Research Article

# Effect of Viola odorata extract on *Pseudomonas aeruginosa* produce β-lactamase enzyme

***Thamer M. Jasim*** *Ph.D.*[1]**, *Suhad F. Hatem*** *MSc.*[1]**, *Ayad M. Raauf*** *Ph.D.*[2]**, *Rawaa Assad*** *MSc.*[3]

[1]*Department of clinical laboratory science, College of Pharmacy, Al-Mustansiriya University, Baghdad, Iraq.*
[2]*Department of Pharmacy chemistry and Pharmacognosy, College of Pharmacy, Al-Mustansiriya University, Baghdad, Iraq.*
[3]*Medical city. Baghdad, Iraq.*

*Date Submitted:5.4.2012*

*Date Accepted:28.5.2012*

**Address for Correspondence:**
Dr. Thamer M. Jasim, Ph.D.
E-mail: thamermutlek@yahoo.com

## Abstract

**Background:** *Pseudomonas aeruginosa* is a classic opportunist pathogen with innate antibiotics and disinfectant. It is physiologically versatile and flourishes as a saprophyte in warm moist situation within human environment, including sinks, drains, respirators, humidifiers and disinfectant solutions.

**Aims:** investigate the antibacterial activity of Viola odorata extract *in vitro*.

**Patients and methods:** alcohol solvent was mainly utilized for such extraction procedure with subsequent application of the extract against resistance *Pseudomonas aeruginosa* isolating from different body sites. Twenty two isolates, out of 222 samples, produced Beta lactamase (β-lactamase) and such isolated bacteria was examined for antibiotic sensitivity test toward ten antibiotics namely ampicillin, Augmentin, cefotaxime, amikacin, gentamicin, ciprofloxacin, tetracycline, trimethoprim, tobramycin, and imipenem.

**Results:** it was revealed that the alcohol extracts of Viola Odorata; range with half concentration (500, 250, 125, 62.5, 31.25, 15.7 mg/ml); exhibited broader spectrum as well as greater activity against resistant *Pseudomonas aeruginosa* among those with/without tested extracts with inhibition zone vary between (10-30 mm).

**Conclusions:** These data suggest that Viola odorata extract could inhibit the growth of *Pseudomonas aeruginosa* strain *in vitro* and this activity may contribute to its chemopreventive effect.

**Keywords:** resistant *Pseudomonas aeruginosa*, Viola odorata, β-lactamase

## INTRODUCTION

β-lactamases are enzymes produced by certain bacteria[1, 2, 3, 4] and are responsible for their resistance to Beta-lactam antibiotics such as pencillin, cephamycin, carbapenem (ertapenem), and cephalosporin. These antibiotics have a common element in their molecular structure with a four–atom ring known as a Beta-lactam. The lactamase enzymes are in charge for breaking and opening that ring along with deactivating such molecules lead to disturbance in their antibacterial properties. In fact, β-lactam antibiotics are ordinarily prescribed to treat a broad spectrum of both Gram positive and Gram negative bacteria.

Viola odorata have long been applied in folk medicine to treat genital tumors, sclerosis of the spleen, inflammatory tumours, cough, and ulcers.[5] Furthermore, Teas prepared

from its flowers or leaves could be used in Phytotherapy and possessing sedative, antispasmodic, diuretic and emmenagogue activities as well.[6, 7] However, no previous studies have been reported to use Viola odorata as antibacterial drug at least in Iraq. It seems that *Pseudomonas.aeruginosa* is primarily an opportunistic pathogen that causes infections in hospitalized patients' e.g those with extensive burn sepsis.[8, 9] Such bacterium is an important pathogen especially in hospital casualty because of its innate resistance to many antibiotics.[9, 10, 11] These pathogens when introduced into airosteroid act against normal defense through which bacterium attaches to and colonizes the mucous membranes of skin locally and produced systemic disease by Pilli, enzymes and toxins.[12] Obviously, burned patients are at high risk for acquiring nosocomial infections due to immunosuppression influence of burn injury. In this regard, *Pseudomonas aeruginosa* accounts for a cardinal role in threatening nosocomial pathogens at burn unit.[13] Here, the aim of present study was to investigate the antibacterial properties of Viola odorata extract against collection of clinical isolate resistant *Pseudomonas aeruginosa* which produces β-lactamase enzyme.

