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Effect of *Aloe vera* gel on some bacterial and fungal causes of conjunctivitis in buffaloes

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Abstract

Pink eye, or conjunctivitis, is a nasty eye disorder that can affect animals of all age's groups. *Aloe vera* is definitely one of nature's best "cure all" medicines, it contains some powerful antiviral, antibacterial and antifungal properties and works exceptionally well for conjunctivitis. Buffaloes suffering conjunctivitis during the period from March 2018 to October 2018 in a farm in El-Fayoum governorate were checked clinically and examined microbiologically. Forty-five infected eyes wabes and twenty feed samples were subjected to the bacteriological and mycological examination based on their culturing, morphology, staining and biochemical tests. The results showed that, bacterial isolates included *Streptococcus* species (44.44% & 25%), *Staphylococcus* coagulase negative (33.33% &15%), *Escherichia coli* spp. (15.56%& 20%), *Staphylococcus aureus* (8.89 %& 30%), *Moraxella bovis* (17.78% & 5%), *Corynebacterium* spp. and *Pseudomonas* spp. (6.67&5 each) and *Klebsiella pneumoniae* (4.44 & 5); while *Aspergillus* spp. and *Candida* species were the most fungal isolates in a percentage of (13.33% & 85%) and (8.88% &35%) from eye swabs and ration samples respectively. The disease induced experimentally by inoculation of a rabbit eye with the infected agents and then treated topically by *Aloe vera* gel. *In vitro* antimicrobial properties of *A.vera* gel extracts were investigated against some bacterial and fungal isolates. The gel extracts of *A.vera* showed promising antimicrobial activity results not only against both Gram-positive and Gram-negative bacterial isolates but also against fungal isolates compared with the traditional antimicrobial one when tested against the same isolates. It can be concluded that the inhibitory activities were found to be dose-dependent. Furthermore, the herbal extracts may be used as antibacterial and antifungal for pathogens resistant to conventional antibiotics and severe infections remedy.

Keywords: *Pink eye, antimicrobial potential of Aloe vera, buffaloes and traditional antibiotics.*

Introduction

Conjunctivitis or pink eye is simply an inflammation of the soft tissues surrounding the eye and eyelids. It has many different causes as virus, bacteria, or an allergic reaction **(Cullen *et al.,* 2016)**. Ruminants such as cattle and goats affected with conjunctivitis will have reddening of the eyeball, swelling of the inner lining of the eyelid and extremely itchy. These animals will have an increased sensitivity to sunlight, which is demonstrated by 'squinting' or closing their eyes in bright sunlight. It can also cause mucus production that's so bad; it cements the eyelids together **(Evans *et al.,* 2004** and **Cullen *et al.,* 2017)**.

Numerous studies recorded the presence of microscopic organisms linked to cases of keratitis and conjunctivitis including *Mycoplasms, Listeria, Moraxella, Streptococcus, Staphylococcus, E.coli, Micrococcus* and *Corynebacterium* **(Barber and Jones, 1989**; **Evans *et al.,* 2004** and **Fernandez-Aguilar *et al.,* 2017**). In addition, the infection by opportunistic fungi has increased significantly especially the *Aspergillus fumigates* which is the important fungi in

causing recurrent infections (**Scott *et al.,* 1977** and **Cullen *et al.,* 2017)**.
Although conjunctivitis is not a fatal disease, it can cause severe economic losses to the cattle producer through reduced growth rates; costs of treatment including labor costs, and a reduced value of affected cattle. The three most common causes of conjunctivitis in cattle would include: 'Pinkeye' or infectious bovine keratoconjunctivitis (IBK); infectious bovine rhinotracheitis (IBR); and foreign bodies in the eye such as dust **(Faez *et al.,* 2017)**.

In the medical industry, the over prescribing and incorrect use of antibiotics has increased drug resistance, making it difficult to treat most of the common microbial infections **(El-Jakee *et al.,*2013** and **Singh *et al.,* 2018)**.The worldwide interest in herbal products has grown significantly, this is not only a general trend towards the usage of natural products for curing illnesses but also due to the availability of considerable evidence regarding the efficacy of herbal remedies as described by **Viegi *et al.* (2010)**.

