ACTIVITY AND ISOLATION OF THE ALLELOPATHIC COMPONENTS OF GMELINA (*Gmelina arborea* Roxb) EXTRACTS AGAINST POPPING POD (*Ruellia tuberosa*) AND CORN (*Zea mays* L.)

Rosalyn P. Alburo and Hemres M. Alburo
Cebu Technological University-Argao Campus
Allan A. Ramal,
Visayas State University
Ma. Ligaya Suico
University of San Carlos, Cebu City.

ABSTRACT

The allelopathic influence of *Gmelina arborea* was determined on the seedling growth of popping pod and corn. Gmelina leaves, roots, fruits and bark were collected and air-dried. The active components were extracted using solvents of different polarities, namely, n-hexane, ethyl acetate, ethanol and water. Bioassay revealed that *G. arborea* Roxb extracts from fruits, leaves and roots using ethyl acetate as solvent inhibited the seedling growth of corn. Allelopathic activity was also noted on popping pod, however, the effect was not comparative to that of the commercial herbicide. Phytochemical screening revealed the presence of saponins, flavonoids and tannins and phenolic compounds in the gmelina fruit extract. Second bioassay done after extraction revealed that tannins and phenolic compounds exhibited the allelopathic effect. Further tests on the fruit extract through thin layer chromatography showed the presence of eight spots in the chromatogram, equivalent to eight types of tannins and phenolic compounds.

Keywords: Allelochemicals, Phytochemical screening, Biological control, Herbicide, Allelopathy

INTRODUCTION

The global population reached 6.705 billion in 2008 and is projected to increase to 9.353 billion in 2050 (Koul, O and Walia, S., 2009). Such change will have a profound implication on the economy, health, environment and quality of life of the people. A huge population in the developing countries requires increased amounts of food and fiber from a shrinking agricultural land base. Intensification of agriculture through expansion of irrigation facilities, introduction of high-yielding varieties and application of increased amounts of agrochemicals has been in progress. In addition, cultural practices such as spacing, crop rotations, sowing times and tillage methods have been modified to achieve maximum productivity per unit time from the available land.

The ever-growing population of the world has triggered the introduction of pesticides in agriculture to control insects and obnoxious weeds that could reduce crop yield and affect food security. Weeds are unwanted plants that compete with crops for nutrients and space resulting to physiological malfunctioning of the crop that develops into a disease thus, eventually reduces its yield. Weeds further harbor some pathogens that will consequently infect the

growing crops at various stages. Weeds pose a recurrent and ubiquitous threat to agricultural productivity. Among the pests, weeds alone are held responsible for nearly 34% reduction in crop yield (Oerke EC., 2006). According to Agrow 2007 report, the total value of world's agrochemical market was between US$31 –35 billion and among the products herbicides accounted for 48%, followed by insecticides (25%) and fungicides (22%).

Due to long term negative effects of inorganic herbicides much focus is set on organic alternative herbicides. One of these is the possible use of allelochemicals. These are chemicals released by plants that can suppress or stunt growth of other plants. The concept of allelopathy received new attention in 1974, after the publication of the first book in English on allelopathy by Elroy L. Rice. He defined allelopathy as the effect(s) of one plant on other plants through the release of chemical compounds in the environment (Rice, 1984a as cited by Bhadoria, 2011). This definition is largely accepted and includes both positive (growth promoting) and negative (growth inhibiting) effects. Many ecologists, however, favor definitions including only negative effects in allelopathy. Lambers *et al.,* (1998) for example, defined allelopathy as the growth suppression of one plant species by another due to the release of toxic compounds. Kohli et al. (1998) and Singh *et al.,* (2001) opined that allelopathy refers to any direct or indirect effect of plants on other plants through the release of chemicals and plays an important role in many agro-ecosystems (Bhadoria, 2011).

The readily visible effects of allelochemicals on the growth and development of plants include inhibited or retarded germination rate; seeds darkened and swollen; reduced root or radicle and shoot or coleoptile extension; swelling or necrosis of root tips; curling of the root axis; discoloration, lack of root hairs; increased number of seminal roots; reduced dry weight accumulation; and lowered reproductive capacity. These gross morphological effects may be secondary manifestations of primary events, caused by a variety of more specific effects acting at the cellular or molecular level in the receiver plants (Rice, 1974). Allelochemicals from several plants have been identified and their activities have also been established (Bhadoria, 2011).

