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To cite this article: Mais E. Ahmed 2018 *J. Phys.: Conf. Ser.* **1003** 012004

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The study of Bacteriocin of *Pseudomonas fluorescens* and *Citrus limon* Effects against *Propionibacterium acnes* and *Staphylococcus epidermidis* in acne patients

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Abstract

Research was carried out antibacterial of (Citrus limon) juice on *Acne vulgaris*. Samples were obtained from individuals having (Pimples) by swabbing their faces. Substances natural from plants are promising to treat disease cause *Acne vulgaris*, the study in vitro biological activity of the juice, as well as bacteriocin cultivated and fruits was investigated on two strain bacteria (*Propionibacterium acnes*, *Staphylococcus epidermidis*). The new antimicrobial (bacteriocin and Citrus juice) is ongoing search. This study used s juice at different concentrations at (20%, 30%, 40%, 60%, 80% and 100%). The Bacteriocin produced from local *P. fluorescens* isolates from wound infection and majority of isolates were found to produce crude Bacteriocin were (P1 and P2) in *Pseudomonas* agar at 37°C for 24 hrs. Crude Bacteriocin and Citrus limon juice against some pathogenic skin bacteria was find to be effective juice Citrus limon against *S. epidermidis* at 100% Concentrations with a range of inhibition zone (18) mm. The isolates of *P. fluorescens* (P2) was positive as producer of Bacteriocin with a wide inhibition growth against gram positive pathogenic bacteria with a range between (10-12) mm.

Key words: Juice extract, Bacteriocin, Pathogenic bacteria

1. Introduction

Pseudomonas fluorescens are gram negative rods aerobic with cells size of from $(2-3) \mu\text{m}$, they are usually occurred in the wild, in the waste water and wound. They occur in contaminated environment with colonization, but no signs of disease [1]. (Pseudomonic acid A) Mupirocin an antibiotic (Bacteriocin) produced by *P. fluorescens*, showed a peaky attributable of activity against Staphylococci and Streptococci and against certain G-ve bacteria, including *Haemophilus influenza* and *Neisseria gonorrhoeae*. There was no cross resistibility between available clinically antibiotics and mupirocin [2]. Proteinaceous toxins are Bacteriocins produced by bacteria to inhibit the growth strain(s) bacterial. Bacteriocins are medicine of interest because they are made by bacteria non-pathogenic in human normally colonize [3].

Citrus limon (*L.*) is a plant from the northeast and north of Brazil with popular name known "limoeiro" [4]. Oranges and Lemon are an expatriate medicaid plant of the family Rutaceae which are having antitumor activities and the antibacterial possibility in crude of disparate parts as follows (flower, root,



stem and leaves) of Lemon against bacterial strains were shown and reported [5] Flavonoids of Citrus have a big spectrum of biological activities including anti-bacterial, anti-fungal, anti-diabetic, anti-cancer and anti-viral activities [6]. The positive health benefits of part to vitamin C juices have been ascribed in [7].

The formed (emergence) of resistance of microorganisms to antimicrobial drugs is one of the world's current challenges. Second hand, plant- based antimicrobials are Attraction as they are often during many aspect effects associated with synthetic antimicrobials. The finding of new antimicrobial compounds from natural sources is, thus, Acne occurs frequently in individuals with high at acne regions bacteria is observed. *Propionibacterium acnes* are considered to cause inflammation around pores, and aggravate the symptoms [8].

The aim of study: this was to test the production of bacteriocin from commune strain. The main objective of study is anti-bacterial activity of Citrus Lemon against isolates from wound from skin infection. Comparable between Bacteriocin and natural juice extract can be used as an effective antibacterial agent against multidrug resistant bacteria skin infection and moreover it is readily available, safe, cheap and has no side effects.

2. Materials and Methods

Bacterial isolates

Ten isolates of *P. fluorescens* (P 1 to P10) were collected from wound cultures (swabs) from Yarmouk hospitals in Bagdad, Iraq and identified by morphological and Bacteriological tests [9].

Indicator isolates:

Clinical bacterial isolates from wound infections like; methicillin resistant *Staphylococcus epidermidis* (MRSE) were used as indicator isolates. These isolates were identified by morphological and biochemical tests according to [10] and *P. acnes* isolates were obtained from College of Science for Women/ Department of Biology (microbiology lab) /University of Baghdad.

