

Antibacterial activities of black tea alcohol ethanolic extract and some antibiotics against isolates *Staphylococcus aureus* and *Staphylococcus epidermidis* isolated from conjunctivitis.

Sundus Adil Naji

Adawia Fadhil Abbas

Esrar H. AL-Hushemi

Department of Biology

Department of Microbiology

College of Basic Education

College of Medicine

College of Dentistry

Diyala Univ.

Diyala Univ.

Al-Murtansiriya Univ.

ABSTRACT

Isolation of bacterial from eye infections (conjunctivitis) was carried out in this study 39 specimens (55.71%) gave positive bacterial culture from totally (70) samples. We found that *staphylococcus aureus* and *staphylococcus epidermidis* in 35.71,20% respectively from the total isolates.

Antibacterial activities of the antibiotics vancomycin, oxacillin, cephalothgin chloramphenicol, tobramycin, ciprofloxacin *Staph. Aureus* and *Staph. Epidermidis* shows resistance rate about (4,12, 16, 28 , 8, 8%) and (7,14,14.28 ,35.7,28.57,42.85,21.42%) respectively.

The inhibition ability of black tea alcoholic extract against *Staph. aureus* and *Staph. epidermidis* isolates at concentration (10, 20, 40, 80, 100%) were (13,15,17,19,27) mm, (10,14,16,18,24) mm of inhibition zones respectively.

-keywords: antibacterial activity , antibiotic, black tea, conjunctivitis.

INTRODUCTION

The conjunctiva is a mucous membrane which covers the under surface of the lids and is reflected from the lids to cover part of the eye ball up to the margin of the cornea (Jain,1990).

Received for publication August 10 , 2009 .

Accepted for publication December 30 , 2009 .

Most conjunctival disorders are self-limiting and seldom affect vision, but some lead to blindness, if left untreated, conjunctivitis is characterized by hyperemia fomites or the patient's own hands and spread from one eye to the others (Smith,1997).

Conjunctivitis can be produced by viral or bacterial infection (Tetz *et al.*,1997) in bacterial conjunctivitis discharge various from mild to severe but usually appears purulent and persists throughout the day (Tarabishy *et al.*,2008). The choice of an antimicrobial is not only influenced by the type and susceptibility of the infecting organism but also by the mode of action when more than one antibiotic is used a part from the spectrum of the individual antibiotic, synergism and antagonism must be kept in mind (Smith,1997).

The purpose of this study is to determine the invitro effect of black tea alcohol ethanolic extract and some antibiotics on *Staph. aureus* and *Staph. epidermidis* isolate from conjunctivitis.

MATERIALS AND METHODS

Patients

This work was done at Baquba general hospital (70) specimens were collected from patient with conjunctivitis by sterile swabs.

Isolation and identification

The clinical samples were added to the brain heart infusion broth then incubated at 37C° for 18 to 24h. and then cultured on 5% sheep blood agar and mac Conkey agar.

Identification of *Staph. aureus* and *Staph. epidermidis* were depend on routine laboratory techniques (Forbes *et al.*, 2007)

Plant extracts

Black tea (*Camellia Sinensis* "Theaceae") leaves were obtained from the markets various aqueous of ethanol alcoholic concentrations (10, 20, 40, 80, 100) mg/ml of leaves extracts were prepared according to Deshmukh and

Borle, (1975) and sterilized by millipore filter technique (diameter 25Mm, pore size 0.45Mm).

Antibiotics

Six antibiotics (as antibiotic disc) were used for comparison their effects. Vancomycin (10 mcg), Oxacillin Ox (1mcg), Cephalothin Cp (5mcg), Chlormphenical C (30mcg), Tobramycin (10mg), Coprofloxacin (5mg). Antibiotic discs were supplied from Bioanalyse (Turkey).

Evaluation the antibacterial activity

agar diffusion method was used to evaluated antibacterial activity of plant extracts and antibiotics on growth of bacterial types isolated from conjunctivitis patients to determine growth inhibition zones (Mm) by using Muller-Hinton agar.

RESULTS AND DISCUSSION

Thirty nine isolates were identified as shown in table(1).72.21% of Gram positive were detected *Staph . aureus* in 46.29% from the positive cases followed by *Staph.edidermidis* in 25.29%. In spite of the fact that the source of infection cannot be determined up to 20% of the bacteria present in this flora might be associated with biofilm formation and, once adhered by electrostatic attraction to the intraocular lense will case infection (Miyanaage,1997). Other studies supported reslts of our study that the *Staph. aureus* and *Staph .epidermidis* associate with conjunctivitis (Hughes and Hill(1994), Tetz *et al.*, (1997); Locatelli *et al.*, (2008); AL-Sammary,(2007).

