Study the effect of *Camellia sinensis* alcoholic extract against Gram negative bacteria isolated from eye infections (conjunctivitis)

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Abstract:

The present study examined the effect of alcoholic extract of *Camellia sinensis* against Gram negative bacteria isolated from eyes infections (conjunctivitis), 25 specimens (39.68%) gave positive bacterial culture from a total of 63 samples. The bacteria detected includes *Pseudomonas aeruginosa* (12.69%), *Enterobacter spp.* (7.93%), *Escherichia coli* (6.34%), *Moraxella spp.* (4.76%), *Proteus mirabilis* (4.76%) and *Klebsiella spp.* (3.17%).

The activity of the alcoholic extract of *Camellia sinensis* was also evaluated in the present study. The inhibitory ability of *Camellia sinensis* alcoholic extract against bacterial isolates at concentration (10,20,40,80,100)% showed the biggest average of inhibition zones diameters (25,23,23,17,15,13) mm at 100% concentration of *P. aeruginosa*, *E.coli*, *Moraxella spp., Enterobacter spp., Klebsiella spp*. and *Proteus mirabilis* isolates respectively.

Different antibiotics were also used to compare their activities with the activity of *Camellia sinensis* alcoholic extracts.

Keywords: *Camellia sinensis* Linn., bacteria, conjunctivitis, antimicrobial activity, antibiotic.

Introduction:

The conjunctiva is a transparent membrane that covers the sclera and lines the inside of the eyelid. It is a protective barrier against invading pathogens[1].

In spite of natural protective mechanism of the conjunctiva as cilia, tears that contain immunoglobulins, Lysozyme and multiple antibacterial enzymes. It is continuously being flushed and renewed creating a physically and immunologically adverse environment for bacterial growth [2].

The conjunctival sac may be infected with different pathogens such as bacteria fungi virus, and viral [3].

The infected microorganism might exhibit resistance to drugs by producing enzymes changing their permeability to the drug, develop an altered in structural larget for the drug and develop an altered metabolic path way [4].

Therefore most of the conjunctivitis cases caused by these infectious microorganism which failed to respond to the treatment.

The aim of this study was to determine the Gram negative bacterial types causing conjunctivitis and study the antibacterial activity of *Camelliai sinensis*.

Materials and Methods:

- Patients

This procedure was carried out at Baquba general hospital under supervision of ophthalmologist Dr. Wissam Ali. Sixty three specimens

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were collected from patients with conjunctivitis by using sterile swabs.

- **Isolation and microorganism identification**

Samples were added to the brain heart infusion broth (Oxiod, England) then incubated at 37Cº for 18 to 24h and then cultured on to 5% sheep blood agar (Oxoid, England), and MacConkey agar (Oxiod, England).

The identification of Gram negative bacteria was carried out depending on routine laboratory techniques according to the references [5], [6].

- **Preparation of Crude Plant extract**

Green tea (*Camellia sinensis* "Theaceae") leaves were obtained from the local market.

Extract was prepared according to Deshmukh and Borle [7] by putting 100g of dried samples of green tea leaves powder in thumble of soxhlet apparatus with 750 ml of ethonal (95%) at 60Cº for 30 h. The final extract was passed through Whatman filter paper No.2 (Whatman Ltd, England).

The filtrate obtained was stored at 4Cº for further use. The working solutions (10, 20, 40, 80, 100 mg/ml) of the extract were prepared from the stock solution using suitable dilution.

- **Antibiotics**

Six antibiotics (as antibiotic discs) were used to compare their effects. Tetracycline TE (30 mcg), Rifampin RA (5 mcg), Ceftazidime CAZ (30 mcg), Neomycin N (30 mcg), Polymixine PB (300 units), Amikacin AK (30 mcg). Antibiotic discs were supplied from Bioanalyse (Turkia). Mu'eller- Hinton agar (Oxiod, England) was used to perform the diffusion test as established in Kirby-Bauer method [8].

- **Evaluation of antibacterial activity**

Agar diffusion method was used to evaluate antibacterial activity of plant extract on growth of bacterial types isolated from conjunctivitis patients to determine growth inhibition zones (mm) by using Muller- Hinton agar (Oxiod, England)

Results and discussion:

A total of 80 samples were collected and cultured, 63 samples (78.75%) were positive to culture while 17 (21.25%) showed no bacterial growth

Table (1) Cultures determination obtained from patients samples

	N of Cases	% from the total cases
Positive cultures	63	78.75%
Negative cultures	17	21.25%
Total n of cases	80	100%

As shown in table (1) a great number (78.75%) of positive cultures were collected this is an important aspect to be takes into consideration that the bacterial population in conjunctivitis increases, Locatelli *et al*., [9] observed 82.6% of positive cultures from samples collected from conjunctivitis cases, Verdayes *et al*., [10] found that the rate of bacterial positive for five years were (88.5%).