Gmelina (*Gmelina arborea* Roxb.) has gained prominence not only in the Philippines but also among our Asian neighbors because of its economic importance. It is a raw material for pulp and paper making, posts, house timbers and poles while rotary cut veneers are utilized for plywood. It is also utilized as fuelwood and sometimes as feed for cattles, being a multiple-use species (Palaypayon and Batalon, 2002). Studies on the allelopathic potential of gmelina have been conducted. Ramirez and Stefano (1994) tested its allelopathic potential on tomato seeds exposed to indirect natural light. Results showed negative effect of the leaf extracts on germination, growth and total biomass. Further results showed that roots were more affected. Allelopathic influence of gmelina was also determined on the seed germination and seedling growth of green gram (Vignaradiata) and black gram (Vignamungo) (Madhan, S., et al, 2009). The *G. arborea* extracts inhibited the germination, seedling growth and the total protein content of both test crops. However, there is still a need to explore the allelopathic effects of gmelina to weeds. Hence, this study was conducted to test the allelopathic activity of Gmelina (*Gmelina arborea* Roxb) and isolate its allelopathic components.

MATERIALS AND METHODS

Collection of Plant Material

Gmelina plant parts were collected from the gmelina plantation of CTU-Argao Campus at the Forestry Station in Canbantug. Parts of approximately the same age were chosen.

Selection of Target Species

One important consideration for bioassays with allelophatic action is the selection of target-species. The major requirements are fast germination, uniformity and sensibility (Ferreira, A.G., and A´ quila, M.E.A., 2000). According to Dayan and Duke (2006), these characteristics are essential for obtaining data without undesirable delays. The objective of these assays is to find substances for weeds control, but the use of these species presents some limitations as not uniform germination, due to the low diaspores homogeneity, unlike what occurs when using species cultivated in commercial scale (Silva *et al.,* 2009). It is for these reasons that popping pod (*Ruellia tuberosa*) and corn (*Zea mays* L.*)* were chosen to represent broadleaf and grass types of weeds, respectively.

Extraction Using Solvents of Different Polarities

Plant extract were prepared following the procedure described by Guevara and Recio (1985) with modification.

Five hundred grams of the ground, dried plant materials were extracted with different solvents of increasing polarity i.e., hexane, ethyl acetate, ethanol and water. Approximately two liters solvent (or enough to cover the sample) was added and the filtrate was collected after 24 hours. Only 1 extraction was done for each solvent. The filtrates were concentrated at 35 degrees – 40 degrees Celsius under vacuum using a rotary evaporator.

Bioassay of Extracts from Different Solvents (Morallo-Rejesus and Decena, 1982)

Bioactivity of solvent extracts from barks, roots, leaves and fruits of gmelina tree was investigated against popping pod and corn seedlings under greenhouse conditions. The treatments were the following:

TPPositive control (Sharpshooter, commercial herbicide)
TS1Water
TS2Hexane
TS3Ethanol
TS4Ethyl Acetate
TB1Bark, water solvent
TB2Bark, hexane solvent
TB3Bark, ethanol solvent
TB4Bark, ethyl acetate solvent
TF1Fruit, water solvent
TF2Fruit, hexane solvent
TF3Fruit, ethanol solvent
TF4Fruit, ethyl acetate solvent
TR1Roots, water solvent
TR2Roots, hexane solvent
TR3Roots, ethanol solvent

 ISSN: 1656-0264

TR4 Roots, ethyl acetate solvent
TL1 Leaves, water solvent
TL2 Leaves, hexane solvent
TL3 Leaves, ethanol solvent
TL4 Leaves, ethyl acetate solvent

Fifteen viable 10 day-old popping pod seedlings were used per treatment and each treatment was replicated three times. Ten seedlings were used for corn. The seedlings were regularly watered. Approximately 100mL of the recommended dose of herbicide, which is 5% concentration, was used for all treatments and applied through a hand-held spray.

