

ANTIMICROBIAL ACTIVITY OF *Aloe vera* EXTRACT ON CASES OF KERATOCONJUNCTIVITIS IN SHEEP (*IN VIVO* AND *INVITRO* STUDY) AND COMPARED WITH PENICILLIN –STREPTOMYCIN.

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ABSTRACT

The study was conducted at the of animal fields in AL-Qassim city, sheep in this fields was suffering from severe eye infection during the period April to October ,2015 .sheep was clinically examined and showed signs copious eye drops, increase body temperatures, swollen of eye with redness, eye pus in some cases, the sheep was checked clinically and eye swabs of 20 keratoconjunctivitis affected sheep carried out on the basis of their culturing,morphology, staining,biochemical tests and antimicrobial susceptibility testing was done by (disc diffusion method and well diffusion method) from present study .percent of infection was *S.aureus*(50%), *M.ovis* (33%)and *Proteus spp.*(17%) was isolated . Result of antimicrobial properties of *Aloe Vera*was evaluated in various *in-vitro* experiments against these species of bacteria *S.aureus*,*M.ovis* and *Proteus spp.* and showed good inhibition activity.*In vivo* study recovery from the conjunctivitis was record after 6 -10 days for animal treated with Pencillin-Streptomycine while group of extract that showed complete healing during period 6-10 day according to type and severity of infection, also there is no recurrent infection after treated with aloe Vera ointment and eye lotion , as well as Gide to done many of research on aloeVera for used in eye problem which consider very important in Veterinary Medicine .

INTRODUCTION

*Aloe Vera*it's an important medical plant belongs to the family *Liliaceae*, it has thick, tapered, spiny leaves growing from a short stalk near ground level(1). Concentrated extracts of Aloe leaves are used for treatment of constipation as laxative and hemorrhoid treatment. *Moreover Aloe* gel can help to stimulate the immunity system in the body (2).Many scientific studies on *aloe Vera* are used analgesic, anti-inflammatory, wound healing, immune modulating and anti-tumor activities as well as antiviral, antibacterial and

antifungal properties (3,4).Also it showed significant *in vitro* antibacterial effect against *Escherichia coli*, *Staphylococcus aureus*,*Staphylococcus epidermis*, *Bacillus subtilis* and *Shigella flexneri*(5).The *A. Vera* plant contains different medical content including Vitamins A, B₁, B₂, B₆, B₁₂, C and E, are to play an important role as antioxidant and inflammation .amylase, lipase and carboxypeptidase these enzyme are found in plant which act as breakdown fat, sugar and starch specially carboxypeptidase act on bradykinin so that potentiate anti-inflammatory response and vasodilatation that may be give chance for treatment of hypertension.Also it contain Anthraquinones as Aloe-emodin, Aloin, Anthranol, Isobarbaloin, Emodin, Barbaloin and aloetic acid. These compounds responsible of purgative effect, antimicrobial activity and analgesic effects(6,7).*Aloe Vera* alone or its simultaneous use with cisplatin exhibits anti-neoplastic effects in breast and cervical cancers by inducing apoptosis and modulation of expression of effector molecules(8).Infectious keratoconjunctivitis (IKC) is a disease of worldwide economic importance causing blepharospasm, corneal opacity and conjunctivitis in ruminants.Recovered animal may develop corneal opacity and blindness (9).Antibacterial properties of *Aloe Vera* was evaluated in various in-vitro experiments against many species bacteria involved like *S.aureus*, *P.aeruginosa*, *E. coli* and *H. pylori* but only a few *in-vivo* studies exist to investigate its antibacterial properties(10).due to lack of studies on the use of *aloe Vera* extracts as antimicrobial in keratoconjunctivitis cases and its importance of the pharmaceutical has conducted this study in order to evaluate the effectiveness of it's in treat these cases.So the aim of this study are: a) The study was aimed to investigate activity of ointment *Aloe Vera* extract on keratoconjunctivitis in sheep *in vitro* and *in vivo* and comparative with activity antibiotic sensitive of the bacteria; b) Determination of susceptibility and resistance pattern of bacterial isolates against *Aloe Vera* gel extracts as well as seven different antibiotics by agar (disc and well) diffusion assay.

MATERIAL AND METHODS

Animal study

Twenty sheep suffering from eye infected was isolated from farmers sheep in AL-Qassim city, eye swab was taken for laboratory diagnosis, the diagnosis was fixed as *Moraxella ovis*, *S.aureus* and *Proteus* spp.

Collection of samples

Sterile swabs were passed over 20 of infected sheep which eye have signs of diseases take immediately to laboratory .

Idea of study

In fact the idea of research still that first study about use of *aloe Vera* alternative treatment for antibiotic and uniqueness of study was derived from herbal medicine of Imam Muhammad AL-Baqir peace be upon him.

A-in vivo study

Infected Animal was divided into four groups:

Group A six Infected animal treated with penicillin streptomycin ointment twice daily for 5 day; B twelve's infected animal treated with *Aloe Vera* extract as ointment and lotion twice daily for 5 day; (C) two infected animal still without treatment as control positive for show completed clinical signs, (D) five healthy sheep was treated with *aloe Vera* extract to evaluation safety of agent there is no any discomfort or any symptoms on eye.

Preparation of the extract

The seeds of *aloe Vera* leaf were collected from local Medicinal plants in AL-Qassim city. *Aloe Vera* leaf 300g was dissected to small portion then put in blender max. the mixture dissolved by 1000 mL of 70% hydro alcoholic solution mechanical shaker (magnetic stirrer) at 55 °C for 6 h. the content was filtered and kept in an incubator at 37°C for 36 h. The concentrate extract was stored dry at -20 °C in deep freezes(11). The percentage yield of the extract 2.5% weighting extract by electrical imbalance according to dose used in this study, finally PH of extract was record (6.3).

Microbial isolation

Culture media used for isolation and purification of bacteria included: blood agar, MacConkey agar and nutrient agar (Oxoid). Inoculated media were incubated aerobically at 37 °C for 24 hours(7,12).

Methods

After positive results of growth were appear, bacterial samples were identified with Gram stain and Biochemical test.

- Biochemical test:

Gram positive bacterial isolates were identified by:

1. Catalase test.
2. Coagulase test (tube and slide method).
3. Mannitol salt agar (for *S. aureus*).

All the tests above done according to(12).