The pathologic markers occurred in conjunctivitis due to looseness of epithelium particularly of the bulbar conjunctiva and made the exudates containing fibrin and leucocytes comes to the surface in form of discharge, also the superficial cell which form the second line of defense phagocytes are invading agents and are themselves desquamated the basal layer of the cell proliferates and makeup of the deficiency [11].

Table (2) Gram negative bacteria isolated from 63 positive cultured cases of patients with conjunctivitis

Bacterial types	No. of cases	% of (25) cases	% of (63) cases
Pseudomonas aeruginosa	8	32%	12.69%
Enterobacter spp.	5	20%	7.93%
Escherichia coli	4	16%	6.34%
Moraxella spp.	3	12%	4.76%
Proteus mirabilis	3	12%	4.76%
Klebsiella spp.	2	8%	3.17%
Total	25	100%	39.68%

Table (2) shows the different isolates of Gram negative bacteria present in 39.63% of the total positive cases.The following microorganisms being isolated: *P. aeruginosa*, *Enterobacter spp.*, *E.coli*, *Moraxella spp.*, *Proteus mirabilis* and *Klebsiella spp.*

The *P. aeruginosa* in our study was found in 32% of the positive cases followed by other bacterial types, the ability of *P. aeruginosa* to cause disease may be due to having a large number of virulence factors such as exoenzymes, exotoxin A, LPS, elastase, Pili, hemolysin, Phosphorylase, Proteolytic enzymes, Leukocidin and exopolysaccharide which may play an important role in their capacity to cause conjunctivitis [12], [6].

Our results agrees with the studies of others which were associated with conjunctivitis [13], [9], [14], [15].

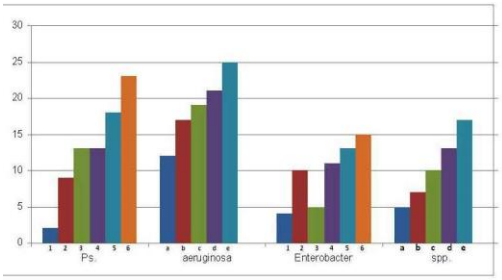
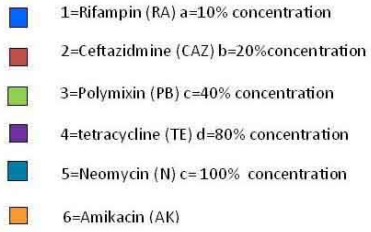


Figure (1)A comparison of antibacterial activity of antibiotics and different concentrations of alcoholic extract of *Camellia sinensis* by measurement of diameter of inhibition zones (mm) to *P. aeruginosa* and *Enterobacter spp.*



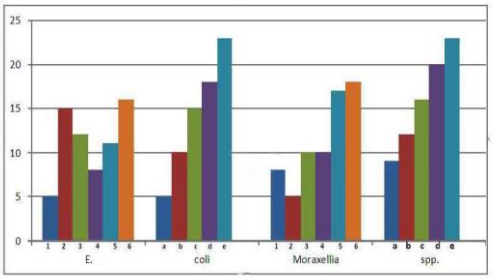
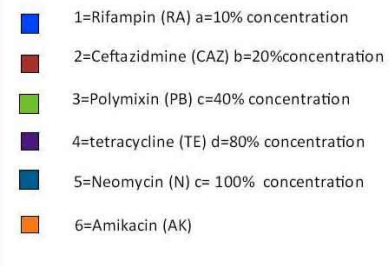


Figure (2) A comparison of antibacterial activity of antibiotics and different concentrations of alcoholic extract of *Camellia sinensis* by measurement of diameter of inhibition zones (mm) to *E.coli and Moraxellia spp.*



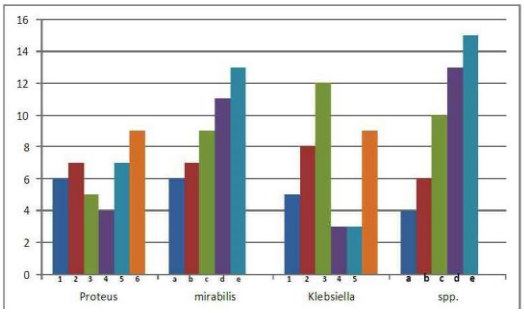


Figure (3) Acomparison of antibacterial activity of antibiotics and different concentrations of alcoholic extract of *Camellia sinensis* by measurement of diameter of inhibition zones (mm) to *Proteus mirabilis* and *Klebsiella spp.*