Data Gathering

Mortality/changes of the seedlings were observed 72 and 96 hours after application of treatments.

Statistical Analysis

Completely Randomized Design (CRD) was used. All allelopathic data were analyzed using Analysis of Variance (ANOVA). The Duncan's Multiple Range Test (DMRT) was used to identify its significance.

Phytochemical Screening

Solvent extract that exhibited the most allelopathic activity was phytochemically screened for secondary metabolites using the methods of Guevarra and Recio (1985).

Screening for Alkaloids

The Culvenor – Fitzgerald laboratory analysis method was used in the screening for alkaloids. Formation of precipitate is an indication of a positive test.

Screening for Flavonoids

The crude ethanol and ethyl acetate extracts were defatted with hexane and further extracted with ethanol using a Soxhlet apparatus. The flavonoid extracts were subjected to the Bate – Smith and Metcalf test and the Wilstatter "Cyanidin" test. Gradual development of strong red or violet color for positive Bate-Smith and Metcalf Test. Positive result is orange to red or crimson and magenta or greenish blue color for Wilstatter "Cyanidin" test.

Screening for Saponins

The presence of saponins in the extracts was tested through the froth test, capillary method and the Liebermann – Burchard test. Positive result is the formation of persistent 1cm froth. For Liebermann –Burchard test positive result is reddish violet color at the junctionof the two layers and a bluish green color in the acetic anhydride layer indicates the presence of unsaturated sterols and or triterpenes.

Screening for Cardenolides and Bufadienolides

The presence of cardenolides and bufadienolides was detected through three test, namely: the Keller – Kiliani test, t he Liebermann – Burchard test and the Kedde test. Positive result for Keller - Kiliani is the presence of reddish brown which may gradually become bluish or purplish color indicates the presence of two deoxysugars. Kedde test positive result is blue violet to purple indicating the presence of unsaturated lactone ring.

Screening for Tannins and Polyphenolic Compounds

Tannins were detected in the plant extract by the gelatin test and confirmed by the ferric chloride test. Positive result for ferric chloride test is the blue to black color for the presence of hydrolysable tannins while brownish green or greenish blue/black may indicate condensed tannins. Polyphenolic compounds give a negative ferric chloride test.

Screening for Anthraquinones

Anthraquinones were detected using Borntrager's test and the Modified Borntrager's test. Positive result is presence of red coloration because of sublimation of yellow crystals of antraquinones for Borntrager's test. For Modified Borntrager's test, positive result is indicated by pink color because of sublimation of yellow crystals of anthraquinones.

Screening for Cyanogenic Glycosides

Grignard's test was used to detect the presence of cyanogenic glycosides in the ethanol and ethyl acetate extract. Positive result is the appearance of various shades of red within 15 minutes.

Extraction of Detected Secondary Metabolites

Standard methods in the extraction for secondary metabolites that tested positive in the phytochemical screening were followed.

Bioassay of Extracted Secondary Metabolites

The extracted secondary metabolites from Procedure H were subjected to another bioassay to determine which of these caused the allelopathic activity of Gmelina. The treatments which were replicated twice were as follows:

TPcommercial herbicide (positive control)
TSsolvent (negative control)
TRextracted secondary metabolite

Ten corn seedlings were used. Corn was chosen basing on the results of the first bioassay. Same procedure of the first bioassay was followed.

Isolation

The bioactive components of the extract that exhibited higher allelopathic activity were isolated and characterized using Thin Layer Chromatography technique using pre coated TLC plates. Different solvent systems were used.

 ISSN: 1656-0264

RESULTS AND DISCUSSION

Extraction of secondary metabolites from bark, leaves, fruits and roots of Gmelina was done using solvents of different polarities, i.e. hexane the most nonpolar, ethyl acetate, ethanol then water as the most polar. The colors of the concentrated extracts using different solvents are presented in Table 1. Based on the polarities of the solvents, it is expected that the non-polar compounds would be extracted by n-hexane. The ethyl acetate fraction would contain mixtures of polar and non-polar components while the ethanol fraction being more polar than ethyl acetate because of its hydroxyl group would contain polar compounds. The most polar would be extracted by water.

Table 1. Color of Extracts.