Bacterial isolates *Moraxella ovis*: suspected isolates were passed twice on Trypticase soy agar containing 5% sheep blood. Field isolates of *M. ovis* were identified by phenotypic and biochemical criteria. Briefly, *M. ovis* colonies were 1 to 3 mm in diameter after 24 h of incubation, firm, and hemolytic on blood agar. Microscopically, the *M. ovis* isolates were gram-negative Cocci arranged in pairs. All *M. ovis* isolates were aerobic and oxidase and catalase positive, and they reduced nitrate but did not ferment carbohydrates or liquefy gelatin(13).

***In vitro* study**

Antimicrobial susceptibility testing of *aloe Vera* extract

Bacterial isolates were inoculated into nutrient broth incubated at 37 °C for 18 hours. The bacterial suspensions were diluted with normal saline. Adjust the turbidity and compare with standard tube (McFarland number 0.5) to yield a uniform suspension containing 1.5×10^8 CFU/mL. These bacterial suspensions which use later in both sensitivity methods (disc and well diffusion).

- A. **Disc diffusion method:** Whitman Filter paper No. 1 was used to prepare discs (6 mm). The discs were then sterilized in autoclaving, and added one drop of *Aloe Vera* extract to each

disc. Prepared discs were stored at 4 °C in the refrigerator till use. After, Muller – Hinton agar plate were inoculate with cotton swab dipping into screw tube containing bacterial suspension and streaking over the surface of plate and put disc contain on extract in the middle of plate then incubated the plate at 37°C for 24hr. After that measuring the inhibition zone around the disc by ruler (43).

B. Wells diffusionmethod

Bacterial suspension was streaking into Mueller-Hinton agar (for all tested bacteria) surface of plates then the plates were left for one 5 -15 minutes at room temperature to dry. Media were cut into one well (5mm diameter) in the middle by cork borer and add 20µ of the *aloe Vera* extracts solutions. All plate of the tested organisms was then allowed to incubate at 37°C for overnight. After 24 hrs.of incubation, then we was noted zone of inhibition of aloe vera extract on each isolate. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm). (43).

Antibiotic susceptibility testing by disc diffusion method

Antimicrobial susceptibility testing of theisolates to various routinely used antibiotics was determined by disc diffusion technique on Muller Hinton agar using commercially available discs following CLSI guidelines. Sterile swab was used to inoculate the suspension by streaking on the prepared and dried Mueller Hinton agar plate evenly. It was then allowed to stay for 3-5 minutes. Sterile forceps was used to place the antimicrobial discs on the inoculated plates. Within 30 minutes after applying the disc, the plate was incubated at 37°C for 18-24 hours by using Meter rule on the underside of plate, the diameter of each zone of inhibition was measured in millimeter. Zone diameter for isolate was compared with CLSI Published Limits; Interpretative chart was then used to interpret the zone sizes of Inhibition. Result was recorded as susceptible, intermediate susceptible, or resistant based on the Zones sizes of each antimicrobial disc used. The susceptibility of the bacteria was determined based on the breakpoints recommended by the Clinical Laboratory Standards Institute (1, 43).

Table 1: Antibiotics disks with standard Zone diameter(CLSI, 2014):

Antibiotic disc	Symbol	Potency	Zone diameter nearest whole mm		
			<i>Sensitive</i>	Medium	Resistant
Tetracycline	TE	30 µg	≥ 19	15~18	≤ 14
Streptomycin	ST	10 µg	≥15	12~14	≤11
Chloramphenicol	CH	30 µg	≥18	13~17	≤12
Amikacin	AK	30µg	≥17	15~16	≤14
Gentamicin	CN	10 µg	≥15	13~14	≤12
Penicillin G	PG	10 IU	≥29	—	≤28
Ampicillin	AMP	10µg	≥17	14~16	≤ 13

Statistical analysis

Bonferroni test was used for statistical analysis ($P \leq 0.05$) to show if there is any significant differences in the results.

RESULT

In vitro study:

The clinical signs of infected eye can be listed by copious eye drops, increase body temperatures, swollen of eye with redness, eye pus in some late cases. the percent of causative keratoconjunctivitis bacteria was (*Staphylococcus aureus* (50%; *M.avis* 33% and *Proteus spp* 17%).as explained with figure1.

causative agent of keratoconjunctivitis in sheep

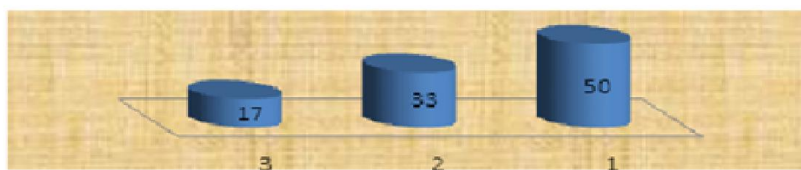
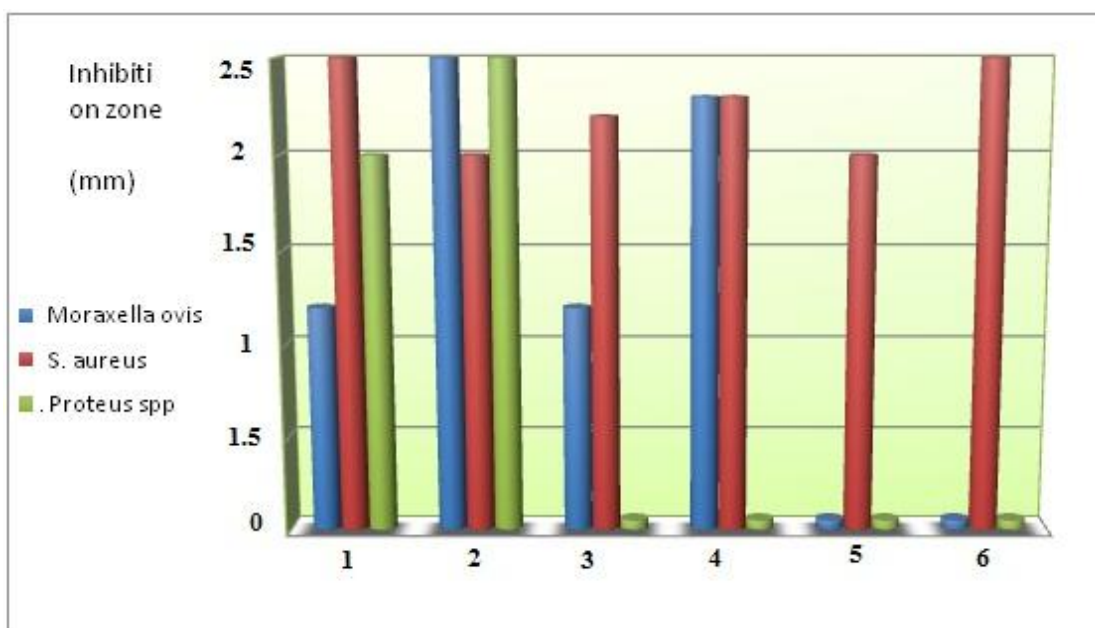


Fig. 1: the causative agents of keratoconjunctivitis infections in p(1(*Staphylococcus aureus*; 2(*M.avis*; 3(*Proteus spp*).

