Phytotoxic effects of *Piper betle* L. extracts on germination of *Eclipta prostrata* L. and *Chloris barbata* Sw. weeds

Kamrai Woranoot[1], Paphitchaya Naree[2], Anupan Kongbangkerd[1], KanjanaWongkrajang[3], Rattikarn Buaruaeng[2], Chonnanit Choopayak[2*]

[1]*Department of Biology, Faculty of Science, Naresuan University*
[2]*Department of Biochemistry, Faculty of Medical Science, Naresuan University*
[3]*Department of Chemistry, Faculty of Science and Technology, Pibulsongkram Rajabhat University 65000, Phitsanulok, Thailand*
([*]Corresponding author; e-mail: chonnanitc@nu.ac.th)

ABSTRACT

Weeds are the major problem in agricultural crop fields. To control weeds nowadays, tremendous hazardous herbicides have been applied into fields, causing harm to farmers, consumers and environment. Alternatively, nature products have been evaluating for allelopathic property and using as clean weed management. In this work, the effect of betel extracts on weed germination was investigated and their potential allelochemicals were identified. Betel oil, hexane, dichloromethane (DCM), ethanol (EtOH) and ethyl acetate (EtOAc) crude extracts were derived from *Piper betle* leaf. The chemical constituents of the extracts were determined using Gas chromatography/Mass spectrometry (GC/MS). Then, the extracts were applied to 2 species of weeds, a broadleaf (*Eclipta prostrata* L.) and a narrowleaf (*Chloris barbata* Sw.), and 3 crop species (*Oryza sativa* L., *Lactuca sativa* L. and *Brassica alboglabra* L.H. Bailey) on filter paper. The results demonstrated that yields of oil, hexane, DCM, EtOAc and EtOH extracts were 0.25%, 1.86%, 8.14%, 11.21% and 9.57% respectively. All extracts contained mainly phenylpropanoids approximately 65% and sesquiterpenoids approximately 13% including β-caryophyllene. All betel extracts had allelopathic effect with different activities. Betel oil at 1 mg/mL was suitable for weed control, since it had a slight effect on crops, but completely inhibited germination of weeds. Hexane extract at 0.5 mg/mL significantly inhibited weeds germination with a little effect on crops germination. EtOH extract and EtOAc extract at 1 mg/mL unaffected crop germination, but totally inhibited weed germination. Taken together, EtOH and EtOAc were the choice of solvents for betel extraction for weed control. These extracts had high recovery yields and had selective action only to weeds without crops interference. Interestingly, the extracts affected more to the narrowleaf weed, the main problem in paddy, than to the broadleaf. The finding suggested *P. betle* as a potential source of natural herbicide.

Keywords:Piper betle L., *Allelopathy, weed control, betel extracts, natural herbicide*

INTRODUCTION

Weeds are defined as useless plants in agricultural fields. They are the main problem for agriculture everywhere worldwide. They reduce the productivity of economic crops. In order to get rid of weeds, farmers must depend on synthetic chemical herbicides which are hazardous compounds. In 2014 farmers spent plenty

of money about 11,294 million bath for herbicides imported from United State of America (Office of Agricultural Economics, 2014). Moreover, those toxic compounds make a lot of problems to users, consumers and environment. They deposit in land and run to river. To avoid toxicity of chemical herbicides, natural ones were considered as an alternative clean way. Moreover, natural products also alleviate weed tolerance due to commercial herbicide. The natural products with allelopathic property have the ability to control other plant germination and growth. This ability is from allelochemicals in the plant. Many works have been studied on plant extracts and allelochemicals. The *Juniperus oxycedrus* L. sub sp. *Macrocarpa* oil completely inhibited seed germination and seedling growth of 3 common weeds: *Phalaris paradoxa, Trifolium campestre* and *Lolium rigidum* (Amri, Hamrouni et al 2011). Water extract of sunflower (*Helianthus annuus* L.) could affect noxious weed spp. such as *H. spontaneum, L. rigidum*and *A. retroflexus* (Nikneshan, Karimmojeni et al 2011). Pine oil (*Thuja orientalis* L.) that contained terpenoids inhibited seed germination and seedling growth of weeds *Sinapis arvensis* L., *Phalaris paradoxa* L. and *Lolium rigidum* Gaud. (Ismail, Mohsen et al 2014). The crude ethyl acetate extract of *Suregada multiflorum* Baill inhibited seed germination and seedling growth of weeds *Amaranthus tricolor* (Armradit P, Chotsang P et al. 2008). Interestingly, plants in Piperaceae family also had allelopathic activity. The methanolic extract of *Piper samentosum* Roxb. 0.01 g/mL inhibited seedling growth of *Medicago sativa* L. (Pukclai and Kato-Noguch 2011). The powder of Kava (*Piper methylsticum* L.) inhibited seed germination of *Lectuca sativa* L. and paddy weeds (*Echinochloa crus-galli*) and *Monochoria vaginalis* Presl. (Hong, Xuan et al. 2002). The allelochemicals in extracts was identified and reported (Amri, Hamrouni et al. 2013). β-Caryophyllene at ≥ 3.0 mg/L significantly inhibited the germination rates and seedling growth of *Brassica camperstris* and *Raphanus sativus* (Wang, Peng et al. 2009).

Piper betle L. is a perennial dioecious, climber. It belongs to the family Piperaceae, commonly known as "Plu". It is extensively grown in South East Asian countries including Thailand. Their leaves and fruits possess a strong pungent and aromatic flavor. It was widely used as medicinal ingredients in traditional Thai pharmacopoeia. The main active compounds in *P.betle* were phenylpropanoids and particularly sesquiterpenes (Choopayak C, Laksuk H et al. 2011).

