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Chemical Composition and Biological Activity of the *Centipeda minima* (Asteraceae)

Surjani Soetardjo[1], Jong Poh Chan[1], Ahmad Mohamad Noor[2], Yoga Latha Lachimanan[3] & Sasidharan Sreenivasan[3]

[1] School of Distance Education, [2] School of Chemical Sciences, [3] School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia.

ABSTRACT

The antimicrobial activity of the *Centipeda minima* L. (Asteraceae) extract was evaluated against seven microorganisms using the disc diffusion method. The extract showed a broad spectrum of antimicrobial activity against all the tested bacterial strains, especially *Enterobacter aerogenes, Klebsiella pneumonia, Staphylococcus aureus* and *Yersinia enterocolitica.* The chemical composition of the extract obtained from *Centipeda minima* was analysed by GC–MS. Twenty-three compounds, constituting about 84.75 % of the total extract, were identified. The main constituents were palmitic acid (7.47%), (Z,Z)-9-,12-octadecatrienoic acid (6.52%), (Z,Z,Z)-9-,12-octadecatrienoic acid (7.01%), phytol (7.01%), naptho[2.3-b]furan-2-(3H)-on (6.21%), 1-(1,2,3,4,7,7a-hexahydro-1,4,4,5-tetramethyl-1,3a-ethano-3aH-inden-6-yl)etanon (7.95%), 1,3,5-tri-tert-butyl-benzene (4.52%), (3Z)-2-methyl-3-octen-2-ol (5.95%) and artemisia ketone (4.98%). The extract was also tested against brine shrimp for toxicity. There was no significant toxicity as it only recorded a LC_{50} value of 4.92 mg/ml. The study shows that the extract is a good antimicrobial agent with potential applications in public health against diseases.

INTRODUCTION

Malaysia has a great wealth of therapeutic plants, which have been used traditionally for many years. It is estimated that numerous medicinal plants have been used. These plants could have immense value in the future especially with the emergence of new infectious diseases and resistance of pathogenic microbes to the commonly used antibiotics (Ali-Shtayeh *et al*., 1998; Primo *et al.,* 2001). In order to overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for infectious diseases.

Centipeda minima (L) A. Braun & Aschers (Asteraceae) is widespread in Malaysia, India, China, Australia and Oceania, but this plant has not been studied extensively for its biological activity. The aerial parts (stems, leaves and flowers) of the *C. minima* are widely used in traditional medicine. For example in Chinese traditional medicine, the aerial parts of the *C. minima* are used to treat headaches, head colds, conjunctivitis, piles and malaria (Perry, 1980). In Malaysia, the plant was reported to be used by the rural people as a kind of snuff to make the eyes water when afflicted with ophthalmia (Burkill, 1935). A decoction from this plant

Correspondence author: Sasidharan Sreenivasan, Email: srisasidharan@yahoo.com

was suggested as a remedy for rickets and children's sores, and for diseases of the digestive system in Taiwan. In the Philippines, the leaves are squeezed between the fingers and inhaled to clear the head by provoking sneezing (Perry, 1980).

Previous research by Robin & Towers (1998) reported that the *C. minima* contains three antibacterial sesquiterpene lactones, identified as 6-O-methylacrylylplenolin, 6-O-isobutyroylplenolin, and 6-O-angeloylplenolin. These compounds had activity against *Bacillus subtilis* and *Staphylococcus aureus,* with 6-O-isobutyroylplenolin being the most active. Our study aims to reevaluate the *C. minima* as an antimicrobial agent, based on their findings and its ethnomedical use.

In this paper, we describe the results of GC/MS analysis of the chloroform extract from the aerial part of *C. minima* and its antibacterial activity and toxicity against *Artemia salina*.