Plant Source	Solvent	Description
Bark	Hexane	Colorless
Bark	Ethyl acetate	Very light yellowish brown
Bark	Ethanol	Brown
Bark	Water	Brown
Roots	Hexane	Colorless
Roots	Ethyl acetate	Very light yellowish brown
Roots	Ethanol	Brown
Roots	Water	Brown
Leaves	Hexane	Colorless
Leaves	Ethyl acetate	Yellowish brown
Leaves	Ethanol	Brown
Leaves	Water	Brown
Fruits	Hexane	Colorless
Fruits	Ethyl acetate	Yellowish with reddish brown insoluble droplets
Fruits	Ethanol	Very dark reddish brown
Fruits	Water	Very dark reddish brown

Allelopathic Activity Test of Gmelina Extracts

The concentrated extracts were subjected to allelopathic activity test to determine which solvent extract and which gmelina plant part had the bioactive components. Analysis of Variance (ANOVA) gave a highly significant difference among treatment means for popping pod and corn 72 and 96 hours after treatment, hence, further test using the Duncan's Multiple Range Test was conducted. The succeeding tables present the bioassay results. As can be seen in Tables 2 and 3, the positive control that is the commercial herbicide resulted in a 100% effect to the popping pod seedlings. Extract from the fruits with ethyl acetate as solvent followed with a mean mortality of 6.67. However, DMRT indicates that this mean is different from that of the positive control that would denote that the extract is not as effective as that of the commercial herbicide.

Table 2. Bioassay result for popping pod (72 hours after treatment).

Treatment	Mean Mortality	DMRT Grouping*	Treatment	Mean Mortality	DMRT Grouping*
Positive Control	15.0	A	Leaves, Ethanol	0.667	E
Fruit, Ethyl Acetate	6.67	B	Roots, Hexane	0.333	F
Roots, Ethyl Acetate	6.00	BC	Leaves, Hexane	0.333	F
Roots, Ethanol	3.33	D	Leaves, Ethyl Acetate	0.333	F
Bark, Ethyl Acetate	3.00	DE	Water	0	
Fruit, Ethanol	3.00	DE	Ethyl Acetate	0	
Fruit, Hexane	1.67	DE	Bark, Water	0	
Roots, Water	1.67	DE	Bark, Hexane	0	
Bark, Ethanol	1.33	DE	Fruit, Water	0	
Ethanol	1.00	DE	Leaves, Water	0	
Hexane	0.667	E			

** Means with the same letter(s) are not significantly different from each other.*

Further tests for the difference between means for allelopathic effect of gmelina extracts to corn seedlings show that the commercial herbicide had 100% effect. This was followed by ethyl acetate extracts from the root part. However, based on DMRT, this result was different from that of the positive control which means that their effects are not comparable 72 hours after treatment (Table 4). However, 96 hours after treatment, all the ethyl acetate extracts with exception for the bark have no significant difference compared to that of the commercial herbicide (Table 5). This would mean that these extracts have comparable allelopathic effect with that of the positive control. It is also worth noting that allelopathic effect is non-existent for all treatments using the solvent. These treatments were incorporated to check in case allelopathic effect is caused by the solvents and not by the extracts.

Phytochemical Screening

The fruit extract with ethyl acetate as solvent was subjected to phytochemical screening on the basis that it gave the best allelopathic activity of all the extracts for both popping pod and corn seedlings

As can be gleaned from Table 6, the fruit extract using ethyl acetate as solvent contained the secondary metabolites flavonoids, saponins, tannins and

 ISSN: 1656-0264

phenolic compounds. The positive "Cyanidin" Test for flavonoids indicates the presence of an x-benzopyrone nucleus which is a characteristic structure of flavonoid compounds. The Bate-Smith and Metcalf Tests indicates the presence of leucoanthocyanins. These are the colorless flavonoids in plants. Harborne (1993) reported that flavonoid potassium bisulphates also occur in the dicotyledons of the Compositae and Umbelliferae families. He added that 6-hydroxy flavones were isolated in *Compositae orastiridae* and concluded that the 6-hydroxy compounds are more effective than the 8-hydroxy compounds as toxins to predating animals.

