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Synthesis, Isolation of Phenazine Derivatives and Their Antimicrobial Activities

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ABSTRACT

Antimicrobial activity of natural phenazine-1-carboxylic acid (PCA) from *Pseudomonas aeruginosa* TISTR 781 and synthetic phenazine-5,10-dioxide (PDO), prepared by oxidation of the phenazine, were evaluated by *in vitro* disc diffusion and minimal inhibitory concentration (MIC) methods. The results indicated that both phenazine derivatives differed clearly in their antimicrobial activity. PCA showed better efficacy against growth of *Acidovorax avenae* subsp. *citrulli*, *Bacillus subtilis*, *Candida albicans, Escherichia coli* and *Xanthomonas campestris* pv. *vesicatoria* than PDO at low concentrations of PCA (MIC; 17.44 - 34.87 ppm) as an antimicrobial agent. In contrast, PDO acted as a stronger inhibitor than PCA when tested against *Pseudomonas syringae* and *Enterobacter aerogenes*. The last bacterial strain, *Ralstonia solanacearum*, can be suppressed by the same concentration of PCA and PDO (MIC; 62.50 ppm). The data provided beneficial information for choosing phenazine types to inhibit some general strains and plant pathogenic bacteria.

Keywords: Phenazine-1-carboxylic acid (PCA), antimicrobial activity, phenazine-5,10-dioxide (PDO), disc diffusion method, minimal inhibitory concentration (MIC) method

INTRODUCTION

Phenazine, a nitrogen-containing heterocyclic redox agent, is 1 group of antibiotic agents. More than 6,000 phenazine derivatives have been identified and described during the last 2 centuries. These compounds can be produced in 2 ways, namely biosynthesis *via* phenazine-producing bacteria and chemosynthesis. Firstly, phenazine compounds secreted by *Pseudomonas aeruginosa* are largely found as phenazine-1-carboxylic acid (PCA), pyocyanin, 1-hydroxyphenazine (1-HP), and phenazine-1-carboxamide (PCN), when incubated in suitable media [1-3]. Biosynthesis of phenazine is up regulated by nutrient depletion, by high cell density and by conversion of the bacterium to the biofilm form [4-7]. Chemical synthesis has also been used to prepare various synthetic phenazines [8]. One interesting procedure is oxidation of the phenazine core using oxidizing reagents such as H_2O_2, $H_2SO_4/K_2S_2O_8$, $HOF{\cdot}CH_3CN$, Tf_2O and $NH_2CONH_2{\cdot}H_2O_2$ [8-10]. This process gives N-oxide phenazines for instance, phenazine-5-oxide, phenazine-5,10-dioxide (PDO), etc. Nitrogen atoms and functional groups of the phenazine ring produced from both processes result in different physicochemical and biological properties of individual phenazine derivatives. From previous papers phenazine compounds are known to possess a broad-spectrum of antibiotic activity toward bacteria, fungi, and animal tissues [11-13]. Phenazine derivatives were also chosen to reduce the use of chemical pesticides in agriculture [14]. Biopesticides, phenazine compounds, can be used either alone or in combination with pesticides to lower the doses of chemicals needed to obtain a profitable crop yield. The production costs of new agrochemicals have increased and stricter safety rules on their use also require alternative pest control methods. Seed coating with biopesticides for wheat, potato, radish, sugar beet and fruits has been proved to result in crop protection and increased crop yields [15-17]. Therefore, this work is concerned with phenazine compounds and in particular natural PCA and synthetic PDO because both phenazines have well-known antibiotic properties and are potent antimicrobial agents. The antimicrobial activities of PCA produced by *Pseudomonas* spp., have been investigated and used to inhibit some bacterial and fungal growth [11,18,19]. Moreover, PDO plays a role as a disinfectant against various plant-pathogenic microorganisms [20]. This study focuses on the synthesis and efficacious antimicrobial activity of phenazine derivatives (PCA and PDO) against general strains (*Bacillus subtilis*, *Candida albicans*, *Enterobacter aerogenes* and *Escherichia coli*) and some plant pathogenic bacteria (*Acidovorax avenae* subsp. *citrulli*, *Erwinia carotovora*, *Pseudomonas syringae*, *Ralstonia solanacearum* and *Xanthomonas campestris* pv. *vesicatoria*). The results of this study may provide primary information for the selection of suitable phenazine types to restrain microbes in agricultural and pharmaceutical applications.

