.12 ± 0.08 b
Cassia alata	6.25	0.06 ± 0.06 b
Centella asiatica	12.5	0.12 ± 0.08 b
Azadirachta indica	6.25	0.75 ± 0.75 b
Costus speciosus	12.5	0.12 ± 0.08 b
Plectranthus zeylanicus	43.75	1.38 ± 0.74 b
Justicia adhatoda	12.5	0.12 ± 0.08 b
Pseudarthria viscida	68.75	8.06 ± 1.85 b
Osbeckia octranda	87.50	4.00 ± 0.44 b
Acalypha indica *	87.5	2.04 ± 0.34
Phyllanthus niruri *	93.75	1.99 ± 0.19
Ipomoea carnea *	75	0.48 ± 0.06
Passiflora edulis *	87.5	2.25 ± 0.31
Ocimum sanctum	100	5.23 ± 0.35
Pisonia grandis	93.75	4.15 ± 0.65
Distilled water	100	4.58 ± 0.77

Values are mean ±SD, according to the one way ANOVA and Turkey's pairwise comparison test mean values are statistically significant at p= 0.000<0.05

Means followed by the same letters in each column are not significantly different.

The allelopathic MeOH extract of *Cassia alata* (25 g) was subjected to column chromatography on silica gel (50 g, Merk Kiselegel 60, 230-400 mesh ASTM) using n-hexane, ethyl acetate and methanol as eluants, yielding fifteen major fractions F-1 to F-15.(Table 2)

Table 2: fifteen fractions separated by column chromatography, gradient elution technique with the help of TLC

Fraction no	Combination of used eluent
F-1	0% to 5% Ethyl acetate with hexane
F-2	5% to 10% Ethyl acetate with hexane
F-3	10% to 15% Ethyl acetate with hexane
F-4	15% to 20% Ethyl acetate with hexane
F-5	20% to 25% Ethyl acetate with hexane
F-6	25% to 30% Ethyl acetate with hexane
F-7	30% to 40% Ethyl acetate with hexane
F-8	40% to 45% Ethyl acetate with hexane
F-9	45% to 65% Ethyl acetate with hexane
F-10	65% to 80% Ethyl acetate with hexane
F-11	80% to 100% Ethyl acetate with hexane
F-12	0% to 10% Methanol with ethyl acetate
F-13	10% to 15% Methanol with ethyl acetate
F-14	15% to 25% Methanol with ethyl acetate
F-15	25% to 100% Methanol with ethyl acetate

The fifteen fractions were tested against lettuce seed germination assay. (Table 3)

Table 3: % Germination, Average radicle length and average hypocotyl length of seedling of lettuce seed under different treatment.

Fraction no	%Germination	Average root length (mm)	Average hypocotyl length (mm)
F-1	87.50	1.875±1.237a	3.458±1.120a
F-2	87.50	1.375±0.530a	3.250±3.182a
F-3	62.50	3.750±1.061a	6.750±2.475a
F-4	50.00	1.667±0.471a	3.167±1.179a
F-5	62.50	1.500±0.707a	4.250±3.889a
F-6	37.50	2.000±1.414a	5.000±2.828a
F-7	25.00	1.000±0.000a	1.000±0.000a
F-8	87.50	2.125±0.177a	3.083±0.589a
F-9	12.50	2.000±0.000a	1.000±0.000a
F-10	37.50	5.000±1.000a	3.667±2.887a
F-11	0.00	-	-
F-12	0.00	-	-
F-13	62.50	2.500±0.707a	2.250±1.768a
F-14	37.50	2.000±0.000a	1.500±0.707a
F-15	62.50	2.250±0.354a	2.667±2.357a
crude	0.00	-	-
control	100.00	2.125±0.177a	7.250±1.414a

Values are mean ±SD, according to the one way ANOVA and Turkey's pairwise comparison test mean values are statistically significant at $p= 0.000<0.05$

Means followed by the same letters in each column are not significantly different.

The final crude of methanolic extract of *Cassia alata* showed complete inhibition of germination of lettuce seeds confirming that it contains the chemical constituents which can act as effective lettuce seed germination inhibitors. The obtained results of different allelopathic activities in different fractions conformed that the chemical compounds which are extracted to the solvents can be varied with the type of used eluent.

