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Performance of Some Thai Weed Extracts on Antioxidants and Atherosclerosis-Related Enzymes

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ABSTRACT

This study used several methods to investigate the performance of aqueous extracts from some Thai weeds on antioxidants and atherosclerosis-related enzyme inhibitors. The inhibitory effect of aqueous extracts was expressed as the percentage of inhibition at a concentration of 500 µg/ml. GS-MS analysis was used to identify isolated compounds in sample extracts. The antioxidant activities were investigated using DPPH, ABTS, nitric oxide radical scavenging and oxidation of LDL. The results showed that the aqueous extract from the leaves of *Bidens pilosa* had greater inhibitory effects than others (71.23, 57.89 and 50.09%, respectively, except ABTS); however, *B. pilosa* had weaker inhibition than the positive controls. Pancreatic lipase and 15-lipoxygenase (15-LO) had inhibitory effects regarding atherosclerosis-related enzyme activities. The research found that *Euphorbia hirta* had stronger inhibitory activity against PL and 15-LO than other extracts (30.47 and 84.66%, respectively). Moreover, *E. hirta* had similar activity to quercetin against 15-LO (89.25%). Finally, isolated compounds were referred by GC-MC, the result presented more than 30 phenolic compounds and quite different characters, which might widely encouraged in antioxidants and inhibition of enzymatic activities. The results indicated that *B. pilosa* and *E. hirta* were the sources of bioactive compounds as antioxidants and anti-atherosclerosis, respectively.

INTRODUCTION

Weeds have been recorded as serious plant pests in agricultural production systems. Nowadays, they are approved to well medicinal sources, a number of researches have contributed to the utilization of some weeds for drugs (Hassan, 2020). *Amaranthus spinosus* is commonly known as a weed in fallow areas. The boiled leaves of this weed were used as antipyretic, anti-diabetic and help to reduce breathlessness (Ganjare & Raut, 2019). *Biden pilosa* is an easy-to-grow weed that distributed in tropical areas. It is noticed as a good herb medicine to against numerous diseases such as antioxidants, antimicrobial and anticancer by against human epidermoid carcinoma cells (Singh et al., 2017). Moreover, *Eclipta prostrata* is one of the traditional medicinal uses. Its chemical constituents are uses as drugs for antihaemorrhagic, antileprotic, antiviral, antimytoxic, antioxidants and anticancer (Mukhopadhyay et al., 2018). Worldwide, people think that herbal medicines are safer to use than synthetic drugs. These matters have attracted the researchers to investigate weeds as medicinal herbs; moreover, using weeds as medicine is an alternative implementation of weed control and can increase income for farmers.

Atherosclerosis is a play role in causing vascular disease worldwide. Its major clinical characteristic includes stroke and heart attack. Uptake and accumulation of low-density lipoprotein (LDL) oxidative modification by macrophages

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initiates several bioactivities that may drive the development of atherosclerosis. Increasing LDL level into the sub-endothelial space by the inlet from bloodstream seems to be one of the critical steps for the development of atherosclerotic lesion which metabolism of cells causes oxidation reactions, by consequent overloading of intracellular lipid (Linton et al., 2019). In this case, reactive oxygen species (ROS) and reactive nitrogen species (RNS) appear in the vascular entourage form a proatherogenic condition exacerbated by owning an unbalance between the oxidizing and reducing ability of the cell (Toledo-Ibelles & Mas-Oliva, 2018). However, inactivation of superoxide anion form to peroxynitrilte and peroxynitrous acid are subsequently decomposed into nitric and hydroxyl radicals, which react with unsaturated fatty acids inducing lipid peroxidation (Phaniendra, Jestadi, & Periyasamy, 2015). Several enzymes have been suggested to possess indirect and direct effects on lipids accumulation within the artery wall causing atherosclerosis viz. pancreatic lipase (PL) and 15-lipoxygenase (15-LO). PL is an enzyme that catalyzes the digestion of triglycerides into free fatty acids and glycerol. Increasing blood glycerol levels was induced to have hypertriglyceridemia and related to proatherogenic risk (Peng, Luo, Ruan, Peng, & Li, 2017). 15-LO is an enzyme that breaks down the oxidation of polyunsaturated fatty acid which associate with the initiation and progression of the atherosclerosis process by binding the endothelium cells and become to foam cell (Zhang et al., 2016). Therefore, the inhibition of PL and 15-LO activity may be effective in the prevention and treatment of atherosclerosis development.