Staph . aureus are able to cause severe infection because they can produces a large numbers of toxins and enzmes such as leucocidin, coagulase, Hyaluronidase and Syndrome toxin (Forbes *et al.*, 2007).

Table 1. The rate of *Staph. aureus* and *Staph. epidermidis* isolated from 54 positive cultured cases patients with conjunctivitis.

Bacterial types	N of cases	% from the +ve cases (54)	% from total isolates (70)
<i>Staph. aureus</i>	25	46.29	35.71
<i>Staph.epidermidis</i>	14	25.92	20
Total	39	72.21	55.71

Table(2) shows that *Staph. Aureus* and *Staph. epidermidis* have a low rate resistance to vancomycin while antibiotics high resistance developed by *Staph. aureus* and *staph . epidermidis* to Cephathin and tobramycin respectively Iwalokun *et al.*, (2007 observed that *staph. aureus* and *staph. epidermidis* have high resistance to streptomycin ,ampicillin and amoxicillin .

Locatelli *et al.*,(2003 observed that vacomycin displayed the best activity followed by cephaothin, ofloxacin and oxacillin .

Petersdorf *et al.*,(1960 stressed a significant ability of Staphylococci to developresistance against chloramphenicol .

It has recently been show that *Staph.aureus* and *Staph.epiderminis* are capable of biofilm formation an important factor for adherence antibiotic resistance and protection from host innate defense (Costerton *et al .*,1999)

Resistance of *Staph.epidermidis* biofilm to some antibiotics of *Staph* to some antibiotics might in part , be due to a poor antibiotic penetration nutrient limitation and slow growth, adaptive stress responses and formation persisted cell in a biofilm, *Staph.epidermidis* is prones and formation persisted cell in a biofilm, *Staph. epidermidis* is protected against attacks from the immune system and antibiotic treatment making *Staph .epidermidis* infections difficult to eradicate (Laikwok *et al.*, 2003).

Table2. Resistance of *Staph.aureus* and *Staph. epidermidis* to some antibiotics

Antibiotics	The rate of from <i>Staph.aureus</i>	Resistance (%) <i>Staph. epidermidis</i>
Vancomycin	4%	7.14%
Oxacillin	12%	14.28%
Cephalothin	16%	35.7%
Choramphenicol	28%	28.57%
Tobramycin	8%	42.85%
Ciprofloxacin	8%	21.42%

Figure (1) shows the zones of inhibition of black tea alcohol ethanolic extract wider range from (13-27) mm in *Staph. aureus* and (10-24) mm in *Staph.epidermidis* .

Michalczyk and Zawislak ,(2008) reported that black and green tea may exert a modeling effect on the balance of the intestinal microflora and *Staphylococcus aureus* was the most sensitive to the addition of tea extracts to the media. (Yam *et al.*, 2007).

Ciraj *et al.*, (2001) studied the antibacterial activity of the alcoholic extract of black tea against salmonella serotypes causing enteric fever and found that 42.19% of *S.typhi* was inhibited by this extract, *Staph. aureus* and *Vibrio parahaemolyticus* which causes diarrhoeal diseases inhibited by tea and coffee extracts (Toda *et al.* , 2008). Tea leaves produce organic compounds that may be involved in the defense of the plants against invading pathogens, these metabolites compound include polyphenolic, catechin, methyl-xanthine, theobromine (Friedman, 2007).

Ikigai *et al.*, (10) indicated that catechin damages the lipid bilayer which partly explains the greater bactericidal effect of catechin to Gram positive bacteria than Gram negative bacteria, polyphenol are contained in green tea at an approximate concentration of 10-15% and 5% in black tea. Tea leaves usually contain 8% of epigallocatechin gallate (EGCG), which is the dominant constituent of polyphenol 705 and is recognized to play a major role in anti-microbial effects. Khalaf *et al.*, (1993) found that the antioxidant activity of black tea due to ascorbic acid and phenolic content.

Laikwok *et al.*, (2001) believe that the flavins in black tea possess at least the same antioxidant potential as catechins present in green tea the glycosides,

tannis and flavonoids in the crude methanolic extract tea leaves are known to posses potent antioxidant activity (Lee *et al.* ,2004).