MATERIALS AND METHODS

Biosynthesis of Phenazine-1-Carboxylic Acid (PCA)

Pseudomonas aeruginosa TISTR 781 was obtained from the Thailand Institute of Scientific and Technological Research (TISTR) and used for PCA production. It was streaked on Luria-Bertani (LB) agar plates and incubated at room temperature for 24 - 48 h [21]. A single colony of *P. aeruginosa* on a LB agar plate was transferred into 100 ml of modified King's A broth (KA): Bactopeptone 15.0 g, NaCl 13.0 g, glycerol 9.0 ml and K_2SO_4 1.0 g in 1,000 ml distilled water; and incubated at room temperature (29 - 30 °C) with an orbital shaker (200 rpm) for 24 h to use as a starter in previous work [22]. For increasing PCA production, the starter was transferred into an Erlenmeyer flask (1,500 ml) containing fresh modified KA medium with 1:50 bacterial dilutions and incubated for 48 h under the same conditions as described above.

An Amberlite XAD-16 resin column was used for the PCA isolation by eluting this column with 70 % (v/v) acetonitrile in water [22]. For purification of phenazine, crude phenazine was attained in 2 steps. Firstly, the pH of the crude phenazine solution was adjusted to 2.5 and residues removed by centrifugation at 3,500 rpm for 15 min. After that, this solution was separated by a liquid-liquid extraction with dichloromethane. The extracted phenazine was then purified on a silica gel column, equilibrated with dichloromethane. The optimum solvent system for the silica gel column was 90 % (v/v) dichloromethane in ethyl acetate.

Chemical Synthesis of Phenazine-5,10-Dioxide (PDO)

A mixture of glacial acetic acid (8.5 ml) and hydrogen peroxide (30 %, 3.8 ml) was heated for 6 h at 40 °C. Phenazine (~0.1 g) was added and the reaction mixture was heated for 22 h at 50 °C. This method was described by Abd EI-Halim and co-workers [23] under modified conditions. After that the obtained precipitate was filtered and then extracted with dichloromethane. The concentrated dichloromethane extract was poured into a silica gel column and eluted by a mixture of solvents (80:20 v/v; dichloromethane:ethyl acetate).

Identification of Phenazine Compounds

UV–vis spectrum of both phenazine compounds were recorded by an Agilent 8453 UV-visible spectrophotometer in the region of 200 - 500 nm when dissolved in 0.2 M HCl and dichloromethane. IR spectra of the phenazines, as KBr discs, were recorded on a Spectrum One FT-IR spectrometer, Perkin Elmer (Germany) from 4,000 - 500 cm^{-1} [18]. Nuclear magnetic resonance spectra (both ^{1}H and ^{13}C NMR) were recorded with samples dissolved in $CDCl_3$ on a Varian Mercury Plus 400 MHz or 360 FT-NMR spectrometer. Moreover, the melting points of the purified phenazines were determined with a melting point apparatus, Gallenkamp, SANYO (U.K.).

In Vitro Antimicrobial Activity of PCA and PDO

PCA and PDO were used to test for antimicrobial activity using various types of microorganisms; general strains including *B. subtilis*, *C. albicans*, *E. aerogenes* and *E. coli* and some plant pathogenic bacteria, including *A. avenae* subsp. *citrulli*, *E. carotovora*, *P. syringae*, *R. solanacearum* and *X. campestris* pv. *vesicatoria*. All strains were obtained from the collection of the Department of Microbiology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand. Each bacterial strain was streaked on LB agar plates. Incubation plates were prepared as single colonies. A single colony was transferred into sterilized water and diluted to match a 1.0 McFarland turbidity standard (~6×10^8 CFU/ml) [22]. Disc diffusion was employed for the screening of antimicrobial activity of a sterilized disc with ethanol (used as the control), PCA (1,000 ppm) and PDO (1,000 ppm). Briefly, a suspension of the tested microorganism was spread over a Mueller Hinton Agar (MHA) surface of 9 cm diameter Petri dishes. Filter paper disks (6 mm diameter) loaded with each sample were placed on the surface of the MHA, which was incubated at room temperature for 24 h, and then inhibition zones were measured in mm. The minimal inhibitory concentrations (MIC) of both phenazines were determined by the serial dilution method at OD_{600} in triplicates as described by Nakai and Siebert [24].