Saponins have detergent properties, hence, are slippery and have lesser capillarity than water. This was clearly evident during the filtration stage. Extracts from the fruits of Gmelina were slippery to the touch and readily formed froth. In plants, saponins serve as anti-feedants and to protect plants againstmicrobes and fungi (Cornell University, 2008). Most saponins, which readily dissolve in water, are toxic to fishes hence they are primarily used to obtain aquatic and marine resources (Forester, H., 2006). These may be the reasons why fruits of gmelina are not being eaten by birds and are just left alone by grazing animals in the field.

Tannins, also known as terpenes or isoprenoids are one of the major groups of natural compounds and perform a variety of important functions in the primary metabolism (Lohr, M. *et al.,* 2012). It is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine (McGee, H., 2004). The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation (Ferell, K.E. and Thorington, R.W., 2006).

Table 3. Bioassay result for popping pod (96 hours after treatment).

Treatment	Mean Mortality	DMRT Grouping*	Treatment	Mean Mortality	DMRT Grouping*
Positive Control	15	A	Leaves, Ethanol	0.667	DE
Fruit, Ethyl Acetate	6.667	B	Roots, Hexane	0.333	F
Roots, Ethyl Acetate	6.00	BC	Leaves, Hexane	0.333	F
Roots, Ethanol	3.33	BCD	Leaves, Ethyl Acetate	0.333	F
Bark, Ethyl Acetate	3.00	DE	Water	0	
Fruit, Ethanol	3.00	DE	Ethyl Acetate	0	
Fruit, Hexane	1.67	DE	Bark, Water	0	
Roots, Water	1.67	DE	Bark, Hexane	0	
Bark, Ethanol	1.33	DE	Fruit, Water	0	
Ethanol	1.00	DE	Leaves, Water	0	
Hexane	0.667	DE			

**Means with the same letter (s) are not significantly different from each other.*

 ISSN: 1656-0264

Table 4. Bioassay result for corn seedlings (72 hours after treatment).

Treatment	Mean Mortality	DMRT Grouping*	Treatment	Mean Mortality	DMRT Grouping*
Positive Control	10.0	A	Ethanol	0	
Roots, Ethyl Acetate	9.00	B	Bark, Water	0	
Fruit, Ethyl Acetate	8.67	BC	Bark, Hexane	0	
Leaves, Ethyl Acetate	7.67	DE	Bark, Ethanol	0	
Bark, Ethyl Acetate	6.00	F	Fruit, Hexane	0	
Fruit, Water	2.00	G	Roots, Water	0	
Ethyl Acetate	0.333	H	Roots, Ethanol	0	
Fruit, Ethanol	0.333	H	Leaves, Water	0	
Roots, Hexane	0.333	H	Leaves, Hexane	0	
Water	0		Leaves, Ethanol	0	
Hexane	0				

Means with the same letter (s) are not significantly different from each other.

Table 5. Bioassay results for corn seedlings (96 hours after treatment).

Treatment	Mean Mortality	DMRT Grouping*	Treatment	Mean Mortality	DMRT Grouping*
Positive Control	10.0	A	Ethanol	0	
Fruit, Ethyl Acetate	9.67	ABC	Bark, Water	0	
Leaves, Ethyl Acetate	9.67	ABC	Bark, Hexane	0	
Roots, Ethyl Acetate	9.33	ABC	Bark, Ethanol	0	
Bark, Ethyl Acetate	7.67	D	Fruit, Hexane	0	
Fruit, Water	2.00	E	Roots, Water	0	
Ethyl Acetate	0.333	F	Roots, Ethanol	0	
Fruit, Ethanol	0.333	F	Leaves, Water	0	
Roots, Hexane	0.333	F	Leaves, Hexane	0	
Water	0		Leaves, Ethanol	0	
Hexane	0				

Means with the same letter (s) are not significantly different from each other.

 ISSN: 1656-0264

Table 6. Results of the detection for secondary metabolites.

