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Antimicrobial, Antioxidant and Cytotoxic Activities of *Cleoma viscosa* Linn. Crude Extracts

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Abstract**—**The bioactivity studies from the weed ethanolic crude extracts from leaf, stem, pod and root of wild spider flower; *Cleoma viscosa* Linn. were analyzed for the growth inhibition of 6 bacterial species; *Salmonella typhimurium* TISTR 5562, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* TISTR 1466, *Streptococcus epidermidis* ATCC 1228, Esc*herichia coli* DMST 4212 and *Bacillus subtilis* ATCC 6633 with initial concentration crude extract of 50 mg/ml. The agar well diffusion results found that the extracts inhibit only gram positive bacteria species; *S. aureus, S. epidermidis* and *B. subtilis*. The minimum inhibition concentration study with gram positive strains revealed that leaf crude extract give the best result of the lowest concentration compared with other plant parts to inhibit the growth of *S. aureus*, *S. epidermidis* and *B. subtilis* at 0.78, 0.39 and lower than 0.39 mg/ml, respectively. The determination of total phenolic compounds in the crude extracts exhibited the highest phenolic content was 10.41 mg GAE/g dry weight in leaf crude extract. Analyzed the efficacy of free radical scavenging by using DPPH radical scavenging assay with all crude extracts showed value of IC_{50} of leaf, stem, pod and root crude extracts were 8.32, 12.26, 21.62 and 35.99 mg/ml, respectively. Studied cytotoxicity of crude extracts on human breast adenocarcinoma cell line by MTT assay found that pod extract had the most cytotoxicity CC_{50} value, 32.41 µg/ml. Antioxidant activity and cytotoxicity of crude extracts exhibited that the more increase of extract concentration, the more activities indicated. According to the bioactivities results, the leaf crude extract of *Cleoma viscosa* Linn. is the most interesting plant part for further work to search the beneficial of this weed.

Keywords**—**Antimicrobial, antioxidant activity, *Cleoma viscosa* Linn., cytotoxicity test, total phenolic compound.

I. Introduction

NOWADAYS, people favour to take care about their health, try to find new products from nature while escaping from synthetic ones. Plants are main source of phytochemicals use as natural antibiotics and antioxidants for drug development and health care products.

Cleome viscosa Linn., wild spider flower, wild mustard, dog mustard, is a small simple weed spread in fields or fallows in Thailand. It belongs to family Cleomaceae. It is an annual herb high about one meter with yellow flower and fury sticky stem [1], [2]. In some countries, it is used to control pest in cowpea [3] or to medicate inflammation and flatulence as medicinal plants [4].

In this study, we examined the antibacterial, antioxidant activity, total phenolic contents and cytotoxicity in leaf, stem, pod and root crude extracts of *Cleome viscos*a to find out health care potential of this weed.

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II. Materials and Methods

A. Plant Materials and Preparation of Crude Extract

The whole fresh sample of *Cleome viscosa* Linn. was collected from Ladkrabang district (13.7300° N, 100.7784° E) Bangkok, Thailand in April, 2014. After cleaned by tap water, it is separated to leaf, stem, pod and root. All plant parts are dried in hot air oven at 45ºC for 3 days, after that mashed into powder, kept in a dark place before extraction.

100 g of the plant powder materials was soaked in 900 ml 95% ethanol at room temperature for 1 week. The supernatants are filtered by Whatmann filter paper, concentrated by rotary evaporator to get all crude extracts, and kept in dark at 4 °C before for bioactivity tests.

B. Source of Bacteria and Antibacterial Assay

All bacterial strains; *Streptococcus epidermidis* ATCC1228, *Staphylococcus aureus* TISTR1466, *Bacillus subtilis* ATCC6633, *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* DMST4212 and *Salmonella typhimurium* TISTR5562 were provided by Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang.

Antibacterial activity was studied by agar well diffusion method using 50 mg/ml crude extract for screening test and serial two-fold dilution of each extract (0.39, 0.78, 1.56, 3.13, 6.25, 12.5 and 25.0 mg/ml) for minimum inhibitory concentration (MIC) against affected strains on preliminary test. We used 20 µg/ml gentamicin as positive control and 95% ethanol as negative control.

We adjusted final density of each bacterial strain to 10^8 CFU/ml compared with standard 0.5 of Mc Farland scale before swabbing bacterial strains onto Mueller-Hinton agar plate. Then, we punched wells into the agar plates with 0.6 mm diameter cork-borer, added 20 µl of prepared extracts into wells, incubated the plates at 37 °C for 24 hr, and measured inhibition zone diameter.

