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***Original research article***

# Anti-Rice Pathogenic Microbial Activity of *Persicaria* sp. Extracts

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**ABSTRACT**

The dichloromethane and methanol extracts, and the essential oil of *Persicaria* sp. were subjected to *in vitro* anti-rice pathogenic microbial activity tests. The essential oil displayed the most potential antimicrobial activity. GC-MS analysis revealed thirteen main compounds as dodecanal (54%), decanal (15%), *trans*-caryophyllene (8%), cyclododecane (7%) and α-humulene (5%). Strong antimicrobial activities of the oil and dodecanal were found against *Rhizoctonia solani* ($IC_{50}$ of 0.066 and 0.851 mg/mL) and *Xanthomonas oryzae* pv. *oryzicola* (MIC/MBC of 0.78/12.50 and 0.78/25.00 mg/mL), and potent activities against *Bipolaris oryzae* ($IC_{50}$ of 3.047 and 3.341 mg/mL) and *X. oryzae* pv. *oryzae* (MIC/MBC of 3.12/12.50 and 3.12/25.00 mg/mL). In terms of structure-activity relationship, 2-dodecanone and 2-dodecanol displayed significantly anti-fungal activity, while 1- and 2-dodecanols expressed potent anti-bacterial activity. The essential oil might be used for new microcides controlling rice pathogenic bacteria and fungi.

**Keywords:** Antimicrobial activity; Essential oil; *Persicaria* sp.; Rice pathogens; Structure activity relationship

## 1. Introduction

The antimicrobial activities of the essential oils and the extracts from various plants have long been utilized and explored towards the discovery of innovative antimicrobial compounds to control pathogenic microorganisms. The essential oil and the extracts from a Thai cultivar of the edible vegetable *Persicaria odorata* (Lour) Sojak (synonym: *Polygonum odoratum* Lour; Vietnamese coriander) and *P. pulchra* (Blume) Sojak (synonym: *Polygonum tomentosum* Lour) were chosen for this study. There were main reasons of our study choosing the plants for the experiment on antimicrobial activity. Some of them were the

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major constituents of the essential oils from *P. odorata* in cultivars from Vietnam, Singapore, Australia and Thailand reported to be decanal, dodecanal, 1-decanol, β-caryophyllene and caryophyllene oxide [1-9]. The antimicrobial activity of the oil was mostly affected on bacteria such as *Salmonella choleraesuis* subsp. c*holeraesuis*, *S. typhi*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae*, *S. pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* [5-7, 9-12]. Meanwhile, the phytochemicals of the whole plant extracts from *P. pulchra* were reported as glycosides, tannins, flavonoids, saponins, sterols, terpenes and alkaloids that could only combat bacteria including multi-drug resistant *Klebsiella* sp. [13, 14]. Therefore, the extracts from the plants should be able to control the growth of rice diseases.

Thailand was previously the largest rice exporting country globally and has been remained in the top three countries. Although rice export is performed entirely by a cartel of private companies, the government subsidizes rice and provides reduced control over rice exports because of the considerable role that rice plays in the welfare of a large sector of the country's poorest people [15]. Nevertheless, rice production and quality absolutely depended on the environmental factors including weeds, soil problems, environmental stresses, insect pests, biological agents, climate changes, relative humidity, rainfall, leaf wetness effects and pathogen have been reported [16-20]. All factors are thus likely that rice yields in Thailand shall be enlarging speedily. From the factors influencing rice production, the most crucial factor is rice pathogens caused by vires, nematodes, bacteria and fungi [21, 22]. Those induced by fungi including sheath blight (*Rhizoctonia solani*) and brown spot (*Bipolaris oryzae*); concurrently, bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*, *Xoo*) and bacterial leaf streak (*Xanthomonas oryzae* pv. *oryzicola*, *Xoc*) have been the bacterial diseases recorded [23]. Rice farmers usually used fungicides and bactericides to suppress rice diseases. Although the chemicals successfully control rice pathogenic microorganisms, those also harm to human and animal health, and environment. Therefore, it is a great need to search for sustainable approaches, particularly natural agents from plants to control rice pathogenic bacteria and fungi. Many studies have described that plant essential oil and extracts provided the proper answer for this posed problem.

