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Short Communication

Evaluating Herbicidal Potential of Aqueous–ethanol Extracts of Local Plant Species against *Echinochloa crus-galli* and *Raphanus sativus*

Thanatsan Poonpaiboonpipat[1*] and Juangjun Jumpathong[1]
[1]*Department of Agricultural Sciences, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok 65000, Thailand*
[*]For correspondence: thanatchasanhap@nu.ac.th

Abstract

Crude aqueous–ethanol leaf extracts of local plant species can be used as natural herbicides. To test this hypothesis, crude aqueous–ethanol leaf extracts of 19 local species were tested for major secondary metabolites (total phenolic and flavonoid contents) and their herbicidal activity, at concentrations of 2.5 and 5 g L^{-1}, based on germination bioassays against radish (*Raphanus sativas* L.) and barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) and distilled water was used as control. *Mitrephora wangii*, *E. scaber* and *Magnolia cathcarii* observed the highest crude yield of metabolites while *Cinnamomum porrectum* and *Zingiber zerumbet* had the minimum metabolites. *Goniothalamus calvicarpa* and *Agapetes lobbii* extract had highest total phenolic contents (143.7 and 145.86 mg GAE g^{-1} crude extract, respectively). Moreover *Ficus microcarpa extrct had* the highest phenolic contents (7,639.70 μg quercetin equivalent g^{-1} crude extract), followed by *Orophea polycarpa* while *Z. zerumbet* extrct had the least total flavonoid contents (607.97 μg quercetin equivalent g^{-1} crude extract). Aqueous-ethanol extracts derived from leaves of *Elephantopus scaber* and *A. lobbii* observed over 50% inhibition on the root growth of barnyardgrass and extracts derived from *Pachyptera hymenaea, Magnolia citrates, Anomianthus dulcis* and *F. microcarpa* observed over 50% inhibition in root length of radish. In conclusion, extracts of *P. hymenaea, F. microcarpa, A. dulcis, E. scaber* and *A. lobbii* observed strong inhibitort effects on germination and early seedling growth of radish and barnyardgrass due to higher phenolic and flavonoid contents. Results of this study might be considered for further studies to develop natural herbicides based on plant extracts. © 2019 Friends Science Publishers

Keywords: Crude extract; Phenolic content; Flavonoid content; Germination assay

Introduction

The continuous use of synthetic herbicides in agricultural and non-agricultural areas has become an increasing concern because these herbicides contaminate agricultural products, water sources, soil and air (Poonpaiboonpipat *et al*., 2013). Therefore, enhancing crop production by using eco-friendly methods is needed; especially *via* use of products based on natural compounds obtained from plants or microorganisms (Dayan *et al*., 2012). Many plant species produce some compounds that influence other species by stimulation or inhibition of growth and development and this phenomenon is called allelopathy. Allelopathic plants can play a beneficial role in weed control in several ways (Poonpaiboonpipat *et al*., 2011), namely, through crop rotation, intercropping system, or as cover crop (Mamolos and Kalburtji, 2001; den Hollander *et al*., 2007; Vasilakoglou *et al*., 2011; Shahzad *et al*., 2016a, b); mulching or incorporating plant material with soil (Laosinwattana *et al*., 2012; Farooq *et al*., 2017); use of plant based herbicides alone or in combination with synthetic herbicides (Jabran *et al*., 2008; Khan *et al*., 2012); use of crude or oil extraction (Kaur *et al*., 2010; Poonpaiboonpipat *et al*., 2013) and isolation of active compound and synthesis of derivative compounds (Heisey and Heisey, 2003). Natural products based on allelochemicals may replace or reduce the use of synthetic compounds because they are safe for humans, pets and the environment. Plant tissues including root, stem, bark, leaves, flower, fruits and seeds, almost contain organic compounds that exhibit allelopathic effects on plant growth (Jilani *et al*., 2008).