RESULTS AND DISCUSSION

Biosynthesis of PCA and Chemosynthesis of PDO

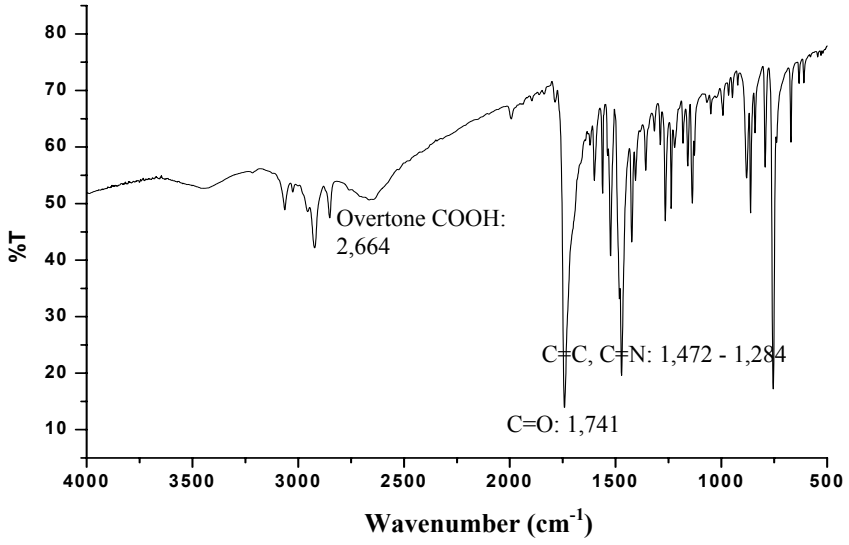
After the isolation process, approximately 1.72 g of crude phenazine per litre of bacterial culture was obtained. It was then purified using adsorption chromatography. The second yellow fraction (main fraction) was collected and it was evaporated to remove solvent by means of rotary evaporation and then kept in the refrigerator (4 °C). Yellow crystals formed with a yield of 10.20 mg/l of bacterial culture.

In the case of PDO, the oxidation process was done under mild conditions eliminating the use of hazardous substances. After the reaction was complete, a silica gel column was used for purification giving an orange-red solution that was collected manually. Orange-red crystals formed in the refrigerator (4 °C) and its weight was approximately 50.60 mg per 0.1 g of phenazine.

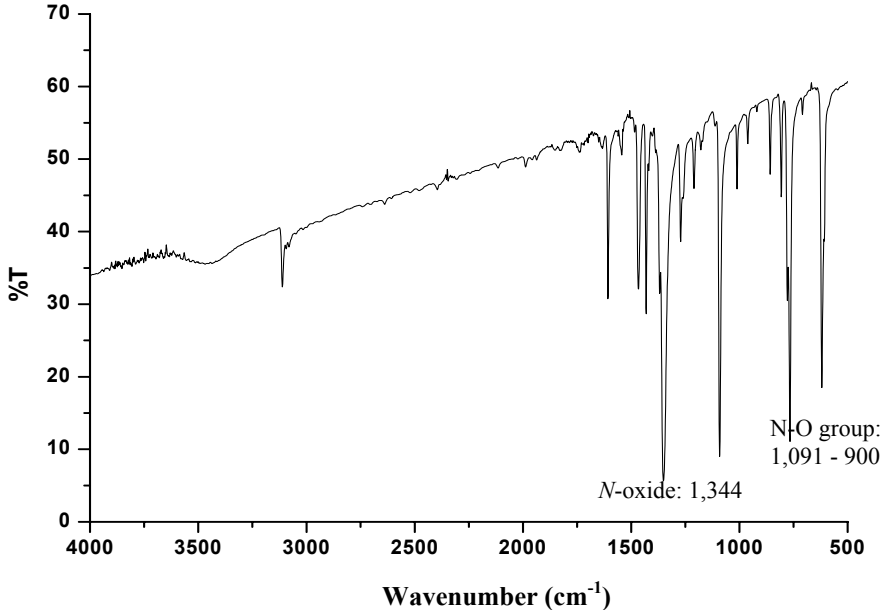
Structural Elucidation of Phenazine Compounds

The structure of biosynthesized PCA was identified, as described by Fernández and Pizarro [25]. The melting point of this pigment was found to be 242 - 243 °C. The absorption maxima of the purified yellow solution appeared at 250 and 369 nm in CH_2Cl_2, corresponding to the previous report [25]. The IR spectrum of the purified yellow crystals (**Figure 1a**) showed bands including the OH of the COOH group (3,446 cm^{-1}), an overtone for the carboxyl group (2,664 cm^{-1}), an intense C=O band (1,741 cm^{-1}) and aromatic CH bends (1,472 - 1,284 cm^{-1}) [18]. In the ^{1}H-NMR spectrum (400 MHz, $CDCl_3$), peaks in the δ = 7.30 - 9.00 ppm region indicated the presence of 7

aromatic protons and at *ca*. 15 ppm the carboxylic acid proton. The ^{13}C-NMR data showed major peaks between 124.95 - 143.95 ppm indicating the presence of 7 aromatic carbons while at 165.86 ppm the carboxylic acid carbon was observed. The purified yellow crystals were proven to be phenazine-1-carboxylic acid (PCA) and its chemical structure is shown in **Figures 2a** and **2b**.



(a)



(b)

Figure 1 IR spectra of PCA (a) and PDO (b).