These result revealed that *S. aureus* were most causative agent than other bacterial isolates. Also *S. Aureus* consider the major cause of IKC in bovines(14). The different types of bacteria isolated in present study correlate with the findings of (11,29)with slight variation .

A methanolic extract of AloeVera showed significant *in vitro* antibacterial efficacy against *Staphylococcus aureus* ,*M.avis*,*Proteus spp*. As shown in fig 2 and 3. Which agree with several studies(5,11).



The No (1,2,3,4,5,6) represent number of sample

Fig. (2) Antimicrobial susceptibility to Aloe vera extract (Disk diffusion method).

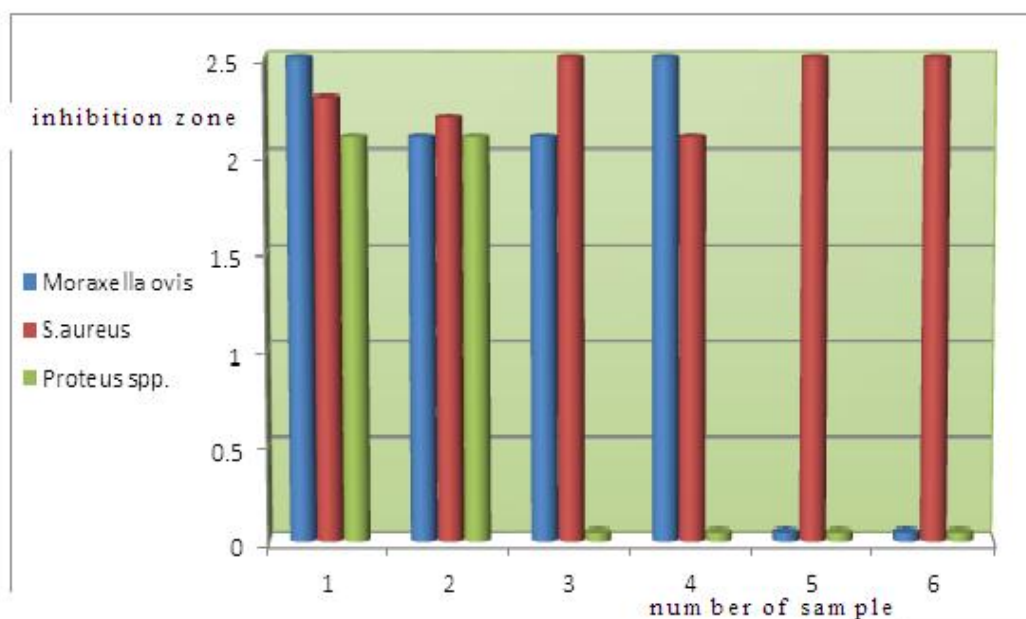


Fig (3) Antimicrobial susceptibility to Aloe Vera extract (well diffusion method).

The antimicrobial property of Aloe Vera gel extracted using different sensitivity methods showed varying degree of response towards isolated bacteria.

The results revealed that *S. aureus* more affect with completely inhibition zonethan other bacterial isolates from antibacterial effect of aloe Vera extract in both methods (disk and wells diffusion) moreover result also indicated that aloe Vera extract more effective on bacterial inhibition zone than aloe Vera gel that may be return to concentration of extract, as shown in pictures below (pic. 1 and pic.2):

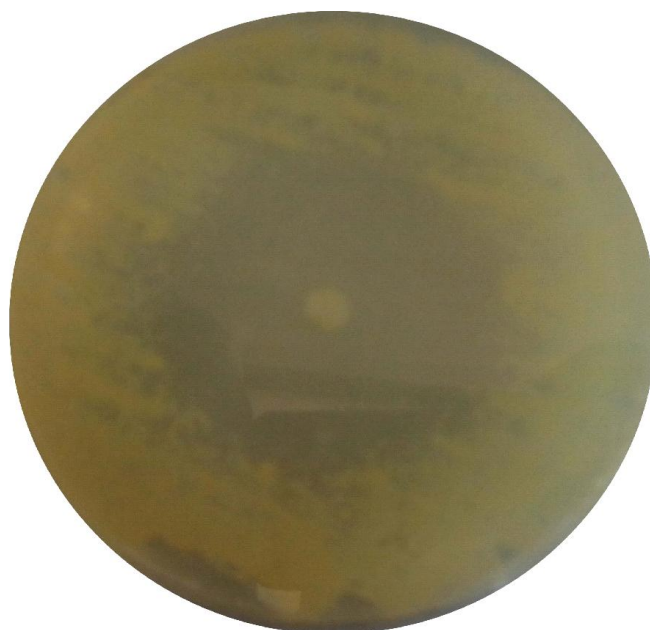


Fig.1 (inhibition zone of *S. aureus* by using disc diffusion method).