## PATIENTS AND METHODS

### *Isolation*

All bacterial isolates applied in this study were obtained from 222 samples collecting from patients who attended Baghdad medical city between July and August 2008.

### *Identification*

Specimens were plated on both blood and MacConkey agars for 18-24h at 37°C. Subsequently, diagnosis was usually based on colonial morphology, Gram stain, oxidase positive, the presence of characteristic pigments, and growth at biochemical activity depending on Api 20 system (Biomerieux, France).

### *Antibiotic assay*

In order to identify sensitive and resistant strain of isolates, samples with positive P. aeruginosa were evaluated toward ten antibiotics namely ampicillin, Augmentin, cefotaxime, amikacin, gentamicin, ciprofloxacin, tetracycline, trimethoprim, tobramycin, and imipenem. Kerby-Bouar methods was performed by using Mueller Hinton agar (Himedia).[13, 14]

### *Detection of B- lactamase production*

β-lactamase production from P. aeruginosa isolates was determined by (Rapid iodometric method). Accordingly, definite solutions had been used as described previously.[15] By this technique, Solution A was prepared by dissolving 0.56 g pencillin G in 100 ml Phosphate buffer with PH (7), whereas Solution B was ready after dissolving 2.03 g Iodide with 5.3 g potassium lodide and completed the volume up to 100 ml with distill water (D.W) and stored at 4 °C. In addition, 1 g of starch was liquefied in water bath with 100 ml D.W. (Solution C).

One colony was transferred to 100 ml of Solution A, and then incubated for 30 min at room temperature. Finally, 20 and 50 ml of Solution B and C, respectively, were added to mixture. Yellow color was regarded as positive result.

### *Antibacterial activity of Viola odorata*

This extract was prepared from flowers part from Viola odorata (25 g with 400 ml 96% ethanol) by using soxhlet apparatus. Later, the extract was evaporated by Rotary evaporate to obtain a thick extract.[13] Many dilutions of this extract was made (500, 250, 125, 62.5, 31.25, 15.75) mg/ml in order to study antibacterial effect of this extract against some isolates of bacteria which produced β–lactamase enzyme by using well diffusion method.[11]

## RESULTS

As shown in table 1, 222 samples were collected from different sites (urine, bone infection, wound swab, bronchial wash, and ear swab), from which the number of isolates revealed positive *P. aeruginosa* was 43 (19.3%). However, it was noted that just 22 (51.16%) samples of *P. aeruginosa* were produced β-lactamase enzyme (Table 3).

**Table 1.** Number of positive *P. aeruginosa* from many specimens.

| Source of isolates | Number of isolates | *P. aeruginosa* (+) | |
|---|---|---|---|
| | | (NO.) | (%) |
| **Urine** | 100 | 6 | 6 |
| **Bone infection** | 50 | 12 | 24 |
| **Wound swab** | 40 | 11 | 27.5 |
| **Bronchial wash** | 22 | 6 | 27.2 |
| **Ear swab** | 10 | 8 | 80 |
| **Total** | 222 | 43 | 19.3 |

**Table 2.** Sensitive and resistant *P. aeruginosa* from many specimens.

| Antibiotics | Code | *P. aeruginosa* isolates | | | |
|---|---|---|---|---|---|
| | | Sensitive | | Resistant | |
| | | (NO.) | (%) | (NO.) | (%) |
| **Ampicillin** | Am | 0 | 0 | 43 | 100 |
| **Augmentin** | AMc | 2 | 4.6 | 41 | 95.3 |
| **Cefotaxim** | CTX | 4 | 9.3 | 39 | 90.6 |
| **Amikacin** | AK | 33 | 76.7 | 10 | 23.2 |
| **Gentamicin** | G | 16 | 37.2 | 27 | 62.7 |
| **Ciprofloxacin** | Cip | 28 | 65.1 | 15 | 34.8 |
| **Tetracyclin** | T | 3 | 6.9 | 40 | 93.01 |
| **Trimethoprim** | Tr | 2 | 4.6 | 41 | 95.3 |
| **Tobramycin** | Tb | 22 | 51.1 | 21 | 48.8 |
| **Imipenem** | IMP | 43 | 100 | 0 | 0 |