Secondary Metabolites	Test Used	Basis	Observation	Result
Alkaloids	Culvenor-Fitzgerald	Turbidity or formation of precipitate	Clear solution	negative
Flavonoids	Wilstatter "Cyandic Test"	Red or violet color	Red color	positive
	Bate-Smith Test	Red color	Strong red color	positive
	Metcalf Test	Red color	Strong red color	positive
Saponins	Froth Test	Persistent froth/foam formation	3.0o cm foam	positive
	Capillary Tube Method	Less capillarity than water	Slippery and lower capillarity level	Positive
	Liebermann – Burchard Test	Orange to red color	Red color	positive
Cardenolides and Bufadienolides	Keller-Kiliani Test	Reddish brown which ma turn blue to purple	No color change	negative
	Liebermann – Burchard Test	Orange to red color	No color change	negative
	Kedde Test	Blue-violet color	No color change	negative
Tannins and Phenolic Compounds	Gelatin Test	Formation of precipitate	Precipitate formation	positive
	Ferric Chloride Test	Blue-black color Brownish-green color	Brownish-green color	positive
Anthraquinones	Borntragger's Test	Red color in the lower layer	No color formation	negative
	Modified Borntragger's Test	Pink color	No color formation	negative
Cyanogenic Glycosides	Guignard Test	Shades of red color on picrate paper	No red color	negative

Extraction of Flavonoids, Saponins and Tannins

Extraction of Flavonoids (Guevarra and Recio, 1985)

A 10-gram equivalent of the plant material from ethyl acetate extract was evaporated to incipient dryness over a water bath. The residue was defatted with hexane until the extract was almost colorless. The hexane extract was discarded.

Extraction of Saponins (Leung, 1980)

A 10-gram equivalent of the plant material from ethyl acetate extract was evaporated to dryness over a water bath. The residue was washed twice with hexane. The hexane-insoluble residue was discarded. The washings were again evaporated to dryness over a water bath. The residue was treated with 5 mL of n-butanol.

Extraction of Tannins and Phenolic Compounds (Harborne, 1989)

Fifty g fresh leaf litters were soaked in 150 mL of distilled water for 24 h at 25°C to extract the allelochemicals. The extract was filtered through two layers of Whatman No.1 filter paper and further extracted as per Harborne *et al.,* (1989) for identification of tannins. The aqueous leachates were mixed with an equal volume of methanol acidified with 1 mL of HCl. This methanolic extract was mixed with 100 mL peroxide free diethyl ether in a separating funnel. The upper organic phase was separated in a 100 mL Erlenmeyer flask. To the aqueous phase twice the volume of ethyl acetate was added and the organic phase was separated using a separating funnel. The process was repeated 3 times. All these organic phases were mixed together and to it 3 g sodium sulfate was added and filtered. The final extract was dried thoroughly in a rotary evaporator and stored in airtight vials.

Allelopathic Activity Test for Flavonoids, Saponins and Tannins

The extracted flavonoids, saponins and tannins were subjected to bioassay to determine which caused the allelopathic activity of Gmelina. Results showed that exhibited the highest mean effect (Table 7.) It can therefore be inferred that the observed allelopathic activity was due to the tannins and phenolic compounds.

Table 7. Bioassay results for flavonoids, saponins and tannin extracts.

Treatment	Mean Mortality
Positive Control	10.0
Flavonoid extract	0
Saponins extract	1.00
Tannins and phenolic extracts	8.50
Ethyl acetate	1.50
n-butanol	0.50

Thin Layer Chromatography

The number of components present in the tannin extract was determined through thin layer chromatography on silica gel G. Samples were spotted on pre-coated plates of silica gel G and separated using chloroform: ethyl-acetate: formic acid (5:4:1, v/v/v) as mobile phase. The phenolic compounds were visualized by spraying the plates with FeCl3 (2% in ethanol). Based on the chromatograms, eight spots were visualized, which could very well represent eight types of tannins and phenolic compounds present in the sample (Table 8.)

Experimental conditions: Solvent system, chloroform: ethyl-acetate: formic acid (5:4:1, v/v/v); absorbent, silica gel G.

Phenolic and terpenoids are principle allelochemicals (secondary plant metabolites) present in leaves, fruits, vegetables and legumes.