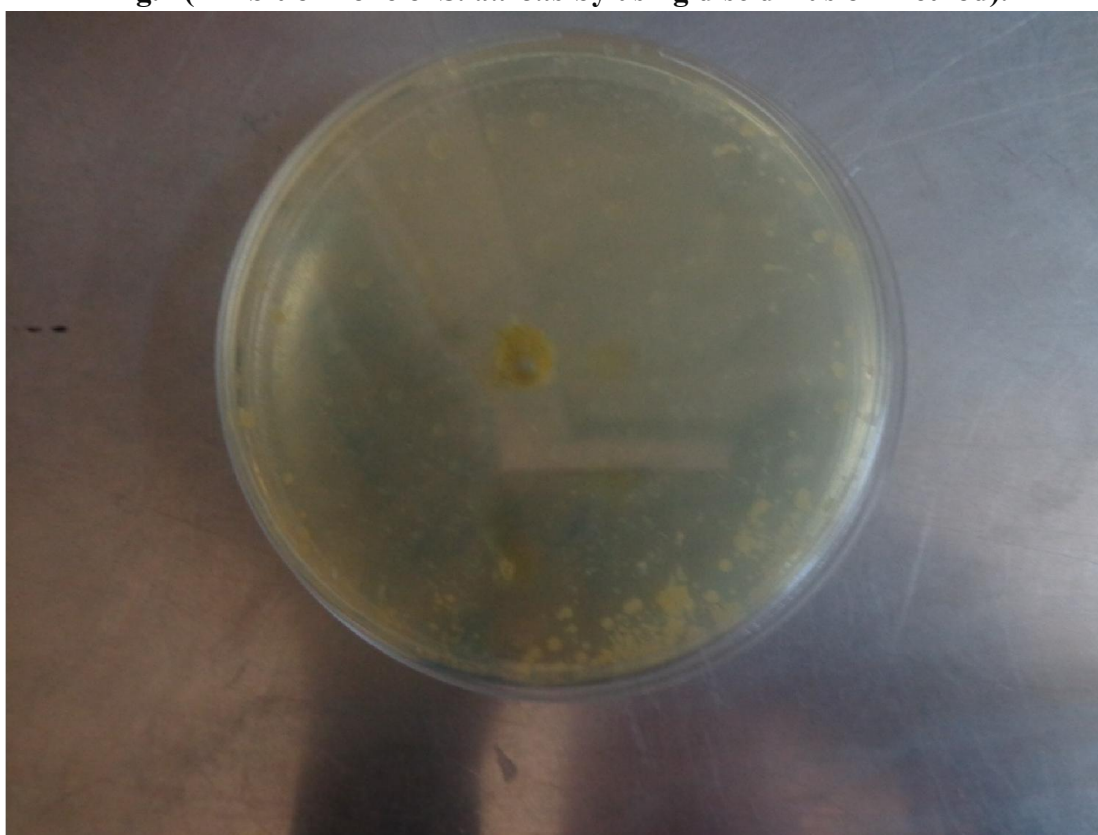


Fig.2 inhibition zone (completely) of *S.aureus* by using wells diffusion method).

The sensitivity of bacterial isolate to the antibiotic by using disk diffusion test:

The result of antibiotic sensitivity of bacterial isolate from sheep with keratoconjunctivitis. *Staphylococcus aureus* are highly sensitive to tetracycline and Penicillin (100%) followed by Chloramphenicol and Ampicillin (83.3%), streptomycin (66.6%) , gentamicin(33.3%) and Amikacin (16.6%), while *Moraxella ovis* are highly

sensitive to tetracycline , and Chloramphenicol(75%) followed by streptomycin, Ampicillin and Amikacin (50%) and gentamicin (25%) , Penicillin are resist. *Proteus spp* are highly sensitive to tetracycline , streptomycin , gentamicin andAmpicillin (50%) while Chloramphenicol, Penicillin and Amikacin are resist. as shown in table 2 and picture 4.

Table 2. Antibiotic sensitivity of the bacterial isolate to different antibiotics by using disk diffusion method .

Isolated bacteria	of isolates tested	Antibiotic sensitivity no.(%)						
		TE	ST	CH	AK	CN	PG	AMP
<i>Staphylococcus aureus</i>	6	6(100)	4(66.6)	5(83.3)	1(16.6)	2(33.3)	6(100)	5(83.3)
<i>Moraxella ovis</i>	4	3(75)	2(50)	3(75)	2(50)	1(25)	0(0)	2(50)
<i>Proteus spp.</i>	2	1(50)	1(50)	0(0)	0(0)	1(50)	0(0)	1(50)

TE= tetracycline, ST=Streptomycin, CH=Chloramphenicol, AK=Amikacin,CN= gentamicin,

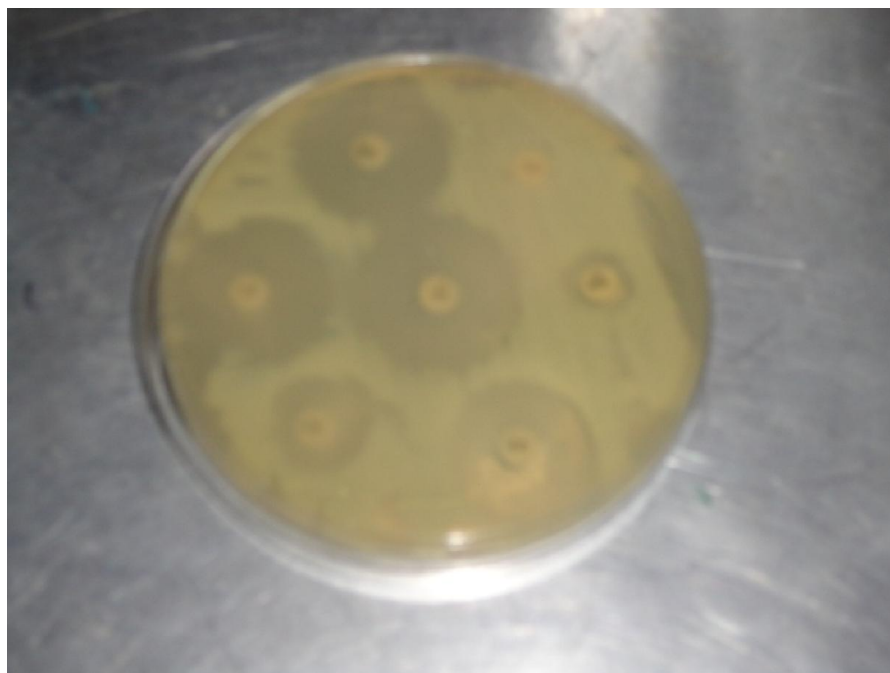
PG=Penicillin, AMP= Ampicillin .

These finding are agreement with result of (15) who reported that most of the bacteria responsible for IBK were highly sensitive to Tetracyclin and Pencillin less sensitive to chlormphencol and other antimicrobial agent.

Table 3; refer to inhibition zone of antimicrobial effect of aloe vera in bacterial isolated from (KC)in two methoed .

bacteria	wells diffusion	disc diffusion mm
Staphyloccocus	80	50
moraxella	36	22
Proteus	29	16

Result showed high inhibition zone for staphylococcus ,followed by Moraxella then proteus



Fig; 3. Antibiotic sensitivity of bacterial isolate by using disc diffusion method, refer to low inhibition zone than aloe Vera extract

In vivo study: Picture refer 8 day of infection with staphylococcus

The aloe Vera extract was found to be effective. All infected sheep which were seriously affected showed complete recovery and (100%) healing to the extract and healing period was record as 5 day for Moraxella , 7 day ,7-10 day for Proteus and Staphylococcus respectively .

Picture A refer eye infected with *Moraxella ovis* (pink eye) .



