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Determination The Inhibitory Effect of the truffle Terfezia sp crude Extract against some types of bacterial species causes Eye infections

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Abstract

150 samples had been collected from eye patients with ages ranged between 1 month to 80 years of both genders. The patients were clients of the consultant clinic of ophthalmology Salahuddin public Hospital for the duration between October 2016 to February 2017. The results of the isolated bacteria were divided into two section: the Gram positive bacteria while represented the highest ratio in a matter of 78 isolates that represent (82%), whereas the number of the Gram negative bacteria reached 22 isolates which represents (18%). The inhibitory effect of the aqueous and spirituous extract of Terfezia sp. Was tested against the bacterial isolates that proved high resistance against antibiotics. Three different concentrations (750,1000 and1500 mg / ml) were used, and The results proved the effectiveness of the aqueous extract against most isolates with inhibitory diameter ranged between (30-9) mm, whereas the spirituous extract proved inhibitive effectiveness against three Staph. Saprophyticus isolates only with inhibition diameters of (28-8 mm).

تحديد الفعالية التثبيطية لمستخلص الكمأ Terfezia sp. تجاه بعض الانواع البكتيرية المسببة لالتهابات العيون

صفا ليث صالح مهدي محمد نظير معروف

الخلاصة

شملت الدراسة جمع 150 عينة من مرضى بأعمار مختلفة من 1 شهر الى 80سنه ومن كلا الجنسين (ذكور واناث) المراجعين الى العيادة الاستشارية لطب وجراحه العيون في مستشفى صلاح الدين العام للمدة مابين شهر تشرين الثاني 2016 الى شهر كانون الثاني 2017 ، وقد قسمت نتائج البكتريا المعزولة الى قسمين, مجموعة البكتريا الموجبة لصبغة كرام وقد كانت النسبة الاكبر بواقع78 عزلة بنسبة 82% بينما بلغ عدد البكتريا السالبة لصبغة كرام 22 عزلة بنسبة 18% تم اختبار الفعالية التثبيطية للمستخلص المائي والكحولي لفطر الكمأ الاحمر Terfezia claveryi تجاه العزلات البكتيرية التي اظهرت مقاومة عالية للمضادات الحيوية اذ تم أستخدام ثلاث تراكيز مختلفة (750,1000,1500) ملغم/مل، وأظهرت النتائج فعالية المستخلص المائي تجاه معظم العزلات بأقطار تثبيطيه تراوحت بين(9-30) ملم بينما اظهر المستخلص الكحولي فعالية تثبيطية تجاه ثلاث عزلات فقط للنوع Staph.saprophyticus بأقطار تثبيطية تراوحت بين(9-28) ملم .

Introduction

Eye is liable for various diseases which can lead to blindness like diabetes mellitus, viral infection, exposure to toxic material s repeated wear of contact lenses (1) and fungal infection which are very serious infection and can lead to blindness if not treated urgently(2,3). Conjunctivitis or red eye is inflammation of conjunctiva (4) is the most common infection of the eye and is often caused by a virus as a complication of upper respiratory tract infection or by bacteria which is associated with pus secretion or allergic conjunctivitis(5,6). One of the serious injuries of the eye is corneal ulceration. Which may be caused by repeated use contact lenses, contact lenses serves as a medium for bacterial colonization which lead to keratitis and corneal ulceration(7). Desert truffles are a rich source of proteins, amino acid, fatty acid ,minerals and carbohydrates (8). Searching for new therapeutic alternatives, in modern medicine, truffles are considered a large source of therapeutic compounds antiflammatory, immunosuppressor, antimutagenic nticarcinogenic antimicrobial properties and antioxidant properties (9,10). Desert truffle have been used as traditional medicine in Arabia for over two millennia without known toxic harmful effect to its users. Truffle water extract is highly recommended by Bedouins for the treatment of the most common eye diseases. This practice developed the following recommendations of the prophet Mohammad (peace be upon him)whom was reported to have said ‘‘Truffle are from man and their water is a cure for the eye’’(11).

