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Antioxidant and antibacterial properties of selected Thai weed extracts

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PEER REVIEW

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Comments

The weeds extracted in this study had phenolics and flavonoids as major constituents. *E. hirta* extracts had high antibacterial activity compared to the other extracts. These weeds may have potential to develop novel bactericidal agents.
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ABSTRACT

Objective: To analyze antioxidant and antibacterial properties of selected weeds commonly found in Northeast Thailand including *Ageratum conyzoides* L., *Alysicarpus vaginalis* L., *Commelina bengalensis* L., *Euphorbia hirta* L., *Hyptis suaveolens* L., *Parthenocissus quinquefolia* L., and *Trianthema portulacastrum* L.
Methods: Ferric reducing antioxidant power and radical scavenging activity of the aqueous and ethanol weed extracts were determined. Phytochemical screening, total phenolic and flavonoid contents were done. Antibacterial activity against *Aeromonas hydrophila*, *Aeromonas caviae*, *Edwardsiella tarda*, *Plesiomonas shigelloides*, *Ralstonia* spp., *Xanthomonas campestris pv. Vesicatoria*, *Salmonella* spp. and *Shigella* spp. was performed by disc diffusion assay.
Results: The results showed that *Euphorbia hirta* extract had the highest total phenolic contents and was the most effective against most of the test organisms compared to the other weed extracts. *Hyptis suaveolens* ethanol extract weakly inhibited *Ralstonia* spp. and *Salmonella* spp. (10.42% and 9.84% inhibition, respectively). *Trianthema portulacastrum* ethanol extract had 20.10% inhibition against *Shigella* spp. *Parthenocissus quinquefolia* aqueous extract strongly inhibited *Aeromonas caviae* and *Aeromonas hydrophila* with 55.90% and 59.68% inhibition, respectively.
Conclusions: These weeds may be serving as a potential source of antibacterial agents.

KEYWORDS
Antioxidant, Antibacterial activity, Weed

1. Introduction

In the tropical region of the world, a wide spread of weeds in agricultural lands interferes in economical crops such as rice, sugarcane, corn and cassava. In Northeast Thailand, these crops are the major export products. Management of weeds are therefore required. However, excessive use of synthetic pesticides[1] and herbicides[2,3] is toxic to humans and the environment. The eco-friendly method is then needed. As weeds are inexpensive sources of material, to develop cost-effective products from weeds may be a method of choice to manage the weeds. This can also help

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reduce the disturbance of weeds to other economical crops.

Weeds are resistant to microbial attack compared to crops[4,5]. This leads to an increasing interest in the investigation of weed extracts as antimicrobial agents for human, animals and crops. Weeds are potentially used in traditional medicine in different parts of the world, such as Thailand, India, and Brazil. There are evidences to support that weeds contain bioactive phytochemicals with antioxidant, antimicrobial and anticancer activities[6-10]. To search for a new source of protectants that interfere with the pathogenicity from bacteria serves as a prototype to develop more effective but less toxic medicine. The aim of this study is to explore antibacterial potency of selected Northeast Thai weeds. The aqueous and ethanol weed extracts were tested against pathogenic bacteria in crops, animals and humans.

2. Materials and methods

2.1. Chemicals and reagents

Ethanol was obtained from SC Science, Thailand. Folin-Ciocalteu reagent, 2,4,6-tri (2-pyridyl)-s-triazine, 1,1-diphenyl-2-picryl hydrazyl (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), and (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid were purchased from Fluka, Germany. Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) and ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) were purchased from Scharlu, Spain. Gallic acid was provided from Sigma, Germany. The other chemicals and solvents used were all of analytical grade.