However, the important source of inhibitory activity is found in leaves as reported in *Suregada multiflorum* (Laosinwattana *et al*., 2010) and *Ageratum conyzoides* L. (Xuan *et al*., 2004). Phenolic compounds form a major group of plant allelochemicals found in the ecosystem (Li *et al*., 2010). Phenolic acids commonly found their potential and putative allelochemical compound which led to a natural herbicide formulation (Chon *et al*., 2005). Besides, these play many functions in different aspects of plant physiology. They influence the transport of plant hormones, especially auxin, which is one of their most essential functions. Their other roles include defense, allelopathy and modulation of the levels of reactive oxygen species (Buer *et al*., 2010).

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Romklao Botanical Garden under the Royal Initiative, Phitsanulok, Thailand is enriched with native plant species and therefore was focused for this study. Thus, this study was designed to analyze the major secondary metabolite contents in the leaves of 19 plant species found in this garden. Moreover, the aqueous-ethanol extracts of these species were tested to evaluate their herbicidal activity against radish and barnyardgrass, the two widely found weeds in paddy fields of Thailand.

Materials and Method

Plant Material and Extraction

Nineteen species *viz. Anomianthus dulcis* (Dunal) J. Sinclair, *Goniothalamus calvicarpa*, *Mitrephora keithii* Ridl, *Mitrephora wangii* Hu, *Orophea polycarpa* A.DC., *Elephantopus scaber* L., *Pachyptera hymenaea* (DC.) A. Gentry, *Garcinia dulcis* (Roxb.) Kurz, *Agapetes lobbii* C.B. Clarke, *Cinnamomum loureiroi* Nees, *Cinnamomum porrectum* Kosterm, *Magnolia cathcarii* (Hook.f. & Thornson) Noot, *Magnolia citrates* (Noot. & Chaoermglin), *Michelia floribunda* Fin. et Gagnep, *Manglietia garrettii* Cralb, *Magnolia henryi*, *Ficus microcarpa* L.f. var. *crassifolia* sheeh Liao, *Zingiber zerumbet* (L.) Smith.) and *Hedychium coronarium* J. Koenig found in Romklao Botanical Garden under the Royal Initiative, Phitsanulok, Thailand were used in this study. Fresh leaves of these species were cleaned several times by water, chopped into 1 cm lengths, dried at 45°C and then ground with electronic grinder into powder. Each dried material was extracted by 50% aqueous-ethanol in a ratio of 10:100 w/v and kept at 25°C for 24 h in dark condition. The extractions were filtered through filter paper (Whatman No.1 Inc.). The residues were re-extracted again in the same condition and filtration. The filtered extractions were mixed and evaporated using a rotary evaporator and then kept in a chamber set to 5°C in the dark until use. After 24 h under a partial vacuum at 45°C, the crude extract was obtained, weighed, and calculated on the dried material percentage.

Seed Germination and Seedling Growth Bioassay

Herbicidal potential of crude aqueous-ethanol extracts of above cited plant species was evaluated against radish (*Raphanus sativus* L.) and barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), widely found weeds in Thailand paddy fields. Seed of these weed species were collected and then firstly examined using seed germination test before starting the experiment. Each crude extract was diluted by 50% aqueous-ethanol giving final concentrations of 2.5 g L^{-1} and 5 g L^{-1}. Petri dishes (90 mm of diameter) were lined with the germination paper, and then 5 mL of aliquot solution was added to each of the petri dish. Twenty seeds per species was sowed on the paper and sealed with parafilm. The treatment with distilled water was used as control. The dishes were placed at room temperature (25°C–30°C) for seven days. The bioassays were done with four replicates. Number of seed germination, shoot length and root length of the indicator species were measured. The inhibition percentage was used for calculating the inhibitory activity on plant growth as followed (Hong *et al*., 2003);

Inhibition percentage sample extracts = [1 – (treatment/control)] * 100.

Analysis of the Total Phenolic Content

The total phenolic content in each of the plant crude extract was determined using the Folin–Ciocalteu method as described by Chumyam *et al*. (2013). In this experiment, each crude extract at concentration of 1 g L^{-1} was prepared in ethanol. Then each of 2 mL diluted crude extract solution was transferred in a test tube before mixing in 10 mL of the Folin–Ciocalteu reagent and 8 mL of 7.5% sodium carbonate. The mixture was kept for 2 h at room temperature and the absorbance of reaction solution was measured at 765 nm by using an ultraviolet (UV) spectrophotometer. The total phenolic content of each crude extract (mg g^{-1}) was calculated using gallic acid (GAE) as standard.