Aim of prescent study

1-isolation and identification of the most important bacterial species that cause eye infection from patients were clients of the consultant clinic of ophthalmology Salahuddin public Hospital

2-study the antibiotic sensitivity against 13 type of antibiotic

3-determination of the inhibitory effect of the aqueous and spirituous extract of Terfezia sp. against the bacterial isolates that proved high resistance against antibiotics

Materials and Mthods of work

Collection of Samples

150 samples had been collected from eye patients with ages ranged between 1 month to 80 years of both genders. The patients were clients of the consultant clinic of ophthalmology Salahuddin public Hospital for the duration between October 2016 to February 2017, The samples were taken from infected eye by a cotton swab sterile and moisturized with normal slain . Information relating to age, previous infections of the eye and antibiotics were recorded.

Insulation and Diagnosis

The bacteria were diagnosed using the blood agar, Macconkey agar, Mannitol salt agar, depending on its Cultural and morphological characteristic and depending on biochemical tests applied and a group of biochemical tests(12). Then, the diagnosis was confirmed using the Vietick 2compact system.

Antibiotics Sensitivity Test Using Disc Diffusion Method

The antibiotic sensitivity test was performed on Agar Muller Hinton Medium using 13 types of antibiotic discs (Co-trimoxazole, Norfloxacin Ceftazidime, Cefepime ,Cefotaxime, Chloramphenicol, Pipraciline, Imipeneme, Nitrofuration, Nitilmicin Amikacin, Tobramycin, Novobiocin). The bacterium genus take one colony to 5 ml from the nutrient agar and incubated at 37 °C for 24 hours. The growth curve was contrasted with the previously recorded standard McFarland solution, (^{8}10 x 1.5) CFU / ml, and then spread 0.1 ml of the bacterial cultured above in Agar Muller Hinton Medium by a sterile glass diffuser and then leave to dry at room its temperature for 10-15 minutes, force the (Forceps) into the dishes by 6 tablets for each dish and then incubate dishes at 37 °C for 24 hours, the diameter measuring Damping areas (including disk diameter) and divided the isolates into 3 categories are sensitive and medium sensitivity and resistance based on global measurements of National Committee for Clinical Laboratory Standards (NCCLS) (13,14).

Collection and preparation of truffle

Truffle *terfezia* sp. were obtained from a local store, Salahuddin - Iraq in March 2017. The samples were then brought to the laboratory of microbiology in the College of Education for Pure Sciences at Tikrit University in the laboratory samples were washed carefully with running water ,blotted on kitchen sucking paper and cut into small pieces. Samples were then dried in hot oven at 35°C pulverized using a mechanical grinder. The obtained powders were stored in clean and dry airtight containers for further studies.

Preparation of truffle extract

Aqueous extraction

Aqueous extraction of truffle were obtained as follows (15) 100g of dry powder from truffle was weight and dissolved in 400ml of sterilized distilled water in a conical flask ,plugged with cotton wool after this had been shaking the solute by using orbital shaker incubator at 40°C for 24 hrs. .then the extract was filtered using whatman filter paper NO1and centrifuged at 1500 rpm for 15 min and the supernatant was evaporated till dryness using lypholizer then stored at 4°C which used for antibacterial testing.

Methanol extract

Methanol extraction of truffle were obtained as follows(16)20 g of dry powder was added to 200 ml of methanol (70%) in a conical flask plugged with cotton wool and kept for 24 hrs., after 24hrs the extract was passed through four layers of cheese cloth to remove the major debris, then evaporated under reduced pressure using Rotary evaporated apparatus and the solute were filtered by Millipore filter unit (0.22µm). the extract was evaporated till dryness using an oven at 40°C then stored at 4°C which used for antibacterial testing.