The objective of this study was to determine the phytotoxic effect of oil, hexane, dichloromethane, ethyl acetate and ethanol extracts of *P.betle* on seed germination of *Chloris barbata* Sw. and *Eclipta prostrata* L. weeds, and *Oryza sativa* L., *Lactuca sativa* L. and *Brassica alboglabra* L.H. Bailey crops. Moreover, the chemical constituents in the betel extracts were determined. The gained knowledge would promote the better understanding of allelopathic mechanisms in plants.

MATERIALS AND METHODS

PLANT MATERIALS

The leaves of *P. betle* were collected from Phitsanulok during February 2015.

Weeds collection and Identification

The seeds of narrowleaf weed *Chloris barbata* Sw. "swallen fingergrass" and broadleaf weed *Eclipta prostrata* L. "false daisy" were collected from paddy in Sukhothai and Phitsanulok. The seeds were identified by plant taxonomist and voucher specimens were deposited at plant specimen collection room at Faculty of Science, Naresuan University. Before using, the seeds were incubated at 60 ^{o}C for 3 days for breaking seed dormancy.Rice seeds (*Oryza sativa* L.) were provided from Phitsanulok rice seed center, Thailand. The seeds of *Lactuca sativa* L. "Lettuce" and *Brassica alboglabra* L.H. Bailey "Chinese Kale" were purchased from Chiatai®, Thailand.

EXTRACTION OF BETEL LEAF

Essential oil extraction

The betel fresh leaves were weighted to 1500 gram and then were blended by electronic blender to tiny pieces. The leaf was subjected to hydrodistillation for 4 hr using a Clevenger type apparatus. The upper layer was partitioned with hexane. The solvent then was evaporated using rotary evaporator, anhydrous sodium sulfate and gas nitrogen. The oil was collected, weighted and stored at 4 ^{o}C in dark. Percentage of recovery yield (% yield) was calculated and reported as extracts weight to fresh-leaf weight.

Solvent extraction

The betel leaves were washed with tap water, dried at 40 ^{o}C for 3 days and grounded to rough powder. One hundred gram dried powder was incubated with 1 L of different solvents, including hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and ethanol (EtOH) at 4 ^{o}C for 48 hr with interval shaking. Afterwards, the suspension was filtered through Whatman paper No.1. The filtrate was collected and evaporated with a rotary evaporator at 40 ^{o}C. The crudes were collected, weighted and stored at 4 ^{o}C in dark. Percentage of recovery yield (% yield) was calculated and reported as extracts weight to dried-leaf weight.

COMPOUND COMPONENTS IDENTIFICATION IN EXTRACTS

The betel oil and solvent extracts of were further analyzed for chemical components identification using Gas-chromatography/Mass spectrometry (GC/MS). The extracts were dissolved in ethanol at a ratio of 1:100 (v/v), filtered through 0.2 μM and then subjected to GC/MS (Perkin-Elmer Sigma-115). The analysis was carried out using HP-5MS, 19091S-433 column (30 m x 0.25 mm i.d.; film thickness 0.25 μm). The operating conditions were as follows: injector and detector temperatures, 150 and 280 °C, respectively; carrier gas, helium; oven temperature program, 5 min isothermal at 40 °C, then at 2 °C/min up to 250 °C and finally held isothermally for 20 min. The identity of components was established from their GC retention times, by comparison of their mass spectra with those reported in literature (Eight Peak Index of Mass Spectra) and by computer matching with the Wiley 5

mass spectra library, as well as, by co-injection with authentic available standards. The amount of compounds reported as percentage of area which represented the percentage of peak area of the compound to total compounds in each extract.

ALLELOPATHIC ACTIVITY ASSAY

Solvent toxicity on seed germination

The solvent toxicity was tested on seed germination. Three solvents at various concentrations including dimethyl sulfoxide, (DMSO 0.2%), Tween 20 (0.5% and 1.0%) and Tween 80 (0.1%, 0.5% and 1.0%) were tested in paper plates. Four mL of each solvent was added to Whatman No.1 paper in 9 cm petri dish. Distilled water was used as negative control. Afterwards, 30 seeds were placed on the paper and kept at 25 ±2°C, 12 hr photoperiod under cool-white fluorescent light (20 μmol $m^{-2}s^{-1}$). The number of germinated seeds was counted every day to 7 days. Seeds with minimal root length of 1 mm were considered as germinated seeds. The solvents which inhibited seed germination more than 70% comparing with seed germination in water were considered as toxic solvent. The non-toxic solvents were selected and used for betel extracts preparation. Experiments were conducted for three replications.

Betel extracts solubility

The crude extracts 1 mg/mL were tested for solubility in different solvents including 0.2% DMSO, 0.1% Tween 20, 0.5% Tween 20, 1.0% Tween 20, 0.1% Tween 80, 0.5% Tween 80 and 1.0% Tween 80. The solvents that could completely dissolve extracts were selected for betel extract dissolution.

Bioassay

Four mL of extracts at different concentrations (0.5, 1.0 and 2.0 mg/mL) was added to Whatman paper No.1 in glass petri dish (9 cm diameter). Distilled water was used as control of seed germination and solvents were used as negative controls of bioassay. Consequently, 30 seeds were placed on paper and incubated at 25 ±2 °C and 12 hr photoperiod under cool-white fluorescent light (20 μmol $m^{-2}s^{-1}$). After 7 days of incubation, the number of seed germination was recorded. Experiments were conducted for 3 replications.

STATISTICAL ANALYSIS

All experiments were done for three replications. Data were subjected to analysis using Duncan's New Multiple Range Test (DMRT) by SPSS Statistics version 17.0. The significant difference was at the 0.05 probability level.

