IDENTIFICATION OF POTENTIAL AND POTENCY OF ALLELOCHEMICALS IN VELVETBEAN (*MUCUNA COCHINCHINENSIS* (WIGHT) BURCK) FOR THE CONTROL OF *STRIGA HERMONTHICA* (DEL.) BENTH.

A. Ibrahim[1*], E. I. Magani[2] and R. I. Ahom[2]

[*1]Deparment of Agronomy, Faculty of Agriculture, Nasarawa State University, Keffi, Nigeria.
[2]Department of Crop and Environmental Protection, University of Agriculture, Makurdi, Nigeria.
[*]**Corresponding Author's email address: ibnibrahims@gmail.com**

Abstract

The experiment was conducted at the Toxicology laboratory, Faculty of Agriculture, University Putra Malaysia, Serdang, Malaysia in 2013. The study was to identify plant growth-inhibitory compounds in M. cochinchinensis as a step to clarifying the weed-suppressing effect of this plant. The treatments consisted of five concentrations (100, 75, 50, 25, 0 ppm); plant parts (leaf, root, seed) and extraction solvents (methanol, water) were replicated three times and arranged as a completely randomized block (CRD) design. In all the treatments, inhibition from root was greater than from leaves. The seed extract showed less inhibition. Six phenolic/flavonone compounds including gallic acid, caffeic acid, L-dopa, tyrosine, quercetin and isovitexin were isolated and identified in velvetbean leaves, root and seed. Concentration of these phenolic compounds in root was higher than in the leaves. These compounds showed different degrees of inhibition against Striga hermonthica and therefore results in the stronger allelopathic activity of M. cochinchinensis. The identification of these substances might provide chemical basis for the development of bio-herbicides for environmentally friendly sustainable agricultural systems.

Keywords: Velvet bean, *Striga*, Identification, Phenolic compounds

Introduction

The phenomenon of biochemical warfare within the plant community has made majority of living organisms to develop adaptability to diverse habitats through chemical signals which have further been exploited as allelopathy. The term "allelopathy" refers to the effect of one plant on another through the release of a chemical compound into the environment (Bhowmik and Inderjit, 2003). The allelochemicals, often considered plant-produced herbicides, might have inhibitory, stimulatory, or no effect on the germination and growth of nearby plants of the same and/or other species. They contain mixtures of many different compounds, the majority of which are the so called secondary products (Swain, 1979).

These compounds, which are products of secondary metabolism, are common constituents of the legume Fabaceae (Geissman and Crout, 1969). Velvetbean (*Mucuna cochinchinensis*), a non-edible tropical legume, produces high amount of a non-protein amino acid, L-3,4-dihydroxyphenylalanine (L-Dopa) (Fujii *et al.*,1991). Other phenolics and isoflavone such as tannins, coumarins and isoschaftoside have also been reported as allelochemicals from the cattle forage legume *Desmodium uncinatum* (Hooper *et al.*, 2010). Previous studies have demonstrated that intercropping subsistence maize with velvetbean potentially reduces *Striga* parasitism and smothers speargrass (*Imperata cylindrica*) with concomitant yield increases, improved soil nitrogen and reduced soil erosion (Avav *et al.*, 2008; Akal *et al.*, 2012). Research on the use of *Mucuna bracteata* as leguminous cover crops on mineral and peat soils in Malaysia and its use in oil palm plantation had been reported (Shaharuddin and Jamaluddin, 2007; Hasnol *et al.*, 2012). Besides improving soil fertility by fixing

nitrogen and making it available to the main crop and reduce competition from noxious weeds, it also improves palm growth and reduces the immaturity period (Hosnol *et al.*, 2012). The development herbicides from naturally occurring plant bioactive chemicals is capable of acting as natural herbicide and can resolve problems with human health hazards, detrimental soil and environmental side-effects, and the evolution of herbicide-resistance biotypes associated with over-use of synthetic herbicide.

Striga hermonthica (Del.) Benth (Orobancheceae), or witchweed, is a hemi-parasitic native to the sub-Saharan Africa. It is a major weed in many countries of Africa, where it is prevalent in the Nigerian savanna, affecting major staple food crops and subsistence farms. It flowers prolifically and the seeds are dispersed by wind, human activities and animals, attached to clothing or possessions. Most control of *S. hermonthica* is achieved through hand-pulling and slashing (Lagoke *et al.*, 1992). However, such methods are labour-intensive and unsustainable for subsistence farmers due to its prolificity and subterranean damage. Chemical control is not feasible for many subsistence farmers due to its high cost relative to the small scale production and low income generated (Day *et al.*, 2012). Therefore, utilization of *M. cochinchinensis* either as a bio-pesticide and intercrop or/and as cover crop seen as the only viable option to control this aggressive weed in most parts of the savannas.

