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Antibacterial and Antifungal Properties of *persicaria odorata* Leaf Against Pathogenic Bacteria and Fungi

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Abstract: *Persicaria odorata* is a common plant and well known locally as "daun kesum" that is commonly used in cuisines and has various medicinal properties. This study was conducted to investigate the antimicrobial activity and the chemical constituent of the most active plant extract. The leaves were extracted using n-hexane, dichloromethane, methanol and water to produce the respective extracts. All extracts were tested against eight bacterial strains which included gram positive and gram negative bacteria and one fungal strain using disc diffusion method. In this research erythromycin 15 µg, vancomycin 30 µg and gentamicin 10 µg were used as the antibacterial standard whereas miconazole 50 µg were used as the antifungal. The antimicrobial activity of the active extract was evaluated quantitatively using broth microdilution assay. Gas Chromatography-Mass (GC-MS) Spectroscopy analysis was used to analyze the chemical constituent of the most active extract. N-hexane extract was found to be the most active extract against *S. aureus* (29.3±0.57), *S. epidermidis* (32.6±1.52), *S. pneumonia* (11.3±1.52) and *S. pyogenes* (15.6±1.15). However, all the extracts were inactive against fungi. The extract produced minimum inhibitory concentration (MIC) of 100 mg/ml against *S. aureus* and 50mg/ml each against *S. epidermidis*, *S. pneumonia* and *S. pyogenes*. Decanal, caryophyllene, dodecanal were the major constituents of the n-hexane extract, found by the GCMS analysis. The results obtained in this study showed that *P. odorata* leaves have high potential to be used as natural antibacterial agent against some bacterial infections.

Keywords: Persicaria odorata, Antibacterial activities, physicochemical properties.

INTRODUCTION

Complementary and alternative medicine (CAM) has become more crucial in medicine because it is proven to be safe, natural and effective when treating diseases. CAM has been used widely in general hospitals to overcome the medical problems such as infections, complications as well as to maintain the patient's health [1]. Research on CAM has been established and the Food and Drug Administration (FDA) have approved several herbs for medical indications [2].

Medicinal plants are considered an important element in various traditional systems of medication such as Traditional Chinese Medicine and Ayuverdic. China used 30% to 50% of total medicinal consumption by the use of traditional herbal medicine among its people. On the other hand, almost 60% African countries such as Zambia, Mali and Nigeria use herbal medicine at home to treat the high fever caused by Malaria among the children [3].

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One of the potential local plants that can be looked into is *P. odorata*. *P. odorata* leaves is one of natural plant that has been traditionally used in worldwide in medicine, cuisines, pharmacy and cosmetics. *P. odorata* can be classified under family of polygonaceae and genus of Persicaria. This plant grows in tropical and subtropical zones, which are warm and damp areas. In stable condition, they can grow up to 15 to 30 cm. Its leaf is dark green and the stem jointed off each leaf. *P. odorata* belongs to a group of fresh culinary herbs, known as the cilantro and mimics the 'cilantro' flavor.

There are many common names for this plant in different countries. English name include Vietnamese cilantro, Vietnamese mint and Vietnamese coriander. However, in Vietnam it is called as "rau ram". While in Malaysia, Brunei and Singapore known as "daun kesom" [4]. *P. odorata* leaves usually used as a flavouring in culinary and it also used as additional flavour and garnish to curries and hot soups.

This plant contains aldehyde and terpene, part of its essential oil [5] that has positive antibacterial activity [6, 7]. Previous studies have shown that different method of extractions may influence the chemical and constituent properties of the plant [8]. This study was aimed to

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Table 1. Selected Microorganism

Selected Microorganism	Group Types:
Bacteria *Staphylococcus aureus* (ATCC 25923)	+
Staphylococcus epidermidis (ATCC 12228)	+
Streptococcus pneumoniae (ATCC 49619)	+
Streptococcus pyogenes (ATCC 19615)	+
Escherichia coli (ATCC 25922)	-
Klebsiella pneumoniae (ATCC 700603)	-
Salmonella typhi (ATCC 14028)	-
Pseudomonas aeruginosa (ATCC 27853)	-
Fungi *Candida albicans* (ATCC 10231)	Yeast

Table 2. Reference Antibiotic and Antifungal

Antibiotics and Antifungal	Concentration (µg) used
Antibiotic Vancomycin	30
Erythromycin	15
Gentamicin	10
Antifungal Miconazole	50

Ref: NCCLS Vol.24 No.1 2004

determine the antibacterial as well as antifungal activities of different method of extractions. The constituent of the most active extract was also evaluated.