Test the Inhibitory Effect of the truffle Terfezia sp. crude Extract against some types of multi resistant bacterial species

Antibacterial activity of the truffle *Terfezia* sp. crude extract against some types of multi-resistant bacterial species causes Eye infections at concentrations (750,1000 and1500 mg / ml) was determined in accordance with agar–well diffusion. The bacterial isolates were first grown in a nutrient broth for 18 hrs. before use and standardized to 0.5 McFarland standards (107 c.f.u.ml-1). Hundred microliter of the standardized cell suspensions were spread on a Mueller-Hinton agar (MHA) by using sterilized cotton swabs (17). Then wells at the distance of approximately 3 cm were made by using sterile 6 mm diameter cork borer.200 µl of test extract (750,1000 and1500 mg / ml), was poured into each well. Before incubation all Petri dishes were kept at 4°C for 4 hrs.

then incubated at 37°C for 24 hrs. Antibacterial activity was evaluated by measuring the diameter of the zones of inhibition (ZOI)against the tested bacteria.

Results and Discussion

Isolation and Identification

The current study included collected of 150 samples from eye patients with ages ranged between 1 month to 80 years of both genders. The patients were clients of the consultant clinic of ophthalmology Salahuddin public Hospital for the duration between October 2016 to February 2017. These samples were cultured on the blood medium in the middle of the Blood ager, Macconkey agar, Mannitol salt agar medium. The bacterial cultivation results showed that a hundred samples that represent 66.66% of the studied samples gave positive bacterial growth, whereas 50 samples represent 33.33% of the studied samples gave a negative growth results as describe in table (1). The results of the isolated bacteria were divided into two section: the Gram positive bacteria group with represented the highest ratio in a matter of 78 isolates that represent (82%) as describe in table (2), whereas the number of the Gram negative bacteria reached 22 isolates which represents 18%. Bacterial not growth may be viral or fungal or anaerobic bacteria that cannot be isolated by conventional transplant methods used in this study, which may require special cultured and development, or because infected patient use doses of antibiotics or may be due to differences in nature and size samples taken as well as variations in grading Heat and humidity (14).

Table(1):- Initial isolation of Samples and Percentages

Results of Transplantation	Number	Percentages
Pathogenic bacteria	100	66.66%
Not Pathogenic bacteria	50	33.33%
Total Number	150	100

Identification

Isolates were diagnosed microscopically and macroscopically based on microscopic and visual tests and then further confirmed using Vitek2 Compact System. The results of the diagnosis showed differences in the types, numbers and proportions of isolated bacteria.

Table(2):-The percentage of gram positive and gram negative bacteria

Group of bacteria	Number	Percentage
Gram positive	78	82
gram negative	22	18
Total number	100	100%

The diagnosis results showed that the bacterium *Staph. aureus* take the highest ratio of isolates represented by 46 isolates (equivalent to 46%) followed by *Staph.*

saprophyticus with18 isolates which represents(18%), *Enterobacter cloacae complex* with 6 isolates (6) , *ps.stutzeri* with 5 isolates (5%), *Staph. haemolyticus* with 4 isolates 4%, *Strep.pyogen*es with 3 isolates (3%), *E.coli, Enterobacter cloacae ssp dissolvens, Ps. aeroginosa* with 2 isolates (2%), while *Enterococcus cassliflvus, Alliococcus otitis, Enterococcus columbae, Staph. xylosus, Ps. fluorescens, Aeromonas. salmonicida, Staph. lentus, P. mirabilis, Ps. putida, Strep. gordonii, Aeromonas sobria* and *Staph. kloosii* with one isolate (1%) respectively as describe in table(3).