 ISSN: 1656-0264

The release of phenolic compounds adversely affects the germination and growth of plants through their interference in cell division, mineral uptake and several other biosynthetic processes (Putnam, A.R. and Duke, W.B., 1978 and Moreland, D.E. and Novitsky, W.D., 1987). The study of Rocha, *et al.,* (2006) on diacetylverrucarol, a terpene produced by the fungus *Myrothecium verrucaria*, showed that is caused growth retardation, wilting, chlorosis, and necrosis in some plants. Rimando *et al.,* 2001 as cited by Madhan, S., 2009 also reportedthat 1,2- benzenedicarboxylic acid, bis(2-ethylhexyl) ester was found in the rice (*Oryza sativa* L.) cv. Taichung Native 1 and it could inhibit the germination of lettuce (*Lactuca sativa* L.). Several other benzenedicarboxylic acids like 1,2-benzenedicarboxylic acid in *Cucumis sativus* (Asao, *et al.,* 1999) and 1,3-benzenedicarboxylic acid (Song, *et al.,* 2002) showed allelopathic effects. Several benzoic acid derivatives are allelopathic to several plant species. Sasikumar *et al.,* 2001) reported the allelopathic effects of benzoic acid derivatives on Redgram.

Similarly several reports are available on the inhibitory effects of terpenoids released from trees. The allelopathic potential of sunflower on wild barley germination and seedling growth was reported by Ashrafi *et al., (*2008). Terzi *et al.,* (2008) has reported the allelopathic effects of Juglone and decomposed walnut leaf juice on muskmelon and cucumber seed germination and seedling growth. Song *et al.,* (2002) has reported that phenolics were involved in the phytotoxicity caused by sugarcane straw. Derivatives of benzoic acids can alter transpiration and ion uptake as well as induce oxidative cell damage (Politycka, B., 1996)).

Table 8. Thin layer chromatogram of tannin extract.

Spot No.	Color	Distance Travelled (mm)	R1
1	Yellow green	10	0.12
2	Yellow green	16	0.19
3	Blue green	23	0.28
4	Light yellow	31	0.37
5	Brownish green	36	0.43
6	Blue green	40	0.48
7	Blue green	47	0.57
8	Yellow	64	0.77
Solvent	-	83	-

CONCLUSIONS

Gmelina (*Gmelina arborea* Roxb) exhibited allelopathic activity to the target species, popping pod (*Ruellia tuberosa*) and corn (Zea mays L.) seedlings. Fruit extract using ethyl acetate as solvent gave the best allelopathic activity compared to bark, roots and leaves. Screening revealed that the fruit extract contained the secondary metabolites flavonoids, saponins and tannins. Of the three metabolites, further bioassay showed that tannins and phenolic compounds caused the allelopathic activity of Gmelina fruit extract. Gmelina fruit extract can be recommended as an alternative herbicide.

ACKNOWLEDGEMENT

The authors are grateful to Cebu Technological University for the financial assistance and the University of San Carlos for the use of facilities.

LITERATURE CITED

Agrow (2007). Agrow's Top 20, Edition-DS258. Informa Health Care, London; 2007.

Asao, T., Pramanik, M.D.H.R., Tomita, K., Ohba, Y., Ohta, K., Hosoki, T. and Matsui, Y. (1999). Influences of phenolics isolated from the nutrient solution nourishing growing cucumber (Cucumis *sativus* L.) plants on f ruit yield. *Journal of Japanese Society of Horticultural Science* 68: 847-853.

Ashrafi, Z.Y., Sadeghi, S., Mashhadi, H.R. and Hassan, M.A. (2008). Allelopathic effects of sunflower (Helianthus *annuus)* on germination and growth of wild barley (Hordeum *spontaneum). Journal of Agricultural Technology* 4: 219-229.

Bhadoria, P.B.S. (2011). Allelopathy: A Natural Way Towards Weed Management. Americal Journal of Experimental Agriculture 1(1): 7-20.

Dayan, F.E., Duke, S.O., (2006). Clues in the search for new herbicides. In: Reigosa, M., Pedrol, N., Gonzalez, L. (Eds.), Allelopathy: A Physiologi cal Process with EcologicalImplications. Springer, Amsterdam, pp. 63–83.