The aims of this study were to investigate antioxidant properties of aqueous leaf extracts from 11 Thai weed species on ROS (DPPH and ABTS radical scavenging), nitric oxide and LDL oxidation and to probe the enzymatic activities of PL and 15-LO inhibitors. Finally, phenolic compounds contained in the aqueous leaf extracts were to identify using GC-MS technique.

MATERIALS AND METHODS

This research was conducted from October 2017 to May 2018. All of the experiments except GC-MS analysis were carried out at the Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasesart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. The GC-MS analysis was done at Salaya Central Instrument Facility, Mahidol University, Nakhon Pathom, Thailand.

Preparation of Aqueous Extracts

Samples were collected at Kasetsart University of 11 species of weeds: *Bidens pilosa* L., *Leucaena leucocephata* (Lam.) de Wit, *Eclipta prostrata* L., *Tribulus terrestris* L., *Acalypha indica* L., *Tridax produmbens* L., *Euphorbia hirta* L., *Gomphrena celosiodes* Matr., *Amaranthus viridis* L., *Synedrella nodiflora* (L.) Gaertn and *Mimosa diplotricha* C. Wright ex Sauvalle). The water was chosen as the solvent for extracting phenolic compounds from the leaves of weeds by boiling fresh samples in water (w/v) for 10 minutes and then filtering through Whatman filter paper No.1. For further analysis, the concentration of aqueous extracts from weeds used was 500 µg/ml.

Determination of Total Phenolic Content

Total phenolic content was determined following the method of Kähkönen et al. (1999). Twenty microliters of sample extract was placed in a 96-well plate and then 50 µl of Folin-Ciocalteu reagent was added. After 1 minute, 80 µl of sodium carbonate solution (7.5%) was mixed and incubated at room temperature (RT) for 30 minutes. Absorbance was measured at 760 nm. The total phenolic content was calculated as gallic acid (GA) equivalents on the basis of a calibration curve of GA ($Y= 0.004x + 0.068$, $R^2= 0.996$) and expressed as mg GA/g extract.

Determination of Total Flavonoids Content

The total flavonoid content was measured using the method of Djeridane et al. (2006). One hundred microliter of aqueous extract was added to a 96-well plate and mixed with 100 µl of aluminum chloride (2%) and incubated at RT. After 15 minutes, the mixture was measured the absorption spectra at 420 nm. The content of total flavonoid was calculated from the calibration plot of rutin (RU) ($Y= 0.004x + 0.039$, $R^2= 0.999$) and expressed as mg RU/g extract.

Antioxidant Assays

DPPH Radical Scavenging Activity Assay

The free radical of DPPH· scavenging activity was determined using Boskou et al. (2006) method. One hundred microliters of sample extract were placed in a 96-well plate and mixed with 40 µl of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) methanol solution (0.5 mM) and 100 µl of sodium acetate buffer (0.1 M, pH 5.5). The mixture was incubated at RT in the dark condition for 30 minutes, and then the absorbance was measured at 517 nm. Tert-butylhydroxytoluene (BHT) was used as the positive control, while water was used as the control for calculation.

ABTS Radical Scavenging Activity Assay

ABTS radical scavenging formation was evaluated as previously report (Hsu, Peng, Basle, Travas-Sejdic, & Kilmartin, 2011). 2,2´-Azino-bis (3-ethylbenzothiazole-6-sulphonic acid (ABTS) solution was prepared by mixing ABTS (7 mM) and potassium persulfate (2.45 mM) in phosphate buffer (0.1 M, pH 7.4) and then incubated in dark at RT until oxidation of ABTS· complete for 16 hours. The ABTS solution was diluted in phosphate buffer to succeed an absorbance of 0.700±0.050 at 734 nm before the test of the antioxidant property of sample extracts. Briefly, 200 µl of ABTS solution was added to 20 µl of sample extract or BHT and stand at RT for 6 minutes and then the absorbance was measured.

Nitric Oxide Radical Scavenging Assay

The reaction mixture was prepared to contain 200 µl of sodium nitroprusside (10 mM), 50 µl of phosphate buffer saline and 50 µl of sample extract or catechin. After incubation at RT for 150 minutes, 50 µl of the reaction mixture was mixed with 100 µl of sulfanilic acid reagent (0.33% dissolved in 20% acetic acid) and placed for 5 minutes. One hundred microliters of N-(1-naphthyl) ethylenediamine dihydrochloride was mixed and put at RT for 30 minutes. The absorbance was measured at 540 nm (Govindarajan et al., 2003).