RESULTS AND DISCUSSION

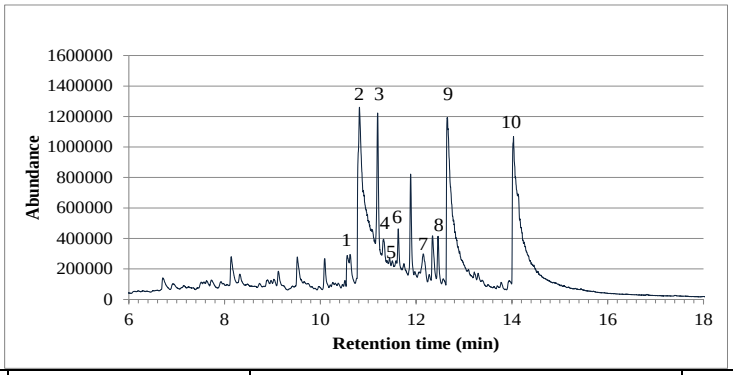
Extracts yield and chemical constituents in extracts

The essential oil from the fresh leaf of *P. betle* leaf was transparent, light-yellowish liquid with pungent smell. For the solvent extracts, all were extracted from dried leaf. The extracts obtained were opaque semisolid with green or brown color. Dicholoromethane extract was dark brown; ethyl acetate extract was dark green, whereas hexane and ethanol extracts were light green. The yields of oil hexane, dichloromethane, ethyl acetate and ethanol extracts were 0.25%, 1.86%, 8.14%, 11.21% and 9.57% (w/w) respectively (Table 1).

Table 1. Yield and physical appearance of betel extracts

Extracts	Physical appearances	% yield (w/w)
Betel oil	transparent, light-yellowish, liquid	0.25
Hexane	opaque, light green, semisolid	1.86
Dichloromethane	opaque, dark brown, semisolid	8.14
Ethyl acetate	opaque, dark green, semisolid	11.21
Ethanol	opaque, light green, semisolid	9.52

Compounds components in extracts were identified by GC-MS. Main compounds were phenylpropanoids and terpenoids. The top-ten most of compounds in betel oil were shown in chromatogram and list in Figure1. The majority of compounds were phenylpropenoids approximately 65.9% including isoeugenol acetate (21.2%), eugenol (20.2%), 4-allylpyrocatechol diacetate (14.4%), trans-propenyl guaiacol (4.8%), eugenol acetate (4.3%) and isoeugenol (1.0%). The second most ones were sesquiterpenoids approximately 13.2% including β-caryophyllene (6.8%), α-humulene (2.4%), α-gurjunene (2.3%) and δ-cadinene (1.7%) (Figure1).



Peak No.	Retention time (min)	Compounds	Percent Area (%)
1	10.56	Eugenol acetate	4.3
2	10.82	Eugenol	20.2
3	11.20	β-caryophyllene	6.8
4	11.32	Trans-propenylguaiacol	4.8
5	11.58	Isoeugenol	1.0
6	11.62	α-Humulene	2.4
7	12.14	α-Gurjunene	2.3
8	12.46	δ-Cadinene	1.7
9	12.65	Isoeugenol acetate	21.2
10	14.03	4-Allylpyrocatechol diacetate	14.4

Figure 1. GC/MS Chromatogram and list of main compounds found in betel oil. Percent area represented the percentage of peak area of the compound to total compounds in betel oil.

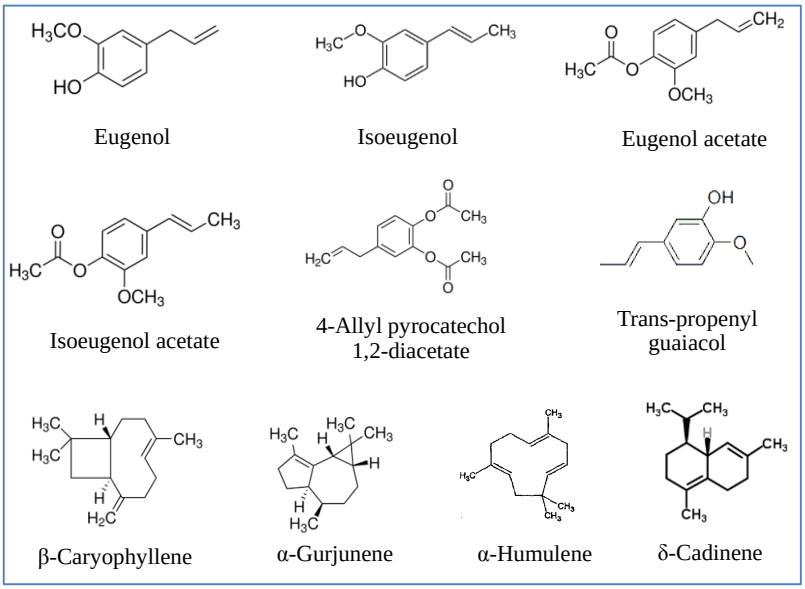
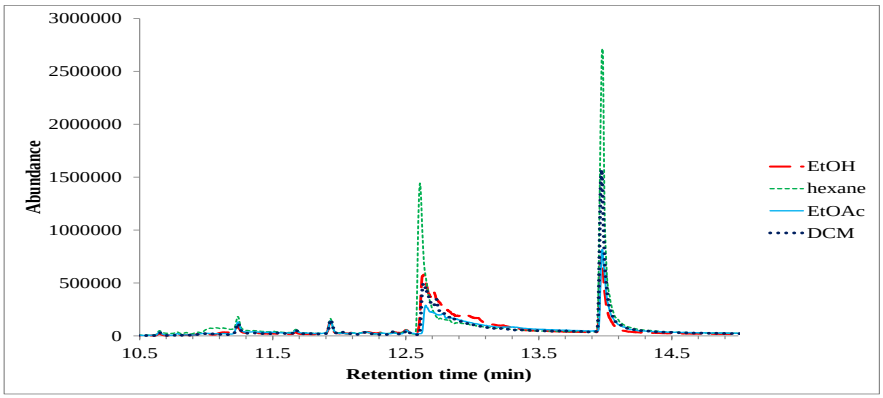


Figure 2. Main compounds found in betel oil were phenylpropanoids and sesquiterpenoids.