It is therefore important to identify the active compound(s) in the cover crop, so that the action mechanisms, potential side-effects, and most efficient method for the use of the plant and the bioactive compounds can be investigated. In the present study, we report the germination bioassay, isolation and identification of the plant growth-inhibitory compounds in *M. cochinchinensis* as a step to clarifying the weed-suppressing effect of this plant.

Materials and Methods

2.1 *Plant materials*

Velvetbean seeds were obtained from the University of Agriculture, Makurdi, Nigeria in 2012 and sown in early March in the Glass house of Faculti Pertanian, Universiti Putra Malaysia in 2013. At growth stage of about 12 weeks after planting (WAP), the plant was harvested and the leaves, stem and root were separated. Seeds of *S. hermonthica* were also collected from naturally and heavily and infested field.

2.2 *Seed sterilization*

About 10 mg of *S. hermonthica* were placed in a 50 ml flask and surface sterilized for 5 minutes with a 1% solution of commercial sodium hypochlorite (NaOCl). Subsequently, the seeds were thoroughly rinsed with distilled water on top of a funnel lined with Whatman no. 1filter paper (Whatman International, Maidstone, UK). For conditioning, about 100 seeds were transferred to glass microfibre filter paper discs (1 cm diameter) moistened with 100 µl of distilled water. On top of the glass microfibre filter paper disc containing the *Striga* seeds, was placed another disc wetted with 100 µl of distilled water as well. Two layers of Whatman no. 1filter papers (90 mm diameter) were placed in a Petri dish (90 mm diameter) and moistened with 5 ml of distilled water to prevent the seeds from drying up during conditioning. Five discs were placed in each Petri dish. The dishes were sealed with parafilm, wrapped in aluminium foil and subsequently kept in an incubator at 28 ± 2°C for two weeks.

2.3 *Sampling and preparation of extracts*

The plant parts were thoroughly washed with distilled water and then divided into two fresh samples. One part of the samples (leaves, stem and root) were immediately oven-dried at 50 ^{o}C for 72 hours, ground with a Wiley Mill to pass through a 1-mm screen, and stored in a refrigerator at 4^{o}C until required. The other fresh parts were crushed and 100 g of pulverized materials of each part were extracted from it. The fresh and dried leaves, stem and root were extracted by soaking in 1 litre of methanol and distilled water to generate two fractions from each part and placed on a shaker for 48 hours at room temperature. The aqueous extracts were filtered through four layers of cheesecloth to remove the fibre debris and then filtered once again through a Whatman no. 1filter. The seed residues were subsequently extracted with hexane in order to remove fat-soluble substances. Each extract was dried *in vacuo* on a rotary evaporator at 45^{o}C and then weighed. The methanol and water-extracted fractions were re-dissolved with 100 ml of sterile distilled water. The final concentration 100 ppm was prepared and distilled water was added to the solutions to make different dilution (75, 50 and 25 ppm). The pH of the extracts ranges from 6.02 to 6.56. Extracts were stored in a refrigerator at 8^{o}C until further used for bioassay tests.

2.4 *Germination bioassays*

The experiment was conducted at the Toxicology laboratory, Faculty of Agriculture, University Putra Malaysia, Serdang, Malaysia. After conditioning, the filter paper discs containing the seeds were dried for 1 hour at room temperature and placed in the middle of Petri dishes lined (only on the edge) with a double layer of filter paper moistened with 2 ml of distilled water. To each cluster of seeds on the filter paper discs, the different dilution (100, 75, 50 and 25 ppm) of the solution containing each test plant parts (leaves, seed and root) was applied. The cluster of seeds on the filter plates in the control treatments received 100 ppm of the GR-24 solution. The dishes were sealed with parafilm, wrapped in aluminum foil and incubated at 28 ± 2°C for five days. At the end of the incubation period, each Petri dish was opened and the germinated and non-germinated seeds counted and the radicle length of *S. hermonthica* was measured using QuickPhoto micro 2-3 (Software) attached to Olympus E-system SLR camera E-450 (Japan). Seeds were considered germinated when germ tubes were clearly visible.