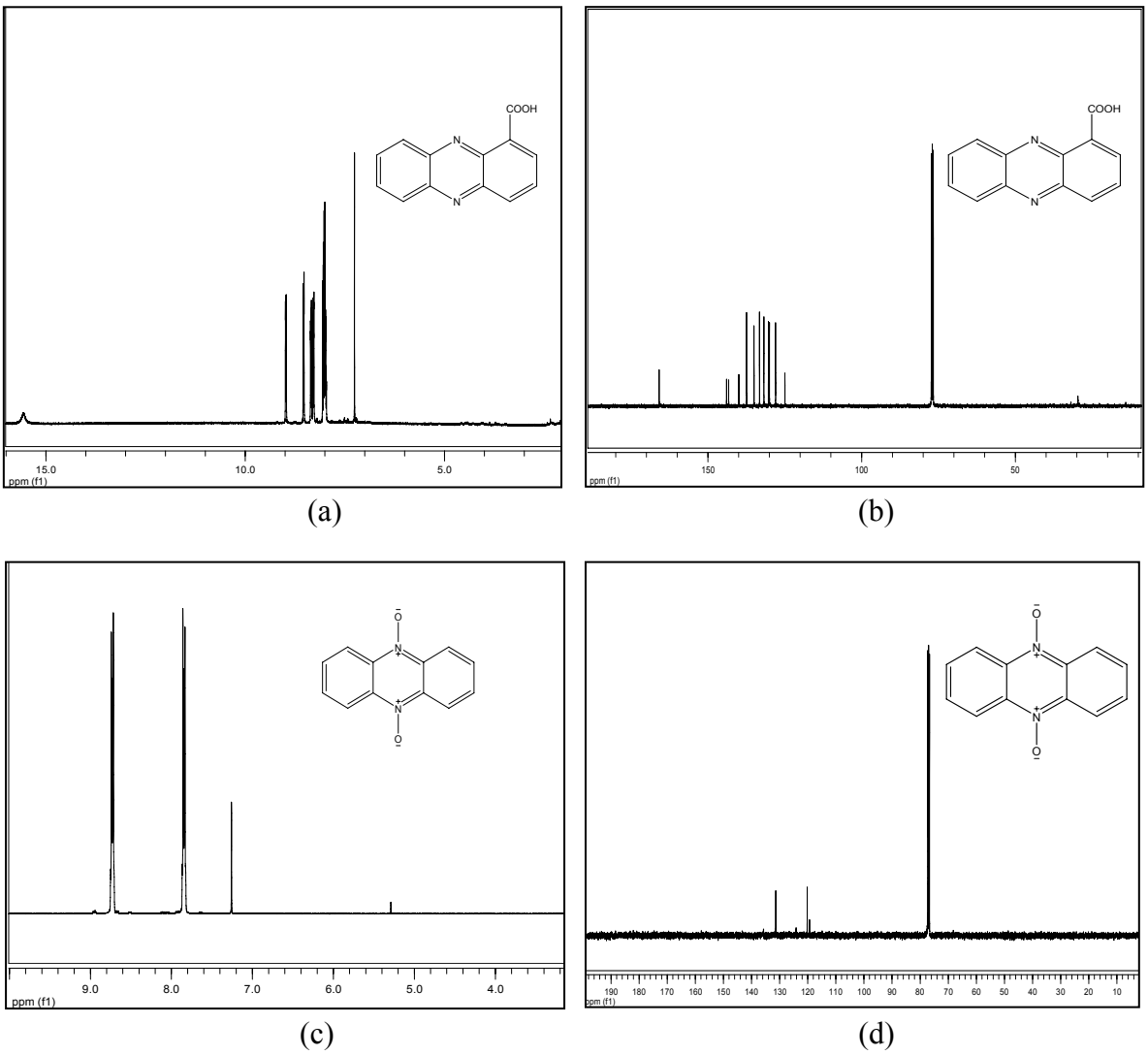


Figure 2 (a) ^{1}H-NMR, (b) ^{13}C-NMR spectra of PCA and (c) ^{1}H-NMR, (d) ^{13}C-NMR spectra of PDO when dissolved in $CDCl_3$.

PDO from chemical synthesis was characterized by spectral and elemental analyses. The absorption maxima for PDO were 277, 395, 439 and 465 nm in CH_2Cl_2, as reported previously [26]. The melting point of purified phenazine was in range from 196 - 198 °C [27]. The IR spectrum of the orange-red crystals shows a strong absorption band at 1,344 cm^{-1} for the N-oxide and the peak at ~1,300 cm^{-1}, characteristic of the N-O group, is either absent or decreased in intensity while a new peak at 1,091 - 900 cm^{-1} is shown in **Figure 1b**, which corresponds to that reported by Andreev and co-workers [26]. The ^{1}H-NMR data also confirmed the structure of this pigment. Peaks between 7.80 - 8.70 ppm are consistent with the presence of signals for aromatic protons. The

^{13}C-NMR spectra showed the peaks between 120.00 - 131.50 ppm indicating the presence of aromatic carbons in the structure. From the data, the purified orange-red crystals were confirmed to be phenazine-5,10-dioxide (PDO) with the structure shown in **Figures 2c** and **2d**.