Table(3):- NO. and percentage of isolated bacteria

Bacterial Isolates	Number of Isolates	percentage For total isolation*%	Percentage For qualitative isolation**%
Staphylococcus. Aureus	46	30.66	46
Staphylococcus. saprophyticus	18	12	18
Enterobacter cloacae complex	6	4	6
Pseudomonas .stutzeri	5	3.33	5
Staphylococcus .haemolyticus	4	2.66	4
Streptococcus. Pogenes	3	2	3
Enterobacter cloacae ssp dissolvens	2	1.33	2
Escherichia. Coli	2	1.33	2
Psedomonas .aeruginosa	2	1.33	2
Alliococcus .otitis	1	0.66	1
Enterococcus .cassliflvus	1	0.66	1
staphylococcus .xylosus	1	0.66	1
Enterococcus. Columbae	1	0.66	1
Aeromonas salmonicida	1	0.66	1
Pseudomonas. Fluorescens	1	0.66	1
Proteus .mirabilis	1	0.66	1
Staphylococcus .lentus	1	0.66	1
Aeromonas .sobria	1	0.66	1
Psedomonas. Putida	1	0.66	1
Streptococcus .gordonii	1	0.66	1
Staphylococcus. Kloosii	1	0.66	1
Total NO.	100	66.56%	100%

* Percentage of total isolation = number of isolates / total number of samples.
** Percentage of qualitative isolation = number of isolates / total number of isolates.

The inhibitive effect of the aqueous and spirituous extract of Terfezia sp. against the bacterial isolates that proved high resistance against antibiotics

The result of the test proved the inhibitory effectiveness of the three concentrations (750,1000 and 1500 mg / ml) aqueous and spirituous extract of *Terfezia* sp. against 25 isolates of gram negative and gram positive bacteria that proved high resistance against antibiotics. The inhibitive effect of the aqueous extract increase with the increment of concentration. The highest inhibition rate at concentration 750 mg / ml for *Staph.haemolyticus* with inhibition diameter of 25 mm and lesser inhibition diameter for *Enterobacter cloacae complex*(10mm).While the inhibitory effectiveness of the aqueous

extract was increased at concentration 1500 mg/ml against the bacterial *Staph.haemolyticus* with inhibition diameter of 30 mm and the least inhibition diameter *Enterobacter cloacae complex*(12mm). The results proved the effectiveness of the aqueous extract against most gram negative and gram positive isolates. The positive bacterial species were more sensitive to the extract with inhibitory diameter ranged between (30 -17)mm at the concentration of 1500 mg / ml as a figure (1), compared with the gram negative bacteria whose inhibitory diameter ranged between (28-12) at the concentration of 1500 mg / ml as a figure(2). This may be due to the fact that the membrane of Gram-positive bacteria is more permeable than the membrane of Grams negative bacteria, which is composed of A homogeneous, thick layer of peptidoglycane containing a low-density lipid. While the membrane of the Gram-negative bacteria is composed of slime layer of peptidoglycane covered with an outer membrane containing lipopolysaccharides, protein, lipopolysaccharides and high amounts of phospholipids. Therefore, the permeability of the entry and interaction of the most antimicrobial agents or microbial agents cross the cell envelope is highly effective in the Gram positive bacteria, and the active substance dissolved in the water is highly capable of penetrating the bacterial cell wall(18). The spirituous extract proved inhibitive effectiveness against three *Staph.saprophyticus* isolates only with inhibition diameters of (28-8 mm) as a figure (4). This may be due to that the active substances extracted have been more interacting and affected by water and this means that most of the active substances in truffle were more soluble in water compared to alcohol. Previous studies(19,9) showed that both aqueous and methanolic extract from *Terfezia* sp. have a significant antibacterial activity against *Staph. aureus* and *pseudomonas. aeroginosa* the authors assumed that the anti-bacterial agent may be a peptide having in view that they noticed as active a protein fraction obtained by ammonium sulphate precipitation and purified by gel filtration and ion exchange chromatography. The observation that only the permeate obtained by ultrafiltration showed antimicrobial activity suggest a molecular weight of the active extract components less than 10kDa. other Previous studies(20)showed the aqueous extract of the truffle *trefezia* sp. contains a potent antibacterial agent with a molecular weight less than 10 which may be used in the treatment of eye infection caused by *Staph. aureus, E.coli staph.epidermidis*, therapy helping to reduce the use of chemically synthesized antibiotics and the development of drug resistance bacteria. Other Previous studies(21)showed the therapeutic effect of *Terfezia* sp. in healing of corneal ulcer they concluded that aqueous extract of *Terfezia* sp. has no toxic effects on biological parameters moreover they obtained good antibacterial activity at 1.5%to3%as observed through the healing of the induced corneal ulcer in rabbits eye. The supernatant crude extract of *Trefezia* may work by different modes of action like antioxidant, antiradical and antimicrobial activities. The reasons for this inhibitory effect against studied bacteria may be due to the effective content in truffle, which includes various effective compounds of organic acids and phenolic compounds such as gallic acid, fumaric acid, ferulic acid, vanillin, coumarin, alligic acid,trans-2-dhiydrox coumarin, catechin hydrate, 2,4-dihydroxy, benzoic acid, Ellagic acid(22). In addition to the content of flavonoids such as In addition to the content of flavonoids such as myricetin, kaempferol, naringin, n aringenin, resveratrol and Saponins (23,24)Which is a candidate the truffle as a source in the medical and pharmaceutical fields. In addition to its nutritional value rich in proteins, carbohydrates, saturated and unsaturated fatty acids, sugars such as Glucose, Inositol, Trehalose Sorbitol Sorbitol, Minerals, Vitamins(25,24).