Culture Media using:

MacConkey agar, Kligler Iron agar and Pseudomonas agar that we used, were purchased from Sigma Company, both media. These media were recommended for differentiation of *P. aeruginosa* while mannitol salt and blood agar for *S. epidermidis* for 24 hours in 37°C. Additional chemicals; Indole, Simmen citrate and Urea test were purchased from Merck Millipore company. Every single colony of different (culture) was tested biochemically.

Antibiotic Sensitivity Test:

By using disk diffusion method for sensitivity test of *P. fluorescens* was carried out according to [11] as follows, Bacterial inoculums pure colonies have been prepared by dissolving in normal saline and the turbidity of the bacterial inoculums should be compared with (10^6 cfu/ml) standard McFarland tube (bacterial suspension in Mcfarland = 1.5×10^8 tube. A volume of 0.1 ml spread on Mueller -Hinton agar medium plates and let at room temperature to dry. Five antibiotics Gentamycine CN (01 mg), Ampiciline AM (01 mg), Amoxiline AX (52mg), Erythromycine E (15 mg) and Tetracycline TE (30) were used, by placing the disks of antibiotics on the inoculated period plate and pressed into the agar with a sterile forceps. Then the plates were incubated at 37 °C for 24 hours in an inverted position. The results have been read after incubation. The diameters of the complete zone of inhibition were noted and measured by a millimeter (mm).

Antibacterial activity of *Pseudomonas fluorescens*:

Antibacterial activity of *P. fluorescens* isolates against indicator isolates were detected by well diffusion agar method [12]. Each isolate of *P. fluorescens* approximately 10^6 CFU was cultured on nutrient agar, and for 24 hr incubated at 37°C. Agar (blocks diameter, 5mm) including growth were cleared excised from the nutrient agar and upside down placed on the surface of Mueller-Hinton agar seeded made with 0.1ml of $\sim 10^6$ cells of indicator isolates. Plates incubated for 24 hr at 37°C. then measuring the diameter of the inhibition zones bacteriocin activity

The Extraction of Juice Lemon:

Lemon fruits were obtained from the local markets of Baghdad, with a bactericidal washed and filets into two halvers. Squeeze the juice out from it immediately and the seeds were removed to avoid contamination of the juice lemon during squeezing.

Antibacterial Activity of Lemon Juice by Agar Well Diffusion Method:

The Lemon juice was double or serial dilution which was prepared by using distilled water to obtain concentrations at (20%, 30%, 40%, 60%, 80% and 100%). Equal volume of lemon juice of Nutrient agar was mixed together. Specifically 0.1ml of the organism inoculated (*Staphylococcus epidermidis* and *Propionibacterium acnes*) was added to each of the tube test containing the different concentrations above. Tubes are incubated at 35°C for 24 hours anaerobic condition, and then

after 24 hours observed of incubation to determine the (MIC), that showed concentration is the lowest and no growths were shown . [13].

3. Results

Ten isolates of *P. fluorescens* (P 1 to P10) were obtained from wound infections and identified with conventional methods Figure (1) and also for further confirmation with API-20 Profile Recognition System.