In this work, the antimicrobial activities of the essential oil and the extracts from two Thai cultivars of *P. odorata* and *P. pulchra* were evaluated against two fungal species (*R. solani* and *B. oryzae*) and two pathovars of the bacterial species *X. oryzae* (*Xoc* and *Xoo*) that are significant rice pathogens in Thailand. This is also the first report to indicate the ability of the extracts to decrease rice pathogenic microorganisms.

## 2. Materials and Methods

### 2.1 Plant materials

The whole plants of *P. odorata* were purchased from Pak Klong Talad Market, Bangkok, Thailand in 2014. The plant was labeled, identified and kept at the herbarium of Kasin Suvathabandhu Herbarium, Faculty of Science, Chulalongkorn University (BCU-Herbarium: BCU 016034, Collector number: Pragatsawat Chanprapai 1). *P. pulchra* was collected from Phranakorn Sri Ayutthaya province in 2014. The plant was also identified by the herbarium of Kasin Suvathabandhu Herbarium.

### 2.2 Rice pathogens

Rice pathogenic fungi (*R. solani*, DOAC1406 and *B. oryzae*, DOAC1760) and bacteria (*Xoo*, TB0006 and *Xoc*, TS8203) were supplied and purchased from the Plant Pathology Research Group, Plant Protection Research and Development Office, Bangkok, Thailand. The fungal stock cultures were maintained on potato dextrose agar (PDA) at room temperature (25–32$^{o}$C), while the

bacteria were maintained on nutrient agar (NA) at 28ºC.

### 2.3 Compounds

Dodecanal (≥95%, main component), 1-dodecanol (reagent grade), 1-dodecene (≥99%) and 2-dodecanol (99%) were purchased from Fluka-Chemika Co (Steinheim, Switzerland). 2-Dodecanone (≥97%) and 1,2-dodecene oxide (90%) were obtained from Sigma-Aldrich, respectively. Nativo 750 WG as the positive control was procured from Bayer Co., Bangkok, Thailand.

### 2.4 Solvent extraction

Ten kilograms of the whole plants of *P. odorata* and *P. pulchra* were air dried, milled to fine powder and extracted with dichloromethane ($CH_2Cl_2$) and then methanol ($CH_3OH$) by Soxhlet extraction for three times. Each extract was filtered, evaporated and kept at room temperature for antimicrobial activities. The percentage yield of extracts was presented in Table 1.

### 2.5 Hydrodistillation

The essential oil was derived as reported [24]. Briefly, the fresh whole plants of *P. odorata* (1.2 kg) was cut into small pieces and hydrodistilled using a Dean-Stark apparatus for 3 h [25-27]. The distillate was dried over anhydrous sodium sulfate and concentrated using rotatory evaporator to leave the essential oil. The pale yellow essential oil was obtained (0.03% yield (w/w), Table 1). The oil was protected from oxidation and photo-degradation of light by bottle wrapping with aluminum foil and stored at 4ºC until further used.

**Table 1.** Percentage yield of the extracts from *Persicaria* sp.

| Plants | % Yield (w/w) | | |
|---|---|---|---|
| | $CH_2Cl_2$ | $CH_3OH$ | Essential oil |
| *P. odorata* | 1.67 | 10.33 | 0.03 |
| *P. pulchra* | 1.19 | 7.13 | - |

**Note:** - means not extraction

### 2.6 Antibacterial assays

The agar diffusion method, modified from Barry and Thornsberry [28], was used to screen for the antibacterial activity of plant essential oil and its principal components. The two bacterial pathovars (*X. oryzae*, *Xoo* and *Xoc*) were cultivated in NA at 37ºC for 18–24 h. Then four or five single colonies of each tested bacteria were selected and cultivated in nutrient broth (NB) at 37ºC for 2–5 h. These bacterial suspensions in NB were then standardized to a cell density of $1.5x10^8$ colony forming units (CFU)/mL (McFarland No. 0.5) using sterile 0.85% (w/v) NaCl solution. The adjusted bacterial suspension (1 mL) was then mixed into NA media (19 mL) and poured into Petri plates and allowed to set. Wells of 6 mm diameter were created with a cork border and 40 µL of the dissolved extract in DMSO (or DMSO only for the solvent control) was added into these wells (in duplicate plates) and incubated at 37ºC overnight. The bacterial lawn (opaque) was then observed and the diameter of the zones of inhibition (clear zone) was measured.