2.2. Weed collection

Seven weeds namely, *Ageratum conyzoides* L. (*A. conyzoides*), *Alysicarpus vaginalis* L. (*A. vaginalis*), *Commelina bengalensis* L. (*C. bengalensis*), *Euphorbia hirta* L. (*E. hirta*), *Hyptis suaveolens* L. (*H. suaveolens*), *Parthenocissus quinquefolia* L. (*P. quinquefolia*), and *Trianthema portulacastrum* L. (*T. portulacastrum*) (Figure 1), which are commonly found in local field crops including sugarcane, corn and cassava, were collected from Chonnabot District, Khon Kaen Province, Thailand in May 2013. They were identified by Prof. Arunrat Chaveerach, Department of Biology, Faculty of Science, Khon Kaen University.

2.3. Preparation of ethanol and aqueous weed extracts

Fresh stems and leaves of weeds were mixed and homogenized by using a Waring blender at room temperature under dimmed light. For ethanol extraction, 5 g of each homogenized weed was added with 10 mL of 70% (v/v) ethanol and mixed with a vortex mixer for 5 min. The mixture was left in the dark overnight. After that, the extracts were centrifuged at 2000 r/min for 10 min at room temperature. The supernatants were adjusted to their final volume to 10 mL. For aqueous extraction, distilled water was used as an extraction solvent instead of ethanol.

2.4. Phytochemical screening

The phytochemical constituents presented in the extracts were screened by standard procedures with slight modification[11,12].

2.4.1. Screening of alkaloids

One milliliter of weed extract was mixed with 1% hydrochloric acid and boiled at 95 °C for 10 min. It was then added with two drops of Wagner′s reagent (1.27 g iodine and 2.0 g potassium iodide in 100 mL distilled water). The reddish-brown precipitate indicated the positive result.

2.4.2. Screening of flavonoid

A piece of magnesium wire was dropped in 1 mL of the weed extract, followed by adding three drops of conc. hydrochloric acid. The positive result was observed by orange color.

2.4.3. Screening of saponin

A foam test was done by boiling 1 mL of weed extract for

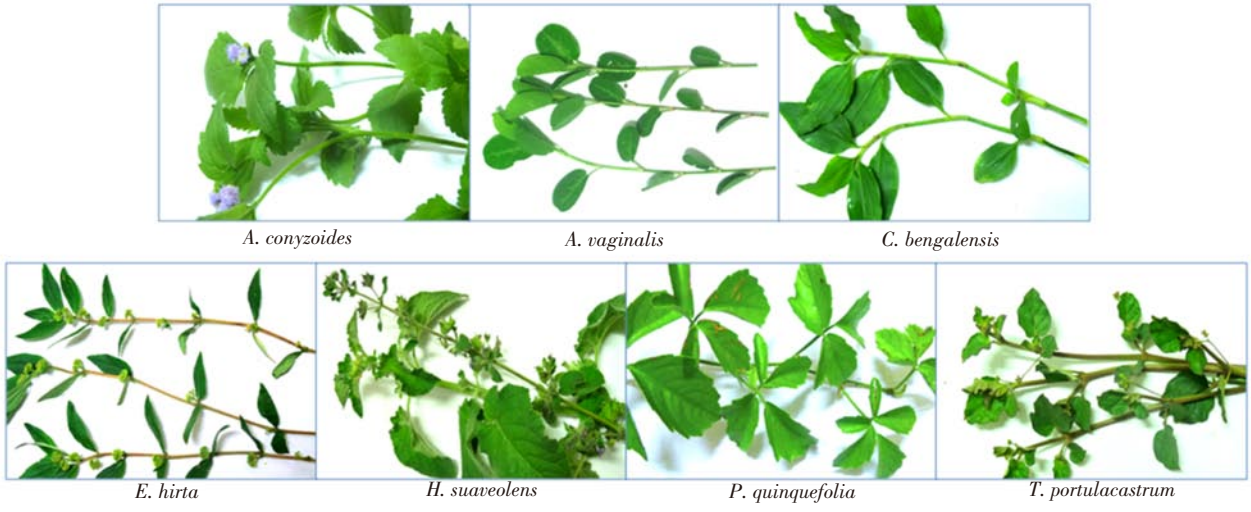

Figure 1. Weeds collected at Chonnabot District, Khon Kaen Province.

