Comparison between aqueous extracts of *Solanum nigrum* and *Allium cepa* on locally isolated bacteria of otitis media

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Abstract

Thirty nine bacterial species were identified among forty one samples that were collected from patients suffering from otitis media. These species were *Pseudomonas aeruginosa*, *Staphyllococcus aureus*, *Proteus mirabilis, Klebsiella pneumonia* and *E. coli*. Antibiotic sensitivity test was performed to these isolates. Then the antibacterial activity of two plant aqueous extracts was performed. The extracts were from *Solanum nigrum* (black nightshade) and *Allium cepa* (onion). Both extracts affect negatively on the growth of selected bacterial isolates but at different levels and at different concentrations. The aqueous extract of black night shade predominate at onion extract especially at concentration 1000mg/ml at $P<0.05$. *S. aureus* was the most affected bacteria especially with black nightshade while *P. mirabilis* was the least. *Klebsiella pneumonia*, *P. aeruginosa* and *E.coli* came as second , third and fourth respectively according to their sensitivity to black nightshade extract.

Introduction

Otitis media is the infection associated with the malfunctioning of the middle ear due to pathogenic micro-organisms that are resident in the middle ear [1]. In otitis media, the middle ear is usually affected due to colonization by pathogenic organisms. The damage causes the deficiency in hearing [2]. Sources of infection in otitis media are dependent on the route by which infection reaches the middle ear and the chief route by which this occurs is the Eustachian tube [3]. Otitis media varies in complication depending on the level of severity and duration of the infection in relationship to the associated microorganism. There are 2 types of otitis media, of which each is subdivided based on the level of complication chronic suppurative otitis media (CSOM) and acute otitis media (AOM) [4]. The organism often isolated in cases of acute otitis media as causative organisms are: *Haemolytic,Streptococci*, *Staphylococci*, *Heamophilus* or *Pneunococci* while gram negative bacilli are commonly associated with

chronic otitis media particularly *Pseudomonas* and *Proteus sp* [5 and 6]. *Solanum nigrum* (Black nightshade) and *Allium cepa* (Onion) have been widely reported to show their antimicrobial activities against Gram negative and Gram positive bacteria [7, 8, 9, and 10]. *Solanum nigrum* contains different active constituents to which the antibacterial activity is related. These includes saponines, flavones, alkaloids and glycosides [11 and 12]. While *Allium cepa* contains many sulfur compounds like S-methyl –Cysteine Sulfoxide, tran –s- (1-propenyl) cystein sulfoxide and propylcysteine sulfoxide -Sin addition to flavones, sterols, saponines, glycosides and little volatile oils [13]. This study aims to compare between the antibacterial activity of both plants concerning their aqueous extracts.

Materials and Methods

Bacterial Sample collection:

Forty-one samples were taken from patients with otitis media from Madinat El-Tib hospital who were never took any antibiotics for at least one week before taking the swab at period from March till June 2011 using a sterile cotton swab. These samples were put in a sterile test tubes contained in a plastic bag.

Isolation and characterization of bacterial isolates:

Samples were plated on blood agar and MacConkey agar, then incubated at 37˚C for 24h. Morphological characteristics including colonial morphology and cultural characteristics were performed followed by biochemical tests for identification of bacterial isolates. Biochemical tests includes Catalase, Oxidase, Indole Production, Vogas-Proskaur, Citrate utilization, Coagulase, Methyl Red test, Urease, TSI test, Phenylalanine deaminase test, Motility test, ability to grow at 4˚C and 42˚C, Growth on EMB and Growth on Mannitol- Salt agar [14, 15, 16, 17, 18 and 19].

Antibiotic sensitivity test:

100µl of bacterial suspension was streaked onto a nutrient agar plate using a sterile cotton swab. After 10-15minutes, antibiotic disks were put onto the agar plates about 5 disks/plate using a sterile forceps then incubated at 37˚C for 24 hours. The diameters of inhibition zones were measured and bacteria was determined as sensitive or resistant according to the standard measurements of [20].