Picture B refer 4th day of mixed infection copious eye drop with acute inflammation of eye swelling and redness

Picture C refer to acute inflammation of eye with pus in 8 day of infection , Picture D reveal eye opacity after mixed infection *Staphylococcus aureus* , *Moraxella ovis*, *proteus* in 10 day



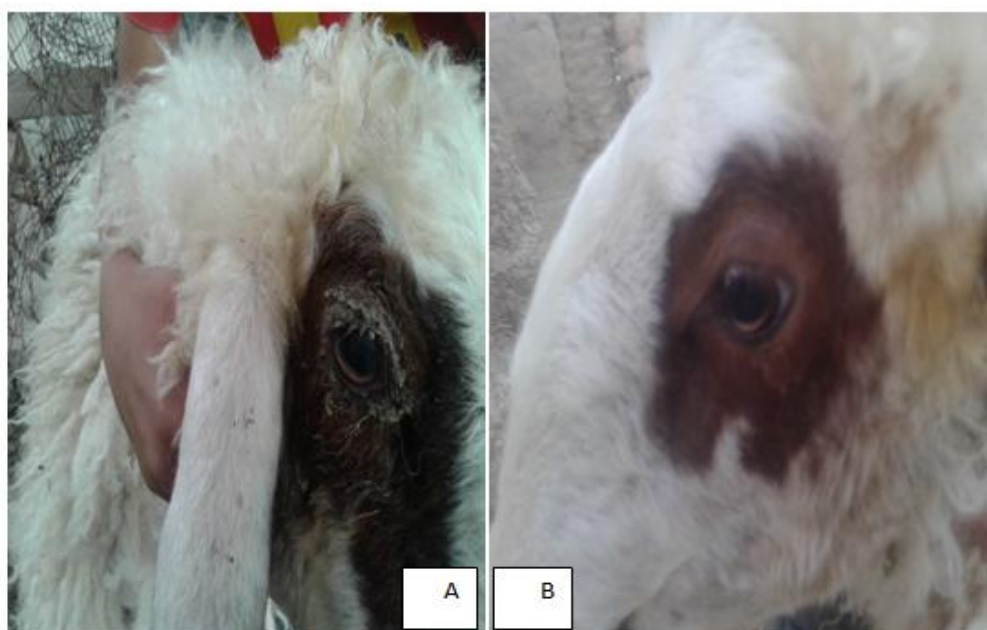
Picture F Eye treated with penicillin -streptomycin ointment there is slightly inflammation after 6 day of infection healing appear at 7-10 day .



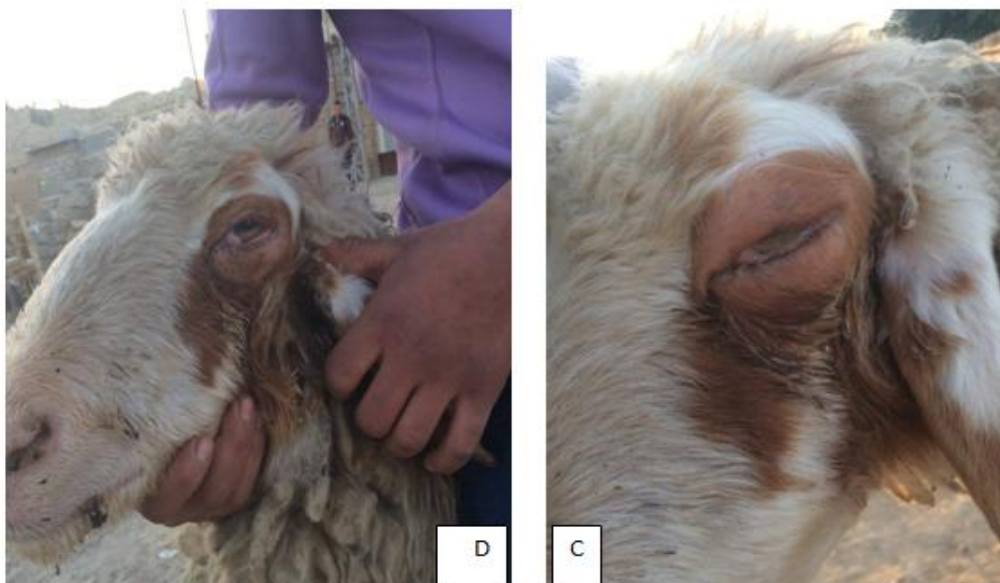
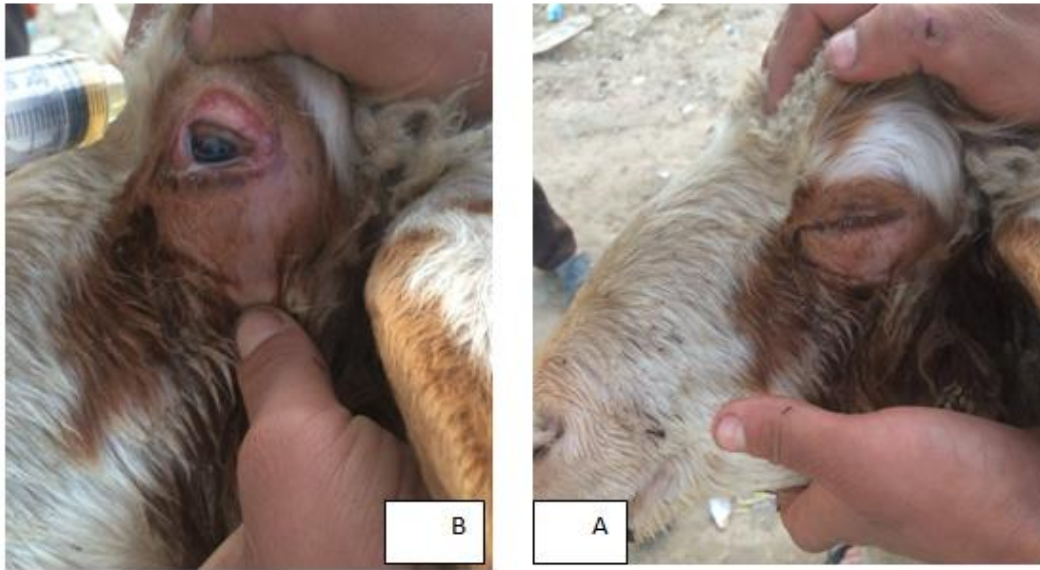
Picture

F

Picture E 5 day of infection after treated with ointment of extract eye show there is complete healing ,absence of inflammation ,no redness, lightly eye drop then disappear and healing from 7-10 day .