The minimum inhibitory concentration (MIC) was measured using the macro-dilution broth susceptibility assay. Stock solutions of the test essential oil and each of the principal components were prepared in DMSO and then ten-fold serial diluted from 100 mg/mL to 0.08 mg/mL with sterile NB. Each serial dilution (1 mL) was pipetted with 1 mL of test bacteria to a concentration of $1.5x10^8$ CFU/mL and incubated at 37ºC for 24 h. The lowest concentration of the tested compound that showed no visible bacterial growth was taken as the MIC and the mean MIC value was calculated from the duplicate replications. In addition, 100 µL of the test media from each MIC broth was then spread over NA plates and incubated at 37ºC for 24 h. The prevention of the visible of bacterial growth at the lowest concentration of each sample (duplication) on top of agar plates was determined as the minimum bactericidal concentration (MBC) [29].

The MIC index (MBC/MIC) was calculated for effective extract and its major constituent to determine whether an extract

and the constituent is bactericidal (MBC/MIC<4) or bacteriostatic (4<MBC/MIC<32) on growth of bacterial organisms [30].

### 2.7 Anti-fungal activity assay

The essential oil and all tested compounds were dissolved in DMSO and then 100 µL was added to PDA to give a final concentration of 1,000 mg/L for the essential oil and the extracts and 1 mmol/L for the pure constituent. A 5-mm diameter agar disc containing mycelia was transferred to the center of the PDA plate containing the extract or compounds, and the plates were then incubated at 25ºC for 3 d (*R. solani*) or 8 d (*B. oryzae*). Nativo 750WG (250 g/kg trifloxystrobin and 500 g/kg tebuconazole) was used as a positive control and 1% v/v DMSO as the solvent negative control. When the mycelium reached the edge of the Petri dish in the solvent control plates, the mycelium growth on all the other plates were measured and the antifungal indices were calculated as the percentage of mycelia growth inhibition = 100[(C–T)/C] with triplications, where C and T are the colony diameter (cm) of the control and test plate, respectively. Inhibition was expressed as $IC_{50}$ value, which is the concentration of the compound to inhibit the measured function by half [31].

### 2.8 Sclerotia and spore germination

The effect of the essential oil on the sclerotia germination of *R. solani* was examined. Batches of ten sclerotia were placed on each of three replicate PDA plates (9 cm diameter) and incubated for 72 h at 25ºC. The germination of sclerotia was then scored by viewing the hyphae under a stereo binocular microscope at x45 magnification. A sclerotium was considered to have germinated when the outgrowing hyphae were equal to or greater than the diameter of the sclerotium [32].

The spores of *B. oryzae* were prepared by cultivation of mycelium on PDA for 14 d or until the highest yield of spores were obtained. After that, 9 mL of sterile water was added on to the mycelia-filled agar, scraped by needle and harvested. The spore concentration in the suspension was then adjusted to approximately $1.5x10^8$ CFU/mL using 0.5 McFaland standards. Batches of 0.1 mL spore suspension were placed on triplicate PDA plates (9 cm diameter) mixing with the tested compounds and incubated at 25ºC for 5 d. The scoring spore germination inhibition was calculated following as % spore germination inhibition = 100[(C – T)/C], where C and T are the colony diameter (cm) of the control and test plate, respectively.

### 2.9 GC-MS analysis

GC-MS analysis was performed using an Agilent 6,890 gas chromatograph in electron impact (70 eV) mode coupled to an HP 5,973 mass selective detector and fitted with a fused silica capillary column (HP-5MS; 30 m x 0.25 mm x 0.25 µm film thickness). Helium (1.0 mL/min) was used as the carrier gas. Samples were injected in the split less mode at a ratio of 1:10–1:100. The injector was kept at 250ºC and the transfer line at 280ºC. The mass spectra were operated in the EM mode at 1,576.5 EM voltage, in the *m/z* range 50–550. The identification of the compounds was performed by comparing the mass spectra with those found in the literature and the Wiley 7n GC/MS libraries.