Preparation of McFarland Turbidity Stranded Solution:

Prepared according to [18] as following: solution (A): 1.175 gm of Barium chloride ($BaCl_2$) was dissolved in 100 ml of distilled water, solution (B): 1 ml of concentrated sulphoric acid (H_2SO_4) was added to 100 ml of distilled water. 0.05ml of solution (A) was added to 9.95 ml of solution (B) in a test tube

and mixed well, then sealed to prevent evaporation and kept in dark till use which gives an estimation of bacterial cell number of 1.5×10^8 cell/ml.

Plant Sample collection:

Black Nightshade were taken from local pharmaceuticals while Onion from local groceries in Baghdad.

Preparation of the powder plant:

Dried plant parts were milled using electric grinder, keeping the powder dry vegetation in containers in the refrigerator degree (4˚C) until use.

Preparation of the plant extracts:

This was performed according to the procedure mentioned by [21]. Plant powder weighted to 10 gm was put in a flask, then 100 ml of distilled water was added to the flask and incubated in shaking incubator at 37˚C for 24 hr. Mixture was filtered using cotton, then filtrate was centrifuged at 5000 rpm for 10 minutes and suspension was filtered using filter paper (Whattman No.1), after that the filtrate was evaporated in an incubator at 37˚C to get the dry powder of the extract, then the powder was put in a dark sealed tube and kept at 4˚C in a refrigerator till use.

Estimation the pH of the plant extract:

10 gm of plant powder was mixed with 50 ml of distilled water with continuous shaking, then stirred using magnetic stirrer for 10 minutes. After that solution was filtered using filter paper of Whattman No.1, then PH was determined using litmus paper and PH meter [22].

Testing the antimicrobial activity of the plant extracts:

Preparation of serial dilutions:

Serial dilutions of the dry aqueous extracts of the two plant were prepared using sterile D.W and as follows(100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000)mg/ml.

Preparation of bacterial suspension:

Isolated bacterial species were cultured on nutrient agar plates for 24hours. By using loop, single culture from each species were picked up and added to sufficient amount of physiological salt solution and compared with McFarland turbidity stranded solution which gives a cell density nearly 1.5×10^8 cell/ml.

Performing the antibacterial activity test:

Using a sterile swab, 0.1ml of bacterial suspension was spread over Mueller-Hinton agar plates and let to dry for 5 minutes. Using cork borer, an 8mm wells were made in the culture and filled with 0.1ml of previously prepared dilutions of the extracts and one well filled with D.W as a control, then incubated at 37˚C for 24hours.

Activity of each concentration was determined by measuring the diameter of inhibition zone around the well [23].

Results and discussion

Bacterial isolation and identification:

Among forty-one samples collected, thirty-nine bacterial species were isolated and identified according to morphological and biochemical tests. **Table 1** lists all these species with their percentage of dominance.

Table 1: Bacterial isolates responsible for otitis media and it's percentage

Isolates	No. of isolates	% of isolates
P. aeruginosa	18	46
S. aureus	10	26
Proteus mirabilis	5	13
Klebsiella pneumonia	4	10
E. coli	2	5
Total	39	100

According to this table, *Pseudomonas aeruginosa* was the most frequent among other bacterial species causing otitis media, and that may be due to its presence in the external ear and spread into the interior of the middle ear through a hole in the tympanic membrane [14] in addition to its highly resistance to a wide variety of antibiotics and antiseptics [24]. These results agreed with [25 and 26].

Followed by *P. aeruginosa*, *Staphyllococcus aureus* which came next according to frequency of occurrence in otitis media. This due to its ability to develop antibiotic resistant strains and production of ß-lactamase enzymes, in addition to its ability to invade middle ear through the external acoustic meatus [27]. *Proteus* came in the third class followed by *Klebsiella*, while *E.coli* was the least among the isolates. This study agreed with [28, 29 and 30] whose referred to *P. aeruginosa* as the most frequent followed by *Staphyllococcus aureus* then *Proteus.*

Antibiotic sensitivity test:

Strains has been subjected to sensitivity test using disk diffusion method. According to **table 2**, Gentamicin, Ampicillin & Imipenem were the most effective antibiotics that they didn't show any bacterial resistance towards them, while Tetracyclin was effective against 2 isolates only (*P.aeruginosa&P. mirabilis*). Also we can see from table that *P.aeruginosa&P. mirabilis* were the most sensitive strains with the selected antibiotics.