Picture A referred infected eye before treatment ,B after twice daily treatment with aloe vera lotion and ointment complete healing showed after 5 day only .



Picture A explain eye conjunctivitis with staphylococcus before treatment , B after 1 Day ,C 3 day ,D 5 day of treatment with aloe vera lotion and ointment twice daily showed good healing and remove inflammation.

DISCUSSION

The different types of bacteria isolated in present study correlate with the findings of (16,11) with slight variation . penicillin –streptomycin treated infected sheep was showed effective and completed healing occur but some cases specially infected with staphylococcus showed recurrent these study was agreement with (13).

A methanolic extract of Aloe Vera showed significant *in vitro* antibacterial efficacy against *Staphylococcus aureus*, *M.avis*, *Proteus spp.* As shown in fig 2 and 3. Which agree with several studies(5).

The using of well diffusion agar technique showed that various components of *Aloe Vera* inhibit growth of *S.aureus* (17, 18).

The antimicrobial agents of Aloe Vera extract was reported to effectively kill or greatly reduce or eliminate the growth of *Staphylococcus aureus* (19,20).Anti-*S.aureus* activity was confirmed by (21) in isolated bacteria from skin infections in well diffusion agar test. *In vitro* study reveal inhibitory effect of aloe Vera extract also confirmed by(22,23,24). But although these studies confirmed antibacterial effects of *Aloe Vera* extract against a different types of bacteria including *S.aureus*, (25) showed this bacterium is resistance to *Aloe Vera*. The antimicrobial activity of aloe Vera extract was showed greater antibacterial activity against Gram-positive (*S.aureus*) as compared to Gram-negative bacteria (*Moraxella ovis* and *Proteus*). These differences may be attributed to the fact that the cell wall in Gram-positive bacteria consists of a single layer, whereas the Gram negative cell wall is a multi-layered structure and quite complex (26). or due to the presence of additional lipopolysaccharide layer in the former(27). It could be believes that presence of greater amount of the anthraquinones, saponins (28,29) and phenolic antioxidants in the extract could be responsible for the high and broad spectrum antimicrobial activity of aloe Vera extract(3). While polysaccharides have been attributed within direct bacterial activity through the stimulation of phagocytic leucocytes to destroy bacteria (27).

(18) study activity of ethanolic and methanolic extracts of Aloe Vera were studied for their antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumonia*, that indicated broad spectrum activity due to contain (coumaric acid, ascorbic acid, pyrocatechol and cinnamic acid were identified by thin layer chromatography(TLC) which act by synergistic manner. anthraquinones and saponins have antibacterial effect(30) .

(11) reported that aloe Vera extract accelerate burning healing induced by sulfuric acid in rabbit infected with *staphylococcus aureus* and non-infected burns that may be given another proven on importance of extract in healing activity. aloin and aloe-emodin chemical constitute of aloe v showed potent inhibitory activity against *Colletotrichum gloeosporides*

and *Cladosporium cucumerinum* (31,10) .improve tissue regeneration and healing through increase blood supply ,reduce oxidative stress and increase oxygenation ,addition to anti-inflammatory due to contain peptidase Brady kinase that destroy bradykinin and prevent synthesis of prostaglandin via inhibition cyclooxygenase that very active in acute case (32)).Antimicrobial activity of *A. Vera* gel was also compared with seven standard antibiotics used in the study.It found activity of Aloe Vera extract healing of eye infected with staphylococcus aureus and other bacteria more active from antibiotic , that may return to extract have multivitamins specially vitamin A and E needed for tissue repair ,on the other side have antibacterial, anti-inflammatory , and antiseptic is due to contain lupeol, salicylic acid, cinnamonic acid, phenols and sulphur. These agent possess inhibitory effect on fungi, bacteria and viruses(33,11).Scientific evidence has brought about the possibility of the utilization of plant extracts in the treatment of bacterial infections and the development of antibacterial products (2,33,11).A. Vera contain anthraquinones as an active compound, which resemble of tetracycline in mechanism of action there for inhibits bacterial protein synthesis by blocking the ribosomal site. Therefore, the bacteria cannot grow in the media containing A. Vera extract(34,35,7) . moreover contain Polysaccharides and phenol reported refer direct bacterial activity through the stimulation of phagocytic leucocytes that killed bacteria. modern studies showed that aloe Vera gel healing ulcer induced by *Helicobacter pylori* through direct effect as anti-bacterial and healing properties , therefor healing activity 100% was demonstrated in our study due to inhibition of bacterial that It was proven during in vitro study to reach high percent of sensitivity of bacteria% (80,36,29 in both methods of wells diffusion while disc diffusion in mm(50, 22,16) for staphylococcus, *Moraxella* and *Proteus* as shown in pictures (pic. 1 and pic.2). Farther current results focus inhibition effect of aloe vera in vitro Antibiotic sensitivity of the bacterial isolate to different antibiotics by using disk diffusion method of *Staphylococcus*, *Moraxella* and *Proteus* (66.6, 50,50mm)respectively for streptomycin while for Penicillin G(100,0,0) and Ampicillin (83.3, 50,50) respectively other result showed in table 2 .our result was agreement with(7). demonstrated antibiotic resistance and susceptibility pattern of four agent against gram positive and negative (Erythromycin, Cefoperazone,Ciprofloxacin and Doxycycline).was proven that most inhibition zone100% for Cefoperazone followed by100% inhibition for positive, ciprofloxacin showed activity (28mm,73mm)for positive and negative respectively .However Gram-positive isolates were found 94% susceptible with methanol extract whereas Gram -negative bacterial

susceptible 100%, *inhibition zone for staphylococcus aerus record* (28mm, 14,20,17,0)for Meropenene ,Erythromycin , Cefoperazone ,ciprofloxacin and doxycycline respectively.

Aloe Vera gel showed greater all these result may be return to aloe vera contain six antiseptic compound can be listed as lupeol, salicylic acid , urea nitrogen, cinnamonic acid ,phenol and sulfate these agent act synergistically to inhibit bacteria so that has antiseptic activity ,addition to calming effect and anti-inflammatory effect due to salicylic acid and cyclooxygenase pathway there for reduce PGE2 beside Vera cyglucan B and C have demonstrated anti-inflammatory(36,37).the main causes of healing in vivo may be explain as due to extract contain many vitamins such as (A,C,E)that responsible of antioxidant activity and immune modulation activity of extract (38).