Analysis of the Total Flavonoid Content

The total flavonoid content from each crude extract was measured by the aluminum chloride colorimetric assay. Aqueous-ethanol extracts that had been adapted to be within the linearity range (*i.e.*, 400 μg/mL) and different dilutions of the standard solution of quercetin (10–100 μg/mL) were added to a 10 mL volumetric flask containing 4 mL of water. In above mixture, 0.3 mL of 5% $NaNO_2$ was added and incubated for 5 min. After that, 0.3 mL of 10% $AlCl_3$ was transferred and allowed the reaction to continue for 6 mins. Later, 2 mL of 1 *M* NaOH was added and the total volume reached 10 mL with distilled water. Then, the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm using a UV spectrophotometer. Finally, the total flavonoid contents obtained from each extract was expressed as the percentage of quercetin equivalent per 1 g dry weight of the crude extract.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) technique to analyze the overall significance of data while least significant difference (LSD) test at 5% was used to compare the means (Steel *et al*., 1997).

Results

Crude Extract Yield of Aqueous-ethanol Extracts of 19 Plant Species

Mitrephora wangii, *Elephantopus scaber* and *Magnolia cathcarii* had the highest crude yield (over 20% w/w) enclosed in the leaves. *Cinnamomum porrectum* and *Zingiber zerumbet* had the least yield (2.91% w/w and 2.95% w/w, respectively) (Table 1).

Table 1: Crude yield, total phenolic and flavonoid contents in the plant species under investigation

Family	Species	Crude yield[a] (% dry weight of leaves)	Total phenolic contents (mg GAE g^{-1} crude extract)	Total flavonoid contents (mg quercetin equivalent g^{-1} crude extract)
Annonaceae	*Anomianthus dulcis* (Dunal) J.Sinclair	8.25	7.21	2.92
Annonaceae	*Goniothalamus calvicarpa*	9.57	143.7	3.16
Annonaceae	*Mitrephora keithii* Ridl	8.33	17.31	2.55
Annonaceae	*Mitrephora wangii* Hu	25.12	46.49	2.04
Annonaceae	*Orophea polycarpa* A.DC.	9.51	6.14	5.61
Asteraceae	*Elephantopus scaber* L.	25.79	69.56	2.31
Bignoniaceae	*Pachyptera hymenaea* (DC.) A. Gentry	7.33	3.48	1.22
Clusiaceae	*Garcinia dulcis* (Roxb.) Kurz	9.44	135.91	3.69
Eeicaceae	*Agapetes lobbii* C.B. Clarke	14.83	145.86	3.62
Lauraceae	*Cinnamomum loureiroi* Nees	13.31	3.8	1.65
Lauraceae	*Cinnamomum porrectum* Kosterm.	2.91	97.79	1.42
Magnoliaceae	*Magnolia cathcarii* (Hook.f. & Thornson) Noot	24.88	136.61	4.74
Magnoliaceae	*Magnolia citrates* (Noot. & Chaoermglin)	7.24	77.13	2.88
Magnoliaceae	*Michelia floribunda* Fin. et Gagnep	16.57	114.74	3.97
Magnoliaceae	*Manglietia garrettii* Cralb	20.11	70.37	2.37
Magnoliaceae	*Magnolia henryi*	11.59	109.13	2.90
Moraceae	*Ficus microcarpa* L.f. var. *crassifolia* sheeh Liao	11.44	143.42	7.64
Zingiberaceae	*Zingiber zerumbet* (L.) Smith.)	2.95	107.3	0.61
Zingiberaceae	*Hedychium coronarium* J. Koenig	11.17	23.63	3.01

[a]Yield of extract per 10 g of plant material

Table 2: Effect of extracts of different plant species on germination, shoot length and root length of barnyard grass