The kind of compounds found in betel solvent extracts were similar to in betel oil, but the amount of the compounds was quite different. All of extracts contained plenty of phenylpropanoids and some sesquiterpenoids. Hexane, DCM and EtOH extracts had profoundly high phenylpropanoids 80-90%, whereas EtOAc extract had them about 50%. The main compounds found in solvent extracts were eugenol acetate, isoeugenol acetate and 4-allylpyrocatechol diacetate. Sesquiterpenes including α-copaene, β-caryophyllene, α-humulene, α-amorphene, δ-cadinene also were found in solvent extracts (Figure 3).

Comparing between solvent extracts, the different numbers of compounds in each extract lead to the difference in percentage of the compound, even though the same amount. The more number of compounds lead to lesser percent of each compound in the extract. For example, among betel solvent extracts, hexane extract had the highest amount of β-caryophyllene (Figure 4), but the percentage of the compounds in hexane extract (2.27%) was less than in EtOAc extract (3.27%)(Figure 3).



Peak No.	RT (min)	Compounds	Percentage of compounds (%)			
			Hexane extract	DCM extract	EtOAc extract	EtOH extract
1	10.65	α-Copaene	0.55	0.57	0.99	-
2	11.24	β-Caryophyllene	2.27	2.17	3.27	1.55
3	11.68	α-Humulene	-	0.56	0.74	-
4	11.94	α-Amorphene	1.83	-	-	-
5	12.5	δ-Cadinene	0.67	1.03	1.25	0.86
6	12.61	Eugenol acetate /isoeugenol acetate	38.53	41.04	10.48	49.89
7	13.98	4-allyl-1,2-diacetoxybenzene	51.96	48.79	40.28	30.59

Figure 3. GC/MS Chromatogram of main compounds found in different solvent extracts; hexane, dicholoromethane (DCM), ethyl acetate (EtOAc) and ethanol (EtOH). Retention time was set between 10.5 to 15 min. Percentage of compounds represented the percentage of peak area of the compound to total compounds in each extract.

Many reports showed that phenylpropenes and sesquiterpenes were found in betle oil with difference kinds and quantities depend on habitat of betel. Among many reports, the study on chemical composition of betle oil in Thailand showed the same pattern with our study (Sukatta, Haruthaithanasen et al. 2003). The major components from betel oil is phenylpropenoids and sesquiterpenoids including isoeugenol, eugenol acetate, 4-allyl-1,2-diacetoxybenzene, copaene, β-caryophyllene, β-gurjunene, isoledene, chavicol acetate, cineole, α- caryophyllene, chavicol, linalool, elemene and eugenol. Another report, *P. betle* leaf oil from Sri Lankan contained safrole (52.7%), allylpyrocatechol diacetate (15.4%), eugenol (6.4%) and eugenol acetate (5.8%) as the major components followed by sabinene (2.2%), myrcene (1.1%), β-caryophyllene (1.2%), α-selinene (1.4%), β-selinene (1.2%) (Mohottalage, Tabacchi et al. 2007). While the essential oil of *P. betle* leaves from India contained 5(2-propenyl)-1,3-benzodioxole (32.79%), eugenol (16.17%),

2-methoxy-4-(2propenyl)-acetate-phenol (8.01%), gurjunene (4.14%), sabinene (3.43%) and germacrene D (3.19%) (Sugumaran, Poornima et al. 2011). However, Philippine betel oil had chavibetol (53.1%), chavibetol acetate (15.5%), caryophyllene (3.79%) as the major composition (Rimando, Han et al. 1986). Essential oil of *P. nigrum* in India contained β-caryophyllene (29.9%), limonene (13.2%), β-pinene (7.9%) and sabinene (5.9%). Whereas, the major component of both ethanol and ethyl acetate extracts contained piperine (63.9 and 39.0%), β-caryophyllene (1.0% and 6.7%), piperolein (5.3% and 5.5%) and piperanine (4.0% and 5.1%) (Kapoor, Singh et al. 2009).