2.5 *Identification of potential allelochemicals in M. cochinchinensis*

Five grams of dried fine-ground powder of velvetbean leaves, seed, stem and root were added with 100 ml of 80% methanol, shaken with 80 times/minute by a bath shaker for 24 hours at 40^{o}C, and centrifuged at 4,000 rpm for 15 minute. The supernatant was passed through a Whatman no. 1 filter and evaporated to dryness by freeze-dried vacuum (Labcono Corporation, USA) at -40^{o}C. The precipitate were calculated and added by MeOH to be 1 mg ml^{-1} (1000 ppm). The fractions were filtered through a 0.20 µm filter (Minisart, Sartorius-Stedim, Germany) for HPLC (High Pressure Liquid Chromatography).

The chromatographic peaks of the analytes were confirmed by comparing their retention time and UV spectrum with those of the reference standards. Recordings of chromatograms and quantitative measurement of peak area were performed with a computer connected to the Empower software (Waters, USA).

2.6 *Samples preparation for analysis*

Dried *M. cochinchinensis* leaves, seed and root powder (1.0 g) was accurately weighed and transferred in a 150 ml conical flask with glass stopper, and 60% aqueous

methanol (15 ml) was added (Perk *et al.*, 2007; Zeraik and Yariwake, 2010). The solution was stirred for 12 hours and sonicated for 30 min. at room temperature and then centrifuged at 4000 rpm for 10 min. The residue was further extracted with 60% methanol (2 × 15 ml) as mentioned above. All the extracted solutions were combined in a 50 ml volumetric flask and diluted to the mark. All solutions were filtered through 0.45 μm membrane filter prior to analysis. An aliquot of 20 μl of solutions were injected for HPLC analysis. The mobile phase used solvents A (H_2O): B (MeOH) in a gradient programme initially 60:40 (A:B), to 70:30 at 3 min., 35:65 at 3.5 min., 80:20 at 6 min. and 40:60 at 10 min. at 1 ml min^{-1}. The treatments consisted of five concentrations (100, 75, 50, 25, 0 ppm); plant parts (leaf, root, seed) and extraction solvents (methanol, water) were replicated three times and arranged as a completely randomized block (CRD) design.

2.*7 Statistical analysis*.

The total germination (TG) was determined, as described by Siddiqui (2007), and the percentage inhibition (1 – Lt/Lc) x 100, Lt = radicle length of the germinated seeds exposed to treatment, and Lc = radicle length of control germinated seed) computed. The collected data were statistically analyzed by using a Genstat Statistical Software (v. 10; VSN, Harpenden, UK) and the Fischer Least Significance Test was used to determine the differences between the treatment means at the 5% probability level. The mean LC_{50} value (the dose for 50% inhibition of seedling growth) was calculated by using a probit analysis, as described by Finney (1971). A logistic equation was fitted to the germination data as a function of the logarithm of the concentrations of the *M. cochinchinensis* leaves, seed and root extracts by using SPSS for Windows (v. 19.0; SPSS, Chicago, IL, USA):

Y= a + bx, where Y = the probit value, a = the intercept, b = the slope of the line, and x = the Log_{10} concentration. The value of x was obtained in order to calculate the LC_{50} values of the concentrations of the *M. cochinchinensis* leaves, seed and root extracts.

Results and Discussions

Results of the probit analysis for the seed germination of *S. hermonthica* to the different concentrations of the leaf extracts are presented in (Table 1). For methanol extract, the LC_{50} was found to be 33.60%; while the water extract was 43.20%. Irrespective of the concentrations, the probability of inhibition was generally higher in the methanol extract (0.33 – 0.95) than the water extract (0.30 – 0.78).

The *M. cochinchinensis* methanol and water extracts (100, 75, 50 and 25 %) decreased the seed germination of *Striga*. However, the methanol root extract caused the highest inhibition in the number of the seed germination. The mean LC_{50} value of the *M. cochinchinensis* methanol and water extracts in relation to the germination inhibition of *Striga* was 25.34 % and 41.05 % respectively, (Table 2). Similarly, the probability of inhibition was generally higher in the methanol extract (0.50 – 0.91) than the water extract (0.25 – 0.89).

Table 1: Probit analysis for the seed germination of *S.hermonthica,* exposed to four concentration of *M. cochinchinensis* leaf aqueous extract.