***In Vitro* Inhibition of Pathogenic Strains by PCA and PDO**

Clear zones and the minimal concentrations of the phenazines required to inhibit various pathogen strains are summarized in **Figure 3** and **Table 1**, respectively. In preliminary studies for antimicrobial activity, PCA and PDO were tested for their ability to inhibit the growth of 1 fungal and 8 bacterial strains by *in vitro* plate inhibition assay and serial dilution. **Figure 3** presents that PCA showed better efficiency against growth of *A. avenae* subsp. *citrulli*, *B. subtilis*, *C. albicans*, *E. coli* and *X. campestris* pv. *vesicatoria* than PDO. The good antimicrobial activity of PCA may be due to acidity because PCA contains a carboxylic group in the structure and a pKa value of 4.24 ± 0.01, which confirms the acidic nature of PCA [18]. From previous research, acids can inhibit the growth of microorganisms [28]. Thus, the antimicrobial activity of PCA was more than PDO because of the combination between the acidity of the carboxylic acid and the potency of the nitrogen atoms in phenazine core. However, PDO exhibited better antimicrobial activity than PCA when tested in *P. syringae* and particularly inhibited in *E. aerogenes*, as shown in **Figures 3** and **4**. One bacterial strain, *R. solanacearum*, however, can be inhibited by the same concentration of PCA and PDO as shown in **Table 1**. Nitrogen atoms in the phenazine structure play an important role in accepting electrons, yielding a relatively stable ion radical that readily undergoes redox cycling in the presence of several reducing agents and molecular oxygen, leading to the accumulation of toxic oxygen species in bacterial cells [13]. In the case of *E. carotovora*, which causes soft rot in cabbage, the bacteria were not sensitive to PCA and PDO antibiotics. However, this strain can already be suppressed by pyocyanin (PYO) and phenazine-1-carboxamide (PCN) [22]. The results suggest important conclusions; the efficacy of phenazine compounds against microorganisms will vary, therefore not every phenazine compound will have activity against all microbes. It also seems clear that substituents on the phenazine core are preferred to increase the antimicrobial activity of the phenazine compound [11].