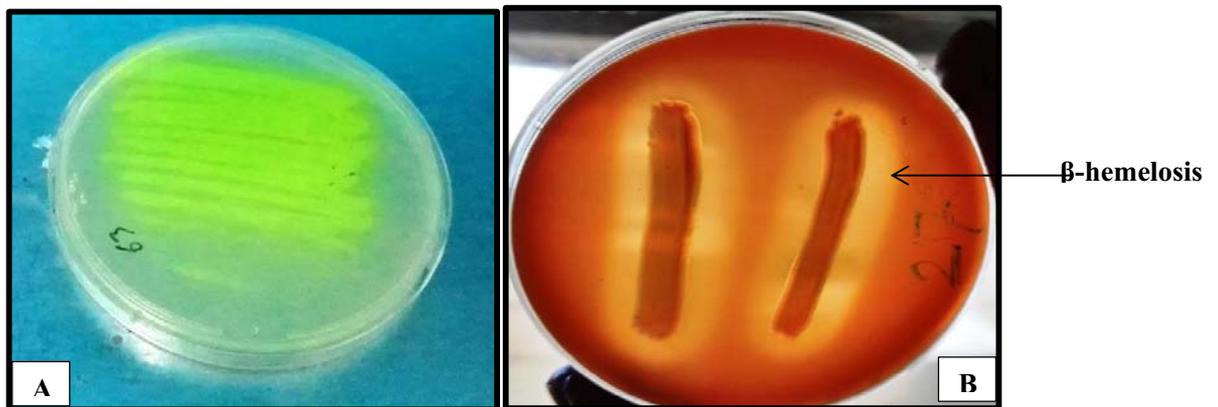


Figure 1. (A) A presumptive *P. fluorescens* on Pseudomonas agar (B) presumptive *P. fluorescens* on blood agar

Antibiotic Sensitivity Tests:

The results of the sensitivity test have shown that (isolates) P2, P1, P4 and P5 more resistant for all antibiotic using (choosing) resistant than P3, P8, P7, P9 and P10 for all type antibiotic using Figure (2), then using resistance *P. fluorescens* (P1 and P2) as bacterocin producers.

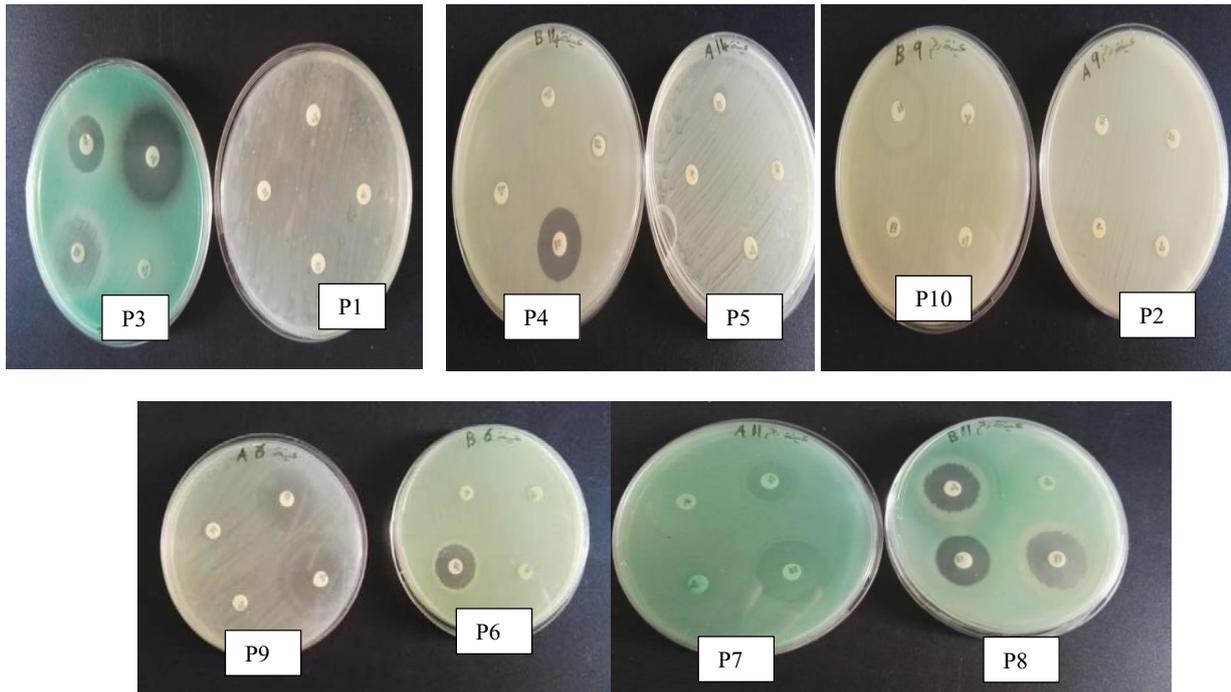


Figure 2. Antibiotic susceptibility test of *P. fluorescens* isolates

The two isolates of *P. fluorescens* the two isolates produced bacteriocin (P1 and P2) with a wide range effect on growth of gram positive *Staphylococcus. epidermidis* as shown in (Figure 3). The P2 (11) strain produced bacteriocin more than P1, while the range of inhibition zones against *Propionibacterium acnes* by P2 was (12) mm. as in (Figure 4).