There are several medicinal plants that contain active compounds that may find their application in veterinary medicine as antibacterial, antimycotics, antiparasitics, disinfectants and immunostimulants **(Laudato and Capasso, 2013)**. *Aloe vera* is one of nature's best "cure all" medicines. It contains some powerful antiviral, antibacterial and antifungal properties and works exceptionally well for conjunctivitis **(Meenatshi *et al.,* 2013)**. The plant's name is derived from the Arabic word 'alloeh' that means 'bitter', referring to the taste of the liquid contained in the leaves. The central bulk of the leaf contains colorless mucilaginous pulp, made up of large, thin-walled mesophyll cells containing the *A.vera* gel itself **(Beckford and Badrie, 2000** and **Strickl *et al.,* 2004)**. It is acclaimed to cure ailments ranging from mild fever, wounds and burns, gastrointestinal disorders, diabetes, sexual vitality, and fertility problems to cancer, immune modulation and AIDS **(Grindlay *et al.,* 1986** and **Mackay and Miller, 2003)**. Also, Aloe has been used for the treatment of skin diseases for more than 5000 years. Among more than 360 *Aloe* species, *A.vera* (*A. barbadensis* miller) has been the most popular in both folk and official medicine **(Kodym and Bujak, 2002; Sun *et al.,* 2002** and **Larry, 2003)**.

Many studies reported the effective use of this plant when applied topically for the treatment of burns, sunburns, inflammatory skin disorders and wounds **(Paulsen *et al.,* 2005; Reider *et al.,* 2005** and **Belo *et al.,* 2006)**; they suggested that beneficial effects of gel are due to its high molecular weight components such as polysaccharide, lectin-like proteins, and prostaglandins. It contains over 70 biologically active compounds and is claimed to have anti-inflammatory, anti-oxidant, immune boosting, anticancer, healing, anti-aging and anti-diabetic properties **(Puke and Ayensu, 1985; Koo, 1994** and **Kodym *et al.,* 2003)**.

The aim of the present study was to evaluate the effects of *A.vera* gel extract son the most predominant bacterial and fungal causes of conjunctivitis in a buffaloes farm in El-Fayoum governorate, either in a single form or in a combination with the most common and effective antimicrobial agents used in the field to reduce the amount of antibiotics used.

Materials and Methods

Collection of samples: A total of 45 infected eye swabs were obtained by retropulsion each eye through the closed upper eyelid and running a sterile swab along the surface of the ventral conjunctival fornix. The procedure was repeated twice to obtain specimens of pathogens. Swabs collected were maintained in 2 ml of a sterile saline solution but with gentamicin (50µl/ml) for mycotic cultures. Special care was taken to ensure that the swab did not come into contact with the vibrissae, eyelids, or eyelashes. All the samples maintained at 4°C **(Quinn *et al.,* 2013)**. In addition, 20feed samples were collected aseptically in sterile bags from rations submitted to the animals under study to detect the source of infection.

Bacteriological examination:
Each conjunctival swab was inoculated in Trypticase Soya broth (TSB), incubated at 37° C for six hours and then streaked onto whole media. The culture media used were: Columbia sheep blood agar, Mannitol Salt agar, Mac-