MATERIALS AND METHODS

Microorganisms

Enterobacter aerogenes, Listeria monocytogenes, Klebsiella pneumonia, Staphylococcus aureus, Salmonella enteritidis, Yersinia enterocolitica and *Shigella sonnei* were used as test organisms and were obtained from the laboratory stock cultures. The bacteria were cultured on nutrient agar slants at 37°C for 18 h. The stock culture was maintained on nutrient agar slants at 4°C.

Centipeda minima sample

C. minima aerial parts were bought from Chuah Huah Herbal Garden Kuching, Sarawak, Malaysia in 2004 and authenticated by the botanist of School of Biological Sciences at Universiti Sains Malaysia, where the herbarium was located.

Preparation of the crude extract

The dried aerial parts of *C. minima* were boiled in a soxhlet with 350 ml of chloroform for 8 h. The entire crude extract of *C. minima* was evaporated to dryness in a rotary evaporator. The dried extract was then re-dissolved in 10% (v/v) dimethylsulphoxide (DMSO) to yield a concentration of 10 mg of extract per ml solution.

Antimicrobial Activity

The agar disc diffusion method was employed to determine the antimicrobial activity of the crude extract (NCCLS, 1997). The test microbes were removed aseptically from the working culture with an inoculating loop and transferred to a test tube containing 5.0 ml of sterile distilled water. Sufficient inoculum was added until the turbidity equaled 0.5 McFarland standards (bioMerieux, Marcy Petoile, France). One ml of the bacterial suspension was added to 15-20 ml of nutrient agar before setting aside the seeded agar plate (9 cm in diameter) to solidify for 15 min. Three paper discs (6-mm diameter, Whatman's No.1) were placed on the surface of the seeded plates to screen the antimicrobial activity for each test microorganism. The sterile discs were impregnated with 20 µl of extracts (corresponding to 10 mg/ml of crude extract). Gentamycin (2 mg/ml) and 10% DMSO (v/v) were used as positive control and negative control, respectively. The plates were placed at 4°C for 2 h, followed by incubation at 37°C for 24 h and examined for zones of growth inhibition which were expressed in millimeters (mm). Each test was performed in three replicates and repeated twice.

Toxicity testing against the brine shrimp

Shrimp hatching

Brine shrimp eggs, *Artemia salina* were hatched in artificial seawater prepared by dissolving 38 g of sea salt in 1L of distilled water. After a 24 h incubation period at room temperature (22°C - 29°C), the larvae were attracted to one side of the vessel with a light source and collected with a pipette. The larvae were separated from the eggs by aliquoting them three times in small beakers containing sea-water.

Brine shrimp assay

The bioactivity of the extract was monitored by the brine shrimp lethality test (Meyer *et al.,* 1982). Samples were dissolved in 10% DMSO (v/v) and diluted with artificial seawater. Nine bottles with the concentration from 8.0 mg/ml to 4.0 mg/ml of the *C. minima* extract were prepared. The last bottle was filled with sea salt water and 10% DMSO (v/v) only, serving as a drug free control. One hundred µl of larvae suspension containing about 10-15 larvae were added into each bottle and incubated for 24 h. The bottles were then examined and the number of dead larvae in each bottle was counted. The total number of shrimp in each bottle was counted and recorded. The mean percentage mortality was plotted against the logarithm of concentrations, and the concentration that could kill 50% of the larvae (LC_{50}) was determined from the graph (Geran *et al.,* 1972).

Data analysis

The mean results of brine shrimp mortality against the logarithms of concentrations were plotted using the Microsoft Excel computer program, which also presents regression equations. The regression equations were used to calculate LC_{50} value. Extracts giving LC_{50} values greater than 20 µg/ml were considered to be non-toxic.

Gas chromatography–mass spectrometry (GC–MS) analysis

The GC/MS analysis was done on a thermo gas chromatograph mass spectrometer (model Shimadzu 2010) equipped with DB-5 capillary column (30 m long × 0.25 mm i.d., film tickness 0.25 µm). The column temperature program was 50°C for 6 min, with 5°C increases per min to 250°C; which was maintained for 30 min. The carrier gas was helium at a flow rate of 1 ml/min (splitless mode). The detector and injector temperatures were both maintained at 250°C. The quadrupole mass spectrometer was scanned over the range 28–400 amu at 1 scan s^{-1}, with an ionising voltage of 70 eV, an ionisation current of 150 Ma and an ion source temperature of 200°C.