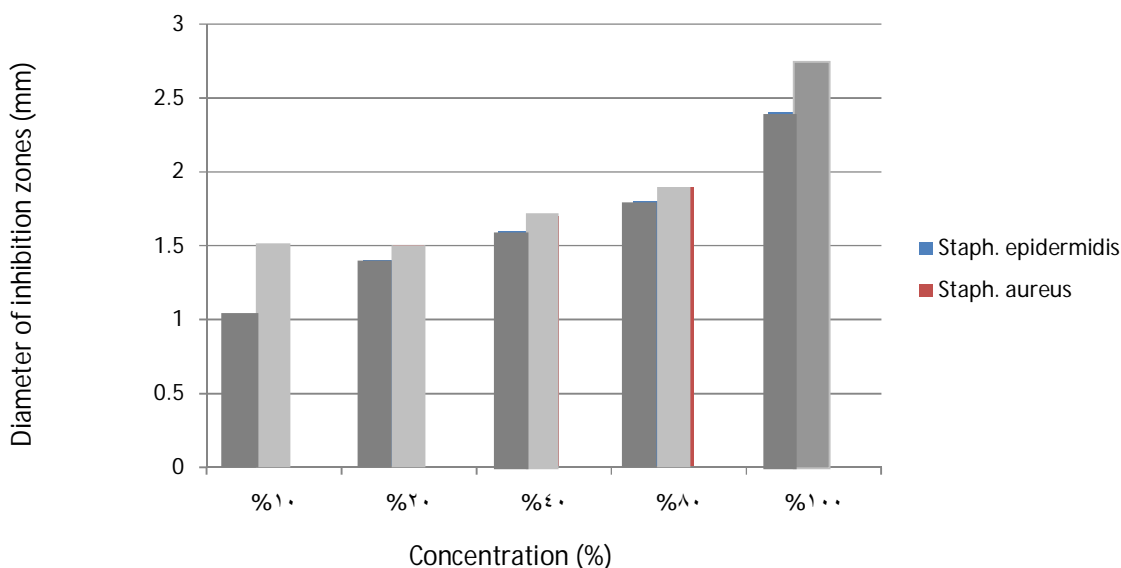


Figure 1. The antibacterial activity of black tea alcohol ethanolic extract on *Stap* and *Staph epidermidis* .

REFERENCES

- Al-Samary, I.E. 2007. A study of antibacterial activity of plant extracts on bacterial pathogens isolated from eyes infections (conjunctivitis). The Internet Journal of Microbiology. 4(1): 1-8.
- Ciraj, A.M.; Sulaim, J. Mamatha, B.; Copalkrishnay B.K. and Shivannanda .2001. Antibacterial activity of black tea (*Camellia Sinensis*) extract against *Salmonella* serotypes causing enteric fever. Indian Journal of Medical sciences. 55 (7): 376-381.
- Costerton, J.W.; Stewart, P.S. and Greenberg.1999. Bacterial biofilms: acommon cause of persistent infection. Science. 284: 1318-1322.
- Deshmukh, S.D. and Borle .1975. Studies on insecticidal properties of indigenous plant products. India, J. Ent. 37 (1): 11-18.
- Forbes, B.A., Sahm, D.F. and Wiessfeld .2007. Diagnostic Microbiology, 12th ed. Baily and Scotts. Mosby company. U.S.A.
- Friedman, M.(2007). Overview of antibacterial, antitoxin, antiviral and antifungal activities of tea flavonoids. Molecular Nutrition and Food Research. 51 (1): 116-134.