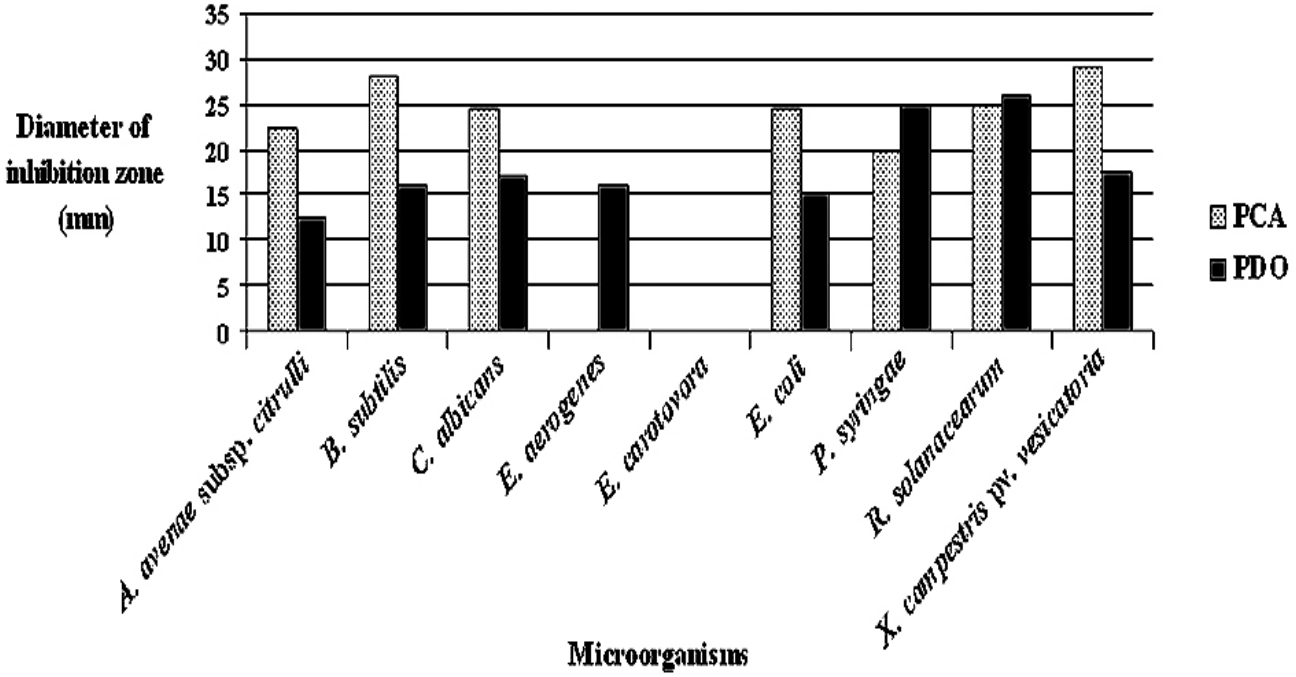
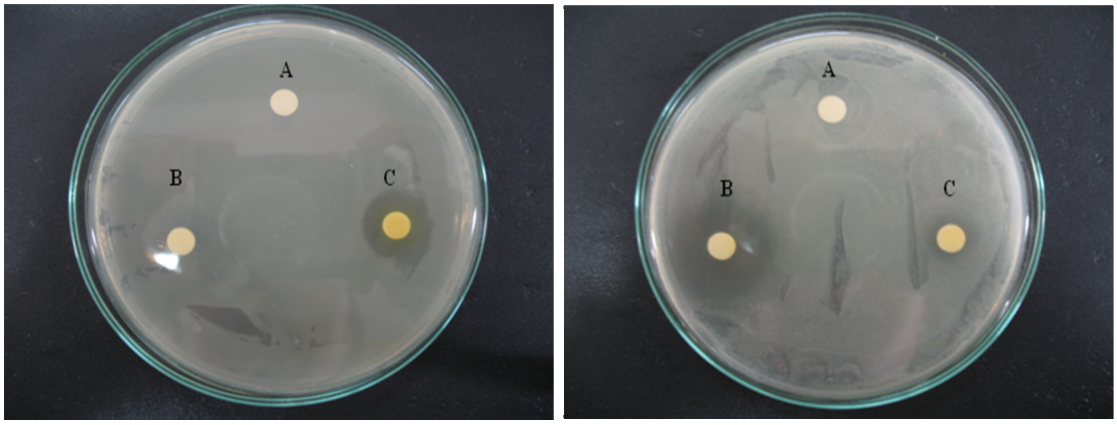


Figure 3 Diameter of inhibition zone (in mm) of PCA and PDO against microbial growth (including disc paper).

Table 1 Comparison of minimal inhibitory concentration (MIC) in ppm of PCA and PDO tested against microorganism strains by the serial dilution method.

Phenazine derivatives	Minimal inhibitory concentration (MIC) in ppm								
	A. avenae* subsp. *citrulli	***B. subtilis***	***C. albicans***	***E. aerogenes***	***E. carotovora***	***E. coli***	***P. syringae***	***R. solanacearum***	***X. campestris* pv. *vesicatoria***
PCA	34.8	17.4	17.4	-	-	34.8	125.0	62.5	17.4
PDO	125.0	62.5	62.5	62.5	-	62.5	62.5	62.5	62.5

Noted; (-) non-inhibited



(a) (b)

Figure 4 Inhibition susceptibility of phenazine derivatives (a) *E. aerogenes* and (b) *E. coli* growth when incubated at room temperature for 24 h, A: the control, B: PCA and C: PDO.

CONCLUSIONS

Natural PCA produced by *P. aeruginosa* TISTR 781 in modified King's A broth had 10.20 mg/l of bacterial culture. An approximate quantity of synthetic PDO was 50.60 mg/0.1 g of phenazine. The chemical structures of both pigments were elucidated using UV-Vis spectra, IR, ^{1}H-NMR and ^{13}C-NMR data. From the results, *in vitro* antimicrobial ability between PCA and PDO by disc diffusion and minimal inhibitory concentration (MIC) methods were compared. PCA showed more potent inhibition against some strains than PDO when it was tested against *A. avenae* subsp. *citrulli*, *B. subtilis*, *C. albicans*, *E. coli* and *X. campestris* pv. *vesicatoria*. Although, PCA had good antimicrobial activity it was not enough to suppress bacteria such as *P. syringae*, *E. aerogenes* and *E. carotovora*. In contrast, these microorganisms can be inhibited by PDO. The inhibitory mechanism of phenazine was actually a result of the toxicity of the superoxide radical and hydrogen peroxide as described in a report by Dwivedi and Johri [13]. Thus, this beneficial data may provide alternatives for choosing suitable phenazine types to eradicate general strains and some plant pathogenic bacteria. Finally, phenazine can restrain bacteria cells and protect agricultural product from many diseases but it can damage human cells too, thus it must be carefully used [29,30].