Species	2.5 g L^{-1}			5 g L^{-1}		
	Germination (%)	Shoot length (cm)	Root length (cm)	Germination (%)	Shoot length (cm)	Root length (cm)
Control	95.00	4.65	4.82	95.00	4.65	4.82
A. dulcis	86.25	5.06	4.89	85.00	5.11	4.57
G. calvicarpa	93.75	5.09	3.82	85.00	5.16	3.37
M. keithii	97.50	5.03	3.59	82.50	5.43	2.92
M. wangii	100.00	3.51	2.77	98.75	2.31	2.98
O. polycarpa	98.75	3.98	3.82	95.00	4.37	3.81
E. scaber	88.75	4.88	1.22	91.25	3.73	1.36
P. hymenaea	98.75	4.97	5.11	91.25	5.01	5.45
G. dulcis	98.75	5.30	2.91	95.00	5.36	1.49
A. lobbii	100.00	5.01	1.81	96.25	4.84	1.41
C. loureiroi	91.25	5.29	4.04	91.25	5.00	4.25
C. porrectum	88.75	4.90	5.50	86.25	4.43	4.84
M. cathcarii	98.75	5.07	5.71	95.00	5.08	5.69
M. citrates	97.50	4.85	7.03	93.75	5.20	5.51
M. floribunda	96.25	5.32	4.25	95.00	5.25	3.72
M. garrettii	100.00	4.06	4.37	100.00	4.05	4.77
M. henryi	97.50	3.84	4.98	96.25	4.11	4.09
F. microcarpa	95.00	4.75	3.67	87.50	4.52	2.54
H. coronarium	97.50	4.06	3.75	97.50	4.01	3.47
Z. zerumbet	85.75	4.94	3.85	80.00	5.14	4.54
LSD $p \leq 0.05$	14.50	2.24	2.14	20.75	2.32	2.86

Seed Bioassay

All crude extracts at both 2.5 and 5 g L^{-1} showed no effect on germination of barnyard grass when compared with control. All crude extracts at 2.5 g L^{-1} were not significantly affected on shoot length of barnyard grass, however at 5 g L^{-1}, only *M. wangii* significantly inhibited shoot length of barnyard grass. *E. scaber* and *A. lobbii* at both concentrations showed the inhibition of root length of barnyard grass. *M. citrates* significantly promoted root length of barnyard grass at 2.5 g L^{-1} (Table 2). Another result of radish was showed in Table 3. *G. calvicarpa*, *P. hymenaea*, *G. dulcis*, *C. loureiroi*, *C. porrectum*, *M. cathcarii*, *M. citrates* and *M. floribunda* at 2.5 g L^{-1} and 5 g L^{-1} significantly inhibited the germination of radish. *P. hymenaea* significantly inhibited the shoot length and root length of radish at both 2.5 g L^{-1} and 5 g L^{-1} (Table 3).

Analysis of Phenolic and Flavonoid Content

G. calvicarpa, *A. lobbii and F. microcarpa* had the highest total phenolic content (143.7, 145.86 and 143.42 mg GAE g^{-1} of crude extract, respectively), whereas *P. hymenaea* and *C. loureiroi* had the least (3.48 and 3.8 mg GAE g^{-1} of crude extract, respectively). In terms of the flavonoid content, *F. microcarpa* had the highest content

Table 3: Effect of extracts of different plant species on germination, shoot length and root length of radish

Species	2.5 g L^{-1}			5 g L^{-1}		
	Germination (%)	Shoot length (cm)	Root length (cm)	Germination (%)	Shoot length (cm)	Root length (cm)
Control	90.00	4.50	5.20	90.00	4.50	5.20
A. dulcis	83.75	4.75	6.87	95.00	4.33	5.10
G. calvicarpa	68.75	5.39	5.97	56.25	3.96	3.58
M. keithii	93.75	4.28	6.95	78.75	4.65	6.17
M. wangii	77.50	3.11	3.47	73.75	2.63	2.70
O. polycarpa	97.50	4.89	6.02	83.75	5.55	6.20
E. scaber	85.00	4.97	6.04	76.25	5.18	4.90
P. hymenaea	38.75	2.22	1.71	25.00	1.18	0.36
G. dulcis	67.50	3.84	1.87	57.50	3.50	0.55
A. lobbii	77.50	5.50	6.28	65.00	5.76	5.59
C. loureiroi	53.75	3.84	4.31	35.00	4.06	4.23
C. porrectum	50.00	5.71	5.36	47.50	3.89	2.98
M. cathcarii	63.75	4.02	3.70	45.00	4.93	2.68
M. citrates	46.25	3.78	2.56	20.00	3.34	1.97
M. floribunda	66.25	4.49	4.47	55.00	4.67	3.39
M. garrettii	88.75	5.32	5.83	82.50	5.61	4.90
M. henryi	91.25	5.24	4.78	68.75	4.40	3.40
F. microcarpa	85.00	4.60	3.51	81.25	2.24	2.32
H. coronarium	91.25	5.56	6.09	81.25	4.33	3.66
Z. zerumbet	82.50	5.04	4.89	70.00	3.77	3.39
LSD $p \leq 0.05$	17.55	2.14	3.22	22.32	2.29	2.57