10 min and then mixed with 5% sodium carbonate solution. The mixture was vigorously shaken. Persistant foam forming indicated saponin content.

2.4.4. Screening of tannin

Tannin was screened by mixing 0.5 mL of the weed extract with 0.1 mL of 1% ferric chloride solution, and the yellow precipitate was observed.

2.5. Determination of total phenolic content (TPC)

The TPC in weeds was determined by modification of the Folin-Ciocalteu spectrometric method[13]. Briefly, 200 μL of the weed extracts at appropriated dilutions were mixed with 1 mL of 0.1 mol/L Folin-Ciocalteu reagent. After being left in the dark at room temperature for 30 min, 800 μL of 7% sodium carbonate was added to the solution. The absorbance of the resulting blue color was measured at 750 nm (Shimadzu, UV mini 1240, Japan) using gallic acid as standard. Phenolic content was expressed as mg of gallic acid equivalent (GAE)/g wet weight of weed.

2.6. Determination of total flavonoid content (TFC)

The TFC was determined by aluminium chloride colorimetric method[14]. Methanol (1.5 mL), 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 mol/L potassium acetate and 2.8 mL of distilled water were mixed with 0.5 mL of the extract solution and then incubated at room temperature for 30 min. The absorbance was measured at 415 nm (Shimadzu, UV mini 1240, Japan) using quercetin as standard. Flavonoid content was expressed as mg of quercetin equivalent (QE)/g wet weight of weed.

2.7. Antioxidant activity assays

2.7.1. Ferric reducing antioxidant power (FRAP) assay

Determination of total antioxidant activity was carried out according to FRAP assay[15] with slight modification. The FRAP reagent was freshly prepared by mixing 300 mmol/L acetate buffer pH 3.6, 10 mmol/L of 2,4,6-tris(2-pyridyl)-1,3,5-triazine solution in 40 mmol/L HCl and 20 mmol/L ferric chloride ($FeCl_3 \cdot 6H_2O$) solution at a ratio of 10:1:1 (v/v), respectively. One milliliter of the extract was added to 1 mL of the FRAP reagent. After 5 min left, the absorbance of the reaction mixtures was recorded at 593 nm (Shimadzu, UV mini 1240, Japan). The standard curve was constructed using ferrous sulfate ($FeSO_4$) solution. The results were expressed as μmol Fe(II)/g wet weight of weed.

2.7.2. Radical scavenging activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The free radical scavenging activity of weed extracts was evaluated by DPPH spectrophotometric assay as modified by Mensor[16]. Briefly, the extracts were diluted with 70% (v/v) ethanol and 20 μL of the diluted extracts were added with 1 mL of 0.25 mmol/L DPPH solution. After mixing, the reaction mixtures were kept in dark for 10 min and the change in absorbance at 540 nm was measured (Shimadzu, UV mini 1240, Japan) by using gallic acid as standard. Radical scavenging activity was expressed as mg of GAE/g wet weight of weed.

2.8. Antibacterial activity study

2.8.1. Bacterial strains

Eight pathogenic bacterial strains tested found in crops [*Ralstonia* spp. and *Xanthomonas campestris pv. Vesicatoria* (*X. campestris pv. Vesicatoria*)], in animals [*Edwardsiella tarda* (*E. tarda*), *Aeromonas hydrophila* (*A. hydrophila*) and *Aeromonas caviae* (*A. caviae*)] and in humans [*Plesiomonas shigelloides* (*P. shigelloides*), *Shigella* spp. and *Salmonella* spp.] were provided by the Department of Clinical Microbiology, Faculty of Associated Medical Sciences and Department of Microbiology, Faculty of Science, Khon Kaen University.