Extract Concentration (%)	Total number of seeds	Number of ungerminated seeds	Expected response	Probability
Methanol (leaf)				
100	50	49	47.26	0.945
75	50	41	44.02	0.880
50	50	37	35.97	0.719
25	50	17	16.55	0.331
Water (leaf)				
100	50	41	39.19	0.784
75	50	31	34.85	0.697
50	50	31	27.72	0.554
25	50	15	15.22	0.304

Regression line parameters (methanol leaf extract): Y = a + bx; Y = -5.17 + 3.382x; diagnostic concentration = 33.67%. Log = 1.56

Regression line parameters (water root extract): Y = a + bx; Y = -3.52 + 2.154x; diagnostic concentration = 43.20%. Log = 1.64

Table 2: Probit analysis for the seed germination of *S. hermonthica,* exposed to four concentration of *M. cochinchinensis* root aqueous extract.

Extract Concentration (%)	Total number of seeds	Number of ungerminated seeds	Expected response	Probability
Methanol (root)				
100	50	49	45.48	0.911
75	50	41	42.85	0.857
50	50	33	37.40	0.748
25	50	27	24.73	0.495
Water (root)				
100	50	45	44.23	0.885
75	50	38	39.57	0.791
50	50	32	30.23	0.605
25	50	12	12.60	0.252

Regression line parameters (methanol root extract): Y = a + bx; Y = -3.18 + 2.265x; diagnostic concentration = 25.34%. log = 1.40

Regression line parameters (water root extract): Y = a + bx; Y = -5.00 + 3.099x; diagnostic concentration = 41.05%. Log = 1.61

All four concentrations of the seed extract of both methanol and water indicated less inhibition of *Striga* germination as compared to the leaves (Table 1) and root (Table 2) extracts (Table 3). Although, the seed extracts at higher concentrations increased the germination inhibition of *Striga* but less inhibition was recorded under low concentrations. The LC_{50} of the methanol and water extract of the seed extract indicated less inhibitory activity with 48.78 and 56.60%, respectively. Irrespective of the concentrations, the probability of inhibition was slightly higher in the methanol extract (0.27 – 0.74) than the water extract (0.19 – 0.73).

Table 3: Probit analysis for the seed germination of *S. hermonthica,* exposed to four concentration of *M. cochinchinensis* seed aqueous extract.

Extract Concentration (%)	Total number of seeds	Number of ungerminated seeds	Expected response	Probability
Methanol ((seed)				
100	50	39	36.84	0.737
75	50	34	32.40	0.648
50	50	19	25.45	0.509
25	50	17	13.91	0.278
Water (seed)				
100	50	37	36.62	0.732
75	50	30	31.03	0.621
50	50	23	22.31	0.446
25	50	9	9.33	0.187

Regression line parameters (methanol seed extract): Y = a + bx; Y = -3.42+ 2.029x; diagnostic concentration = 48.74%. Log =1.69

Regression line parameters (water seed extract): Y = a + bx; Y = -4.40+ 2.509x; diagnostic concentration = 56.60%. Log = 1.75

3.1 *Effective biocides and concentrations on Striga germination*

The allelopathic effects of the five different chemical compounds tested on *Striga* germination and radicle inhibition representing the most active groups applied at five different concentrations is shown in (Table 4). L-dopa, gallic acid and isovitexin actively and significantly suppressed *Striga* germination which differed significantly with caffeic acid and quercetin. Among the phenolics, thyrosine was less effective on *Striga* germination and subsequent inhibition of the radicle length than all other chemical compounds.

Table 4: Effects of chemical compounds and concentrations on the *S. hermonthica* germination.

Phenolics	Germination %	Radicle length (mm)
Caffeic acid	43.90	0.63
Gallic acid	23.90	0.50
Isovitexin	26.67	0.45
L-dopa	21.40	0.47
Quercetin	43.30	0.56
Thyrosine	66.10	1.00
$LSD_{(0.05)}$	12.65	0.10
Concentration (ppm)		
100	5.83	0.04
50	8.61	0.15
25	23.61	0.35
10	45.00	0.53
1	55.56	1.01
0 (control)	86.70	1.55
$LSD_{(0.05)}$		0.10
Analysis of variance		
Phenolics x Concentration	*	ns