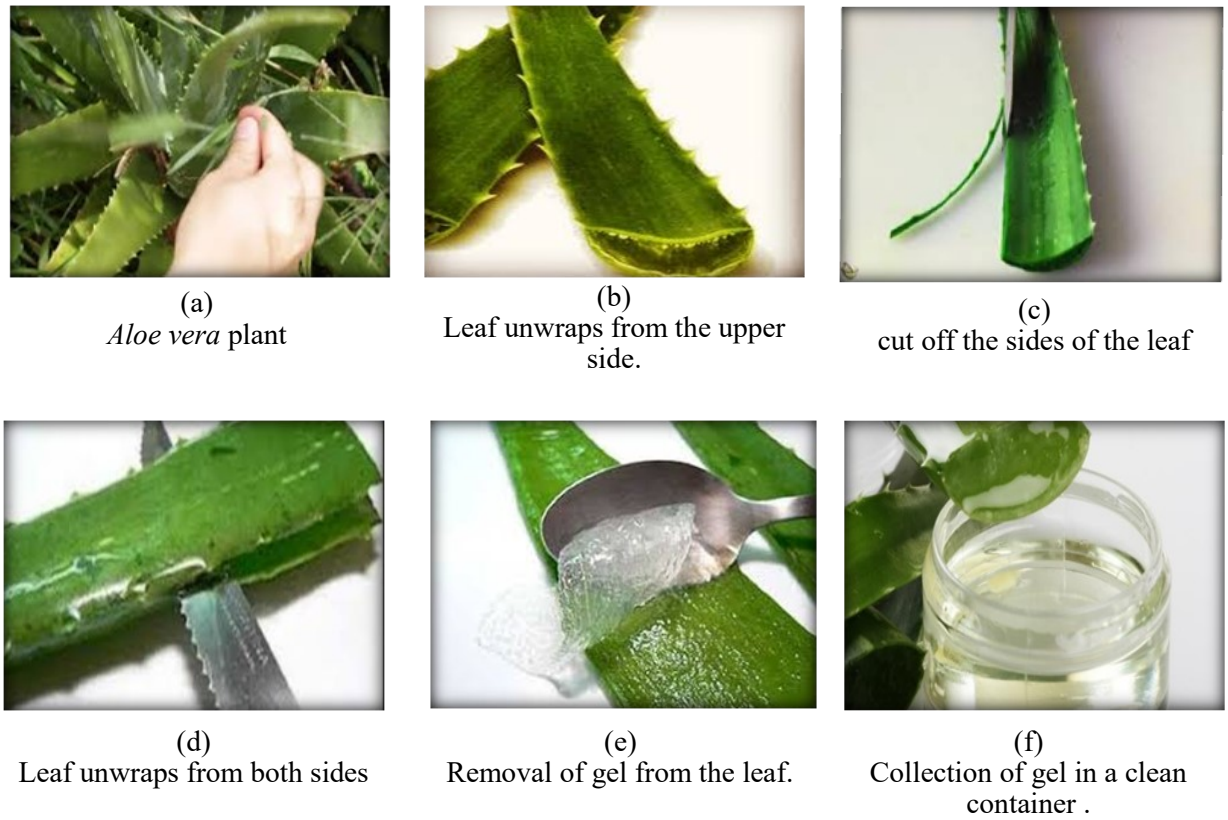
Conkey agar. Inoculated plates were incubated at 37°C and examined for growth at 24, 48 and 72 hours. Representative colonies of bacteria were sub-cultivated onto Columbia blood agar plates and identified by morphological assessment, Gram staining and biochemical tests. Stock cultures were maintained in vials by growing the isolates in 3 ml nutrient broth and the next day overlaying with 3 ml 40% glycerol. Vials were then frozen at -70°C**(Gul *et al.,* 2004**and **Richardson *et al.,* 2005)**.

Mycological examination: Samples were plated onto Sabouraud dextrose agar (SDA), incubated at 25°C and examined daily from day 4 post incubation, over 21 days to identify slow-growing organisms **(Rosa *et al.,* 2003)**. Identification of colonies of filamentous fungi was achieved at the genus level based on the macroscopic and microscopic features of colonies. *Aspergillus* species were identified and yeast colonies were highlighted based on keys provided by **Refai *et al.* (2012)**.