2.8.2. Preparation of test samples

To prepare the extracts for testing antibacterial activity, each fresh homogenized weed was added with solvent [70% (v/v) ethanol or distilled water] at a ratio of 1:2 and mixed vigorously. After centrifugation at 2000 r/min for 10 min at room temperature, the supernatant was collected. It was filtered through Whatman No. 4 filter paper and the filtrate was evaporated by using a rotary evaporator and kept at 4 °C until analysis.

2.8.3. Determination of antibacterial activity

Antibacterial activities of the weed extracts were determined by paper disc diffusion method as described by Bauer *et al*[17]. For all the test organisms, overnight culture grown in broth was adjusted to an inoculums size of approximately 10^5-10^7 CFU/mL, followed by spreading of a 0.5 McFarland turbidity standard over the surface of nutrient agar medium in 9-cm diameter Petri dishes by sterile swab sticks. The 50 mg/mL of dried weed residue was reconstituted in 10% (v/v) dimethyl sulfoxide. Twenty microliters of each reconstituted residue were added to 6-mm diameter sterile paper disc on dried Petri dishes and incubated at 37 °C for 24 h. Diameters of the inhibition zones were measured in millimeters (mm). Negative control was 10% (v/v) dimethyl sulfoxide. A mixture of 10% penicillin and 10% streptomycin were used as positive control. The experiment was carried out in triplicate and the results were expressed as mean± SD. The percentage of inhibition was calculated by using the following formula:

Inhibition (%)=(diameter of sample test-diameter of negative control)×100/diameter of positive control

3. Results

3.1. Phytochemical screening

The results indicated the presence of tannins and alkaloids in all the weed extracts. Saponin was present in these

weeds except *E. hirta* and *P. quinquefolia* aqueous and ethanol extracts. Flavonoid test gave positive results for all the ethanol extracts of the weeds but not aqueous extracts. Only *E. hirta* showed positive results for flavonoid for both extraction solvents.

3.2. Total phenolic and flavonoid contents

TPC of ethanol extracts were present in a wide range from (84.87±0.01) (*H. suaveolens*) to (1 554.93±0.01) (*E. hirta*) μg GAE/g wet weight (Table 1). For aqueous extracts, *P. quinquefolia* had the lowest and *E. hirta* had the highest TPC [(75.78±0.01) and (781.13±0.01) μg GAE/g wet weight respectively]. TFC ranged from (6.43±0.01) in *H. suaveolens* to (104.84±0.01) in *A. vaginalis* μg QE/g wet weight of ethanol extracts. *E. hirta* had a moderate amount of flavonoid content (39.42±0.01) μg QE/g wet weight of aqueous extract.

Table 1

Total phenolic, flavonoid content and antioxidant activity of various extracts of weeds.

Scientific name	Common name in Thai	Extraction solvent	TPC	TFC	FRAP	DPPH Inhibition
A. conyzoides	(Sab-lang-sab-ka)	Aqueous	245.05±0.01	0.00±0.00	322.83±0.01	433.64±0.01
		Ethanol	264.41±0.01	11.95±0.01	207.83±0.01	568.29±0.01
A. vaginalis	(Thua-li-song-naa)	Aqueous	151.95±0.01	0.00±0.00	120.00±0.01	309.98±0.01
		Ethanol	139.03±0.02	104.84±0.05	48.00±0.00	345.70±0.01
C. bengalensis	(Phak-prab-bai-guang)	Aqueous	132.11±0.01	0.00±0.00	355.00±0.01	543.56±0.01
		Ethanol	182.28±0.01	13.52±0.01	231.83±0.01	627.37±0.01
E. hirta	(Nom-rad-cha-see)	Aqueous	781.13±0.01	39.42±0.01	4 295.00±0.02	1 079.81±0.01
		Ethanol	1 554.93±0.01	76.55±0.02	4 945.00±0.03	1 853.92±0.01
H. suaveolens	(Kra-pao-phee)	Aqueous	103.20±0.01	0.00±0.00	245.50±0.01	436.39±0.01
		Ethanol	84.87±0.01	6.43±0.01	138.17±0.05	327.84±0.01
P. quinquefolia	(Thao-kan-kawn)	Aqueous	75.78±0.01	0.00±0.00	227.33±0.01	322.34±0.01
		Ethanol	194.95±0.01	13.19±0.01	413.17±0.01	1 398.85±0.01
T. portulacastrum	(Phak-bea-hin)	Aqueous	125.20±0.01	0.00±0.00	266.17±0.01	509.21±0.02
		Ethanol	121.70±0.02	7.84±0.01	352.50±0.02	531.19±0.01