There was a significant interaction between phenolics and concentrations (Table 5). The activity of the compounds was on a dose – dependent manner relative to a particular compound. The most active compounds at 100 ppm were caffeic acid, gallic, isovitexin, L-dopa and quercetin, all of which completely inhibited *Striga* germination (Table 5). Caffeic acid, gallic acid, isovitexin, L-dopa and quercetin were highly active with inhibitory activity ranging between 70 and 90%. Tyrosine was slightly active, causing around 20% inhibition of germination. At 1 ppm gallic acid, isovitexin and L-dopa still caused a dramatic reduction in seed germination. It was observed that the most active compounds were not only able to reduce or completely inhibit seed germination, but effectively altered both the size and shape of the radicle (Table 4). This could further reduce the capability of the parasite reaching the host root as an escape mechanism. Yang *et al.* (2005) found that gallic acid concentrations of 40 mg/l had complete growth-inhibitory effects on *Alexandrium tamarense* in an obvious dose-dependent manner.

Table 5: The Effect of interaction between Phenolic compounds and concentration on percentage germination of *S. hermonthica*.

Concentration of phenolic compounds	Caffeic acid	Gallic acid	Isovitexin	L-Dopa	Quercetin	Thyrosine
100	0.00	0.00	0.00	0.00	0.00	35.00
50	6.67	0.00	1.67	1.67	1.67	40.00
25	40.00	6.67	5.00	3.33	23.33	63.33
10	63.33	21.67	28.33	11.67	66.67	78.33
1	66.67	28.33	38.33	25.00	81.67	93.33
0 (control)	86.67	86.67	86.67	86.67	86.67	86.67
$LSD_{(0.05)}$				5.27		

3.2 *Identification of potential allelochemicals in M. cochinchinensis.*

By analyzing the recorded DAD-UV spectra, one of the five numbered peaks were assigned tohydroxycinnamic derivatives (4, λ_{max} at approx. 220 nm), two flavonoids (3 and 5, λ_{max} at approx. 220 nm) and two amino acids (1and 2, λ_{max} at approx. 220 nm) in the leaf extract of *M. cochinchinensis* (Fig. 1). The results from the HPLC analysis showed that phenolic acids, flavone *C*-glycosides and amino acids were identified in *M. cochinchinensis* leaves, stem and root including caffeic acid, gallic acid, isovitexin, quercetin, L-Dopa and L-tyrosine (Table 6). Caffeic acid was found in smaller amount in the seed and root extracts. Gallic acid was also found in the leaves, seed and root extracts of *M. cochinchinensis* with the highest recorded in the root extract (0.39 mg g^{-1}) (Fig. 3). These phenolic acids had also been detected in the leaves, stem, and root of *A. conyzoides* as putative allelochemicals which show effects (promotion or inhibition) on paddy weeds (Xuan *et al.*, 2004). The amount of tyrosine was the greatest in the leaves, seed and root extracts (131.95, 136.73 and 17.84 mg g^{-1} respectively) (Figs. 1, 2 and 3). The 3,4-dihydroxy-L -phenylalanine (L-dopa or DOPA) is a non-essential amino acid and synthesized from L-tyrosine, metabolized to alkaloids, lignin and phenylpropanoids in plant cells (Fujii, 1994). The allelopathic effect could be as a result of lignification of the cells during the process of germination. Two flavone *C*-glycosides, isovitexin and quercetin compounds were observed in the leaves, seed and root of *M. cochinchinensis* with isovitexin at the concentration of 0.12, 0.05 and 0.65 mg g^{-1} respectively. However, quercetin was found in only the leaves and seed extracts (0.91 and 0.77 mg g^{-1}, respectively) (Figs. 2 and 3). Generally, the HPLC results showed that higher concentrations of the phenolic compounds were found in the root. However, quercetin was not found in the root; while caffeic acid was not found in the leaves (Table 7). Previous studies had reported that a C-glycosylflavone, isoschaftoside, in *Desmodium uncinatum* as the pertinent allelochemical that suppressed *Striga* parasitism (Hooper *et al.*, 2010). Gallic acid and caffeic acid were found in *Ageratum conyzoides* (Xuan *et al.*, 2004), as putative allelochemicals which inhibited weeds on paddy field. Moreso, research findings had also reported the presence of L-Dopa in *Mucuna prupriens* as a potential allelochemical to suppressed weed germination (Fujii *et al.*, 1991).