C. Total Phenolic Content

Total phenolic assay was analyzed by Folin-Ciocalteu method [5] for leaf, stem, pod and root crude extracts. We dropped 20 µl of 0.1 mg/ml crude extracts into 96 well-plate after that added 100 µl of Follin-Ciocalteu reagent and filled wells with 80 µl of 7.5% Na_2CO_3. The absorbance of the resulting color was measured at 765 nm after incubation for 30 min at room temperature. We determined total phenolic

contents by using a standard curve prepared with gallic acid and expressed contents of total phenolic in each crude extract in terms of milligram GAE (gallic acid equivalent) per gram plant dry weight.

D. Antioxidant Activity

Antioxidant scavenging activity was carried out by DPPH assay. Dissolved twelve mg of leaf, stem, pod and root of *C. viscosa* extract in 1 ml absolute ethanol for stock solution. A sequential dilution of samples was used (1.5, 3.0, 6.0 and 12.0 mg/ml). 500 mM of α –tocopherol was adopted as a positive control. DPPH (2,2 diphenyl-1-picrylhydrazyl) solution was fresh prepared at concentration of 100 µM in absolute ethanol and kept in the dark until use. We mixed DPPH solution with plant extracts, then shook vigorously and incubated the mixture in dark chamber at 37ºC for 30 min. The solution was placed in microtiter plate and the absorbance was measured at 517 nm.

$$\% \text{ scavenging} = \frac{[\text{A control} - \text{A sample}]}{\text{A control}} \times 100$$

A sample = absorbance of samples; A control = absorbance of control

E. Cytotoxicity Test

The cell inhibition assay was performed by MTT colorimetric method against MCF-7 adenoma cells. The MCF-7 cells in mid-log phase at approximately 1.0 $x10^5$ cells/ml were left to attach onto bottom surface of the 96-well plates for 24 hr before treated with extracts. The MCF-7 cells were treated with ten-fold serial dilution concentration of *C. viscosa* extracts (0.1, 1, 10, 100, 1000 µg/ml) and incubated in a 5% CO_2 incubator at 37 ºC for 72 hr. We replaced crude extract with 5 mg/ml of MTT (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphynyl tetrazolium bromide) solution and left for 4 hr. Then, we dissolved the formazan crystal with DMSO. The level of formazan derivative colored was analyzed by microtiter plate at wavelength of 540 nm. The percentage of cell inhibition was calculated according to:

$$\% \text{ cytotoxic} = \frac{[\text{A control} - \text{A sample}]}{\text{A control}} \times 100$$

A sample = absorbance of treated cells; A control = absorbance of control

The IC_{50} value was obtained by plotting the percentage of cell inhibition versus the concentration. Each sample was tested at least five independent experiments and the mean values were reported.

III. Results and Discussion

A. Antimicrobial Activity

The antibacterial activity of leaf, stem, pod and root of *C. viscosa* crude extract was assayed against gram positive bacteria; *Streptococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis* and gram negative bacteria; *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhimurium* at 50 mg/ml. The inhibition of bacterial growth by crude extracts of four parts of *C. viscosa* is summarized in Table I.

TABLE I
ANTIBACTERIAL ACTIVITY OF *C. VISCOSA* EXTRACTS AT 50 MG/ML

crude extract	inhibition zone (diameter in mm)					
	S. aureus	*S. epidermidis*	*B. subtilis*	*S. typhimurium*	*E. coli*	*P. aeruginosa*
leaf	13.21^{a}±0.22	15.05^{a}±0.38	16.04^{b}±0.50	6.00^{a}±0.00	6.00^{a}±0.00	6.00^{a}±0.00
stem	9.11^{b}±0.85	9.68^{b}±0.16	13.26^{c}±0.70	6.00^{a}±0.00	6.00^{a}±0.00	6.00^{a}±0.00
pod	12.85^{a}±1.33	8.19^{c}±0.17	18.69^{a}±0.47	6.00^{a}±0.00	6.00^{a}±0.00	6.00^{a}±0.00
root	9.51^{b}±0.25	8.22^{c}±0.26	14.31^{c}±0.87	6.00^{a}±0.00	6.00^{a}±0.00	6.00^{a}±0.00
gentamicin	16.23	19.72	18.78	15.49	13.38	14.71
95%EtOH	6.00	6.00	6.00	6.00	6.00	6.00

* The same alphabet are not statistically significant (Tukey's test, $p < 0.05$) in each crude extract