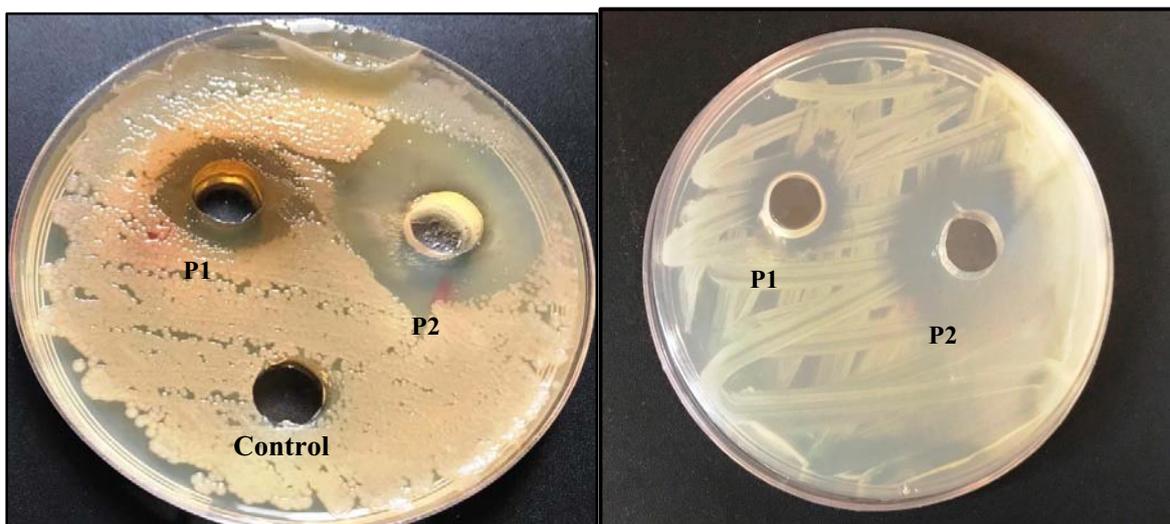


Figure 3. Crude Bacteriocin against *S. epidermidis* P1 (4mm), P2 (11mm) at 37°C for 24 hr on nutrient agar

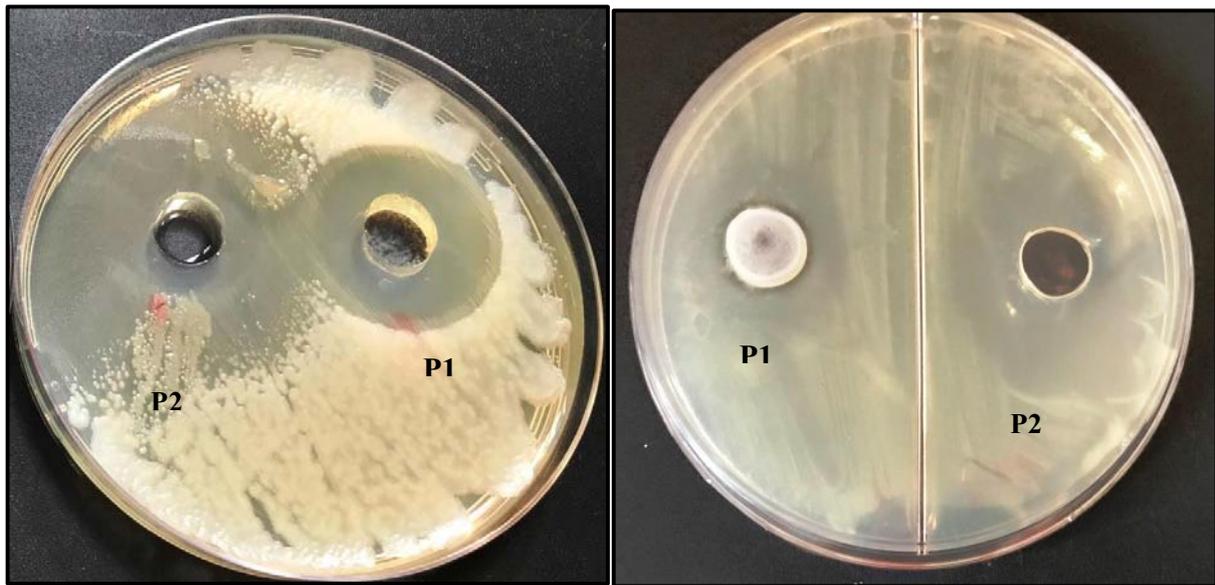


Figure 4. Crude Bacterocin on nutrient agar at 37°C for 24 hr against *Propionibacterium acnes* P1 (5mm), P2 (12 mm)