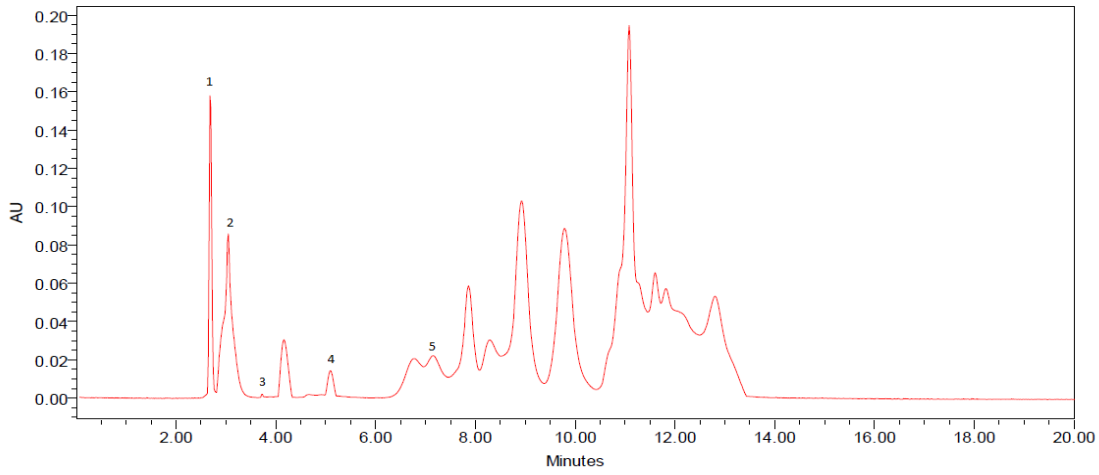

Figure 1: HPLC chromatogram of the leaf extract using diode array detection at 220 nm. (1) L-Dopa, (2) tyrosine, (3) isovitexin, (4) gallic acid, and (5) quercetin.

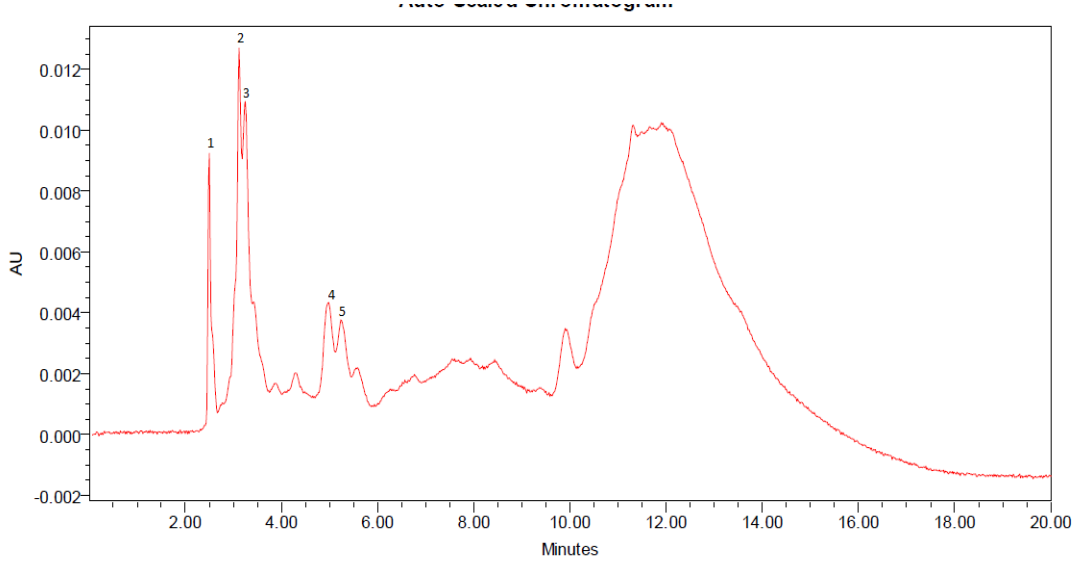

Figure 2: HPLC chromatogram of the root extract using diode array detection at 220 nm. (1) L-Dopa, (2) tyrosine, (3) isovitexin, (4) caffeic acid, and (5) gallic acid.