LDL oxidation (ox-LDL) Inhibition Assay

Oxidized-LDL by $CuSO_4$ was generated as described previously (Rattan & Arad, 1998). Low-density lipoprotein (LDL) was dissolved in PBS buffer (10 mM, pH 7.4) at a concentration of 220 µg/ml and mixing with sample extract or curcumin. Before incubation at 37°C for 24 hours, LDL solution was added into CuSO4 (55 µM). The reaction was stopped by 50 µl of EDTA (1 M) and stored at -20°C until thiobarbituric acid reactive substance (TBARS) assay was done. The LDL reaction was mixed with 1.0 ml of thiobarbituric acid (0.67%) and 1.5 ml of trichloroacetic acid (20%) and placed at 100°C for 30 minutes. And then, the reaction was stand at RT for 30 minutes and centrifuged at 4°C for 15 minutes. The supernatant measured the absorbance at 532 nm (Steinbrecher, Parthasarathy, Leake, Witztum, & Steinberg, 1984). The calibration curve of malondialdehyde (MDA) was used for calculation of LDL oxidation, the result was expressed as nanomol of MDA (Y= 0.06x + 0.121 (R^2= 0.995).

The antioxidant properties of aqueous leaf extracts from 11 Thai weed species on DPPH, ABTS, and nitric oxide radical scavenging and inhibition of LDL oxidation were calculated using the following formula:

$$\mathrm{Ie}\ (\%) = \frac{(\mathrm{O.D.control} - \mathrm{O.D.sample})}{\mathrm{O.D.control}}\ \mathrm{X}\ 100 \quad \text{.........1)}$$

Where:
Ie : Inhibitory effect
O.D. : the optical density in the presence or absence of the samples

Enzymatic Inhibition Assays

Pancreatic Lipase (PL) Activity Assay

The performance of aqueous extracts of the 11 weed species to inhibit PL activity was measured as described previously (Kim, 2007). The enzyme solution was prepared containing 30 µl of lipase from porcine pancreatic (10 units dissolved in 10 mM MOPS and 1 mM EDTA, pH 6.8) and 850 µl of Tris buffer (100 mM Tris-HCl and 5 mM $CaCl_2$, pH 7.0). Briefly, 880 µl of enzyme solution was added to 100 µl of sample extract or curcumin as the reaction mixture and then incubated at 37°C for 15 minutes. Twenty microliters of p-nitrophenyl butyrate in dimethyl formamide (10 mM) as substrate was put in the reaction mixture. After incubation at 37°C for 15 minutes, the PL activity was determined by absorbance measurement at 400 nm.

15-Lipoxygenase (15-LO) Activity Assay

The inhibition of 15-LO activity was determined as described previously (Lyckander & Malterud, 1996). Five hundred microliter of 15-lipoxygenase from soybean (500 units/ml dissolved in DMSO) was mixed with 50 µl of sample extract or quercetin. After 5 minutes of incubation at RT, 500 µl of linoleic acid (134 µM) as the substrate was added to the reaction mixture. The activity of 15-LO measured the absorbance at 234 nm after incubation at RT for 5 minutes.

The percentage inhibition of PL and 15-LO activities were calculated using the formula:

Enzyme inhibition activity (%) = (1 - B/A) x 100 …2)

Where:
A is the enzymatic activity without the test sample
B is the enzymatic activity with the test sample

GC-MS Analysis

The phenolic compound analytical column was a HP-5MS with 5% phenyl methyl siloxane (30 m x 0.25 mm i.d., 0.25 μm; Agilent J&W, Folsom, CA, USA). Helium was used as the carrier gas at a flow rate of 1 ml/min. The GC oven temperature program was: held at 50°C for 1 minute, then raised at 10°C/min to 220°C, followed by raised to 300°C at 40°C/min and held for 6 minutes. The temperatures of the injector and detector were set at 280°C and 300°C, respectively. Each aqueous extract of 2.0 μl was injected into the GC-MS. Mass spectra were scanned from m/z 50-500 amu and the electron impact ionization energy was 70 eV. Phenolic compounds were determined quantitatively based on peak area measurements and determined relative to the retention times of a series from the NIST08 Mass Spectral Library.

Statistical Analysis

Data were processed to analyze variance followed by Duncan's new multiple range test at a significance level of 0.01 ($p \leq 0.01$). The relationship between variables was determined using Pearson's correlation. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 16.0 (International Business Machines Corporation, New York, NY, USA).