- Hara, Y. 2001. Antibacterial action of green tea. Marcel Dekker, Inc. Newyork.
- Hughes, D.S. and Hill .1994. Infectious endophthalmitis after cataract surgery. *Brit. J.Ophthalmot*, 78: 227-232.
- Jain, M.R. (1990). Diagnosis and management of Ocular inflammations. Znded. India.
- Ikigal; H. Nakae, T.; Hara, Y. and Shimamura.1993. Bctericidal catechins damage the lipid bilayer *Biochim. Biophys. Acta.* 1147: 132-136.
- Iwalokun, B.A.; Usen, V.A.; Otunba, A.A. and Olukoya .2007. Comparative Phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus Ostreatus*. *African Journal of Biotechnology.* 6 (15): 1732- 1739.
- Khalaf, N.A.; Shakya, A.K.; Othman, A.A., Al-Agbar, and Faran .2008. Antioxidants avtivity of some common plants. *Turk. J. Biol.* 32: 51-55.
- Konig, C.; Schwank, S. and Blaser .2001. Factors compromising antibiotic activity against biofilms of *Staph. epidermidis*. *Eur. J. Clin. Microbrol. Infect. Dis.* 15: 259-261.
- Laikwok, L.; Yalun, S.U.; Ruoyun, C.; Zesheng, Z.; Yu,H. and Chen .2001. Theaflavins in black tea and catechins in green tea are equally effective antioxidants. *J. Nutr.* 131: 2248- 2251.
- Lee, J.; Koo, N. and Min .2004. Reactive oxygen species, aging and antioxidative neutraceuticals: *Comp. Rev. Food Sci. Food safety.* 3: 21- 33.
- Locatelli, C. I.; Kwitko, S. and Simonetti .2003. Conjunctival endogenous microbial in patients submitted to cataract surgery. *Brazilian Journal of Microbiology.* 34: 203- 209.
- Michalczyk, M. and Zawislak .2008 . The effect of tea infusions on the proliferation of selected bacteria important for the human intestnal tract. *Acta. Sci. Pol, Technol. Aliment.* 7 (1): 59-65.
- Miyanaga .1997. New perspective in ocular infection and the role of antibiotics. *Ophthalmologrca*, 211 (1): 9- 14.

- Petersdorf, R.G.; Rose, M.C.; Minchew, H.B.; Keene, W.R. and Bennett 1960 . The sensitivity of hemolytic Staphylococci to a series of antibiotics. Arch. Int. Med. 105: 398-412.
- Smith, M.A. 1997. Treatment of experimental methicillin resistant Staph. epidermidis. J. Infect. Dis. 175: 462-466.
- Srivastava, U. and Kolhapure. 2004. Evaluation of efficacy and safety of ophthacare eye drops in acute and chronic conjunctivitis. The antiseptic. 101 (6): 211- 217.
- Tarabishy, A.B.; Bennie, H. and Jeng. 2008. Bacterial conjunctivitis. Cleveland clinic Journal of medicine. 75 (7): 507- 512.
- Tetz, M.R.; Klen, U. and Volcker. 1997. Staphylococcus- associated blepharokerato- conjunctivitis. Ophthalmology. 94: 186- 190.
- Toda, M. Okubo, S.; H. Yoshi, R. and Shimamura .2008. The bactericidal activity of tea and coffee. The society for applied Microbiology. 8 (4): 123- 125.
- Yam, T.S.; Shah, S. and Hamilton, J.M. 1997. Microbiological activity of whole and fractionated crude extracts of tea (*Camellia Sinensis*) and of tea components. FEMS. Microbiol. Lett. 152: 169- 174.

الفعالية ضد الميكروبية لمستخلص الايثانول الكحولي للشاي الأسود وبعض المضادات الحيوية ضد عزلات *Staphylococcus aureus* & *Staphylococcus epidermidies* المعزولة من التهاب الأجنان

إسراء حسن الهاشمي	عدويه فاضل عباس	سندس عادل ناجي
كلية طب الأسنان	قسم الأحياء المجهرية / كلية الطب	قسم العلوم / كلية التربية الأساسية
الجامعة المستنصرية	جامعة ديالى	جامعة ديالى

الخلاصة

أظهرت نتائج الزرع البكتريولوجي لـ (70) عينة جمعت من حالات التهاب الأجنان التلوث بكتريا *Staph. aureus* و *Staph. epidermidies* ونسبة (35.71 و 20%) من المجموع الكلي للعزلات . أوضحت دراسة حساسية العزلتين *Staph. aureus* و *Staph. epidermidies* للمضادات الحيوية Chloramphenicol Oxacillin , Cephalothin , Vancomycin , Ciprofloxacin , Tobramycin , chloramphenicol (8, 8,28,) إن معدلات المقاومة كانت (21. 42, 42.85,28.57,35.7,14.28,7.14) و (16, 12, 4 %) على التوالي .

كما واطهر استخدام مستخلص الايثانول الكحولي للشاي الأسود بتركيز (100,80,40,20,10)% تأثيراً وبمعدل (27,19,17,15,13) ملم و (24,18,16,14,10) ملم لكل من *Staph. aureus* و *Staph. epidermidies* على التوالي .