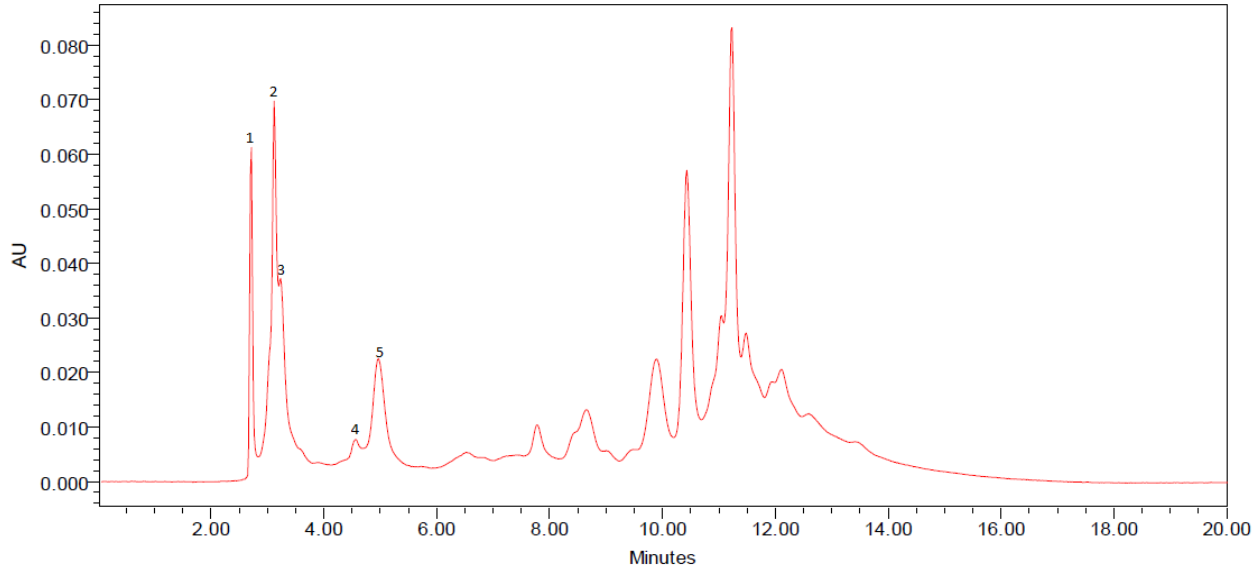

Figure 3: HPLC chromatogram of the seed extract using diode array detection at 220 nm. (1) L-Dopa, (2) tyrosine, (3) isovitexin, (4) caffeic acid, and (5) gallic acid.

Table 6: Retention time and concentration of phenolic compounds of *M. cochinchinensis* identified by HPLC.

Phenolic compounds	Retention time (min)			Concentration (mg mL^{-1})			
	Standard	Leaves	Seed	Root	Leaves	Seed	Root
Caffeic acid	4.77	-	5.25	4.97	-	0.45	0.44
Gallic acid	4.91	5.01	4.97	5.25	0.36	0.26	0.39
Isovitexin	3.42	3.62	3.27	3.24	0.12	0.05	0.65
L-Dopa	2.69	2.69	2.71	2.6	0.42	0.11	0.42
Quercetin	7.08	7.16	7.42	-	0.91	0.77	-
Tyrosine	2.97	3.05	3.12	3.12	131.95	136.73	17.84

Table 7: Regression equation and linear range used for the quantification of compounds in extractive samples.

Phenolic compounds	Linear range (µg/ml)	Regression equation	Correlation coefficient (r)
Caffeic acid	10 – 1000	Y= 42974x - 335020	0.9999
Gallic acid	1 – 500	Y= 107799x + 883486	0.9976
Isovitexin	1 – 500	Y= 7621.6x + 21194	0.9998
L-Dopa	50 – 1000	Y= 31383x + 305479	0.9995
Quercetin	10 – 1000	Y= 22130x - 317586	0.9963
L-Tyrosine	50 – 1000	Y= 241.82x + 7863	0.9991

CONCLUSION

The results from this study showed that the methanol and water aqueous extracts of the leaves, seed and root of *M. cochinchinensis* possess allelochemicals that was found to suppress the germination and root growth of *S. hermonthica*, and that the inhibition was concentration and extraction solvent-dependent. The identification of allelochemicals in *M. cochinchinensis* has now demonstrated another biological activity from a C-glycosylflavone, isovitexin and quercetin detected in the leaves and root of the plant. Henceforth, the results of this study had confirmed that *M. cochinchinensis* contained bioactive compounds that can inhibits the germination and growth of weeds in arable crops especially *S.*

hermonthica. This discovery provides the potential for transferring biochemical traits of *M. cochinchinensis* into edible legumes through genetic introgression and the formulation of bio-herbicides that could be used as environmentally friendly herbicides to control weeds.