Data are expressed as mean±SD. TPC and TFC are expressed as mg GAE/g wet weight; FRAP is expressed as mmol/L Fe(II)/g wet weight; DPPH inhibition is expressed as mg GAE/g wet weight.

3.3. Antioxidant activity

Antioxidant activity assayed by FRAP method showed that ethanol extract of *E. hirta* had the highest total antioxidant activity [(4 945.00±0.03) μmol/L Fe(II)/g wet weight] compared to the other ethanol extracts (Table 1). Its aqueous extracts also exhibited the highest antioxidant activity [(4 295.00±0.02) μmol/L Fe(II)/g wet weight]. *A. vaginalis* had low FRAP in both ethanol and aqueous extracts [(48.00±0.00) and (120.00±0.01) μmol/L Fe(II)/g wet weight, respectively]. The highest radical scavenging as reported by DPPH inhibition were found in *E. hirta* ethanol and aqueous extracts [(1 853.92±0.01) and (1 079.81±0.01) μg GAE/g wet weight, respectively].

3.4. Antibacterial activity

Four of seven weed extracts, including *E. hirta*, *H. suaveolens*, *P. quinquefolia* and *T. portulacastrum* exhibited antibacterial activity against the tested pathogenic bacteria. All these four weed extracts had no potential to inhibit *X. campestris pv. Vesicatoria* and *P. shigelloides*. Both aqueous and ethanol extracts of *E. hirta* exhibited antibacterial activity against most of the tested bacterial strains (Figure 2).

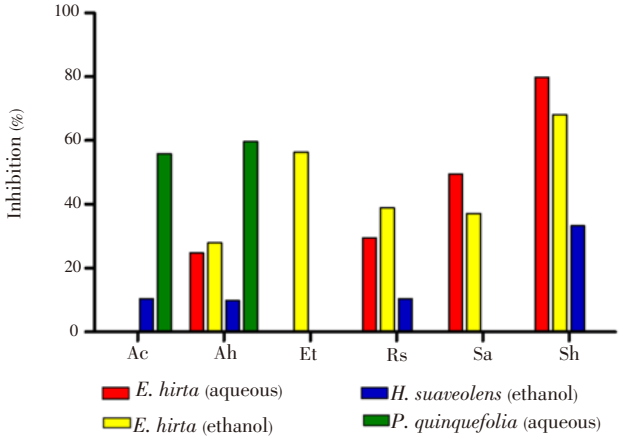

Figure 2. Comparison of inhibition (%) of weed extracts against pathogenic bacteria.
Ac: *A. caviae*; Ah: *A. hydrophila*; Et: *E. tarda*; Rs: *Ralstonia* spp.; Sa: *Salmonella* spp.; Sh: *Shigella* spp.

Aqueous and ethanol extract of *E. hirta* moderately inhibited *A. hydrophila* (24.76% and 27.94% inhibition, respectively) but could not inhibit *A. caviae*. Both aqueous and ethanol extracts of *E. hirta* inhibited *Ralstonia* spp. and *Salmonella* spp. whereas only ethanol extract of *E. hirta* highly inhibited *E. tarda* (56.25% inhibition). *Shigella* spp. were inhibited by aqueous and ethanol extracts of *E. hirta* with 79.90% and 68.14% inhibition.

H. suaveolens ethanol extract weakly inhibited *Ralstonia* spp. and *Salmonella* spp. (10.42% and 9.84% inhibition, respectively). *H. suaveolens* and *T. portulacastrum* ethanol extracts had 33.33% and 20.10% inhibition against *Shigella* spp.