### 2.10 Statistical analysis

All data were analyzed using the SPSS for windows version 20 (SPSS Inc: Chicago, IL, USA). Comparison of means was performed using Duncan's Multiple Range Test (DMRT) and significance was taken at the $p$ value <0.05 level. The experiment was designed as a general linear model within a completely randomized design with duplications for bacteria and triplication for fungi.

**Table 2.** Antimicrobial activity from *Persicaria* sp. extracts

| Plants | Extracts | Mean of % mycelia growth inhibition[*,***] | | Mean of Clear zones[**,***] | |
|---|---|---|---|---|---|
| | | ***R. solani*** | ***B. oryzae*** | ***Xoo*** | ***Xoc*** |
| ***P. odorata*** | $CH_2Cl_2$ | 19.63±1.95[c] | 21.48±1.16[b] | 0.23±0.04[b] | 0.30±0.01[c] |
| | $CH_3OH$ | 0.00[e] | 0.00[c] | 0.15±0.02[b] | 0.15±0.03[c] |
| | Essential Oil | 77.00±0.87[a] | 59.63±0.32[a] | 0.72±0.10[a] | 1.17±0.35[a] |
| ***P. pulchra*** | $CH_2Cl_2$ | 36.11±2.00[b] | 18.33±7.71[b] | 0.00[c] | 0.00[d] |
| | $CH_3OH$ | 17.96±10.43[cd] | 18.89±6.19[b] | 0.23±0.02[b] | 0.50±0.01[bc] |

**Note:** [*]Values were determined by: (control – treatment)/control x 100%, and represent the mean values of triplicates (± SD) at 1,000 mg/L of each fungi. [**]Values, an average of the mean inhibition zones (cm) ± standard deviation (SD) of duplicates of the extracts at 10,000 mg/L of each bacteria. [***]Mean values within a column followed by a different letter are significantly different ($p$<0.05; DMRT).

## 3. Results and Discussion

### 3.1 Antimicrobial activity

The essential oil from *P. odorata* was found to have moderate antifungal activity against *R. solani* and *B. oryzae*, with 77.00±0.87% and 59.63±0.32% mycelia growth inhibition at 1,000 mg/L. The percentage of mycelia inhibition was calculated as presented in Table 2. Similarly, the essential oil displayed significantly antibacterial activity. The diameter of the inhibition zone was 0.72±0.10 cm and 1.17±0.35 cm for *Xoo* and *Xoc*, respectively, at an oil concentration of 10,000 mg/L. This study examined the antimicrobial activity from the essential oil of *P. odorata* for controlling four rice pathogenic diseases. However, the extracts from both plant species revealed resemble antimicrobial activity on the rice pathogenic fungi and bacteria. According to preliminary screening, the essential oil displayed significantly the highest potential antimicrobial activity. The study indicated that the hydrophobic components in the essential oil had an influence in the growth of tested rice pathogenic microorganisms.

Interestingly, the antimicrobial activity of the extracts from *P. odorata* and *P. pulchra* was closed to proximity, even if both of *Persicaria* sp. were different. According to previous reports [5-7, 9-11], the extracts from these two species were confirmed to reveal antimicrobial activity. Therefore, the essential oil from *P. odorata* was suitable for further studies.

### 3.2 Chemical composition of the essential oil

The GC-MS analysis revealed 13 principal constituents of this essential oil extract. The major constituent was dodecanal (54%), followed by six other main components (15 to 2% molar composition) of decanal, *trans*-caryophyllene, cyclododecane, α-humulene, cyclodecane and β-eoclovene (Table 3). The main compound of the essential oil from Vietnamese coriander were a long chain aliphatic aldehyde-dodecanal. The molar proportion of dodecanal was higher than that previously reported in *P. odorata* from Australia (44.1%), Vietnam (11.4%) and another Thai place as Thungkru district of Bangkok (19.9-27.5%) [1-5, 7].