References

Akal, P.S., Avav, T. & Magani, I. E. (2012). Effects of Time of Intercropping of Mucuna (*Mucuna cochinchinensis*) in Maize (*Zea mays*) for Weed and Soil Fertility Management. International Journal of Agriculture and Biology, 1814–9596.

Avav T., Shave, P. A. & Hilakaan, P. H., (2008). Growth of Mucuna accessions under fallow and their influence on soil and weeds in a sub-humid savanna environment. Journal of Applied Biosciences, 10: 224 -228.

Bhowmik, P. C. & Inderjit , J. (2003). Challenges and opportunities in implementing allelopathy for natural weed management. Crop Protection, 22: 661-671.

Day, M. D., Kawi, A., Kurika, K., Dewhurst, C. F., Waisale, S., Saul-Maora, J., Fidelis, J., Bokosou, J., Moxon, J., Orapa, W. & Senaratne, K. A. D. (2012). *Mikaniami crantha* Kunth (Asteraceae) (mile-a-minute): Its distribution and physical and socioeconomic impacts in Papua New-Guinea. Pac. Sci. 66:213-223.

Finney, D. J. (1971). Probit Analysis. Cambridge University Press, Cambridge. 333 pp.

Fujii, Y. (1994). Screening of allelopathic candidates by new specific discrimination, and assessment methods for allelopathy, and the inhibition of L-DOPA as the allelopathic substance from the most promising velvetbean (*Mucuna pruriens*) Bull. Nat. Inst. Agro-Environ. Sci. 10:115-218 (in Japenese with English summary).

Fujii, Y., Shibuya, T., & Yasuda, T. (1991). L-3,4-dihydroxypheny -lalanine as an allelochemical candidate from *Mucuna pruriens* (L.) DC. var. *utilis*. Agric. Biol. Chem, 55:617–618.

Geissman, T. A, & Crout, D. H. G. (1969). Organic chemistry of secondary plant metabolism. First edition. San Francisco: Freeman-Cooper. 429 pp.

Hasnol, O., Farawahida, M. D. & Zulkifli, H. (2012). Best management practices for oil palm cultivation on peat: *Mucuna bracteata* as a cover ground crop. Malaysia Palm Oil Board (MPOB) Information Series. ISSN 1511-7871. p. 588-592.

Hooper, A. M., Tsanuo, M. K., Chamberlain, K., Tittcomb, K., Scholes, J. Z., Hassanali, A., Khan, Z. R. & Picketta, J. A. (2010). Isoschaftoside, a C-glycosyl flavonoid from *Desmodium uncinatum* root exudate is an allelochemical against the development of *Striga*. Phytochemistry. 71: 904–908.

Lagoke, S. T. O., Parkinson, V. & Agunbiade, R. M. (1991). Parasitic weeds and control methods in Africa in S. K. Kim (Ed). Proceedings of International Workshop Organized by IITA, ICRISAT and IDRC, 22nd – 24th August, Ibadan, Nigeria.

Park, H. S., Ju, H. L., Hyun, J. K. Hyun, J. C. & Lee, I. S. (2007). Antioxidant flavone glycosides from the leaves of *Sasa borealis*. Archives of Pharmacal Research. 30 (2):161–166.

Shararudin, B. & Jamaluddin, N. (2007). Golden Hope's experiences with establishing *Mucuna bracteata* under oil palm. *Mucuna bracteata*: A cover crop and Living Green Manure (Goh, K. J. and Chiu, S. B. eds.). Agricultural Crop Trust. 97 – 109 pp.

Siddiqui, Z. S. (2007). Allelopathic effects of black pepper leachings on *Vigna mungo* (L.) Hepper. Acta Physiol. Plant. 29:303–308.

Swain, T. (1979). Secondary compounds as protective agents. Annu. Rev. Plant Physiol, 28: 429-501.

Xuan, T. D., Shinkichi, T., Hong, N. H., Khanh, T.D. & Min, C. (2004). Assessment of phytotoxic action of *Ageratum conyzoides* L. (billy goat weed) on weeds. Crop Protection, 23: 915 – 922.

Yang, W. D., Zhang, X. L., & Liu, J. S. (2005). The influence of allelochemical on the growth of *Alexandrium tamarense*. China Environmental Science, 25(4): 417–419.

Zeraik, M. L. & Yariwake, J. (2010). Quantification of isoorientin and total flavonoids in *Passiflora edulis* fruit pulp by HPLC-UV/DAD. Micro-chemical Journal, 96 (1): 86–91