In order to determine the Kovats index of the components, a mixture of alkenes (C_9–C_{24}) was added to the crude extract before injecting in the GC–MS equipment and analysed under the same conditions as above. The compounds were identified by computer searches in commercial libraries of NIST (*National Institute of Standard and Technology*) and by their Kovats retention indexes.

RESULTS

Antimicrobial Activity

The results of antimicrobial activity of the crude extract against the tested bacteria are given in Table 1. The extract exhibited a favorable activity against all the bacteria tested. The zone of clearance produced by the commercial antibiotic disc was larger than those produced by the extract disc. The solvent-only negative control disc produced no zone of clearance.

Table 1. Antibacterial activity of the *Centipeda minima* L. (Asteraceae) crude extracts

Microorganisms	*Zone of inhibition (mm)*[a]	
	Crude Extract (10 mg/ml)	*Gentamycin (2 mg/ml)*
Enterobacter aerogenes	16.6	21.3
Listeria monocytogenes	15.8	27.6
Klebsiella pneumonia	19.6	23.2
Staphylococcus aureus	17.4	20.2
Salmonella enteritidis	14.2	23.2
Yersinia enterocolitica	16.4	20.6
Shigella sonnei	13.2	21.2

[a]Values, including diameter of the filter paper disc (6.0 mm), are means of three replicates.

Table 2. The result of toxicity study of crude extract of *Centipeda minima* L. (Asteraceae) against brine shrimp larvae (*Artemia salina*)

Concentration (mg/ml)	*Log_{10}Concentration*	*Number dead*	*Number survived*	*Percent mortality (%)*
Control	-	0.00	15.00	0
8.0	0.903	17	3	85.0
7.5	0.875	17	3	85.0
7.0	0.845	15	5	75.0
6.5	0.813	14	6	70.0
6.0	0.778	12	8	60.0
5.5	0.740	12	8	60.0
5.0	0.699	8	10	44.4
4.5	0.623	8	12	40.0
4.0	0.602	8	12	40.0

The LC_{50}[a] was obtained by linear regression equations. LC_{50} value lower than 1.0 mg/ml was considered to be non-toxic.

[a]LC_{50} value of crude extract of *Centipeda minima* L. (Asteraceae) was 4.91 mg/ml.

Toxicity testing against the brine shrimp

The result of the toxicity evaluation against the brine shrimp of the crude extract is shown in Table 2. The extract showed no significant toxicity against brine shrimp as the LC_{50} value was 4.92 mg/ml.

GC–MS analysis

Twenty-three main compounds were identified in the crude extract (Table 3). Of these components, the 11 most intense, corresponding to 65.05% (w/w) of the crude extract, were: palmitic acid (7.46%), (Z,Z)-9-,12-octadecatrienoic-acid (6.52%), (Z,Z,Z)-9-,12-octadecatrie-

Table 3. Chemical composition of crude extract of *Centipeda minima* L. (Asteraceae)