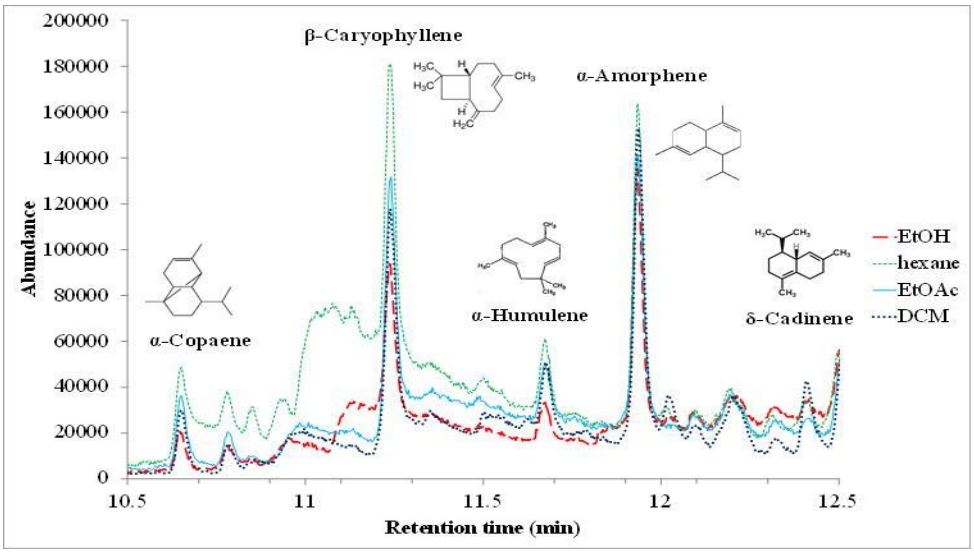


Figure 4. GC/MS Chromatogram of main compounds found in different solvent extracts; hexane, dicholoromethane (DCM), ethyl acetate (EtOAc) and ethanol (EtOH). Retention time was set between 10.5 to 12.5 min.

Allelopathic activity of betel extracts

Toxicity of solvents and solubility of extracts

To test the effect of betel extracts on seed germination, all five extracts were diluted to different concentrations. The solvent for extract dissolution was selected from 2 criteria. 1) It was not toxic on seed germination and 2) it could dissolve betel extracts very well. To complete the criteria for solvent of choice, toxicity of solvents and solubility of extracts were tested.

First of all, toxicity of candidate solvents were tested on seed germination comparing to distilled water (Table 2). Percentage of germination more than 70% was defined as non-toxic. The result showed that 0.2% DMSO and 0.5% Tween 80 was non-toxic solvents and could be used for germination assay. 0.2% DMSO had no effect on seed germination at all (85-90% germination). 0.5% Tween 80 had no effect on *O. sativa*, *B. alboglabra* and *E. prostrata* germination (90% germination),

but a little effect on *L. sativa* (71.11% germination) and *C. barbata* germination (73.33% germination).

In addition to toxicity test, the solvent of choice have to completely dissolve the extracts (Table 3). The results showed that 0.2% DMSO could dissolve only betel oil, but not others. 0.1% Tween 20 could not dissolve any extract at all. 0.1% Tween 80 could dissolve only ethanol extract, 0.5% Tween 20 and 0.5% Tween 80 could dissolve all extracts except DCM. 1.0% Tween 20 and 1.0% Tween 80 could dissolve all extracts.

Using 2 criteria, 0.2% DMSO was used for betel oil preparation at 0.1, 0.5, 1.0, 2.0 mg/mL for bioassay, since it could totally dissolve oil and it was no toxic on all seed tested. 0.1% Tween 20 and 0.1% Tween 80 was not suitable because they could not dissolve extracts well. 1.0% Tween 20 and 1.0% Tween 80 was not selected because they were toxic to seed tested. 0.5% Tween 20 was also toxic due to completely inhibited *E. prostrate* seed germination. Then the solvent of choice for crude extracts dissolution was 0.5% Tween 80, since it could dissolve all extracts except DCM and there was a little effect on seed germination. For bioassay, extracts of hexane, EtOAc and EtOH was diluted in 0.5% Tween 80 to 0.5, 1.0 and 2.0 mg/mL.

Table 2. Solvent toxicity on seed germination

Solvents	**%germination**				
	O. sativa	***L. sativa***	***B. alboglabra***	***E. prostrata***	***C. barbata***
Distilled water	93.33	86.67	97.78	92.22	85.55
0.2% DMSO	91.11	85.55	94.44	84.44	90.00
0.5% Tween 20	86.67	73.33	93.33	0	63.33
1.0% Tween 20	83.33	73.33	90.00	0	80.00
0.1% Tween 80	83.33	66.67	96.67	70.00	90.00
0.5% Tween 80	91.11	71.11	91.11	92.22	73.33
1.0% Tween 80	90.00	100.00	50.00	0	53.33

Table 3. Extracts solubility in different solvents

Extracts	**Solvents**						
	0.2% DMSO	**0.1% Tween 20**	**0.5% Tween 20**	**1.0% Tween 20**	**0.1% Tween 80**	**0.5% Tween 80**	**1.0% Tween 80**
Betel oil	✓	-	-	-	-	-	-
Hexane	×	×	✓	✓	×	✓	✓
Dichloromethane	×	×	×	✓	×	×	✓
Ethyl acetate	×	×	✓	✓	×	✓	✓
Ethanol	×	×	✓	✓	✓	✓	✓

✓soluble, ×insoluble, - not tested

Allelopathic assay

The allelopathic potentials of *P. betle* oil and solvent extracts on seed germination was evaluated against 5 plant species; 3 crops and 2 weeds, including a monocot (*O. sativa*), dicot crops (*L. sativa* and *B. alboglabra*), a narrowleaf weed (*C. barbata*) and a broadleaf weed (*E. prostrata*). Betel extracts were tested at different concentrations including 0.1, 0.5, 1.0 and 2.0 mg/mL of betel oil, and 0.5, 1.0 and 2.0 mg/mL of hexane extract, ethyl acetate extract and ethanol extract, using distilled water as seed germination control, 0.2% DMSO as betel oil solvent control and 0.5% Tween 80 as solvent control. After treatment for 7 days, the germination of seeds was counted. Percentage of seed germination under extracts treatment was calculated as normalized value to seed germination in the solvent. The results demonstrated that all betel extracts had allelopathic activity at different concentrations (Table 4).