(Table 1) and (figure 5 and 6) showed activity the antibacterial on pimples of *Citrus limon*. The results of *Citrus limon* as the concentration increases the inhibition zone showed that all concentrations used between (20-100)% were effective on *S. epidermidis* and *Propionibacterium acnes*. The result at concentrations at 20% indicated that there was no clear inhibition of zone. While higher zone was at concentrations of 60%, 80% and 100%.

Table 1: Percentage (%)Citrus limon juice of Antibacterial Activity of (*Propionibacterium acnes* and *Staphylococcus epidermidis*)

Organism	Citrus limon Juice concentrations (%)	Diameter of zone of inhibition (mm)
<i>P. acnes</i>		3
<i>S. epidermidis</i>	20	2
<i>P.acnes</i>		7
<i>S. epidermidis</i>	40	7
<i>P. acnes</i>		10
<i>S. epidermidis</i>	60	11
<i>P. acnes</i>		12
<i>S. epidermidis</i>	80	14
<i>P.acnes</i>		14
<i>S. epidermidis</i>	100	18
LSD value	11.894 **	4.275 **
P-value	0.0001	0.0001

Lemon on *Propionibacterium acnes* and *Staphylococcus epidermidis* of the (MIC), the results indicated as Lemon increases observed concentration the broth less turbid becoming an absence of growth was. Also it showed the (MIC) of cleanser on *Propionibacterium acnes*.

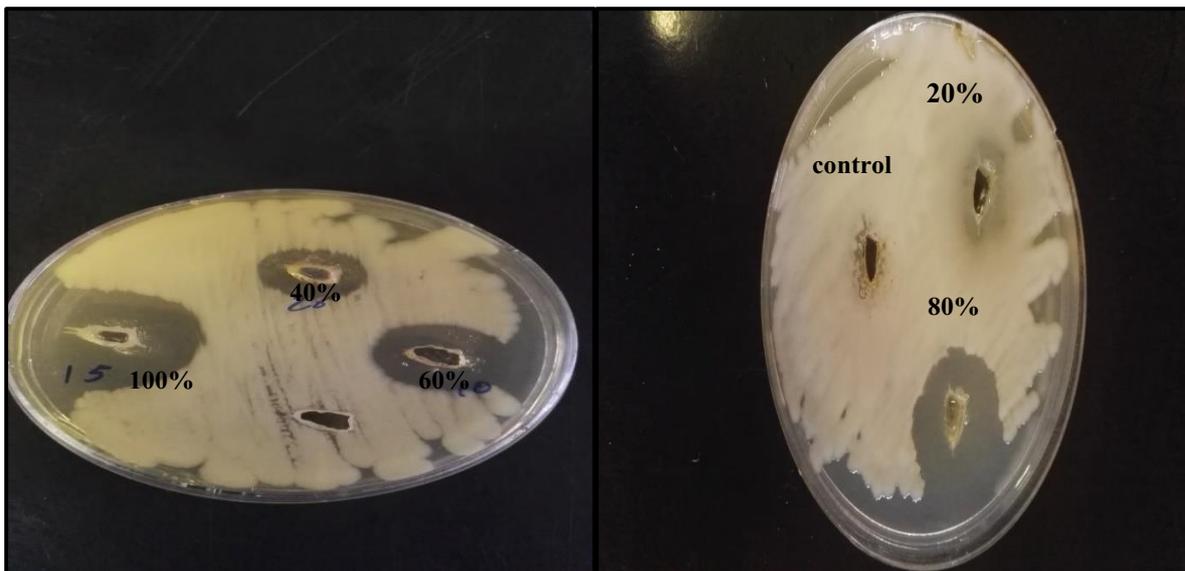


Figure 5. Percentage (%)Citrus limon juice of Antibacterial Activity on *Propionibacterium acnes*