RESULTS AND DISCUSSION

The Amounts of Total Phenolic and Flavonoid Content

Total phenolic and flavonoid contents in the aqueous extracts from the leaves of the 11 Thai weed species are shown in Table 1. The leaf extract of *L. leucocephala* contained a high amount of total phenolics (49.30±0.54 mg GA/g extract) followed by *E. prostrata* and *A. viridis* (35.76±1.05 and 31.69±0.11 mg GA/g extract, respectively). The aqueous extract from *L. leucocephala* also contained a high amount of total flavonoids (165.33±6.66 mg RU/g extract) followed by *E. hirta* and *A. indica* (61.13±0.23 and 57.14±0.36 mg RU/g extract, respectively). Phenolic compounds are one of the vital groups of secondary metabolites present in plants. They represent important compounds which can act as antioxidants to against the free radicals in the human body. Numerous researches have indicated that phenolic compounds are wealthy in ordinary human diets that can help treat and protect a variety of human illnesses (Lin et al., 2016). Ozcan, Akpinar-Bayizit, Yilmaz-Ersan, & Delikanli (2014) reported that phenolic compounds are remarked to manifest for the prevention of infectious and degenerative diseases via the mechanism of antioxidant and protein or enzyme modulation/ neutralization. In nature, flavonoids are a group of natural substances with phenolic compounds that have broad-spectrum in nutraceutical, cosmetic and pharmaceutical applications. They are now attributed as medicines for against oxidative, inflammatory, mutagenic, and carcinogenic activities (Panche, Diwan, & Chandra, 2016).

Antioxidant Properties of Aqueous Extracts from Thai Weeds

The antioxidant properties of the aqueous extracts at a concentration of 500 μg/ml from the 11 Thai weed species are shown in Fig. 1. For DPPH assay, the aqueous extract of *B. pilosa* and *A. viridis* had the best inhibitory activity (71.23 and 70.76%, respectively) followed by *T. terrestris* and *S. nodiflora* (53.69 and 52.82%, respectively) (Fig. 1A). Regarding the nitric oxide activity, the aqueous extracts of *B. pilosa* and *A. viridis* had higher percentages of inhibition than the other extracts (57.89 and 56.07%, respectively) (Fig. 1C). In the ox-LDL assay, both the *B. pilosa* and *T. terrestris* extracts were better inhibitors than the other extracts (50.09 and 49.24%, respectively) (Fig. 1D). However, these extracts had a weaker inhibitory effect than the positive control BHT on DPPH, catechin on nitric oxide and curcumin on ox-LDL assays (84.40, 91.25 and 83.86%, respectively). In contrast, with ABTS radical scavenging activity, all sample extracts had similar inhibition capacity with BHT (more than 80%) (Fig. 1B). Singh et al. (2017) reported that *B. pilosa* is well known for its folkloric medicinal use against various diseases from many decades that the leaves extract showed strong antioxidant on scavenging of DPPH and ABTS. Moreover, Goudoum, Abdou, Ngamo, Ngassoum, & Mbofung (2016) study on antioxidant capacity of the essential oil from leaves of *B. pilosa* exhibits radical scavenging ability on DPPH and power reducing system. Free radicals are harmful molecules that attack the cells in human body, causing the structure and functions of cells to fail. The appearances of free radicals have been major contributed to various diseases. Hence, antioxidants play important role in treatment and protection of free radical formations, they can help in reducing the risk of diseases such as aging, cancer, cardiovascular diseases, hyperglycemia, hyperlipaemia and immune system atrophy (Pooja & Sunita, 2014).

Table 1. Total phenolic and flavonoid contents of aqueous extracts from leaves of 11 Thai weed species

Weed species	Total phenolics (mg GA/g extract)	Total flavonoids (mg RU/g extract)
Acalypha indica	17.36±0.02^{g*}	57.14±0.36^{b}
Amaranthus viridis	31.69±0.11^{c}	12.76±0.44^{f}
Bidens bilosa	9.68±0.68^{j}	18.76±1.52^{e}
Eclipta prostrata	35.76±1.05^{b}	47.52±1.00^{c}
Euphorbia hirta	20.91±0.02^{e}	61.13±0.23^{b}
Gomphrena celosioides	18.89±0.80^{f}	3.84±0.17^{g}
Leucaena leucocephala	49.30±0.54^{a}	165.33±6.66^{a}
Mimosa diplotricha	13.54±0.21^{h}	43.61±1.93^{c}
Synedrell nodiflora	11.58±0.12^{i}	8.25±1.81^{fg}
Tribulus terrestris	22.80±0.57^{d}	30.78±3.20^{d}
Tridax procumbens	4.29±0.07^{k}	7.73±0.56^{fg}

Remarks: *Mean values ± SE within a column followed by the same letters are not significantly different at $p \leq 0.01$ according to Duncan's multiple range test