Inhibitory effect of betel extracts were tested on crops and weeds seeds germination. The results revealed that 1.0 and 2.0 mg/mL of betel oil inhibited all tested seeds (Figure 5a). Betel oil at concentration of 0.1 and 0.5 mg/mL did not affect or slightly affected on crops seeds but oil at 0.5 mg/ml could completely inhibited seed germination of both weeds. Hexane extract at 0.5 and 1.0 mg/mL completely inhibited *C. barbata*, but not *E. prostrata* (70% inhibition) and no inhibitory effect on crops. Hexane 2.0 mg/mL inhibited 2 weeds and a lettuce, but had a little effect on rice (20% inhibition) and chinese kale and (30% inhibition) (Figure 5b). Ethyl acetate and ethanol extracts acted similarly on phytotoxic effect (Figure 5c-d). At 0.5 mg/mL, they completely inhibited *C. barbata* with some effect on *E. prostrata* (60% inhibition) and no inhibitory effect on crops. At 1.0mg/mL, they perfectly inhibited both weeds, and no effect on crops. At 2.0 mg/mL, both extracts inhibited all weeds, but had some inhibitory effect on crops. Taken together ethyl acetate and ethanol extracts at 1.0 mg/mL were choices of weed control, since they unaffected crop seed germination, but totally inhibited weed germination.

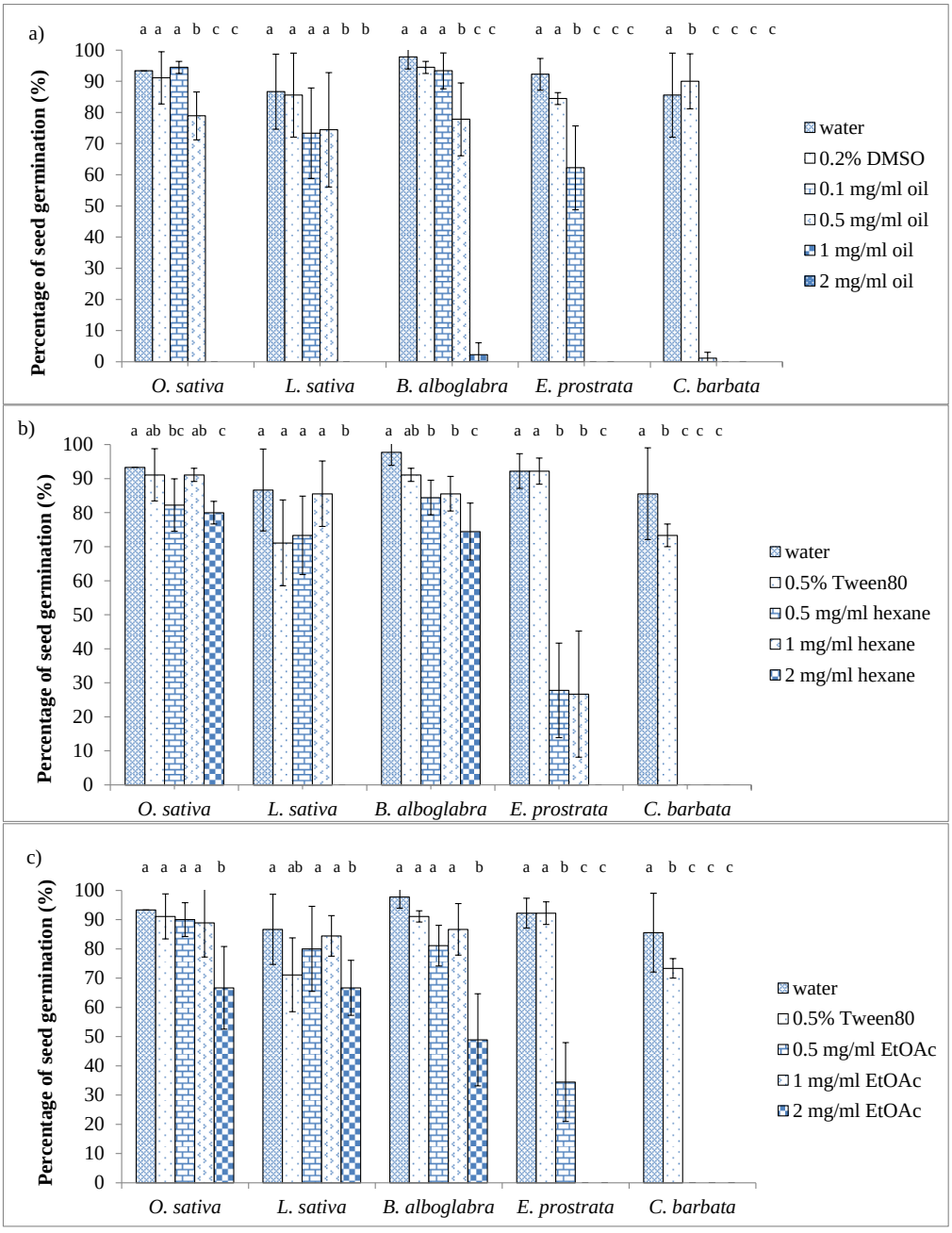
Our study demonstrated that betel oil and solvent extracts had phytotoxic activity, especially selectively more effect on narrowleaf weed than on broadleaf weed and no effect on crops. There were several reports about allelopathic property in plants. Methanol extract from hexane fraction of lettuce (*Lactuca sativa* L.) showed the most inhibition on alfalfa root growth and followed by ethyl acetate, butanol and water fractions (Chon, Jang et al. 2005). Moreover, plants in Piperaceae (the same family with *P.betle*) also had allelopathic activity. *P. methysticum* L. had allelopathic effect by inhibiting germination and growth of lettuce (*Lactuca sativa* L), barnyardgrass (*Echino chloacrus-galli* Beauv var. *formosensis* Ohwi.), and duck-tongue weed (*Monochoria vaginalis* Presl var. *plantaginea* Solms-Laub.) (Hong, Xuan et al. 2002). The ethyl acetate and n-butanol extracts of *P. nigrum* showed inhibitory activity on *Zea mays*, *Glycine max*, *Cucurbita moschata*, *Echinochloa crus-galli* and *Digitarias anguinalis* (Yan, Zhu et al. 2006). Moreover, *P. sarmentosum* Roxb. inhibited growth of narrowleaf (*Echinochloa crus-galli* Beauv, *Echinochloa colona* Link., *Festucamyuros* L., *Lolium multiflorum* Lam., *Lolium rigidum* Gaud., *Digitaria sanguinalis* L., *Leptochloa chinensis* L. Nees.) and broadleaf (*Eriogonum compositum* Douglas ex Benth.) (Pukclai and Kato-Noguch 2011).