**Table 3.** Chemical composition of essential oil from *P. odorata* Lour.

| Rt[*] | Components | %[**] | Chemical formula |
|---|---|---|---|
| 12.48 | Decanal | 15 | $C_{10}H_{20}O$ |
| 13.45 | 1-Decanol | 3 | $C_{10}H_{22}O$ |
| 13.97 | Undecanal | 1 | $C_{10}H_{22}O$ |
| 15.39 | Dodecanal | 54 | $C_{12}H_{24}O$ |
| 15.67 | *trans*-Caryophyllene | 8 | $C_{15}H_{24}$ |
| 16.12 | α-Humulene | 5 | $C_{15}H_{24}O$ |
| 16.18 | Cyclododecane | 7 | $C_{15}H_{24}$ |
| 16.38 | α-Curcumene | 1 | $C_{15}H_{22}$ |
| 17.14 | α-Cedrene | 1 | $C_{15}H_{24}$ |
| 17.33 | (*E*)-Farnesene | 1 | $C_{15}H_{24}$ |

| | | | |
|---|---|---|---|
| 17.74 | β-Neoclovene | 2 | $C_{15}H_{24}$ |
| 18.40 | Euparone | 1 | $C_{12}H_{10}O_4$ |
| 19.80 | Drimenol | 1 | $C_{15}H_{26}O$ |
| | Yield (%) | 100 | |

Note: *Rt = Retention time (minutes)
**% content in terms of the peak area relative to the total peak area (molar composition)

### 3.3 Antimicrobial assays

The essential oil displayed high antibacterial activity against *Xoo* and *Xoc* with MIC values of 3.12 and 0.78 mg/mL, respectively, and MBC values of 12.50 mg/mL for both pathovars. The MIC indices of *Xoo* and *Xoc* were 4.0 and 16.0. The antifungal activity of the essential oil appeared somewhat lower, with an $IC_{50}$value of 0.066 and 3.047 mg/mL against *R. solani* and *B. oryzae*, respectively, in the mycelia plate assay. The level of inhibition of sclerotia germination in *R. solani* was 75.02±7.11% at 2,000 mg/L and of spore germination in *B. oryzae* was 91.52±6.32% at 1,000 mg/L. These results were similar to the essential oils from *Cymbopogon parkeri*, *Origanum minutiflorum*, *Satureja cuneifolia*, *Thymbra spicata*, *Orthosiphon stamineus*, *Calocedrus macrolepis* and its main constituents T-muurolol and α-cadino, *Syzygium aromaticum*, *Ocimum gratissimum* and *Myristica fragrans* inhibited the mycelia growth of the pathogenic fungi *R. solani*, *Pyricularia oryzae* and *Fusarium oxysporum*, with a half maximal inhibition ($IC_{50}$) of 39.8, 72.0 and 43.6 μL/L, respectively [33-36]. The inhibition of *B. oryzae* mycelium growth and spore germination of the oil revealed better antifungal activity than the essential oils from *O. basilicum*, *Citrus hystrix*, *Melaleuca alternifolia* and *O. gratissimum* [37-39]. In addition, the current study displayed similarly the potential antibacterial activity *in vitro* against the rice pathogenic *X. oryzae* isolates *Xoc* and *Xoo* the essential oils from *Spiraea alpine*, *O. gratissimum*, *Thymus vulgaris*, *Metasequoia glyptostroboides* and *Piper sarmentosum* [40-43].

The MIC index (MBC/MIC ratio) has been used to determine if a substance possess bactericidal (MIC index≤4) or bacteriostatic (MIC index>4) properties. With MIC ratios of 4.0 and 16.0 for *Xoo* and *Xoc*, respectively, the essential oil likely acted principally as a bactericidal agent that could actively kill bacterial cells, perhaps as a cell wall synthesis inhibitors, but may well also have a partial bacteriostatic mode of action [44]. The MIC indices of the essential oil and dodecanal on both bacteria were variation. Bactericidal agents are generally not toxic to non-bacteria and can be administered at high doses [45].