Table(4): The inhibitive effect of the aqueous and spirituous extract of *Terfezia* sp. against the bacterial isolates from eye infection

Bacterial Isolates		Aqueous extract of *Terfezia* sp. Concentrations mg/ml			spirituous extract of *Terfezia* sp. Concentrations mg/ml		
		750	1000	1500	750	1000	1500
1	***Staphyloccocus saprophyticus***	20	26	28	12	15	20
2	***Staphyloccocus saprophyticus***	20	23	24	9	11	14
3	***Staphyloccocus saprophyticus***	15	17	20	-	-	-
4	***Staphyloccocus saprophyticus***	14	16	19	-	-	-
5	***Staphyloccocus saprophyticus***	13	16	20	20	25	28
6	***Staphyloccocus saprophyticus***	22	25	29	-	-	-
7	***Staphylococcus aureus***	19	20	22	-	-	-
8	***Staphylococcus aureus***	20	22	25	-	-	-
9	***Staphylococcus aureus***	12	14	17	-	-	-
10	***Staphylococcus aureus***	25	28	30	-	-	-
11	***Staphylococcu haemolyticus***	22	25	28	-	-	-
12	***Aeromonas salmonicida***	-	-	-	-	-	-
13	***Aeromonas sobria***	11	12	13	-	-	-
14	***Pseudomonas putida***	14	19	24	-	-	-
15	***Pseudomonas stutzeri***	19	22	28	-	-	-
16	***Pseudomonas fluorescens***	16	19	25	-	-	-
17	***Pseudomonas aeroginosa***	16	18	22	-	-	-
18	***Proteus mirabilis***	14	16	19	-	-	-
19	***Enterobacter cloacae complex***	19	23	25	-	-	-
20	***Enterobacter cloacae complex***	10	11	12	-		
21	***Enterobacter cloacae complex***	9	10	12	-	-	-
22	***Enterobacter cloacae complex***	14	15	17	-	-	-
23	***Enterobacter cloacae ssp dissolves***	11	13	15	-	-	-

24	***Enterobacter cloacae ssp dissolves***	-	-	-	-	-	-
25	***Escherichia coli***	15	19	22	-	-	