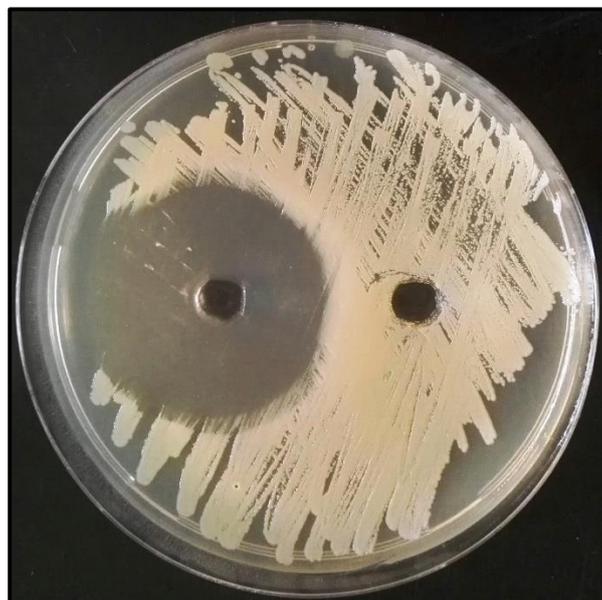


Figure 6. Antibacterial activity of Citrus limon (%) on *Staphylococcus epidermidis*.

4. Discussion

P. fluorescens isolate (1 and 2) as shown inhibited the growth of pathogenic bacteria and this study agrees with the results of the agar spot method, the substance of *P. fluorescens* isolates from wound inhibit the growth of *S. aureus* isolates at 37°C for 24hr [14]. Bacteriocin is released action ability of a lysis protein from the cell [15] The research showed the (Lemon juice) is not only an astringent but it is also a very good antimicrobial agent against *P. acnes*, *S. epidermidis* with high inhibition zone camper baterocin. This bacteria is not surprising because they are part of the skin (normal flora) on the skin. The study carried out indicated that (Citrus limon juice) is a very active treatment against the organism that causes Acnevulgaris as seen from the sensitivity test results which showed that Lemon was very effective at all concentrations used in the study (20%,40%,60%,80% and 100%). A similar study conducted by [16] Citrus limon peel and juice extract works as a detoxifying and as an antibacterial agent activity against bacteria also, *P. cnes* inclusive because it contains the citrus acid main that acnes.Acne scars or even marks are also effectively cured with the help of *Citrus limon* juice. Juice can also be very effective in treating Acne when taken orally, apart from treating Acnes when taken orally, it helps to eliminate acid waste from the body, cures constipation, improves digestion process and reduces infections [15]. Cleanser used in the study was also found to be effective, but not as effective as juice. Cleanser was only effective as its concentrations were increased (60%, 80% and 100%).

When limon citrus is applicatively to the skin, it's known to excess oil remove and get rid helps of cells dead [17]. Juice citrus that is very rich in vitamin c also contains acid citric an acid, the citric acid acts to husk the skin a very important treating step in Acne vulgaris [18]. Works as study a detoxifying *Citrus limon* as agent an anti-bacterial. Also effectively cured of juice with the help Acne scars or even marks are juice of citrus limon can also be consumedly in treating Acne effective when orally taken, apart Acnes from treating when taken orally, it helps to acid waste eliminate from the body, treatment constipation, uptrend process digestion and infections reduces [19]. The result agrees with [20] shown juice and extracts from lemon had good antibacterial effect against the two *staphylococci* tested and on *Klebsiella pneumoniae*.

In experiments juice natural had good antibacterial activity against (gram positive) bacteria the natural components against acne-inducing bacteria refer to medicinal plants. The antimicrobial effect of commercial and wild fruits was investigated against these bacteria. Their inhibitory potential on bacterial growth may be utilized in the development of natural drugs or cosmetics to treat acne vulgaris [21].

5. Conclusion

The present study showed that the bacterial specie that causes Acne vulgaris (Pimples), identified as *P. acnes* and *S. epidermidis* was very sensitive to Citrus limon more than crude bacteriocin from *P. fluorescens* so as to the very acidic normalizes of *Citrus limon*.

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