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REFERENCES

[1] GM Denning, SS Iyer, KJ Reszka, YO Malley, GT Rasmussen and BE Britigan. Phenazine-1-carboxylic acid, a secondary metabolite of *Pseudomonas aeruginosa*, alters expression of immunomodulatory proteins by human airway epithelial cells. *Am. J. Physiol-Lung. C.* 2003; **285**, L584-L592.

[2] R Wilson, T Pitt, G Taylor, D Watson, J MacDermot, D Sykes, D Roberts and P Cole. Pyocyanin and 1-hydroxyphenazine produced by *Pseudomonas aeruginosa* inhibit the beating of human respiratory cilia *in vitro*. *J. Clin. Invest.* 1987; **79**, 221-9.

[3] TFC Chin-A-Woeng, GV Bloemberg, AJ Van der Bij, KMGM Van der Drift, J Schripsema, B Kroon, RJ Scheffer, C Keel, PAHM Bakker, H Tichy, FJ De Bruijn, JE Thomas-Oates and BJJ Lugtenberg. Biocontrol by Phenazine-1-carboxamide producing *Pseudomonas chlororaphis* PCL 1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *Radicis-lycopersici*. *Mol. Plant Microbe In.* 1998; **11**, 1069-77.

[4] SM Delaney, DV Mavrodi, RF Bonsall and LS Thomashow. *phzO*, a gene for biosynthesis of 2-hydroxylated phenazine compounds in *Pseudomonas aureofaciens* 30-84. *J. Bacteriol.* 2001; **183**, 318-27.

[5] GS Byng, DC Eustice and RA Jensen. Biosynthesis of phenazine pigments in mutant and wild-type cultures of *Pseudomonas aeruginosa*. *J. Bacteriol*. 1979; **138**, 846-52.

[6] C Darby, CL Cosma, JH Thomas and C Manoil. Lethal paralysis of *Caenorhabditis elegans* by *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci.* 1999; **96**, 15202-207.

[7] JR Kerr. Phenazine pigments: antibiotics and virulence factors. *Infect. Dis. Rev.* 2000; **2**, 184-94.

[8] M González, H Cerecetto and A Monge. Quinoxaline-1,4-dioxide and phenazine-5,10 dioxide: chemistry and biology. *Top. Heterocycl. Chem*. 2007; **11**, 179-211.

[9] S Caron, NM Do and JE Sieser. A practical, efficient, and rapid method for the oxidation of electron deficient pyridines using trifluoroacetic anhydride and hydrogen peroxide–urea complex. *Tetrahedron Lett.* 2000; **41**, 2299-302.

[10] X Zhu, KD Kreutter, H Hu, MR Player and MD Gaul. A novel reagent combination for the oxidation of highly electron deficient pyridines to *N*-oxides:

trifluoromethanesulfonic anhydride/sodium percarbonate. *Tetrahedron Lett.* 2008; **49**, 832-4.

[11] JB Laursen and J Nielsen. Phenazine natural products: Biosynthesis, synthetic analogues, and biological activity. *Chem. Rev*. 2004; **104**, 1663-85.

[12] DV Mavrodi, W Blankenfeldt and LS Thomashow. Phenazine compounds in fluorescent *Pseudomonas* spp. biosynthesis and regulation. *Annu. Rev. Phytopathol*. 2006; **44**, 417-45.

[13] D Dwivedi and BN Johri. Antifungals from fluorescent pseudomonads: Biosynthesis and regulation. *Curr. Sci*. *India* 2003; **85**, 1693-703.

[14] TFC Chin-A-Woeng, GV Bloemberg and BJJ Lugtenberg. Phenazines and their role in biocontrol by *Pseudomonas* bacteria. *New Phytol*. 2003; **157**, 503-23.

[15] TJ Burr, MN Schroth and T Suslow. Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. *Phytopathol.* 1978; **68**, 1377-83.

[16] TV Suslow and MN Schroth. Rhizobacteria of sugar beets: effects of seed application and root colonization on yield. *Phytopathol.* 1982; **72**, 199-206.

[17] FP Geels and B Schippers. Reduction of yield depressions in high frequency potato cropping soil after seed tuber treatments with antagonistic fluorescent *Pseudomonas* spp. *Phytopathol. Z.* 1983; **108**, 207-14.

[18] PG Brisbane, LJ Janik, ME Tate and RFO Warren. Revised structure for the phenazine antibiotic from *Pseudomonas fluorescens* 2-79 (NRRL B-15132). *Antimicrob. Agents Chemo*. 1987; **31**, 1967-71.

[19] KJ Kim. Phenazine 1-carboxylic acid resistance in phenazine 1-carboxylic acid producing *Bacillus* sp. B-6. *J. Biochem. Mol. Biol.* 2000; **33**, 332-6.