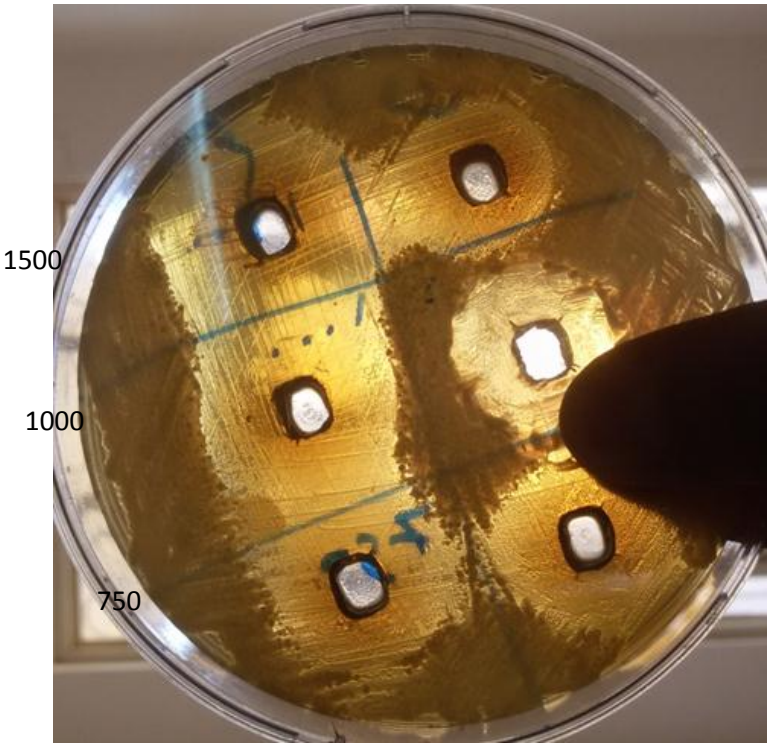

Fig.(1):- The inhibitive effect of three different concentration (750,1000 and 1500mg/ml) of the aqueous extract of *Terfezia* sp. against *Staph.aureus* isolate

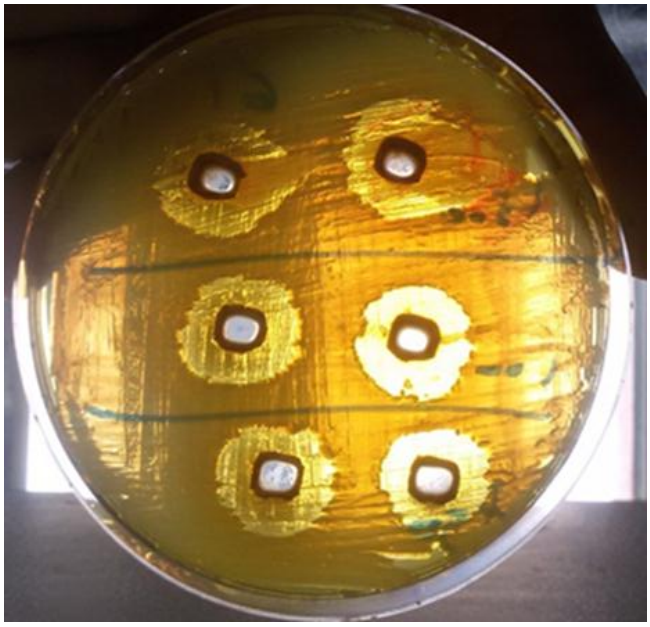

Fig.(2):- The inhibitive effect of three different concentration (750,1000 and 1500mg/ml) of the aqueous extract of *Terfezia* sp. against *Pseudomonas stutzeri* isolate

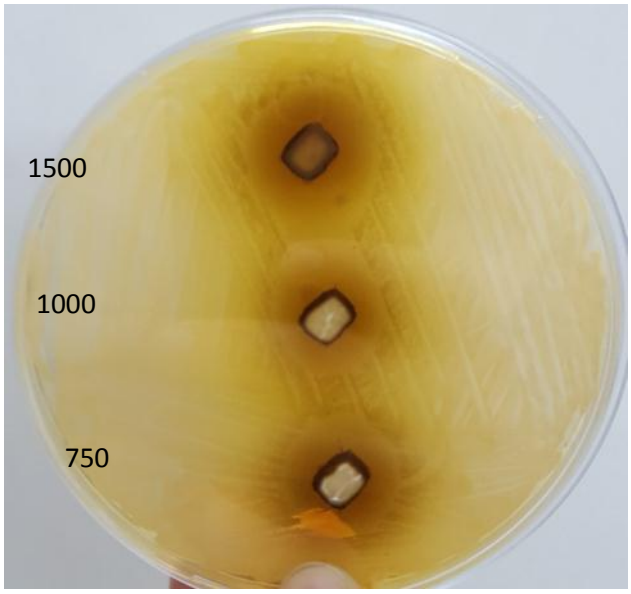

Fig.(3):- The inhibitive effect of three different consentration (750,1000 and 1500mg/ml) of the aqueous extract of *Terfezia* sp. against *E.coli* isolate