From the MIC ratio values of dodecanal, its apparent nature against *Xoc* and *Xoo* pathovars was bacteriostatic (data not shown), which is consistent with the reported MIC range in Gram-positive bacteria of 125–250 g/mL and in anti-wood decay fungi activities of 25–75 g/mL [28]. In addition, the mode of action of this bacteriostatic agent was reported to obstruct the genetic products of the bacterial nucleus [46]. Dodecanal showed low antifungal activity against *B. oryzae* with $IC_{50}$ value of 3.341 mg/mL, while it showed potent activity against *R. solani* with the $IC_{50}$ value of 0.851 mg/mL. Strong antibacterial activity against *Xoc* was observed with MIC and MBC values of 0.78 and 25 mg/mL, respectively, (MIC index of 32.1). On the other hand, less inhibition against *Xoo* with MIC and MBC values of 3.12 and 25 mg/mL, respectively, (MIC index of 8.0). Nevertheless, these results revealed potential antimicrobial activity against bacteria and filamentous fungi and were differed from the previous reports that affected only bacteria [5-7, 9-11].

**Table 4.** Antimicrobial activity of dodecanal and related compounds

| Compounds | Fungal growth assay (% Mycelia growth inhibition; Mean±SD)[*,***] | Agar diffusion assay (Clear zone in cm; Mean±SD)[**,***] | |
|---|---|---|---|
| | *R. solani* | *Xoo* | *Xoc* |
| Dodecanal | 56.94±0.74[e] | 0.23±0.04[c] | 0.45±0.21[b] |
| 1-Dodecanol | 70.37±1.16[d] | 0.35±0.07[b] | 0.48±0.04[b] |
| 1-Dodecene | 30.74±1.95[f] | 0.00[d] | 0.00[c] |
| 2-Dodecanone | 85.19±5.01[b] | 0.00[d] | 0.00[c] |
| 2-Dodecanol | 83.52±1.70[b] | 0.33±0.04[b] | 0.45±0.07[b] |
| 1,2-Dodecene oxide | 78.71±1.95[c] | 0.00[d] | 0.00[c] |
| Nativo 750 WG | 100.00[a] | 1.25±0.07[a] | 1.30±0.14[a] |
| DMSO | 0.00[g] | 0.00[d] | 0.00[c] |

**Note:** [*]Values were determined by: (control – treatment)/control x 100%, and represent the mean values of triplicates (± SD) at 1 mmol/L. [**]Values, an average ± standard deviation of duplicates (cm ± SD), of the mean inhibition zones of compounds at 1 mmol/L. [***]Mean values within a column followed by a different letter are significantly different ($p$<0.05; DMRT).

### 3.4 Structure activity relationship

Dodecanal, 1-dodecanol, 1-dodecene, 2-dodecanone, 2-dodecanol, and 1,2-dodecene oxide have been reported to exhibit antimicrobial activity with a different specificity and level of activity (Table 4). 2-Dodecanone and 2-dodecanol displayed a significant antifungal activity against *R. solani* at 1 mmol/L compared with Nativo 750WG and DMSO as the positive and negative controls, respectively. The highest zone of inhibition was observed from 1-dodecanol against *Xoo* (0.35 cm) and *Xoc* (0.48 cm), followed by 2-dodecanol and dodecanal, while 1,2-dodecene oxide, 2-dodecanone and 1-dodecene did not express any detectable growth inhibition. In addition, 1- and 2-dodecanols, and dodecanal gave no significant difference in activity ($p \geq 0.05$) for two *X. oryzae* pathovars (*Xoo* and *Xoc*) (Table 4). Polar functional groups (alcohol and ketone) were reported to be able to attach to the microbial cell wall and cell membrane and so perturb cell metabolism and growth. With respect to the hydroxyl group of alcohol, the second position (such as in 2-dodecanol) appeared to be more important in determining the antifungal activity than the first position (such as 1-dodecanol) [28,47].

## 4. Conclusion

The essential oil of *P. odorata* showed antimicrobial activity against representative rice pathogenic fungi (*B. oryzae* and *R. solani*) and bacteria (*X. oryzae* pathovars *Xoc* and *Xoo*). The major constituent, dodecanal, exhibited antimicrobial activity. Aldehyde revealed less antibacterial activities than alcohol. 2-Dodecanone and 2-dodecanol displayed better antifungal activity than the major constituent. The essential oil could be feasible to use as agrochemicals for suppressing rice pathogens in the future.

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