Determination of antimicrobial sensitivity profile: Isolates were subjected to antibiotic resistance screening by disk diffusion method. For this purpose, inoculate were prepared by diluting overnight cultures in sterile sodium chloride (0.9%) suspension and then match with the 0.5 standard MacFarland index to yield a uniform suspension containing 1.5×10^{8}CFU/ml. 0.1 ml from each of these suspensions were plated onto TSA and SDA media, and then commercially used discs enumerated in Table (3&4) were placed on the lawn of culture. Bacterial plates were incubated overnight at 37°C while the fungal one at 25°C for 1-3 days **(Hoeger, 2004** and **Veronica and Keelan, 2006)**. Sensitivity was determined by the zone of complete growth inhibition around each disk according to **Clinical and Laboratory Standards Institute (CLSI, 2010)**.

Preparation of *Aloe vera* gel extracts:
Fresh *A.vera* plant was purchased from one of the nurseries in El-Fayoum governorate. As can be seen from the photograph (1), *Aloe vera* gel (AVG) is the mucilaginous jelly obtained from the center of the plant leaf of *A.vera*. The gel portion of the plant was prepared by the method as described by **Rajasekaran *et al.,* (2005)** and **Olaifa (2017)**. In brief, leaves of *A.vera* were collected, washed with water to removed mud and other debris, dried, cut longitudinally using sharp knife, and then thick epidermis was selectively removed and the inner gel-like pulp in the center of the leaf was separated into a clean container using large spoonful. These fillets of gel were cut into small cubes, washed with de-ionized water, minced; homogenized in a home blender, filtrate by using Whatman filter paper (No 42) to separate from fibers, and then refrigerated at 4° C and used such as. On the other hand, a group of small cubes of fillets of gel was dried in oven at temperature 60 °C and further extracted with ethanol (95%) and sterile distal water. The extracts (aqueous and ethanolic) were filtrate, and dried with the help of rotary evaporator at temperature 55 °C until the solvent was completely removed and dried ethanol and aqueous extracts were re-dissolved in different concentrations in their respective solvents (ethanol and distal water) for preparing different concentrations (25, 50, 100 and 200 µg/ml).

Photo. (1): Preparation of *Aloe vera* gel extract.



(a) *Aloe vera* plant

(b) Leaf unwraps from the upper side.

(c) cut off the sides of the leaf

(d) Leaf unwraps from both sides

(e) Removal of gel from the leaf.

(f) Collection of gel in a clean container .

Antimicrobial susceptibility testing of *A. vera*: Sterile trypticase soy agar (TSA) and-Sabouraud dextrose agar (SDA) at 45°C was poured into sterile Petri dishes, which had been inoculated with one ml of the *Streptococcus, E. coli*, *S. aureus*, *M.bovis*, *A. flavus, A. niger* and *C. albicans* (1.5×10^8 CFU/ ml). Then the plates were left for 5-15 minutes at room temperature to solidify. Wells of 6 mm in diameter were made with a sterile tip of pasture pipette on the surface of the agar plates. About 40µlof the prepared gel extracts concentrations (25, 50, 100, and 200 µg/ml) was delivered using micropipette into each of the wells in separate plates for bacteria or fungi. Whereas in a separate welled plate the most effective traditional antibiotics resulting in the previous antibiotic resistance profile (danofloxacin 30µg and miconazole 10µg) were included as positive antibacterial and antifungal controls, and also combination effects of the gel and these two antibiotics were performed but 40 µl was added from each in the same well. These plates were incubated for 24h at 37°C and for 1-3 days at 25°C for bacterial and fungal isolates respectively. The presence of zones of inhibition was regarded as the presence of antimicrobial action, which was expressed in terms of the average diameter of the zones of inhibition measured **(Agarry *et al.*, 2005)**.