MATERIALS AND METHODS

Plant Materials

A 10 kg sample of *P. odorata* leaves was collected from Taman Pertanian Jubli Perak Indera Mahkota, Kuantan Pahang, Malaysia in Jun 2012. A taxonomist identified the plant and the identification voucher specimen was numbered as PIIUM 264. The leaves were washed to remove the foreign substances and oven dried at 40°C for 24 hours to remove the water content.

Extractions

7 kg of fresh leaves were collected and were oven dried for 24 hours. The dry leaves were crushed and grinded using a blender to get the powder. The powders of *P. odorata* were extracted in a round bottom flask in solvent [n-hexane (non-polar), dichloromethane (semi-polar), methanol & water (polar)] at room temperature. After two days, the extracts were filtrated through filter paper No. 2 and dried using rotary evaporator. The final extractions were kept in a refrigerator at 4°C for antibacterial screening and GC-MS analysis.

Microorganisms and Media

The American Type Culture Collection (ATCC) as well as wild type strains from the clinical isolates were used in this study (Table **1**). Media that was used were Blood Agar (BA), Mueller-Hinton Agar (MHA) and Mueller-Hinton Broth (MHB).

Antibiotics and Antifungal

Reference antibiotics and antifungal from National Clinical Control and Laboratory Standard (NCCLS) were used in study was listed in Table **2** that was obtained from Oxoid Ltd England.

Antimicrobial Screening

The antimicrobial screening was performed using disk diffusion method. The agar plates were divided into four regions and labeled with different concentrations. The fungi and bacteria were cultured by spreading evenly a full loop of bacteria and fungi. Then, disks were loaded with *Persicaria odorata* extract and were placed on the plate at an equidistant position. The plates were incubated at 37°C for 24 hours, the sensitivity of the microbes was determined by measuring the diameter (mm) of zone of inhibition [9]. Standard antibiotic and antifungal were used for control.

Minimum Inhibition Concentration (MIC)

MIC value was carried out in microdilution assay. Plants extracts dissolved in 0.25% (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) were first diluted to obtain 100 µl/ml concentration and a serial two-fold dilutions was done. The lowest concentration of each extract that exhibited inhibition was taken as the MIC levels. Erythromycin and miconazole were used for antibiotic control.

Gas Chromatography-Mass Spectrometry Analysis (GC-MS)

GCMS analysis was done only on the most active extract which was n-hexane. An aliquot of 1 µl of n-hexane extract (dissolved in n-hexane) was adjusted to 100 ppm after that it was injected into GC-MS Aligent system. Fitted with 30m x 0.25mm id x 0.25µm film thickness, HP-5ms capillary column. Carrier gas was helium at flow rate 0.57ml/min. Both injector and detector temperature were 250 ºC. The running methods were splitless mode, pressure 3psi, oven temperature 70 ºC to 140 ºC at the rate of 10 ºC /min, and then up to 240 ºC at the rate of 5ºC/min.

RESULT AND DISCUSSION

Antimicrobial Screening

The result of antibacterial screening showed strong antibacterial activities. Crude extract were tested for susceptibility at four different concentrations, which were 400, 200, 100 and 50 mg/ml of n-hexane, Dichloromethane (DCM), methanol and aqueous crude extracts using disc diffusion method. The bacterial strains that were susceptible to n-hexane extract were *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumonia* and *Streptococcus pyogenes*. The most susceptible bacteria for DCM extraction were *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes* and *Salmonella typhi*. *Salmonella typhi* was only susceptible to DCM extract 400 mg/ml concentration while negative results were obtained for the other concentrations. The methanol