Ferreira, A.G., and A´ quila, M.E.A., 2000). Allelopathy: an emerging field of ecophysiology. Rev. Bras. Fisiol. Veg. 12, 175–204.).

Ferrell, K.; Thorington, Richard W. (2006). *Squirrels: the animal answer guide*. Baltimore: Johns Hopkins University Press. p. 91).

Foerster, Hartmut, (2006). "MetaCyc Pathway: saponin biosynthesis I". Guevara, B. and B. Recio. 1985. (eds.). Phytochemical, Microbiological and Pharmacological Screening of Medicinal Plants, (Revised edition).A Supp. of the ActaManilana, Research Center, University of Santo Tomas, Manila. 81.

Harborne, J.B. (1989). General procedures and measurements of total Phenol ics. In: *Methods in Plant Biochemistry. Vol* 1. *Plant phenolics.* pp. 10-11 Academic press, New York.

Leung, A.Y. 1980. Alkaloids in drugs. Grzimek Encyclopedia of Comon Natural Ingredients. John Wiley and Sons, Inc. New York.

Madhan, S. S. R., R. Gritish, N. Karthik, R. Rojendran, and V.S. Mahendran. (2009). Allelopathic effects of phenolics and terpenoids extracted from *Gmelinaarborea* on germination of black gram (*Vignamungo*) and green gram (*Vignaradiata*). Allelopathy Journal. 2009. 23:2, 323-332.

Martin Lohra,□, Jörg Schwenderb, Jürgen E.W. Pollec,□, (2012). Plant Science Journal. 185-186, 9-22. www.elsevier.com

McGee, Harold (2004). *On food and cooking: the science and lore of the kitchen*. New York: Scribner. p. 714).

Morallo-Rejesus, B. and A. Decena. (1982). The activity, isolation, purification and identification of the insecticidal principles from Tagates. Phil. J. Crop Science. 7:31-36.

 ISSN: 1656-0264

Moreland, D.E. and Novitsky, W.D. (1987). Interference by luteolin, quercetin and taxifolin with chloroplast □mediated electron transport and phos phorylation *Plant and Soil* 98:145-150.

Oerke EC. Crop losses to pests. Journal of Agricultural Sciences 2006;144:31–43.

Palaypayon, C. M. and J. M. Batalon. (2002). Issues and Facts on Yemane. Re search Information Series on Ecosystems. 14:3 Sept-Dec. 2009.

Politycka, B. (1996). Peroxidase activity and lipid peroxidation in roots of cu cumber seedlings influenced by □derivatives of cinnamic and benzoic acids. *Acta Physiologia Plantarum* 18: 365-370.

Putnam, A.R. and Duke, W.B. (1978). Allelopathy in agricultural ecosystems, *Annual Review of* □*Phytopathology* **16:** 431-451).

Rimando, A.M., Olofsdotter, M. and Duke, S.O. (2001). Searching for rice allelochemicals. *Agronomy* □*Journal* **93:** 16-20.

Rocha, O.; K. Ansari; F. M. Doohan (April 2005). "Effects of trichothecene mycotoxins on eukaryotic cells: A review". *Food Additives and Contaminants* (University College Dublin: Taylor & Francis) 22 (4): 369–378."Saponins". Cornell University. 14 August 2008. Retrieved 23 February 2009.(http://sdbs.db.aist.go.jp)

Silva, H.L., Trezzi, M.M., Marchese, J.A., Buzzello, G., Miotto, E., Jr., Patel, F., Debastiani, F., Fiorese, J., (2009). Determinac-a~o de espe´cie indica dora e comparac-a~o de geno´tipos de girassol quanto ao potencial alelopa´tico. Planta Daninha 27, 655–663.

Song, H.K., Ahn, J.K., Ahmad, A., Hahn, S.J., Kim S.H. and Chung, I.M. (2004). Identification of allelochemicals in rice root exudates at various phono logical phases and their influence on □barnyardgrass. *Allelopathy Jour nal* **13:** 173-188.

Terzi, (2008). Allelopathic effects of Juglone and decomposed walnut leaf juice on muskmelon and □cucumber seed germination and seedling growth. *African Journal of Biotechnology* **7:** 1870-1874