Table 2: Antibiotic sensitivity test

	Bacterial isolates and zone of inhibition (mm)				
Antibiotic Disc	*P. aeruginosa*	*S. aureus*	*Proteus*	*Klebsiella*	*E. coli*
Streptomycin	6	-	-	6	3
Erythromycin	7	-	5	-	5
Gentamycin	5	5	4	4	5
Tetracyclin	6	-	6	-	-
Chloramphenicol	4	7	6	-	-
Ampicillin	7	8	3	7	7
Ciproflaxin	7	7	6	6	-
Imipenem	8	7	7	7	6
Nitrofurantoin	5	-	5	-	4

The antibiotic activity of both Ampicillin & Imipenem is due to their being a broad spectrum antibiotics effective against G+ve and G-ve bacteria, while Gentamicin, which belongs to Aminoglycosides, interrupt protein synthesis by inhibiting ribosomal function. [31].

Estimation the PH of the plant extract:

PH of the aqueous extracts of both plants was measured. For black nightshade aqueous extracts the value of PH was (6.1), while for onion aqueous extracts the value of PH was (4.6).

Antibacterial activity of aqueous extracts of black nightshade and onion:

As shown in figures (1,2,3,4 and 5), there is a negative effect on the growth of bacterial species under study (*P. aeruginosa, S. aureus, Klebsiella pneumonia, E.coli and Proteus mirabilis*) through the significant increase in zone of inhibition by increasing in the concentration of the aqueous extracts of both black nightshade and onion (except in *Klebsiellapneumonia* and *Proteus mirabilis* in onion extracts). The concentration 1000mg/ml for black nightshade gave the best value compared with other concentrations (50, 100, 150, 250, 500 and 750 mg/ml) and differed significantly with these concentrations which gave inhibition zones for above bacterial species (31.85, 40.25, 31.88, 31.9 and 31.7 mm) respectively. Also for onion extract, the concentration 1000mg/ml gave it's best value compared with other concentrations and differed significantly with these concentrations that the zones of inhibition for (*P. aeruginosa, S. aureus* and *E. coli*) were (13.88, 15.15 and 13.25 mm) respectively.

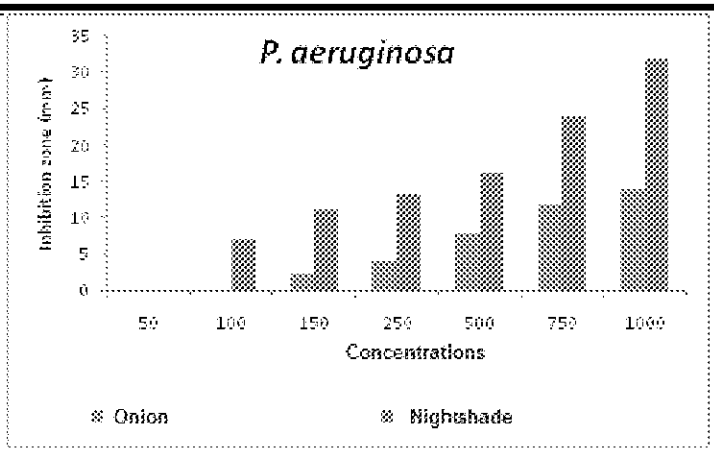

Fig 1 Antibacterial activity of aqueous extracts of black nightshade and onion against *P. aeruginosa*. L.S.D (0.05)=0.4