**Table 3.** Number and percentage of *P. aeruginosa* which produced β-lactamase enzyme.

| Source of isolates | NO. of positive *P. aeruginosa*. | β-lactamase (+) | |
|---|---|---|---|
| | | (NO.) | (%) |
| **Urine** | 6 | 2 | 33.3 |
| **Bone infection** | 12 | 8 | 66.6 |
| **Wound swab** | 11 | 6 | 54.5 |
| **Bronchial wash** | 6 | 5 | 83.3 |
| **Ear swab** | 8 | 1 | 12.5 |
| **Total** | 43 | 22 | 51.16 |

In order to assess the sensitivity of *P. aeruginosa* to antibiotics, the collected specimens with positive *P. aeruginosa* results were exposed to certain antibiotics namely ampicillin, Augmentin, cefotaxime, amikacin, gentamicin, ciprofloxacin, tetracycline, trimethoprim, tobramycin, and imipenem (Table 2).

It seems that all isolates resist to penicillin (100%) while complete sensitivity to imipenem were observed. Other involved antibiotics varied in their responses towards *P. aeruginosa* species (Table 2).

The antibacterial effect of ethanol extract of Viola odorata at concentrations (500, 250, 125, 62.5, 31.25, 15.7 mg/ml) was evaluated against resistant *P. aeruginosa*. As illustrated in table 4, there was Viola odorata extract inhibitory trend towards *P. aeruginosa* at 500 and 250 mg/ml with nearly similar pattern for both concentrations. Wide range of inhibition was established among various isolated samples (10-30 mm), although no inhibitory influence had been seen on some of *P. aeruginosa* obtained from wound infection and bronchial wash. The inhibitory pattern decreased at 125 mg/ml of Viola odorata extract. In contrast, at the lower concentrations there appeared to be no inhibitory effects of various dilution extracts.

**Table 4.** Antibacterial effect of alcohol extract of Viola odorata against some isolates of *P. aeruginosa*.

| Isolates | Inhibitory zone | | | | | |
|---|---|---|---|---|---|---|
| | Concentration of alcohol of extract (mg/ml) | | | | | |
| | 500 | 250 | 125 | 62.5 | 31.25 | 15.7 |
| **L1 (B.W)** | 30 mm | 25 mm | ----- | ----- | ----- | ----- |
| **L2 (B.W)** | 25 mm | 20 mm | 16 mm | ----- | ----- | ----- |
| **L3 (W)** | 30 mm | 25 mm | 15 mm | 12 mm | ----- | ----- |
| **L4 (E)** | 22 mm | 20 mm | 15 mm | ----- | ----- | ----- |
| **L5 (U)** | 21 mm | 19 mm | 15 mm | ----- | ----- | ----- |
| **L6 (B.W)** | 10 mm | ----- | ----- | ----- | ----- | ----- |
| **L7 (W)** | ----- | ----- | ----- | ----- | ----- | ----- |
| **L8 (W)** | ----- | ----- | ----- | ----- | ----- | ----- |
| **L9 (W)** | ----- | ----- | ----- | ----- | ----- | ----- |
| **L10 (B.W)** | ----- | ----- | ----- | ----- | ----- | ----- |

L= isolate, BW (Bronchial wash), W (wound), E (ear), U (urine).

## DISCUSSION

This study has evaluated the antibacterial activity of Viola odorata. Ethanol was proved to be good solvents extracting inhibitory substances from plant. The result is in agreement with Ali et al.[16] who found that the ethanolic extracts 400 mg per disk of the two plants were inactive against both G-ve and G+ve bacteria. However, Eloff[17], and Ojala[18] reported that methanol extract from plant had more inhibitors than ethanol. For best of our knowledge, the present study is the first positive report that addresses the issue of using ethanol extract of Viola

odorata as antibacterial compound. The examined Viola odorata exhibited antibacterial activity against resistant *Pseudomonas aeruginosa*. Similar result were reported in literature[19, 20] at which oxygenated monoterpenes such as alcohol, display strong antimicrobial activity especially pronounced on whole cell while hydrocarbon derivatives possess lower antimicrobial properties as their lower water solubility limits their diffusion through the medium. This result agreed with Skaltsa et al.[21] who described that *Pseudomonas aeruginosa* was found to be the most resistant strain, as none of the essential oils was active against this strain.