Table 3. The Result of Antibacterial Screening

Type of extract	[ ] (mg/ml)	Zone of Inhibition in mm									
		S.aureus		S.epidermidis		S.pneumonia		S.pyogenes		S.typhi	
		ATCC	Wild	ATCC	Wild	ATCC	Wild	ATCC	Wild	ATCC	Wild
n-Hexane	400	29.33±0.58	27.33±1.15	32.67±1.53	32.67±4.62	11.33±1.53	9.33±2.31	15.67±1.15	16.67±1.15	-	-
	200	21.00±1.00	20.67±3.06	29.67± 0.58	28.67±1.15	8.00±1.00	6.00±2.00	11.00±1.00	10.67±2.31	-	-
	100	18.00±1.00	18.00±2.00	23.67±1.53	20.00±2.00	4.67±0.58	4.67±1.15	7.67±0.57	7.33±1.15	-	-
	50	14.33±1.53	13.33±2.31	20.00±1.00	16.67±2.31	0.67±1.15	1.33±1.15	7.00±1.00	4.67±1.15	-	-
DCM	400	13.00±1.00	13.33±2.31	13.33±2.08	12.00±2.00	-	-	12.33±1.52	13.33±1.15	20.00±1.00	18.00±2.00
	200	9.00±0.00	11.33±2.31	9.00±1.00	9.33±1.15	-	-	11.00±1.00	9.33±1.15	-	-
	100	8.33±1.53	9.33±1.15	8.33±1.15	8.67±2.31	-	-	6.00±1.00	5.33±1.15	-	-
	50	7.33±0.58	7.33±1.15	4.33±1.15	6.67±1.15	-	-	3.67±0.58	4.00±2.00	-	-
MeOH	400	15.33±0.58	14.00±2.00	19.00±1.00	16.67±1.15	-	-	-	-	-	-
	200	11.00±1.00	13.33±1.15	18.00±1.00	11.33±2.31	-	-	-	-	-	-
	100	10.33±1.53	10.00±2.00	9.00±1.00	8.00±2.00	-	-	-	-	-	-
	50	8.33±1.53	7.33±2.31	8.00±1.73	7.33±2.31	-	-	-	-	-	-
Aqueous	400	10.00±2.00	9.33±2.52	-	-	16.00±2.00	16.67±4.16	14.33±1.52	15.67±0.58	-	-
	200	9.33±1.15	8.33±0.58	-	-	15.33±2.31	12.00±1.00	11.00±1.00	11.67±0.58	-	-
	100	8.00±2.00	5.67±1.15	-	-	12.00±1.00	11.00±1.00	9.33±1.15	10.00±0.00	-	-
	50	4.00±0.00	18.00±1.00	-	-	9.00±1.00	8.67±0.58	8.00±0.00	9.00±0.00	-	-

Value are represented as Mean ± SD
Key:
(-), No activity.

Table 4. Minimum Inhibitory Concentration (mg/ml) of *Persicaria odorata* Extract

Extraction method	*S.aureus*	*S.epidermidis*	*S.pneumonia*	*S. pyogenes*
n- Hexane	100	50	50	50
DCM	100	100	12.5	12.5
Methanol	50	50	12.5	3.125
Erythromycin as Control antibiotics	25	12.5	12.5	12.5

extract was only effective against two bacteria which were *Staphylococcus aureus* and *Staphylococcus epidermidis*. The result is shown in Table **3**. Finally, inhibition zones were also observed on *S.aureus, S.pneumonia* and *S.pyogenes* cultures when tested against aqueous extract. However, none of the extracts were active against *Candida albicans*.

Minimum Inhibitory Concentration (MIC) Test

Extraction of *Persicaria odorata* using methanol exhibited the most significant antimicrobial activities as compared to DCM and n-hexane extracts with MICs of 3.125 mg/ml and 12.5 mg/ml for *S.pyogenes* and *S.pneumonia* respectively. While, n-hexane extract showed 100 mg/ml for *S.aureus* and 50 mg/ml for the others. MICs for DCM extracts showed 100 mg/ml for *S.aureus* and *S.epidermidis*. The result is shown in Table **4**.

GC-MS Result

There were 28 major volatile compounds from n-hexane extract of *P. odorata* leaves were isolated. However, only eight active compounds were selected because the quantity of the compound was highest in percentage as shown in the Table **5**. The major volatile compounds from n-hexane extract were dodecanal (27.11%), decanal (4.11%), α-citral

Table 5. Constituent of the n-Hexane Extract of *Persicaria odorata* Leaves.

Peak No.	Volatile Compound	RT	Area Pct	Composition (%)
1	Dodecanal	9.9714	31.9069	27.11
2	Decanal	6.8661	4.84	4.11
3	α-Citral	7.787	2.9779	2.53
4	Drimenol	17.0242	2.0323	1.73
5	Z-Citral	7.3883	2.0127	1.71
6	Caryophyllene	10.3195	1.4721	1.25
7	Euparone	14.531	1.2395	1.00
8	2,4-Heptadiene,2,6-Dimethyl	23.3807	0.9833	1.03

(2.53%), drimenol (1.73%), Z-citral (1.71%), caryophyllene (1.25%), euporone (1.00%), and 2,4-heptadiene,2,6-dimethyl (1.03%). The result were shown Table **5**.

CONCLUSION

In conclusion, *P. odorata* possessed strong antibacterial activity with significant amounts of active compounds in its leaves. Therefore, using *P. odorata* leaves may have beneficial health effect especially for bacterial infections.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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