P. quinquefolia aqueous extract strongly inhibited *A. caviae* and *A. hydrophila* with 55.90% and 59.68% inhibition, respectively.

Inhibition zone of *Salmonella* spp. and *Shigella* spp. tended to increase with the increasing polarity of the extraction solvent (Table 2). The positive control showed zones of inhibition ranging from (0.48±0.05) mm (for inhibition of *E. tarda*) to (1.67±0.10) mm (for inhibition of *Ralstonia* spp.) against all the test organisms. All the extracts were found to be less effective than positive control.

Table 2

Antibacterial activity of the various extracts of weeds using disc diffusion assay.

Bacterial strain	Diameter of inhibition zone (mm)				
	E. hirta (aqueous)	*E. hirta* (ethanol)	*H. suaveolens* (ethanol)	*P. quinquefolia* (aqueous)	Positive control
A. caviae	NI	NI	0.10±0.03	0.54±0.04	0.96±0.14
A. hydrophila	0.26±0.03	0.29±0.06	0.10±0.03	0.63±0.03	1.05±0.08
E. tarda	NI	0.27±0.02	NI	NI	0.48±0.05
Ralstonia spp.	0.49±0.02	0.65±0.05	0.17±0.02	NI	1.67±0.10
Salmonella spp.	0.33±0.07	0.25±0.03	NI	NI	0.67±0.03
Shigella spp.	0.54±0.03	0.46±0.06	0.23±0.02	NI	0.68±0.06

NI: No inhibition zone observed.

4. Discussion

To control weeds, prevention of weed growing, distribution of weeds from other areas and destroying parts of weeds in

farms are considered. Using herbicides is preferred to control the weeds by the farmers because of their high efficacy, convenience and rapidity to use in wide areas. Herbicides have negative effects to the farmers and consumers due to their toxic residues[2,3]. Worldwide, phytochemicals with unknown biological and pharmacological activities have been extensively investigated for a new and potential source of medicinal agents. Approximately 25% of drugs are from phytochemical origin[18]. Plant-based medicines for primary health care are increasing interest due to its cost-effective[19]. Many medicinal plants are used to treat ailments caused by microorganisms. There are a few studies that explored the use of Northeast Thai weeds as antibacterial agents. The selected weeds are available in plenty because they are not fed to cattle. If they have antibacterial property, they will be cost-effective as sources of therapeutic agents.

In the present study, *A. conyzoides*, *A. vaginalis* and *C. bengalensis* showed no antibacterial activity against the test organisms. *A. conyzoides* extract was reported to have antioxidant and anticancer against human non-small cell lung (A-549), gastric (SGC-7901), colon (HT-29), golima (U-251), breast carcinoma (MDA-MB-231), prostate carcinoma (DU-145), hepatic carcinoma (BEL-7402), and also mouse leukemia (P-388) cancer cell lines[9]. But it did not inhibit growth of *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Proteus* spp., and *Shigella* spp[10]. *A. vaginalis* was reported to have antioxidant and antiproliferative activity[20]. This supports our study for its moderate ferric reducing activity and radical scavenging activity determined by FRAP and DPPH assays, respectively. There are a few reports about antifungal activity of *C. bengalensis* against *A. fumigatus*[8].

E. hirta, found in India and Africa, has been mostly studied. It inhibited the growth of *E. coli*, *P. aeruginosa*, *Proteus mirabilis* (*P. mirabilis*), and *S. aureus*[6]. In the present study, *E. hirta* extracts had the highest TPC and was the most effective against most of the test organisms compared to the other weed extracts. In this study, inhibition zone of *Salmonella* spp. and *Shigella* spp. tended to increase with the increasing polarity of the extraction solvent. This finding was correlated with previous reports by Bendini *et al*.[21] and Rodriguez *et al*[22]. This indicated that its phenolic compounds may play a role in antibacterial activity. It also contained a moderate amount of total flavonoids which corresponded with the study of Singh *et al.* that flavonoids (free and bound) of *E. hirta* had antimicrobial activity against *E. coli*, *P. aeruginosa*, *P. mirabilis*, and *S. aureus*[6].