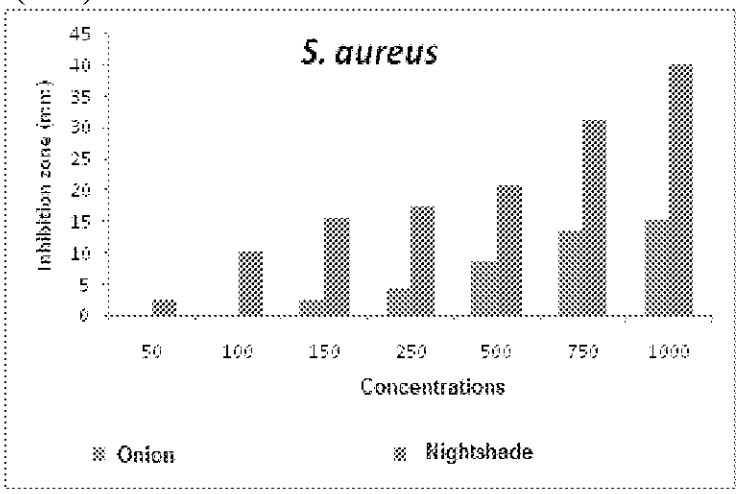

Fig 2 Antibacterial activity of aqueous extracts of black nightshade and onion against *S. aureus*. L.S.D (0.05)=0.27

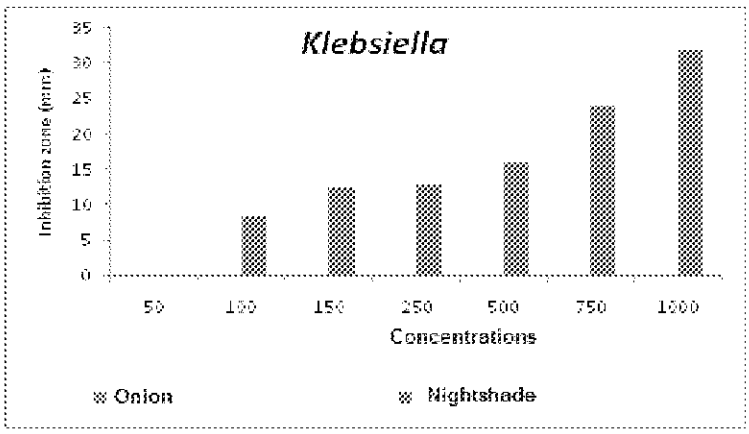

Fig 3 Antibacterial activity of aqueous extracts of black nightshade and onion against *K. pneumonia*. L.S.D (0.05)=0.13

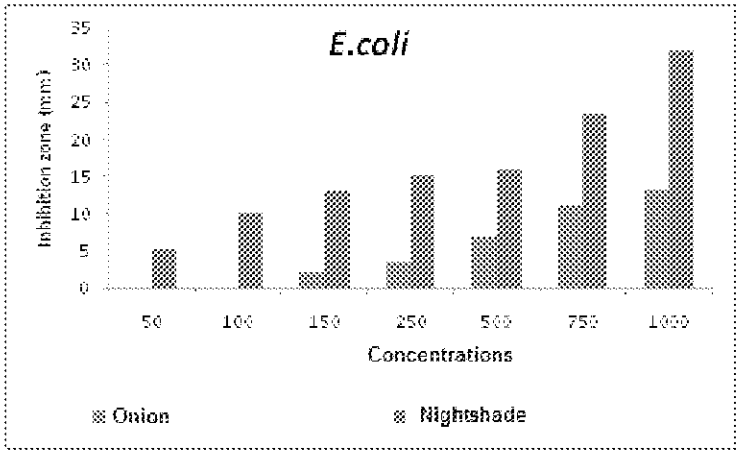

Fig 4 Antibacterial activity of aqueous extracts of black nightshade and onion against *E. coli.* L.S.D (0.05)=0.21