(7,639.70 μg quercetin equivalent g^{-1} of crude extract), followed by *O. polycarpa* (5,614.19 μg), *M. cathcarii* (4,743.00 μg) and *M. floribunda* (3,971.38 μg). *Z. zerumbet* had the least total flavonoid content (607.97 μg quercetin equivalent g^{-1} of crude extract) (Table 1).

Discussion

The results of different crude extracts on germination and seedling growth of barnyard grass and radish were depended on the concentrations and plant bioassay species. Barnyardgrass was likely to be more tolerant than radish. For bioassay allelopathic activity, radish and barnyard grass are always used as bioassay species by petri dish test method because the previous reports indicated that radish is highly sensitive to allelochemicals, whereas barnyard grass is tolerant (Laosinwattana *et al*., 2012). The crude extracts at 5 g L^{-1} had more inhibitory effect on both species than at 2.5 g L^{-1}. The crude extracts from plants, especially allelopathic plant, always show an inhibition of germination and seedling growth of plant bioassay with increasing on a concentration. However, allelopathic compounds sometimes show growth stimulation with a lower concentration (Einhellig, 1986).

The yield of crude extracts and amount of total phenolic and flavonoid contents showed in Table 1 indicated that the inhibitory effect of crude extracts probably depended on the amount of total phenolic and flavonoid contents. *F. microcarpa* exhibited the highest total phenolic and flavonoid contents, inhibiting the germination and growth of radish. However, the results were complicated in case of *P. hymenaea* extract, which contained a small amount of trivial total phenolic content and a moderate amount of total flavonoid content. The extract had a stimulatory effect on the shoot and root length of barnyardgrass and the highest inhibitory effect on radish.

Phenolic acids found in plant tissues appear in two different forms *i.e*., as free compounds such as benzoic acid and cinnamic acid derivatives and in bound forms such as glycosidic phenylpropanoid ester (Deba *et al*., 2007). The major compounds of allelochemicals such as *p*-hydroxybenzoic, vanillic, *p*-coumaric, syringic and ferulic acids are reported (Chon *et al*., 2005). Apparently, phenolic compounds in allelopathy and their modes of action appeared in similar actions such as inhibiting phytopathogens and plant growth causing on non-specific permeability changes on cell membrane, interacting with several phytohormones and enzymes during the biosynthesis pathway resulting deviations from typical patterns in plant (Einhellig, 2004).

Flavonoids are also a major group of allelopathic compounds that estimated as a secondary most of allelochemicals in plants (Einhellig, 2004). Several flavonoids from plants have been reported to have allellopathic activity. For example, isoschaftoside, flavonoid compound released by root exudation from the *Desmodium uncinatum* showed a inhibition of the root growth and development of *Stiga* spp. (Hooper *et al*., 2010). The incorporation of mango leaves in soil had an inhibitory allelopathic effect on the seed germination and seedling growth of *Parthenium hysterophorus* L. and five flavonoids were found in its allelochemical composition (Javaid *et al*., 2010). The flavonoids act as electron transport inhibitors through perturbation of the mitochondrial inner membrane (Moreland and Novitsky, 1987).

Conclusion

In conclusion, *P. hymenaea, F. microcarpa, A. dulcis, E. scaber* and *A. lobbii* represented the high inhibitory effect against radish and barnyard grass due to higher phenolic and flavonoid contents in their leaf extracts.

Therefore, these species should be included in future programs to develop natural plant based herbicides.

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