Table 4. Effects of betel extracts on seed germination

Treatments	Concentration (mg/mL)	% Germination				
		O. sativa	***L. sativa***	***B. alboglabra***	***E. prostrata***	***C. barbata***
Distilled water	-	93.33 ±0.00	86.67 ±12.02	97.78 ±3.85	92.22 ±5.09	85.55 ±13.47
0.2% DMSO	-	91.11 ±8.39	85.55 ±13.47	94.44 ±1.93	84.44 ±1.93	90.00 ±8.82
0.5% Tween 80	-	91.11 ±7.70	71.11 ±12.62	91.11 ±1.92	92.22 ±3.85	73.33 ±3.34
Betel oil	0.1	94.44 ±1.93	73.33 ±14.53	93.34 ±5.77	62.22 ±13.47	1.11 ±1.92
	0.5	78.89 ±7.70	74.44 ±18.36	77.78 ±11.70	00.00	00.00
	1.0	00.00	00.00	2.22 ±3.85	00.00	00.00
	2.0	00.00	00.00	00.00	00.00	00.00
Hexane extract	0.5	82.22 ±7.70	73.33 ±11.55	84.44 ±5.09	27.78 ±13.88	00.00
	1.0	91.11 ±1.92	85.56 ±9.62	85.56 ±5.09	26.67 ±18.56	00.00
	2.0	80.00 ±3.33	00.00	74.44 ±8.39	00.00	00.00
Ethyl acetate extract	0.5	90.00 ±5.77	80.00 ±14.53	81.11 ±6.94	34.45 ±13.47	00.00
	1.0	88.89 ±11.70	84.45 ±6.94	86.67 ±8.82	00.00	00.00
	2.0	66.67 ±14.14	50.00 ±9.43	48.89 ±15.75	00.00	00.00
Ethanol extract	0.5	87.78 ±3.85	56.67 ±23.33	88.89 ±11.70	34.56 ±5.06	00.00
	1.0	90.00 ±8.82	90.00 ±8.82	76.67 ±14.53	00.00	00.00
	2.0	41.11 ±5.09	13.33 ±12.02	2.22 ±3.85	00.00	00.00

The main components in extracts, phenylpropanoids or sesquiterpenoids, might act as allelopchemicals. Our results presented the moderate amount of β-caryophyllene in all extracts. It is well-known volatile with allelopathic activity. It was reported as inhibitor of growth and development of seedlings of various plants. β-Caryophyllene from hexane extraction of *Senecio salignus* inhibited *Physalis ixocarpa* and *Echinochloa crus-galli* growth (Sanchez-Munoz, Aguilar et al. 2012). Another report, β-caryophyllene had allelopathic effect by inhibiting the

development of crops seedlings such as *Raphanus sativus*, *Lactuca sativa* and *Brassica campestris* (Wang, Staehelin et al. 2010).



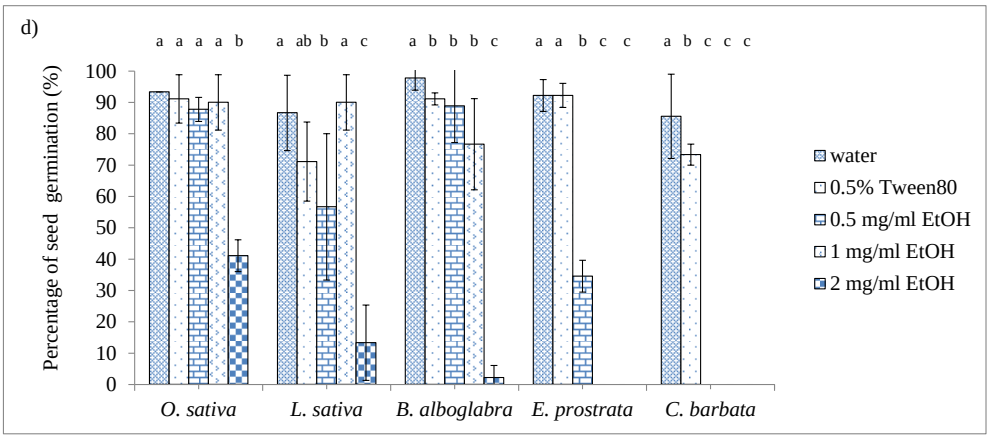


Figure 5. Effects of essential oil and solvents extracts at different concentrations on crops and weeds germination. a) betel oil b) Hexane c) EtOAc d) EtOH Distilled water, 0.2% DMSO and 0.5% Tween 80 were used as control. The statistic values were compared between different concentration of betel extracts within a plant species at *p*-value ≤0.05.

CONCLUSION

Taken together, ethyl acetate and ethanol were the best choice of solvents for betel extraction for weed control. These solvents obtained high recovery yields from betel leaf. The extracts had selective action only to weeds without crops interference. Interestingly, the extracts affected the narrowleaf weed which is the main trouble in paddy more than to the broadleaf one.