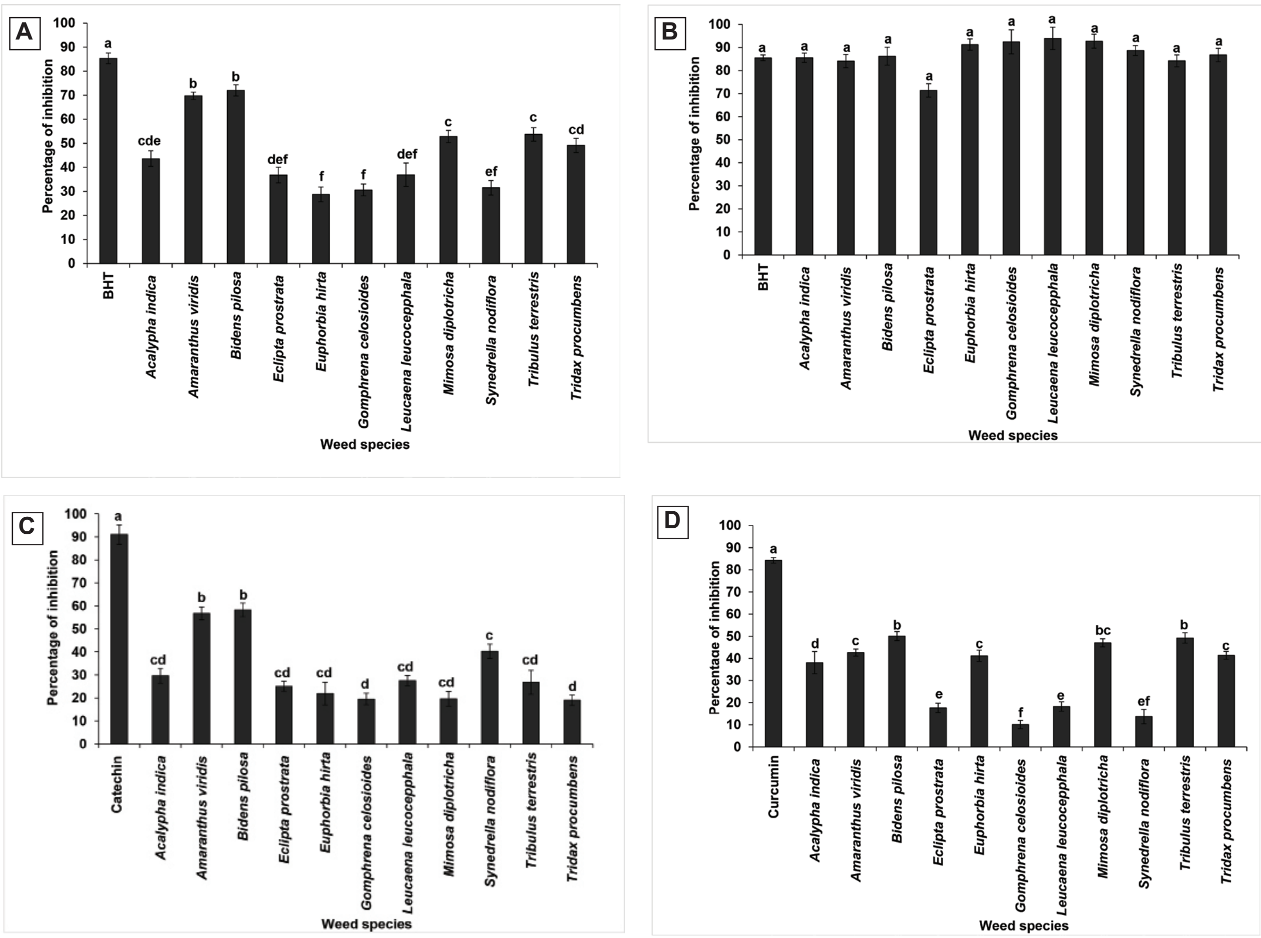

Fig. 1. Effect of aqueous extracts from 11 Thai weed species on (A) DPPH (B) ABTS (C) nitric oxide radical scavenging and (D) LDL oxidation inhibition. Vertical bars represent the standard error