The PH of healthy tears is record between (7.3-7.7) and influenced by drugs used topically additional to eyelids closer for long period lead to lower PH so that long opening increase PH through loos of CO₂ .some drugs used topically cause alkaline burns ,other cause acid eye burns , alkaline drugs high penetration to eye surface can cause damage of cornea while acid low penetrate cause destruction external layer of eye lens so cause blindness(39,40).Our study showed that PH of aloe Vera was 6.3 that consider near form neutralize rather than drugs may be high acidity , that PH give good chance for improvement healing , addition to aloe Vera contain vitamin C act as cofactor of collagen synthesis that reduce ulceration , eye treated with extract showed healing with short period less than 10 day due to emollient nature with increase corneal, conjunctive epithelial and keratocyte proliferation that aid to complete re-epithelization , so the main cause of K.C due to loss of eye fluid mucous due to damage of conjunctival and goblet cell that reduction of mucous that worsen case if not treated (41,42). healthy group of sheep treated with aloe Vera extract 5 day to evaluation safety of agent showed there is no any discomfort or any symptoms on eye that may prove that extract not have any adverse effect on tissue eye.

CONCLUSION

Aloe Vera extract as showed high inhibition zone for staphylococcus ,followed by Moraxella then proteus . on the other aspect animal treated with aloe Vera ointment with ye lotion showed complete healing during period 6-10 day according to type and severity of infection, and there is no recurrent infection after treated so that we recommended to used in eye problem which consider very important in Veterinary Medicine .

دراسة الفعالية المضادة للبكتريا لخلاصة نبات الصبار ومقارنتها مع عقار البنسلين – ستربتومايسين في علاج مسببات التهاب ملتحمة العين سريريا ومختبريا

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الخلاصة

أجريت هذه الدراسة في الحقول الحيوانية التابعة لمدينة القاسم وكانت الأغنام المصابة في هذه الحقول تعاني من التهاب شديد في العين خلال الشهر الرابع – الى الشهر العاشر من السنة. حيث لوحظ تدمع العين بغزارة العين وارتفاع الحرارة وانتفاخ العين واحمرارها وتفتح العين في بعض الحالات اجري الفحص السريري للأغنام وتم اخذ عشرون خزعة مختبريه من الأغنام المصابة لغرض اجراء الفحوصات المختبرية من زرع بكتيري واختبارات الكيموحيوية واختبار حساسية البكتريا بطريقتي طريقة الانتشار القرصي وطريقة الانتشار بالحفر 0 وهدفت الدراسة الى تقييم تأثير مرهم الصبار والغسول في اصابات ملتحمة العين في الاغنام ومختبريا من خلال اختبار طريقة الانتشار القرصي وطريقة الانتشار بالحفر ومقارنتها مع المضادات الحيوية. وجدت الدراسة ان نسبة الاصابة هي 50% للمكورات الذهبية و الموراكزيبلا 33% و بروتيوس 17% وظهرت الدراسة المختبرية نسبة تثبيط عالية بالمقارنة مع المضادات الحيوية. سجلت الدراسة داخل الجسم من خلال استخدام المرهم والغسول نتائج واضحة حيث لوحظ شفاء العين بعد العلاج بمرهم البنسلين –ستربتومايسين وخلال فترة 10-6 وحسب الاصابة بينما اظهرت المجموعة المعالجة بالمستخلص كمرهم وغسول نتائج شفاء تام للعين خلال فترة 10-6 وحسب نوع وشدة الاصابة وعدم رجوع الاصابة لذا يوصى بأجراء العديد من البحوث واستخدام النبات في علاج التهابات العين التي تعتبر مهمة في الطب البيطري

REFERANCE

- 1.Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). (2014). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. 34 , 1.
- 2.Prajapati, M., Patel, P. and V,yas, P. (2011).Phytochemical Analysis of Aloe Vera and Study of Mixing Antibiotic with Aloe Vera and Its Antibacterial Activity. Asian J of Bioch and Pharma Res2,1.
- 3.Grindlay D, Reynolds T.(1986).The Aloe veraPhenomenon: A Review of the properties and morden uses of the leaf parenchyma gel. Journal of Ethanopharmacology.16, 117-151
- 4.Anilkumar KR, Sudarshankrishna KR, Chandramohan G, Ilayaraja N, Khanum F, Bawa AS(2010). Effect of Aloe vera gel extract on antioxidant enzymes and azoxymethane induced oxidative stress in rats. Ind J ExpBiol 48, 837–842.
- 5.Mhya DH , Mankili K .(2015). Bacterial Enzymes: A Good Alternative to ConventionalChemicals in Leather Processing. *Int.J.Biosci.Nanosci.2 ,1*: 20-23

6. KaithwasG, Kumar A, Pandey H, Acharya A K, Singh M, Bhatia D, MukerjeeA. (2008). investigation of comparative antimicrobial activity of ALOE VERA GEL AND JUICE. *Pharmonline*. 1, 239-243.
7. Chatterjee , Rudrangshu; DushyantSingh; AmitaGauravDimri; AnkitaPandita;ShiwaniChaudhary and M.L.Aggarwal. (2015). Comparative study of antimicrobial activity of aloe vera gel and antibiotic against isolates from fast food. *wjpps*, 4, 1058-1073.
8. Hussain Aref, Chhavi Sharma¹, Saniyah Khan¹, Kruti Shah¹, Shafiqul Haque. (2015). Aloe vera Inhibits Proliferation of Human Breast and Cervical Cancer Cells and Acts Synergistically with Cisplatin. *Asian Pac J Cancer Prev*, 16, 7, 2939-2946.
9. Vaid RK, Anand T, Bera BC, Shukla BN, Nagar DK, Singh G, Virmani N, Barua S Singh B K Singh, R K. (2014). Isolation and molecular identification of *Moraxella ovis* and *Moraxella* spp. from Infectious .
10. Ghasemi S, Emami MR, Maleki M. (2009). Histopathologic evaluation of curative impact of Aloe vera L. fresh gel on healing of experimental infected full-thickness open wounds induced with staphylococcus aureus in dogs. *IJVS*. 4, 2, 103-113.
11. Adnan MJ, Hameedah HA¹-Ahbabi¹, Rabab Alhussani A. (2015). Study of the Efficacy of Aloe Vera Extracts in Treatment of Non-Infected Wounds Induced by Sulfuric Acid and Infected Wounds with *Staphylococcus aureus*. *Intern J of Adv Res* ., 3, 1: 593-601.
12. Forbes BA, Sahm DF, Weissfeld AS. (2007). (Bailey and Scotts' Diagnostic microbiology) 12th ed. Elsevier .
13. Naglic T, D. Hajsig, J Frey, B Seol, K Busch, M Lojkic. (2000). Epidemiological and microbiological study of an outbreak of infectious keratoconjunctivitis in sheep. *Vet. Rec.* 147: 72-75.
14. Mehrotra S, Srivastava AK, Nandi SP. (2010). Comparative antimicrobial activities of Neem, Amla, Aloe, Assam tea and Clove extract against *Vibrio cholerae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *J Med Plant Res*. 4, 18, 2473-2478.
15. Rahamatun Nabi M, Ali MR, Islam MA, Tossani, M. T. (2005). Isolation and characterization of bacterial from keratoconjunctivitis affected cattle with their pathogenicity and in vitro antibiotics sensitivity. *Bangl J Vet. Med.* 3, 1: 25-31.