ACKNOWLEDGEMENTS

This work was financially supported by Naresuan University Research Fund. Thank Associated Professor Dr. Kornkanok Inkaninun for technical support and Dr. Pranee Nangngam for plant identification and collection in plant specimen collection room.

REFERENCES

Amri, I., Hamrouni, L., Hanana, M. and Jamoussi, B. (2011). Chemical Composition of *Juniperous oxycedrus* L. subsp *macrocarpa* Essential Oil and Study of Their Herbicidal effects on Germination and Seedling Growth of Weeds. *Asian Journal of Applied Sciences*, 4(8), 771-779.

Amri, I., Hamrouni L., Hanana, M. and Jamoussi, B. (2013). Reviews on phytotoxic effects of essential oils and their individual components: news approach for weeds management. *International journal of applied biology and pharmaceutical technology*, 4(1), 96-114.

Armradit, P., Chotsang, P., Charoenying, P. and Laosinwattana, C. (2008). Effects of *Suregada multiflorum* Baill leaf extracts on germination and seedlings growth of *Amaranthus tricolor*. *Agricultural Science Journal*, 39(3), 488-491.

Chon, S.U., Jang, H.G., Kim, D.K., Kim, Y.M., Boo, H.O. and Kim, Y.J. (2005) Allelopathic potential in lettuce (*Lactuca sativa* L.) plants. *Scientia Horticulturae*, 106(3), 309-317.

Choopayak, C., Laksuk, H. and Kongbangkerd, A. (2011). Enhanced β-caryophyllene Production in *Piper betle* Callus upon Stresses. *The 3rd BMB International Conference*, 267-271.

Hong, N.H., Xuan, T.D., Eiji, T., Matsuo, M. and Yuichi, O. (2002). Evaluation of the allelopathic potential of Kava (*Piper methysticum* L.) for weed control in rice. *Weed Biology and Management*, 2(3), 143-147.

Ismail, A., Mohsen, H., Bassem, J. and Lamia, H. (2014). Chemical composition of *Thuja orientalis* L. essential oils and study of their allelopathic potential on germination and seedling growth of weeds. *Archives of Phytopathology and Plant Protection*, 1-9.

Kapoor, I.P.S., Singh, B., Singh, G., Heluani, C.S.D., Lampasona, M.P.D. and Catalan, C.A.N. (2009). Chemistry and *in vitro* antioxidant activity of volatile oil and oleoresins of black pepper (*Piper nigrum*). *Journal of Agricultural and Food Chemistry*, 57, 5358-5364.

Mohottalage, S., Tabacchi, R. and Guerin, P.M. (2007). Components from Sri Lankan *Piper betle* L. leaf oil and their analogues showing toxicity against the housefly, *Musca domestica*. *Flavour and fragrance journal*, 22, 130-138.

Nikneshan, P., Karimmojeni, H., Moghanibashi, M. and Hosseini, N. (2011). Allelopathic potential of sunflower on weed management in safflower and wheat. *Australian Journal of Crop Science*, 5(11), 1434-1440.

Office of Agricultural Economics. (2014). http://www.oae.go.th (15 July 2015).

Pukclai, P. and Kato-Noguch, H.I. (2011). Allelopathic activity of *Piper sarmentosum* Roxb. *Asian Journal of Plant Sciences*, 10(2), 147-152.

Rimando, A.M., Han, B.H., Park, J.H. and Cantoria, M.C. (1986). Studies on the constituents of Phillippine *Piper betle* leaves. *Archives of Pharmacal Research*, 9(2), 93-97.

Sanchez-Munoz, B., Aguilar, M., King-Diaz, B., Rivero, J.F. and Lotina-Hennsen, B. (2012). The sesquiterpene β-caryophyllene and caryophyllene oxide isolated from *Senecio salignus* act as phytogrowth and phtosynthesis inhibitors. *Molecules*, 17, 1437-1447.

Sugumaran, M., Poornima, M., Venkatraman, S., Lakshmi, M. and Srinivasansethuvani. (2011). Chemical composition and antimicrobial activity of sirugamani variety of *Piper betle* Linn leaf oil. *Journal of Pharmacy Research*, 10, 3424-3426.

Sukatta, U., Haruthaithanasen, V., Jangchud, A., Kongkathip, N. and Dilokkhunanan, U. (2003). Development of rice straw paper coated with Betle (*Piper betle* Linn.) leaf oil for extending shelf life of durian paste. *Proceedings of 41th Kasetsart University Annual Conference*, 251-258.

Wang, R., Peng, S., Zeng, R., Ding, L., Wen, L. and Xu, Z. (2009). Cloning, expression and wounding induction of *β-caryophyllene synthase* gene from *Mikania micrantha* H.B.K. and allelopathic potential of β-caryophyllene. *Allelopathy Journal*, 24(1), 35-44.

Wang, R.L., Staehelin, C., Peng, S.L., Wang, W.T., Xie, X.M. and Lu, H.N. (2010). Responses of *Mikania micrantha*, an Invasive Weed to Elevated CO_2: Induction of β-Caryophyllene Synthase, Changes in Emission Capability and Allelopathic Potential of β-Caryophyllene. *Journal of Chemical Ecology*, 36(10), 1076-1082.

Yan, G., Zhu, C., Luo, Y., Yang, Y. and Wei, J. (2006). Potential allelopathic effects of *Piper nigrum*, *Mangifera indica* and *Clausena lansium*. *Ying Yong Sheng Tai Xue Bao*, 17(9), 1633-1636.