Effect of Aqueous Extracts from Thai Weeds on Enzymatic Activity

The effect of the aqueous extract of the 11 Thai weed species on atherosclerosis-related enzyme activity (PL and 15-LO) showed that *A. indica* had stronger inhibitory activity on PL than the other extracts (30.47%) followed by *E. hirta* and *T. terrestris* (20.20 and 14.86%, respectively). However, the test samples had weaker inhibition than the positive control curcumin (98.36%) (Fig. 2A). Regarding the inhibition of 15-LO activity, the aqueous extract of *E. hirta* had the highest inhibitory effect (84.66%). Besides, this extract had a similar result to the positive control quercetin (89.25%) and it had a stronger inhibitory effect than the *E. prostrata* and *L. leucocephala* extracts (61.44 and 60.88%, respectively) (Fig. 2B). The potency of *E. hirta* has been reported to inhibition of 15-LO activity. Paguigan & Chichioco-Hernandez (2014) reported that *E. hirta* had the highest inhibitory activity (48.5%) and this plant may contain new 15-LO inhibitors. Moreover, Salehi et al. (2019) presented the *Euphorbia* genus were found numerous phytochemicals, which showed medicinal properties on abundant human diseases such as anti-inflammation, anti-cancer and antimicrobial. Moreover, *E. hirta* is an important medicinal source and its active substances revealed wide-ranging pharmacological capacities viz. antidiarrheal, antipyretic, antimalarial, diuretic, antitumor and antiasthmatic (Kale, 2016).

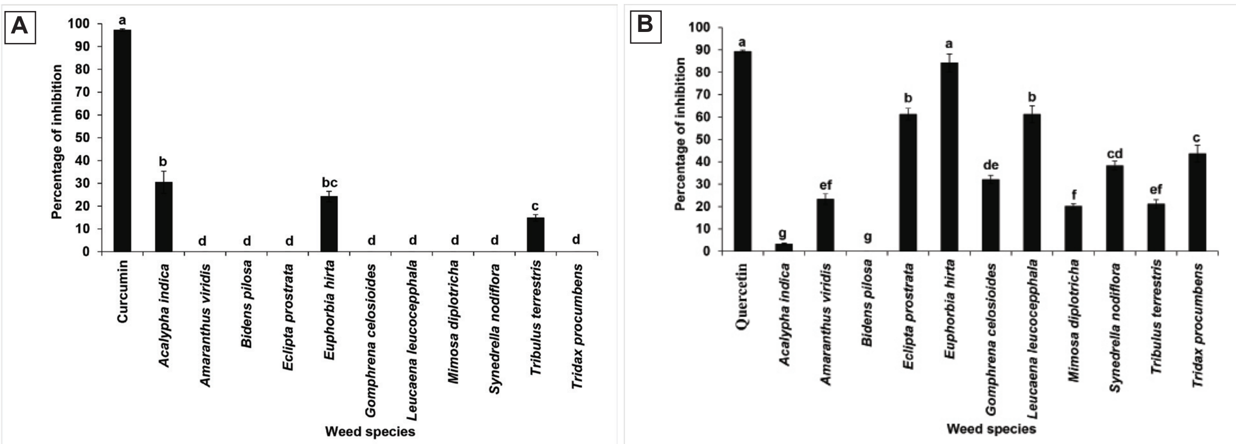

Fig. 2. Inhibitory effect of aqueous extracts from 11 Thai weed species on the activities of (A) pancreatic lipase and (B) 15-lipoxygenase. Vertical bars represent the standard error

Table 2. Correlations between phytochemical contents and antioxidant capacity and inhibitory effect on enzymatic activity

	FLA	PHE	DPPH	ABTS	NO	LDL	PL	15-LO
FLA	1.000							
PHE	0.735*	1.000						
DPPH	0.038	-0.188	1.000					
ABTS	-0.149	0.250	-0.178	1.000				
NO	-0.424	-0.128	0.032	-0.085	1.000			
LDL	-0.367	-0.174	0.574	-0.002	0.119	1.000		
PL	-0.081	0.143	-0.058	-0.001	-0.056	0.316	1.000	
15-LO	0.422	0.395	-0.402	0.010	0.033	-0.389	-0.079	1.000

Remarks: * Significance level at $p<0.05$; FLA = total flavonoids; PHE = total phenolics; DPPH = DPPH radical scavenging; ABTS =ABTS radical scavenging; NO = nitric oxide radical scavenging; LDL = oxidation of low density lipoprotein; PL = pancreatic lipase activity; 15-LO = 15-lipoxygenase activity

Correlation Among Antioxidant/Enzymatic Activity Assays and Phytochemical Contents