Out of fifteen fractions fraction no 11 (80% - 100% ethyl acetate with hexane) and fraction no 12 (0% - 10 % methanol with ethyl acetate) are showed complete germination inhibition. The fraction of F7 showed the minimum root length of 1.00 mm and the fraction of F-10 showed maximum root length of 5.00 mm while it having the considerable germination inhibition (37.5 %). The average root length of F-10 showed higher value than that of control.

The maximum average hypocotyl length of 7.25 mm was showed in control which treated only with water while the minimum average hypocotyl length of 1.00 mm was showed in fraction of F-9 (45% -65% ethyl acetate with hexane) and F-7 (30% - 40% ethyl acetate with hexane)

3. Experimental

3.1 Identification of plants and Collection of plant leaves

The mature, fresh leaves of the plants were collected in the month of April 2016-March-2019 from different locations including North Central province, Eastern province and Central province of Sri Lanka.

3.2 Preparation of plant leaves for extraction

Plant leaves were washed thoroughly with running tap water followed by rinsing with distilled water to remove sand particles and other debris. They were shade dried at room temperature for 45 days. Then they were pulverized into powder using a mechanical grinder.

3.3 Preparation of Crude Extract

Powdered plant leaves (100g) were separately macerated in methanol (250 ml) for 24 hours at room temperature and mixed well for 6 hours by using mechanical stirrer at 800 rpm. The extracts were filtered through a funnel containing a filter paper and a clear filtrates were obtained. The filtrates were evaporated by using a rotary evaporator under reduced pressure at $(40\text{-}45)^0$c. Thick greenish black residues were obtained and they were subjected to the Radish seed germination bioassay. For the chemical investigation, the dried *Cassia alata* leaves (500g) was finely powdered and extracted three times with methanol (1000 mL) by using mechanical stirrer. After filtration, the MeOH extract was evaporated to give 50 g of dry extract. The MeOH extract was subjected to column chromatography (CC) on silica gel.

3.4 Separation by using column chromatography technique and Thin Layer Chromatography Techniques.

Dried crude extract (25 g) was dissolved in minimum quantity of methanol and mixed with silica gel G (60-120) (50 g) and dried by using rotary evaporator under reduced pressure at (30-40) ^{0}C. The obtained residue was finely powdered by using mortar and pastel and stored for column chromatographic separation. Silica gel G (60-120) was used as the stationary phase. Sample was eluted with organic solvents hexane, ethyl acetate and methanol. Sample was eluted initially with increasing polarity of hexane in combination with ethyl acetate ranging from 5% ethyl acetate in hexane to 100% ethyl acetate. Afterwards, increased polarity was obtained by combination of methanol with ethyl acetate, ranging from 5% methanol in ethyl acetate to 100% methanol. After this process 15 fractions was separated by using Thin Layer Chromatography techniques. TLC system was used with silica gel plates using hexane, dichloromethane and methanol in different ratios as the developing solvent and finely methanol with dichloromethane in 1:1 ratio. Spots were detected under UV and by spraying with para anisaldihyde and ceric sulphate.

3.5 Radish Seed Germination Bioassay

The crude extracts of different plant species were tested by Radish seed germination bioassay.

The germination rate in distilled water was examined at random before the experiment and it was found to be >85%. Solutions of crude extracts (1000 ppm) of twenty seven medicinal plants were prepared at room temperature. All the seeds were surface sterilized with 1.5 (v/v) bleach (NaOCl) for 1 min and after they were washed (three times, 3 min/ wash) with sterile distilled water. Four replicates each with 4 seeds were prepared for twenty seven plant extracts using sterile petri dishes (90mm) lined with one sterile filter paper. 5 ml of aqueous plant extracts were added separately in to each petri dish. The negative control groups were treated with sterilized distilled water. Prepared plates were then placed in a growth chamber at 25 ^{0}C in a dark room for 5 days (120 hours). The effect of each extract was observed by calculating the percentage seed germination and by measuring radicle length to the nearest mm. (The radicle is the embryonic root).