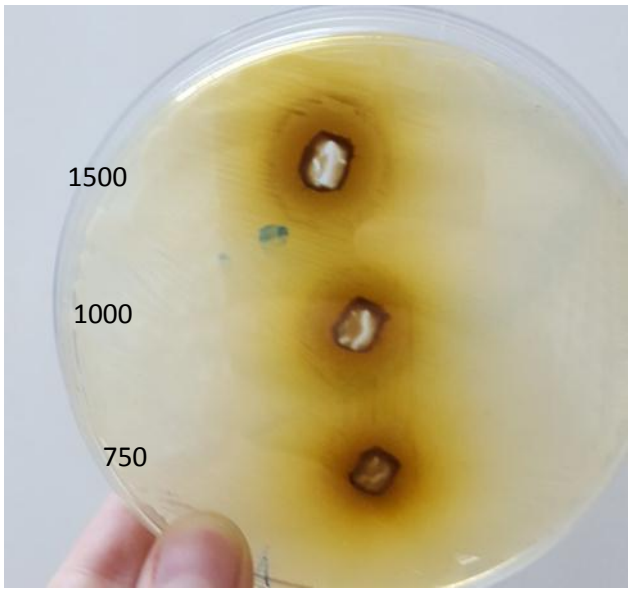

Fig.(4):- The inhibitive effect of three different concentration (750,1000 and 1500mg/ml) of the spirituous extract of *Terfezia* sp. against *Staph.saprophyticus* isolate

References

1. Lemp, M.A.; Bron, A.J. and Foulks, G.N. (2007). The definition and classification of dry eye disease, The ocular surface ISSN: 1542 – 0124; 5 (2) p: 75 – 92.
2. Miller, K.V.; Eisley, K.M.; Shanks, R.M.Q, Lahr, R.M; Lathrop, K.L. kowalski, R..P and Noeckre, R.J. (2012). Recurrent enterococcal endophthalmitis seeded by an intraocular lenses biofim. J. cataract Refract surg. Auther manuscript, 37, (7): 1355 – 1359.
3. Prasad, R. (2008). Fungal infection of the eye – laboratory diagnosis and treatment. Nepal Med. Coll. J. ;10, (1),: 48 – 63.
4. Turner, R. and Martron, (2012). Treatment for conjunctivitis, Oxford Radcliffe Hospital, NHS Trust.
5. Oilver G.F.; Wilson, G.A. and Everts, R.J (2009). Acute infection conjunctivitis Evidence and review and mangment adviece for new Zealand practitioners.J.of The New Zealand medical Association, 122, (1298),: 69 – 75.
6. Mallika, p; Asok, T; Faisal , H. Aziz, S; Tan, A. and Intan, G (2008). Neonatal conjunctivitis- areview. Malaysian Family physician; 3, (2),: 77 – 81.
7. Hoddendach JG, Bokhoorn SS, Wubbels R. Vreugdenhil W Van Rooij J, et al (2014). Clinical presentation and morbidity of contact lens associated bacterial keratitis: a retrospective study. Graefes Arch clin Exp ophthamol; 252 : 299 – 306.
8. Bokhary H.A parves S(1993). Chemical composition of desert truffles Terfezia clarergi Journal of food composition and Analysis,; 6: 285 – 293.
9. Janakat. S. AL – Fakhiri , S, Sallal, A. (2005). "Evaluation of antinacterial activity of aqueous and methanolic extracts of the truffle *Terfezia clavreyi* against *pseudomonas aeruginosa*" . J. Saudi medical joural ;26 (6): 952 – 5.
10. Murcia M.A, Honrubia M, Parras,(2002) Antioxdant activity of edible fungi (truffle and mushrooms): losses during industrial processing. Journal of food protection, 2002: 65: 1614 – 1622.
11. Holt J.G,Krieg N.R,Sneath P.H.A.Staley J.T,Wiliams S.T,1994,Bergs Manuale of determinative bacteriology, Ninth Edition Williams&Wilkins, Baltimor.