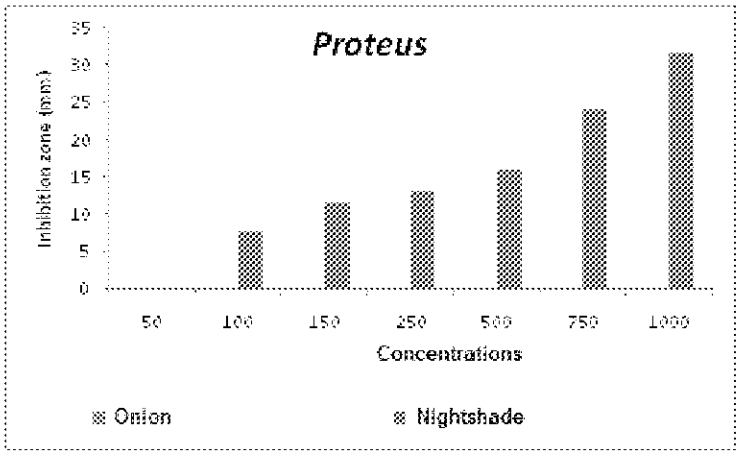

Fig 5 Antibacterial activity of aqueous extracts of black nightshade and onion against *P. mirabilis.* L.S.D (0.05)=0.12

Also it can be noticed from above figures the predomination of black nightshade extract over the onion extract specially at concentration 1000 mg/ml that the significant increase for black nightshade extract compared with onion extract for above concentrations in *P. aeruginosa* (129.47%), *S. aureus* (165.68%) and for *E.coli* (140.75%). It can be inferred from that the *S. aureus* is the most affected bacteria with the extracts specially black nightshade extract while the opposite can be seen in *Proteus mirabilis* which gave the least inhibition zone. On the other side, *Klebsiella pneumonia*, *P. aeruginosa* and *E.coli* came as second , third and fourth respectively according to their effectiveness by black nightshade extract.

Solanum nigrum contains many active constituents like alkaloids to which the antibacterial activity may be attributed to it [32 and 33] also it contains phenols which can form complexes with sulphydryl group leading to damage of bacterial cell [33 and 34]. Phenols also act on oxidation of phosphopeptide layer in the cytoplasmic membrane causing increase in permeability and defects in bacterial enzymes [35].

On the other hand. Antibacterial activity of *Allium cepa* (onion) attributed to its content of active constituents like alkaloids, phenols, allicin and flavones. Alkaloids act through penetration into cell membrane inside the cell and interacts with DNA, while flavones act through it's conjugation with bacterial adhesions at the bacterial cell surface and form complex with bacterial cell wall [36].

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مقارنة بين تأثير المستخلصات المائية لنباتي عنب الذيب والبصل على بكتريا التهاب الاذن الوسطى المعزولة محليا

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الخلاصة

تم تشخيص 39 نوع بكتيري من بين 41 عينة تم جمعها من مرضى يعانون من التهاب الاذن الوسطى. الانواع البكتيرية كانت *Pseudomonas aeruginosa*، *Staphyllococcus aureus*، *Proteus mirabilis*، *Klebsiella pneumonia* و *E. coli*. تم اجراء اختبار الحساسية للمضادات الحيوية لهذه العزلات. بعد ذلك تم اجراء اختبار الفعالية الضد ميكروبية للمستخلصات المائية لنباتين. كانت المستخلصات تابعة لكل من نبات *Solanum nigrum* (عنب الذيب) ونبات *Allium cepa*(البصل). اثرت المستخلصات المائية لكلا النباتين تأثيرا سلبيا على نمو العزلات البكتيرية ولكن على مستويات مختلفة وبتراكيز مختلفة. كانت المستخلصات المائية لنبات عنب الذيب اكثر فعالية من مستخلصات نبات البصل وبخاصة في التركيز 1000ملغم/مل وبمستوى احتمالية P<0.05. كانت *S. aureus* الاكثر تأثرا بالتثبيط وبخاصة من مستخلص عنب الذيب بينما *P. mirabilis* كانت الاقل تأثرا. جاءت بالمرتبة الثالثة من ناحية التثبيط بكتريا *Klebsiella pneumonia* تليها *P. aeruginosa* ثم *E.coli*.