For the purpose of understanding the relationship between the antioxidant activities and phytochemical contents, all aqueous extracts were used in an analysis of the correlation among flavonoid and phenolic contents and DPPH, ABTS, nitric oxide and oxidation of LDL inhibition formation. With reference to Table 2, the flavonoid content was negatively correlated with ABTS, nitric oxide and LDL inhibition, based on the Pearson's correlation coefficients ($p \leq 0.05$), while there was a positive correlation for DPPH radical scavenging. These results suggested that the aqueous extracts with low flavonoid content had great free radical scavenging assay, except for DPPH radical formation. The phenolic content was negatively correlated with DPPH, nitric oxide and oxidation of LDL effectiveness which was an inverse of the relationship between the phenolic content and ABTS radical scavenging. These data suggested that the aqueous extracts with low phenolic content did not have a high antioxidant capacity, except for the oxidation of ABTS. Moreover, we found no significant ($p \leq 0.05$) correlation for the phenolic content with either PL or the 15-LO inhibitory activities, while a negative correlation was observed between the flavonoid content and PL inhibition. Tungmunnithum, Thongboonyou, Pholboon, & Yangsabai (2018) reported that flavonoids are outstanding as antioxidants and many other bioactive compounds that have been used for benefits human health. In case of phenolic content, Galvão, Arruda, Bezerra, Ferreira, & Soares (2018) reported that unspecific phenolic compounds could be reacted with Folin-Ciocalteu reagent, thus, antioxidant performances may be disturbed with some non-phenolic compounds. Additionally, the lack of a positive correlation between phenolic content and antioxidant properties could be described that was the result of differences in phenolic composition.

Phytochemical Components of the Aqueous Extracts

Phytochemical compounds of aqueous extracts from the leaves of the 11 Thai weed species were compared with the chemical compounds from the NIST data library based on at least 90% similarity. The identified compounds with their retention time and percentage amount are listed in Table 3, based on their retention time. The data showed that *B. bilosa* had more phytochemical compounds than other species, while *S. nodiflora*, *E. hirta* and *E. prostrata* contained high amounts of phytochemicals (67.99, 66.35 and 64.06%, respectively). The GC-MS analysis of the different active biological components of *B. bilosa* which had high antioxidant properties on oxidation of ABTS, nitric oxide and LDL consisted of menthol (1.968%), τ-muurolol (1.819%), dihydro-cis-α-copaene-8-ol (0.508%), α-cadinol (1.672%), caffeine (0.593%) and i-propyl tricosanoate (0.626%). Miri (2018) found that menthol was the main antioxidant compound which presented from *Lavandula officinalis*. Likewise, τ-muurolol and α-cadinol was also responsible for direct antioxidant activity (Zuccolotto et al., 2019). Moreover, caffeine has been reported in large amounts in *B. pilosa*, so, this plant has been frequently used as a tonic and stimulant in traditional medicine (Bartolome, Villaseñor, & Yang, 2013). The effect of caffeine was studied on the liver of mice, Cappelletti, Piacentino, Sani, & Aromatario (2015) found that it protected cell damage by exhibits process of lipid peroxidation and protects the membrane from reactive oxygen species. As the result of *A. viridis*, that showed had high antioxidant performances on DPPH, ABTS and nitric oxide radical scavenging, its aqueous extract contained β-myrcene (0.596%), benzene, 1,3-bis(1,1-dimethyethyl)- (1.110%), eugenol (1.585%), phenol,4-(1,1-dimethylpropyl)-(1.292%) and phytol (1.566%). de Menezes Patrício Santos et al. (2013) presented that phytol showed strong antioxidant capacities, it was able to remove hydroxyl and nitric oxide radicals in the reaction of lipid peroxidation, and it also had medicinal capacity as an antinociceptive drug. Moreover, β-myrcene cloud be used to prevent cardiac tissue from ischemic stroke. It demonstrated inhibition of oxidative radical formations and protected ischemia reperfusion injury causing heart tissue damage (Burcu, Osman, Aslı, Namik, & Neşe, 2016). Nejad, Özgüneş, & Başaran (2017) reported that eugenol has been used in medicine as antiseptic and anesthetic. It wide range to use as drugs for antimicrobial, anti-inflammatory, analgesic and antioxidant. For *E. hirta* had a high inhibitory effect on enzymatic activities of this study, its aqueous extract contained menthol (2.656%), undecanal (5.727%), 2-undecanal (3.067%) and octadecanal (2.279%).