[20] M Oda, Y Sekizawa and T Watanabe. Phenazines as disinfectants against bacterial leaf blight of the rice plant. *Appl. Microbiol.* 1966; **14**, 365-7.

[21] ME Hernandez, A Kappler and DK Newman. Phenazines and other redox-active antibiotics promote microbial mineral reduction. *Appl. Environ. Microbiol*. 2004; **70**, 921-8.

[22] K Saosoong, S Tongtumma, S Chanthai, W Wongphathanakul, W Bunyatratchata and C Ruangviriyachai. Isolation and study of chemical properties of pyocyanin produced from *Pseudomonas aeruginosa* TISTR 781 (ATCC 9027). *KKU Res. J.* 2007; **12**, 24-32.

[23] MS Abd EI-Halim, AS EI-Ahl, HA Etman, MM Ali, A Fouda and AA Fadda. A new route for the synthesis of phenazine di-N-oxides. *Monatsh. Chem*. 1995; **126**, 1217-23.

[24] SA Nakai and KJ Siebert. Validation of bacterial growth inhibition models based on molecular properties of organic acids. *Int. J. Food Microbiol*. 2003; **86**, 249-55.

[25] RO Fernández and RA Pizarro. High-performance liquid chromatographic analysis of *Pseudomonas aeruginosa* phenazines. *J. Chromatogr. A* 1997; **771**, 99-104.

[26] VP Andreev, EG Batotsyrenova, AV Ryzhakov and LL Rodina. Intramolecular charge transfer processes in a series of styryl derivatives of pyridine and quinoline-N-oxides. *Chem. Heterocycl. Comp*. 1998; **34**, 941-9.

[27] H Hikoharu, O Reiichi, S Masatoshi and H Tadao. Processing of photographic silver halide light-sensitive materials. US Patent 3,642,481, 2008.
[28] RJ Lambert and M Stratford. Weak-acid preservatives: modelling microbial inhibition and response. *J. Appl. Microbiol.* 1999; **86**, 157-64.
[29] R Wilson, DA Sykes, D Watson, A Rutman, GW Taylor and PJ Cole. Measurement of *Pseudomonas aeruginosa* phenazine pigments in sputum and assessment of their contribution to sputum sol toxicity for respiratory epithelium. *Infect. Immun.* 1988; **56**, 2515-17.
[30] J Nutman, M Berger, PA Chase, DG Dearborn, KM Miller, RL Waller and RU Sorensen. Studies on the mechanism of T cell inhibition by the *Pseudomonas aeruginosa* phenazine pigment pyocyanine. *J. Immunol.* 1987; **138**, 3481-87.

บทคัดย่อ

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การสังเคราะห์ การแยก และคุณสมบัติต้านจุลชีพของสารอนุพันธ์ฟีนาซีน

การศึกษาฤทธิ์ของฟีนาซีน-1-คาร์บอกซิลิก แอซิด (พีซีเอ) ที่ผลิตได้จากวิธีทางชีวภาพ และฟีนาซีน-5-10-ไดออกไซด์ (พีดีโอ) ซึ่งสังเคราะห์โดยวิธีทางเคมี ในการต้านการเจริญเติบโตของจุลชีพทั่วไปและแบคทีเรียที่ก่อโรคพืชบางชนิด ด้วยเทคนิค disc diffusion และเทคนิค minimal inhibitory concentration พบว่าพีซีเอมีฤทธิ์ยับยั้งการเจริญของ *Acidovorax avenae* subsp. *citrulli*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli* และ *Xanthomonas campestris* pv. *vesicatoria* ได้ดีกว่าพีดีโอ เมื่อใช้พีซีเอเพียงปริมาณเล็กน้อย (ความเข้มข้นต่ำสุดอยู่ในช่วง 17.44 - 34.87 มิลลิกรัมต่อลิตร) ในทางตรงกันข้ามพบว่า พีดีโอเป็นสารยับยั้งเชื้อที่แรงกว่าพีซีเอ เมื่อทดสอบกับ *Pseudomonas syringae* และ *Enterobacter aerogenes* เมื่อนำพีซีเอและพีดีโอทดสอบกับแบคทีเรีย *Ralstonia solanacearum* พบว่า ความเข้มข้นต่ำสุดที่สามารถยับยั้งการเจริญเติบโตของแบคทีเรียนี้ได้ คือ 62.50 มิลลิกรัมต่อลิตร ผลการศึกษาครั้งนี้ได้ให้ข้อมูลที่เป็นประโยชน์สำหรับการเลือกใช้ชนิดของฟีนาซีน เพื่อยับยั้งการเจริญของจุลชีพทั่วไปและแบคทีเรียที่ก่อโรคพืชบางชนิดได้

[1]ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002

[2]ภาควิชาชีวเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002