H. suaveolens, mostly found in Brazil, is used as anti-inflammation, treatment of gastric ulcer and infection[7]. In the present study, it had moderate phenolic and flavonoid contents. The antioxidant activity assessed by FRAP and DPPH assays was high. Its phytochemical constituents and antibacterial property of the extract may confirm the therapeutic value.

P. tricuspidata leaves were reported to have weak antimicrobial activity against *S. aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus luteus* (ATCC 10240), *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6633BB), *Bacillus cereus* (ATCC 11778), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (NCIMB 9111), and *Candida albicans* (ATCC 10259 and 24433)[23]. *P. quinquefolia* has antidiabetic activity in the rat model[24]. *A. hydrophilia* is resistant to most common antibiotics. Chloramphenical, tetracycline and sulfonamide are used to control its infection. The present study reported inhibitory effect of *P. quinquefolia* against *A. caviae* and *A. hydrophila* which cause gastroenteritis. This antibacterial activity may be from some phonolic compounds in these weed extracts which need further elucidation of bioactive compounds.

T. portulacastrum is growing throughout tropical countries. It has several traditional uses in India such as analgesic activity[25]. In the present study, *T. portulacastrum* ethanol extracts had 20.10% inhibition against *Shigella* spp. This corresponded with the previous reports that ethanolic extract of its leaves could inhibit *Shigella flexneri*, *E. coli*, *S. aureus*, *Proteus vulgaris*, *P. aeruginosa* and *Salmonella typhi* whereas the least antibacterial activity was found in aqueous extract[26].

All the studied weed extracts could not inhibit *X. campestris pv. Vesicatoria* and *P. shigelloides*. The results were supported by the previous studies. *X. campestris pv. Vesicatoria* usually infects tomato and pepper plants and it can spread quickly in 3-5 d after infection. Streptomycin is used for controlling this pathogen but it developed resistant strains while antibiotic is no longer effective[27]. *P. shigelloides* is often found in fresh and brackish water of Thailand, Japan and China. It is an opportunistic pathogen causing gastroenteritis infection in humans. It is resistant to several antibiotics including penicillin[28]. Thus antimicrobial susceptibility testing for these organisms by the other natural compounds should be further studied.

Weeds in the present study are widely available in Northeast Thailand in all seasons and easy for collection at low costs. Phenolics and flavonoids were the major constituents of the weed extracts. *E. hirta* extracts showed high potency of antibacterial activity compared to the other weed extracts. These weeds may serve as potential sources for the development of novel bactericidal agents in the future.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Weeds are wide spread in tropical country. They can resist to microbial attack compared to crops. They are inexpensive sources to develop cost-effective products.

Research frontiers

This work investigated antibacterial potency of selected

Northeast Thai weeds (both aqueous and ethanol extracts) against pathogenic bacteria in crops, animals and humans.

Related reports

Weeds contain bioactive phytochemicals with antioxidant, antimicrobial and anticancer activities. *E. hirta* can inhibit growth of *E. coli*, *P. aeruginosa*, *P. mirabilis*, and *S. aureus*.

Innovations and breakthroughs

In the present study, *E. hirta* extracts had the highest TPC and was the most effective against most of the test organisms compared to the other weed extracts.

Applications

This study leads to find a new source of phenolic compounds with antibacterial activity. This may be developed to be antibacterial agent in the future. And using weeds for making drugs can also help reduce the disturbance of weeds to other economical crops.

Peer review

The weeds extracted in this study had phenolics and flavonoids as major constituents. *E. hirta* extracts had high antibacterial activity compared to the other extracts. These weeds may have potential to develop novel bactericidal agents.

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