The result of antibacterial activity found that in all crude extracts, we could not measure inhibition zone in gram negative bacteria, they could inhibit only gram positive bacteria. Due to the component of gram negative bacterial cell wall consists of proteins such as porin, gram negative bacteria are more tolerant to uncommon or antibiotic substances than gram positive bacteria [6], [7]. Pod crude extract gave the highest clear zone with *B. subtilis* at 18.69 mm while leaf extract gave the highest clear zone with *S. epidermidis* and *S. aureus* at 16.04 and 13.21 mm, respectively. The results of bacterial growth inhibition from this study indicated that all crude extracts restrain only gram positive bacteria that contrast with a former report [9]. it studied 500 µg of aerial part methanolic crude extract of *C. viscosa,* carried out by agar well diffusion against *B. subtilis*, *P. aeruginosa* and *E. coli*; diameters of inhibition zone were 18.0, 25.0 and 12.0 mm, respectively [8]. On the other hand, *C. viscosa* leaf methanolic extract at 200 mg/ml against *S. epidermidis* and *S. aureus* had smaller inhibition zone were 11.45 and 10.80 mm.

MIC study exhibited (Table II) that leaf crude extract gave the best result of bacterial growth inhibition than other three extracts. The lowest concentrations of leaf extract inhibited growth of all bacterial strains; *S. epidermidis*, *S. aureus* and *B. subtilis* were 0.39, 0.78 and lower than 0.39 mg/ml of leaf crude extract, respectively.

B. Total Phenolic Content

Phenolic compounds are very significant constituents in plants because they express antioxidant activity by inactivating lipid free radicals or preventing decomposition of

hydroperoxides into free radicals. Total phenolic compounds are implicated with antioxidant activity and have an important role on stabilizing lipid oxidation [10]. Total phenolic compounds exhibited as GAE of dry weight are as Table III.

TABLE II
ANTIBACTERIAL ACTIVITY BY DETERMINATION OF MIC OF *C. VISCOSA* EXTRACTS AT 25, 12.5, 6.25, 3.13, 1.56, 0.78 AND 0.39 MG/ML

crude extract	Concentration (mg/ml)	inhibition zone (diameter in mm)		
		S. epidermidis	*S. aureus*	*B. subtilis*
leaf	25.00	14.00^{a}±0.26	12.06^{a}±0.56	15.10^{a}±0.39
	12.50	12.81^{b}±0.30	11.02^{a}±0.71	13.09^{b}±0.35
	6.25	11.91^{c}±0.36	9.93^{b}±0.65	11.44^{c}±0.06
	3.13	10.49^{d}±0.20	9.07^{b}±0.64	9.50^{d}±0.44
	1.56	8.84^{e}±0.30	7.61^{c}±0.58	9.98^{d}±1.33
	0.78	7.26^{f}±0.27	6.00^{d}±0.00	7.13^{e}±0.13
	0.39	6.00^{g}±0.00	-	6.47^{e}±0.45
stem	25.00	8.22^{a}±0.55	8.17^{a}±0.22	11.81^{a}±0.91
	12.50	6.80^{b}±0.70	6.87^{b}±0.76	10.21^{b}±0.66
	6.25	6.00^{b}±0.00	6.00^{b}±0.00	8.86^{c}±0.24
	3.13	-	-	7.92^{cd}±0.74
	1.56	-	-	7.16^{d}±0.25
	0.78	-	-	6.00^{e}±0.00
	0.39	-	-	-
pod	25.00	7.82^{a}±0.39	12.06^{a}±0.50	17.50^{a}±0.09
	12.50	6.65^{b}±0.57	10.29^{b}±0.60	16.45^{a}±0.68
	6.25	6.00^{b}±0.00	7.75^{c}±0.22	14.12^{b}±0.92
	3.13	-	6.00^{d}±0.00	11.32^{c}±0.59
	1.56	-	-	9.31^{d}±0.33
	0.78	-	-	6.85^{e}±0.75
	0.39	-	-	6.00^{e}±0.00
root	25.00	6.00±0.00	8.76^{a}±0.40	11.81^{a}±0.59
	12.50	6.00±0.00	7.15^{b}±0.12	9.57^{b}±0.32
	6.25	-	6.00^{c}±0.00	8.08^{c}±0.23
	3.13	-	-	7.45^{d}±0.31
	1.56	-	-	6.00^{e}±0.00
	0.78	-	-	6.00^{e}±0.00
	0.39	-	-	-
gentamicin		18.69	17.10	17.95
95% EtOH		6.00	6.00	6.00