- 16.RakshaBawankar, V C Deepti,PoojaSingh,RathinasamySubashkumar,Govindasamy Vivekanandhan1 , Subramanian Babu.(2013). Evaluation of Bioactive Potential of an Aloe vera Sterol Extract., *Phytother. Res.* 27: 864–868 .
- 17.Lawless J, Allan J (2000). The Clinical Composition of Aloe vera, In: Aloe vera natural wonder cure. Thorsons, Publishing Ltd., London,United Kingdom. 4,161-171.
- 18.Lawrence R, Tripathi P, Jeyakumar E(2009) .Isolation, purification and evaluation of antibacterial against from Aloe vera. *Braz J Microbiol.* 40, 906-915.
- 19.Lawrence JG, Hendrickson H. (2004). Chromosome structure and constraints on lateral gene transfer. *Dev Genet* 2004:319–336.
- 20-PughN, Ross SA ,ElSohly MA, Pasco DS. (2001). Characterization of Aloeride, a new high molecular weight polysaccharide from Aloe vera with potent immunostimulatory activity. *J. Agri. Food. Chem.* 49,2, 1030-1034.
- 21.Bashir A, Saeed B, Mujahid TY. (2011).Comprative study of antimicrobial activities of Aloe vera extracts and antibiotics against isolated from skin infections. *Afr J Biotechnol.* 10 ,19, 3835-3840.
- 22.Agarrry OO,Olaleye MT, Bello-Michael CO. (2005). Comparative antimicrobial activities of aloe vera gel and leaf. *Afri J of Biotech.* 4 ,12, 1413-1414.
- 23.Ilaiyaraja N, Khanum F, Anilakumar KR.(2010). Anti-ulcerative coloitis and anti-bacterial properties of hydroalcoholic extract of Aloe vera (L) gel. *J Herbal MedToxicol.* 4,1, 197-206.
- 24.Mehrotra S, Srivastava AK, Nandi SP.(2010). Comparative antimicrobial activities of Neem, Amla, Aloe, Assam tea and Clove extract against *Vibrio cholerae*, *Staphylococcus aerous* and *psedumonasaeruginosa*. *J Med Plant Res.* 4 ,18,2473-2478.
- 25.Cock IE.(2008). Antimicrobial activity of Aloe barbadensis Miller leaf gel componets. *The Inter J Microbiol.* 4,2.
- 26.Matu EN, J. Van Staden (2003). Antibacterial and anti-inflammatoy activities of some plants used for medicinal purposes in Kenya. *J. Ethnopharmacol.* 87 ,1, 35-41.
- 27.RodríguezD,D. Hernández-Castillo, R Rodríguez- García, J. L. AngulSanchez.(2005).Antifungal Activity *in Vitro* of Aloe veraPulp and Liquid Fraction against Plant Pathogenic Fungi,” *Industrial Crops and Products*, 21,1: 81-87.

- 28.Reylond T, Dweak A.(1999).Aloe vera leaf gel :a reviewer update .j
ethanppharmacol. 68,3-37.
- 29.Urch. D. (1999). Aloe vera the plant. In: Aloe vera nature's gift. Blackdown
Publication, Bristol, United Kingdom, 8-17.
- 30.Reynolds T, Dweck AC. (1999). Aloe vera leaf gel: a review update. J.
Ethnopharmacol. 68, 3-37.
31. Sebastian E, Nidirj J, Ganeshan G, Lokesha AN.(2011). Antifungal activity of some
extractives and constituents of Aloe vera. Res J Med Plant .5, 196–200.
- 32.sahu PK ,GirPP,singhP,PanderyP,GuptaS,shivaastvaAK, Kumar A,paundery
KP.(2013).therapeutic and medicinal use of aloe versa :reviewer
.j.pharmapharma .pharmacol.4,599-610.
- 33.Barandozi F, Nejat zadeh. (2013). Antibacterial activities and antioxidant capacity of
Aloe vera. Org Med ChemLett, 3, 5.
- 34.Habeeb F, Shakir E, Bradbury F.(2007). Screening methods used to determine the
anti-microbial properties of Aloe vera inner gel. Methods.42,315-320.
- 35.Radha, MaharjanH,NampoothiriP,Laxmipriya .(2015). Evaluation of biological
properties and clinical effectiveness ofAloevera: A systematic review.
Journal of Traditional and Complementary Medicine .5, 21-26.
- 36.Esua MF, Rauwald j w.(2006).Novel biactivemaylolglucanfromaloeveragel:isolation
structure elucidation and in vivo bioassays , dermatol . 143,923-929.
- 37.Surjushe A,Vasani R, Saple D G.(2008). Aloe vera short review .indian .j .dermatol
.,53,163-166.
- 38.Choche T,shendersK.(2014).extraction and identification of bioactive compound from
aloe barbaders , Res.Rev .j .pharmacophytochem .2, 14-23.
- 39.Willam H C , Patricia A J.(1984).Dynamics of ocular surface pH British Journal of
Ophthalmology, 68, 549-552.
- 40.Ingrida Januleviciene , LinaSiaudvytyte and RutaBarsauskaite.(2012).Ophthalmic
Drug Delivery in Glaucoma—A Review . Pharmaceutics . 4, 243-251.
- 41.Adeola Kosoko, ; Omofolasade K.L, MSPH, M.M .(1984).Chemical Ocular Burns: A
Case Review American J. of linal edicine,4,3 ,42-49.
- 42.Havens S, Kosoko-Lasaki O, Palmer M.(2009). Penetrating eye injury: a case study.
American Journal of Clinical Medicine; 6,1, 42-49.
43. JEHAN BAKHT1, AMJAD ISLAM1 AND MOHAMMAD SHAFI.(2011).
antimicrobial potentials of *eclipta alba* bywell diffusion method., *Pak. J.*
Bot., 43: 169-174. .