Table 3. Main phytochemical composition of aqueous extracts from leaves of 11 Thai weed species

No.	Compound	Retention Time (min)	Peak area (%)										
			Acalypha indica	*Amaranthus viridis*	*Bidens bilosa*	*Eclipta prostrata*	*Euphorbia hirta*	*Gomphrena celosioides*	*Leucaena leucocephala*	*Mimosa diplotricha*	*Synedrell nodiflora*	*Tribulus terrestris*	*Tridax procumbens*
1	β-Myrcene	8.560	-	0.596	-	-	-	-	-	-	-	-	-
2	Pyridine, 2,4,6-trimethyl-	8.369	-	0.833	-	0.864	-	-	-	-	-	-	0.588
3	Acetophenone	10.807	-	-	0.192	-	-	-	-	-	-	-	-
4	Phenol, 2-methoxy-	11.974	-	-	-	-	-	12.751	-	-	-	-	-
5	Menthol	14.486	-	-	1.968	0.793	2.656	-	-	-	-	-	-
6	Benzoic acid	14.476	-	-	0.347	-	-	-	0.722	-	-	-	-
7	Benzothiazole	16.198	-	-	0.269	-	-	-	-	-	-	-	-
8	Undecanal	16.956	-	-	-	-	5.727	-	-	-	-	-	-
9	Benzene, 1,3-bis(1.1-dimethylethyl)-	17.272	-	1.110	-	1.043	-	-	1.021	-	-	-	-
10	Ethanone, 1-(4-ethylphenyl)-	19.950	-	-	-	-	-	-	0.279	-	-	-	-
11	2-Undecanal	20.017	-	-	-	-	3.069	-	-	-	-	-	-
12	Butanoic acid, butyl ester	21.215	-	-	0.507	1.484	-	-	-	-	-	1.727	1.473
13	α-Cubebene	21.335	-	-	-	-	-	-	-	-	2.034	-	-
14	Eugenol	21.589	-	1.585	-	0.713	-	2.032	-	-	2.174	-	0.615
15	Oxirane, decyl	22.155	-	-	-	-	7.322	-	-	-	-	-	-
16	Copaene	22.243	-	-	-	-	-	-	-	-	1.884	-	-
17	Caryophyllene	22.689	-	-	-	3.675	-	-	-	-	3.180	-	-
18	Phenol, 4-(1,1-dimethylpropyl)-	22.933	-	1.292	-	-	-	-	-	-	-	-	-
19	α-Caryophyllene	23.737	-	-	-	9.072	-	-	-	-	18.518	-	-
20	Epizonarene	25.102	-	-	-	-	-	-	-	-	0.463	-	-
21	Pentadecane	25.133	-	-	-	3.489	-	-	-	-	-	-	-
22	Isoledene	25.501	-	-	-	-	-	-	-	-	0.785	-	-
23	Benzophenone	28.858	-	0.804	0.220	0.825	-	-	-	-	-	-	-

Table 3. *(continue)*

No.	Compound	Retention Time (min)	Peak area (%)										
			Acalypha indica	*Amaranthus viridis*	*Bidens bilosa*	*Eclipta prostrata*	*Euphorbia hirta*	*Gomphrena celosioides*	*Leucaena leucocephala*	*Mimosa diplotricha*	*Synedrell nodiflora*	*Tribulus terrestris*	*Tridax procumbens*
24	τ-Muurolol	29.330	-	-	1.819	-	-	-	-	-	-	-	-
25	α-Cadinol	29.678	-	-	1.672	-	-	-	-	-	0.443	-	-
26	Caffeine	34.649	-	-	0.593	-	-	-	-	-	-	-	-
27	Octadecanal	40.823	-	-	-	-	2.279	-	-	-	-	-	-
28	Phytol	43.086	-	1.566	-	-	-	-	-	-	-	-	-
29	i-Propyl tricosanoate	47.838	-	-	0.626	-	-	-	-	-	-	-	-
30	Others	-	49.353	54.055	27.282	42.392	45.297	38.636	39.844	33.805	38.518	41.823	43.724
	Total		49.353	61.841	35.495	64.350	66.350	53.419	41.866	33.805	67.999	43.550	46.400

Mandal and Mandal (2015) found that (E)-2-tetradecenal, 2-decenol, (E2-undecenal, dodecanal, (E)-2-tridecenal, and (E)-2-hexadecenal were the major identified volatile compounds in *Cariandrum sativum* leaf essential oils which had excellent antioxidant activity and antibacterial, antifugal and antioxidative activities. *E. hirta* has been previously evaluated a wide range of medicinal properties to treat inflammatory, diabetes, muscle spasm, epilepsy and wound healing (Tuhin et al., 2017).

CONCLUSION

Aqueous extracts from the weed species *Bidens bilosa* and *Amaranthus viridis* had strong antioxidant activity, while *Euphorbia hirta* gave good results in the activity of 15-lipoxygenase. GC-MS analysis indicated there were important compounds in the aqueous extracts of these plants which may act as antioxidants and also act against atherosclerosis-related enzyme activity. Studies are ongoing to isolate and identify these bioactive compounds so that they may be used to develop biopharmaceuticals against infectious diseases and provide a source of antioxidants in the future.

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