* The same alphabet are not statistically significant (Tukey's test, p< 0.05) in each crude extract
* - : not tested

TABLE III
TOTAL PHENOLIC CONTENT OF *C. VISCOSA* EXTRACTS

crude extract	Total phenolic content (mg GAE/g dry weight)
leaf	10.40
stem	5.62
pod	8.54
root	4.50

We analyzed the amount of total phenolic compounds in the leaf of *C. viscosa* extract. It is shown that the highest total phenolic contents among pod, stem and root crude extract are as 10.40, 8.54, 5.62 and 4.50 mg GAE/g dry weight, respectively. There was a previous experiment to find total phenolic and flavonoid contents in leaf and stem methanolic crude extracts. The results exhibited that level of total phenolic contents in leaf was higher than in stem [11].

C. Antioxidant Activity

DPPH is a stable free radical with characteristic absorption at 517 nm and antioxidant in crude extract reacts with 2,2 diphenyl-1-picrylhydrazyl (DPPH) and converts it to 2,2-diphenyl-1-picrylhydazine. The degree of discoloration indicated the scavenging potential [12]. A smaller IC_{50} value corresponds to a higher antioxidant activity of the plant extract. DPPH radical scavenging activity of extracts was investigated as shown in Table IV.

The more increased of crude extracts concentration, the more scavenging effect expressed. The 50% inhibitory effect of the crude extracts was calculated and found that the lowest was 8.32 mg/ml from leaf extract.

TABLE IV
DPPH SCAVENGING ACTIVITY OF DIFFERENT PARTS OF *C. VISCOSA* EXTRACTS

Concentration	% scavenging			
(mg/ml)	leaf	stem	pod	root
12.0	55.14^{a}±1.06	44.82^{a}±0.68	23.16^{a}±2.36	17.56^{a}±0.31
6.0	40.21^{b}±0.84	31.86^{b}±0.13	15.42^{b}±0.59	8.58^{b}±0.14
3.0	21.63^{c}±0.34	13.95^{c}±0.32	3.15^{c}±2.51	1.01^{c}±0.72
1.5	7.96^{d}±1.40	6.11^{d}±0.38	0.08^{d}±0.05	0.29^{d}±0.24
α– Tocopherol	91.13			
IC_{50} (mg/ml)	8.32	12.26	21.62	35.99

* The same alphabet are not statistically significant (p< 0.05)

Total phenolic contents and scavenging activity showed that leaf crude extract was the most interesting choice for aim to find antioxidant substance.

D. Cytotoxicity Assay

Cytotoxicity of *C. viscosa* extracts against MCF-7 carcinoma cell was carried out by MTT assay and the result was shown in Table V.

TABLE V
INHIBITORY RESPONSE OF MCF-7 CELLS TO *C. VISCOSA* EXTRACTS

Concentration	% Cytotoxicity			
(µg/ml)	leaf	stem	pod	root
1000	80.31^{a}±2.17	58.72^{a}±1.97	81.19^{a}±0.91	62.53^{a}±0.80
100	58.25^{b}±2.63	50.54^{b}±1.00	66.30^{b}±3.89	50.08^{b}±1.25
10	41.09^{c}±2.28	36.86^{c}±2.11	53.36^{c}±4.23	29.29^{c}±5.39
1.0	34.64^{d}±3.89	29.17^{d}±2.79	30.28^{d}±5.08	23.36^{d}±1.88
0.1	20.65^{e}±0.74	25.08^{e}±0.75	21.23^{e}±9.48	22.83^{d}±4.53
CC_{50} (µg/ml)	56.92	115.9	32.41	132.08

* The same alphabet is not statistically significant (p< 0.05)

The cytotoxicity against MCF-7 cells depends on crude extracts concentration. 50% of cell death caused by the crude extracts was analyzed and it was shown that the lowest was 32.41 µg/ml from pod extract. The result of this assay was similar to the result in a previous study about lethality of brine shrimp (*Artemia salina*) against *C. viscosa* leaf metanolic extract. LC_{50} and LC_{90} were 28.18 and 112.20 µg/ml, respectively [9].

IV. CONCLUSION

The bioactivities of leaf, stem, pod and root crude extracts of *C. viscosa* were investigated. The results revealed that leaf

ethanolic extract has high level of phenolic contents that are potential antibacterial and antioxidant activities. This study provides the information for the use of this weed to added value. Therefore, the further study aims to investigate the qualitative and quantitative of phytochemicals in *C. viscosa*.

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