12. Abu H. The ajwah dates come from paradise and contain a cure for poison truffles are akind of manna and their juice is amedicine for the eye. Al – Tirmidhi Hadith, No. 1127.
13. National Committee for Clinical Laboratory Standareds (2003). Performa nce Standared for antibiotic susceptibility testing NCCLS. Villanova P.A.
14. National Committee for Clinical Laboratory Standareds (2004). Performa nce Standared for antibiotic susceptibility testing NCCLS. Villanova P.A.
15. Salah,G.A;Elsayed,K.B.and Usama M.A(2013).Antibacterial activities of Thymus vulgaris and Rifampicin resistant Staphylococcus aureus. J.Eco.Health. Env,1(1):19-24
16. Simo,A. A, A. D. Correa, T. C. L. de Carvalho, P. H. S. Cesar, C.H de Moura Oliveira and S.Marcussi,(2015).Pharmaco-toxic characterization of the aqueous extract from Pereskia grandifolia leaves. J. Med. plant; 9:216-222
17. Clinical and Laboratory Standards Institute (CLSI). (2009). *Performance standards for antimicrobial susceptibility testing*, 19th informational supplement, CLSI document M100-S19. Wayne, PA: CLSI; 29(3).
18. Prescott; H.L.M; Harley, J.P. and Klein, D.A. (2007) microbiology 6th ed. Published by MGgraw – ltill companies Inc. PP. 531 – 532.
19. Janakat, S. S.; AL Fakhiri and A.Sallal,(2004).A promising peptide antibiotic from *Terfezia claveryi* aqueous extract against *Staphylococcus aureus* in vitro. phytothery Research; 18(10):810-813.
20. Casarica,A.; Moscovici, M., Daas, M.; Nicu,I.; Panteli, M.; Rasit, I.(2015).Apurified Extract from Brown Truffles of the Species *Terfezia claveryi* Chatin and its Antimicrobial Activity, Faramcia,Vol 64,2.
21. Yousef H. Aldebasi, Wael G.Nouh,Nashwa M. Abdl Atti, Mounir M. SalemBekhit, MazoorA. Qureshi, SalahM. Aly(2012),. Coparative Pathological Studies on the Healing Effect of Natural (*Terfezia claveryi*) and Synthetic (Vigamox) Antimicrobials on Corneal Ulcer in Rabbits J. pharm.Biomed. Sci,2(6)66-77.
22. Cayan,G. T; Deveci, E.; Cayan, F.; Emin, M. (2018) Comparative assessment of phytochemical composition, antioxidant andanticholinesterase activities of two Basidiomycota Truffle Fungi from Turkey; Marmara PharmJ; 22(1):59-65.
23. Bouatia, M., 1Touré, H. A., 2Cheikh, A., 3Eljaoudi, R., 4Rahali, Y., 1Oulad Bouyahya Idrissi, M., 5Khabar, L. and 1Draoui, M.(2018) Analysis of nutrient and ant nutrient content of the truffle *(Tirmania pinoyi)*from Morocco; I.F.R.J; 25(1):174-178.
24. Akyüz, M., Onganer, AN, Erecevit P., Kirbag, S(2012).a. Flavonoid contents and 2,2-diphenyl-1- picrylhydrazyl radical scavenging activity of some edible mushrooms from Turkey: *A. bisporus* and *Pleurotus* spp. Curr. Top. Nutraceut. R. 10, 133-163.
25. Yan, X.; Wang,Y.; Sang, X; Fan, L. (2017). Nutritional value, chemical composition from and antioxidant activity of three *Tuber* specie China; AMB; 7:136.