Peak number	*Compound*	*Rt*[a]	*Percentage*
1	Palmitic acid	32.71	7.46
2	(Z,Z)-9-,12-octadecatrienoic acid	32.83	6.52
3	(Z,Z,Z)-9-,12-octadecatrienoic acid	32.84	7.01
4	Phytol	32.97	6.21
5	octadecanoic acid	32.27	0.98
6	Naptho[2.3-b]furan-2-(3H)-on	39.03	6.21
7	1-(1,2,3,4,7,7a-hexahydro-1,4,4, 5-tetramethyl-1,3a-ethano-3aH-inden-6-yl)etanon	39.03	7.95
8	1,3,5-tri-tert-butyl-benzene	40.53	4.52
9	(3Z)-2-methyl-3-octen-2-ol	41.42	5.95
10	3,3,6-trimethyl-1,5-heptadien-4-ol	43.26	4.21
11	Artemisia ketone	43.67	4.98
12	Tetracosanoic acid	44.59	1.11
13	7,7-dimethyl-4-methylenebicyclo[4.1.0]-hept-3-yl-oxobutanoate	45.12	2.10
14	1-isopropenyl-3,3-dimethyl-5-(3-methyl-1-oxo-2-butenyl)cyclopentane	4.91	1.12
15	3β-acetyloxy-5 α-androstan-7-one	26.37	3.02
16	Propanoic acid	31.47	1.02
17	8,14-cedranoxide	36.08	1.01
18	9-dodecynyl-2-thienlaceate	36.3	4.03
19	Cholest-5-ene3,16,22,26-tetrol	37.23	3.03
20	lumiprogestrone	38.25	2.42
21	6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8a-hexahydro-7-methyl-methylene-2H-cyclohepta[b]furan-2-one	41.26	2.83
22	2-(2-bromoethyl)-3-methyl)-oxirane	41.45	0.61
23	2,6-di-t-butyl-4-methylphenyl ester	46.95	0.45
Total		-	84.75

[a]Retention time (min)

noic acid (7.01%), phytol (6.21%), naptho[2.3-b]furan-2-(3H)-on (6.21%), 1-(1,2,3,4,7,7a-hexahydro-1,4,4,5-tetramethyl-1,3a-ethano-3aH-inden-6-yl) etanon (7.95%), 1,3,5-tri-tert-butyl-benzene (4.52%), (3Z)-2-methyl-3-octen-2-ol (5.95%), 3,3,6-trimethyl-1,5-heptadien-4-ol (4.21%), artemisia ketone (4.98%) and 9-dodecynyl-2-thienlaceate (4.03%).

DISCUSSION AND CONCLUSIONS

Antimicrobial activity of *C. minima* crude extract was evaluated against seven bacterial species, which are known to cause infections in humans. As summarised in Table 1, the extract exhibited antimicrobial activity against all the microorganisms tested. This antimicrobial result is in concordance with other studies. Robin & Towers (1998) also found the extract of *C. minima* to be active against *Staphylococcus aureus*.

Most of the studies on the mechanism of active compounds focused on their effects on cellular membranes. Active compounds of *C. minima* might attack the cell wall and cell membrane, thereby destroying its permeability barrier and causing the release of intracellular constituents (ribose, Na glutamate, etc.) (Souza *et al.,* 2004). They might also interfere with membrane function e.g. electron transport, nutrient uptake, protein and nucleic acid synthesis and enzyme activity (Shunying *et al*., 2005; Freiberg *et al*., 2006; Zhang, White & Rock, 2006). Hence, crude extract of *C. minima* might have one of the above mechanisms to attack the bacterial cell tested and exhibit the antimicrobial activity.

The antimicrobial properties of *C. minima* are suspected to be associated with the artemisia ketone, which was detected by the GC–MS analysis in this study (Table 2). Artemisia ketone has been tested previously and was reported to have a significant antimicrobial activity (Setzer *et al*., 2004; Juteau *et al.,* 2002). It is possible that this compound was mainly responsible for the observed antibacterial effects in this study. The result on brine shrimps assays indicate that the crude extract has LC_{50} value greater than 1 mg/ml (Table 3); the recommended cutoff point for detecting cytotoxic activity (Simionatto *et al*., 2005). This suggests that this plant is not toxic to humans. The results of the present study agree with the use of this plant in traditional medicine.

The results detected in this *in vitro* study provide further evidence that the plant is potentially a rich source of antimicrobial agent against many microorganisms especially *Staphylococcus aureus* and *Klebsiella pneumonia*. Hence, the extracts of *C. minima* may be useful as an alternative anti-infective agent in natural medicine for the treatment of many infectious diseases.

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