1=Rifampin (RA) a=10% concentration

2=Ceftazidmine (CAZ) b=20%concentration

3=Polymixin (PB) c=40% concentration

4=tetracycline (TE) d=80% concentration

5=Neomycin (N) c= 100% concentration

6=Amikacin (AK)

The figures (1,2,and 3) shows that all isolates were found to be more susceptible to the alcoholic extract of *Camellia sinensis*, the zones of inhibition were large ranging from (4-25) mm on Muller- Hinton agar supplemented with (10, 20, 40, 80, 100) mg/ml of alcoholic extract of *Camellia sinensis* compared to the zones of inhibition with the antibiotics ranging from (2-18) mm on Muller-Hinton agar supplemented with 5 mcg of Rifampin, 30 mcg of Tetracycline, Ceftazidime, Neomycin and Amikacin, 300 units of Polymixin.

The strongest inhibition of bacterial growth was shown in green tea and the weakest from antibiotic discs among the pathogenic microorganism from our samples *P. aeruginosa* appeared to be very sensitive to the addition of alcoholic extract than with the other isolates. Similar results were obtained for were antibacterial activity of green tea: Toda *et al*.,[16] showed the inhibiting effect of green tea on various diarrheas causing bacteria. Tiwari *et al*., [17] reported inhibition of *S.typhimurium, S.dysenteria, Y. enterocolitica* and *E.coli* using green tea extract.

Susceptibility of bacterial strains to the green tea extract has been shown to produce differences in cell wall components, catechins partition in the lipid bilayer membrane resulted in loss of cell structure and function and finally the cell death. [10], [18].

Michalczyk and Zawislak [19] showed that the inhibiting effect of green tea is due to connection between antioxidant and antimicrobial properties. Khalaf *et al*., [20] found that the strongest antioxidant properties were found in green tea, they also showed that the presence of alkaloids, glycosides and flavonoids in crude methanolic extract are known to possess potent antioxidant activity. Friedman [21] believe that the tea leaves produce organic compounds including polyphenolic compounds, catechins and methyl-xanthine alkaloids, caffeine, theobromine and the ophylline that may be involved in the defense of plants against invading pathogens including bacteria. Iwalokun *et al*.,[22] reported that the green tea has greater phenolic content as compared with *pleurotus ostreatus*.

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دراسة فعالية المستخلص الكحولي للشاي الأخضر Camellia sinensis تجاه البكتريا السالبة لصبغة كرام المعزولة من إصابات العيون (التهاب الملتحمة)

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الخلاصة

بحثت الدراسة الحالية تأثير المستخلص الكحولي للشاي الأخضر *Camellia sinensis* تجاه البكتريا السالبة لصبغة كرام المعزولة من إصابات العيون(التهاب الملتحمة) ، وقد أعطت (25) عينة و بنسبة (39.68%) ايجابية للزرع البكتيري من المجموع الكلي للعينات البالغ (63) عينة ،وقد اشتملت البكتريا المكتشفة على (12.69%) *Pseudomonas aeruginosa* و (7.93%) *Enterobacter spp.* و (6.34%) *Eschericha coil* و (4.76%) *Moraxella spp.* و (4.76%) *Proteus mirabilis* و (3.17%) *Klebsiella spp.* .

وقد تم تقديم قيمة المستخلص الكحولي لـ *(Camellia sinensis)* ، وقد اظهرت القدرة التثبيطية للمستخلص الكحولي تجاه العزلات البكتيرية عند التراكيز (10 ، 20 ، 40 ، 80 ،100) % اكبر معدل لأقطار التثبيط وقدره (25 ، 23، 23 ، 17 ، 15 ، 13) ملم عند التركيز (100%) لعزلات كل من *Pseudomonas aeruginosa* ، *Eschericha coil* ، *Moraxella spp.* ، *Enterobacter spp* ، *Klebsiella spp.* ، *Proteus mirabilis* ، على التوالي وقد استخدمت بعض أقراص المضادات الحيوية القياسية لمقارنة فعاليتها مع فعالية المستخلص الكحولي للشاي الأخضر *Camellia sinensis* .