Recently, it has been used cyclotide cycloviolacin cyo2 which isolated by HPLC from Viola odoratam.[22] Cyo2 killed all G-ve bacterial strain including important humans' pathogens such as *P. aeruginosa* and Multidrug resistant (MDR) *Klebsiella pneumoniae.* Therefore, cyclotide could have biological role in protection against bacterial infections, because it disrupts cell and lipid membrane. In addition, cyclotides have certain biological activities with anti-HIV, antifouling antibacterial, insecticidal and cytotoxic pharmaceutical and agricultural application.[20] These results will encourage us to undertake further studies regarding the isolation and characterization of the active principles present in the active extracts, moreover clinical studies are required to understand the mechanism along with the actual efficacy of these herbal extracts in treating various infections.

## REFERENCES


1. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. ***Antimicrob Agents Chemother*** 1995;39:1211-33.
2. Ambler RP. The structure of beta-lactamases. ***Philos Trans R Soc Lond B Biol Sci*** 1980;289:321-31.
3. Knothe H, Shah P, Krcmery V, Antal M, Mitsuhashi S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of Klebsiella pneumoniae and Serratia marcescens. ***Infection*** 1983;11:315-7.
4. Emery CL, Weymouth LA. Detection and clinical significance of extended-spectrum beta-lactamases in a tertiary-care medical center. ***J Clin Microbiol*** 1997;35:2061-7.
5. Hartwell JL. Plants used against cancer: a survey. Lawrence, MA: Quarterman Publications; 1982. pp 438-39.
6. Duke JA. Handbook of medicinal herbs. Boca Raton: CRC Press; 1985. pp 457.
7. Lewis WH, Elvin-Lewis MPF. Medical Botany: Plants affecting man's health. New York, NY: Wiley Interscience Publication John Wiley and Sons; 1977. pp 389-390.
8. Levinson W. and Jawetz E. Medical Microbiology and Immunology. 6th ed. McGraw Hill; 2000.
9. Lyczak JB, Cannon CL, Pier GB. Establishment of Pseudomonas aeruginosa infection: lessons from a versatile opportunist. ***Microbes Infect*** 2000;2:1051-60.
10. Sleigh JD, and Timbury MC. Notes on medical Bacteriology. 5th ed. Edinburgh: Churchill Livingstone; 2000.
11. Brooks G, Butel J, Morse S. Medical Microbiology. Lange Medical Books, 21th ed. New York: McGraw-Hill Compaines inc.; 1995.
12. Takesue Y, Yokoyama T, Kodama T, Murakami Y, Sewake H, Miyamoto K, et al. Beta-lactamase in gram-negative rods: the relationship between penicillinase and R plasmids in gram-negative rods. ***Hiroshima J Med Sci*** 1990;39:65-9.
13. Perez C, Pauli M, Bazevque P. An antibiotic assay by the agar well diffusion method. ***Acta Biologiae et Medicine Experimentalis*** 1990;15:113-5.
14. Nair R, and Chanda S. Anticandidal activity of Punica granatum exhibited in different solvents. ***Pharm Biol*** 2005;43:21-5.
15. Mackie and McCartney. Practical medical microbiology. 14th ed. Singapore: Churchill Livingstone; 1996.
16. Ali NA, Jülich WD, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. ***J Ethnopharmacol*** 2001;74:173-9.
17. Eloff JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants?. ***J Ethnopharmacol*** 1998;60:1-8.
18. Ojala T, Remes S, Haansuu P, Vuorela H, Hiltunen R, Haahtela K, et al. Antimicrobial activity of some coumarin containing herbal plants growing in Finland. ***J Ethnopharmacol*** 2000;73:299-305.
19. Leta Aboye T, Clark RJ, Craik DJ, Göransson U. Ultra-stable peptide scaffolds for protein engineering-synthesis and folding of the circular cystine knotted cyclotide cycloviolacin O2. ***Chembiochem*** 2008 ;9:103-13.
20. Thongyoo P, Tate EW, Leatherbarrow RJ. Total synthesis of the macrocyclic cysteine knot microprotein MCoTI-II. ***Chem Commun (Camb)*** 2006;27:2848-50.
21. Skaltsa HD, Demetzos C, Lazari D, Sokovic M. Essential oil analysis and antimicrobial activity of eight Stachys species from Greece. ***Phytochemistry*** 2003;64:743-52.
22. Pränting M, Lööv C, Burman R, Göransson U, Andersson DI. The cyclotide cycloviolacin O2 from Viola odorata has potent bactericidal activity against Gram-negative bacteria. ***J Antimicrob Chemother*** 2010;65:1964-71.