3.6 Lettuce Seed Germination Bioassay

The germination of lettuce seeds in methanol was examined in randomly before the experiment and it was found to be >80%. Methanolic solutions (1000 ppm) of dried fifteen fractions and initial methanolic extract of plant were prepared at room temperature. All the seeds were surface sterilized with 1% (v/v) NaOCl solution for 1 min and then they were washed (three times, 3 min / wash) with distilled water and soaked in distilled water for 2 hours. 5 ml of methanolic solution of each fractions were applied separately on sterilized petri dishes (90 mm) lined with two sterilized filter paper. Methanol was evaporated in a lamina flow. The filter paper discs were wetted with 4 ml of distilled water. Five seeds, which presoaked for 2h, were placed on each paper discs and germination was carried out in an incubator at 25 ^{o}C. The effect of each fraction and initial crude of plant extract was observed by calculating the percentage of seed germination and by measuring radicle length and hypocotyl length to the nearest mm (the radicle is embryonic root) after 7 days. All treatments were performed in duplicate and methanol was used as negative control(Tang and Young, 1982, El Ayeb-Zakhama and Harzallah-Skhiri, 2016, Islam and Kato-Noguchi, 2013).

The percentage of seed germination (G %) was calculated relative to the control using following formula (Islam and Kato-Noguchi, 2013).

$$G\% = (a/b)*100$$

Where, a is the average no of germinated seeds in the treatment and b is the average no of germinated seeds in the control.

3.6 Data analysis

One-way ANOVA test was carried out for the average radicle length and average hypocotyl length measured from various fractions in Minitab version 17.0 Mean comparison test – Turkey's pair wise comparison was carried out to compare the average radicle length and average hypocotyl length among the various fractions.

4. Conclusion

The present study suggests that the methanolic leaf extract of *Cassia alata* possess potent allelopathic activity against the germination of lettuce seed. According to the analysis of allelopathic activity fractions of 80% - 100% ethyl acetate with hexane and 0% - 10 % methanol

with ethyl acetate showed complete inhibition of lettuce seed germination. The knowledge gained from this study will be helpful in isolation, purification and characterization of highly active allelochemicals which can be used to develop environmental friendly and well effective herbicides in future. In addition, some of the medicinal plants tested showed significant allelopathic activity against seed germination and thus they can be used as sources of natural herbicides in their crude form.

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References

AMINI, S., AZIZI, M., JOHARCHI, M. R. & MORADINEZHAD, F. 2016. Evaluation of allelopathic activity of 68 medicinal and wild plant species of Iran by Sandwich method. *International Journal of Horticultural Science and Technology,* 3**,** 243-253.

DEVKOTA, A. 2013. Evaluation of allelopathic potential and phytochemical screening of some medicinal plant species of Nepal. *International Journal of Pharmaceutical & Biological Archive,* 4.

EL AYEB-ZAKHAMA, A. & HARZALLAH-SKHIRI, F. 2016. Allelopathic activity of extracts of Citharexylum spinosum L. from Tunisia. *Journal of Plant Breeding and Crop Science,* 8**,** 189-196.

GNIAZDOWSKA, A. & BOGATEK, R. 2005. Allelopathic interactions between plants. Multi site action of allelochemicals. *Acta Physiologiae Plantarum,* 27**,** 395-407.

INDERJIT, WESTON, L. A. & DUKE, S. O. 2005. Challenges, achievements and opportunities in allelopathy research. *Journal of Plant Interactions,* 1**,** 69-81.

ISLAM, A. M. & KATO-NOGUCHI, H. 2013. Plant growth inhibitory activity of medicinal plant Hyptis suaveolens: could allelopathy be a cause? *Emirates Journal of Food and Agriculture***,** 692-701.

NATH, S., YUMNAM, P. & DEB, B. 2016. Allelopathic Effect of Lemon Plant Parts on the Seedling Germination and Growth of Lettuce and Cabbage. *Int J Plant Biol Res,* 4**,** 1-5.

TANG, C.-S. & YOUNG, C.-C. 1982. Collection and identification of allelopathic compounds from the undisturbed root system of bigalta limpograss (Hemarthria altissima). *Plant Physiology,* 69**,** 155-160.