Experimental Pathogenicity (virulence) testand treatment by *Aloe vera* gel: A rabbit with an average weight of about1-1.5 kg were infected with 0.1 ml (one drop) of the last prepared (1.5×10^8 CFU/ ml) suspension of all bacterial and fungal isolates after pooling of them, then installed into the conjunctiva of the rabbit. Severe conjunctivitis within 24-36h was recorded **(Richard, 2018)**. The day of conjunctivitis creation marked day zero of the experiment. The infected eye was topically treated with the *A.vera* gel dispensed with a syringe 3-4 time/ day while the other eye treated with one drop of 0.9% normal saline and leave as a control. This eye treatment was done daily from day zero until recovery representing the termination of the experiment **(Al-Ahbabi *et***

***al.*, 2016)**.

Result and Discussion

Eye infections are quite irritating and may alter the mood of animals; they are commonly reported in domestic buffaloes and cattle **(Abdullah *et al.*, 2015)**. The most common causes of pink eye are viral because of frequent colds, followed by bacterial infections. Both the viral and bacterial causes can spread from animal to another quite fast. The most common allergic reasons for pink eye are pollen and animal hair allergies **(Ali *et al.*, 2011)**. The higher incidence of pinkeye occur in the late summer or early falls corresponds to the increase in flies, plant growth, pollen production, and hay feeding that is occurring in the animal's environment **(Wilcox, 1970)**.

Culture and susceptibility testing are advised before any treatment is carried out because antibiotic susceptibility may vary depending on different geographical regions **(Faez *et al.*, 2017)**. So in our study the affected buffaloes showed signs copious eye drops, increase body temperatures, swollen of eye with redness, eye pus in some cases in the farm were properly restrained in cattle crush and 45 swabs were collected from conjunctivae of affected eyes and also 20 samples of their ration were subjected to bacteriological and mycological examination using standard culture technique to determine the causal organism according to **Quinn *et al.* (2002)** and **Singh (2009)**. Positive bacterial culture was obtained in all samples (100%) either in a single form or combined with other bacteria or fungi.

On the basis of isolation and identification, high ratios of bacteria was obtained from the conjunctival and ration samples in our study as shown in Table (1). *Streptococcus* spp.(44.44% &25%); *Staphylococcaus* spp.(42.22% &45%); followed by *M.bovis* (17.78%&5%).*E. coli*spp. (15.56%& 20%), *Corynebacterium* spp. and *Pseudomonas* spp. (6.67% &5%) were the most predominant isolated bacteria. Similar studies were conducted, **Sarma *et al.* (1989)** isolated mostly *Streptococcus* spp. and to a lesser extent, *Mannheimia haemolytica*, *Corynebacterium bovis*, *Micrococcus* spp. and *Candida* spp. from conjunctiva of 27 cows in England, and also the same result were recorded by **Nadra-Elwgoud and Manal (2010)** in Egypt. In a large sample epidemiological study carried out in Australia **(Maggs *et al.*, 2008)**, it was shown that, Gram-positive bacteria predominated (54.4%), other bacteria were *Corynebacterium* spp. (27.4%), *Moraxella non -liquefaciens* (26.9%), *N. catarrhalis* (10.5%), *Acinetobacter* spp. (8.0), *M.bovis* (6.5%), *Coliform spp.* (6.5%) and *Bacillus* spp. (1.3%). In France **Turnes and Albuquerque (1984)** isolated *M.bovis* (82.3**%)** from conjunctiva swabs of 16 cows with infectious bovine keratoconjunctivitis**.**

Table (1). Incidence of bacterial pathogens isolated from examined samples:

Isolated bacteria	Eye swabs (45)		Ration (20)	
	No.	%	No.	%
***Streptococcus* spp.**	20	44.44	5	25
***Coagulase negative staphylococcus* (CNS)**	15	33.33	3	15
***Escherichia coli* spp.**	7	15.56	4	20
Staphylococcus aureus	4	8.89	6	30
Moraxella bovis	8	17.78	1	5
***Corynebacterium* spp.**	3	6.67	1	5
***Pseudomonas* spp.**	3	6.67	1	5
Klebsiella pneumoniae	2	4.44	1	5

%: was calculated according to the total number of samples of each item (45, 20) NO: Number of positive samples.
N.B: Positive bacterial culture was (100%) in all conjunctival and rations samples

Fungi are ubiquitously found in hay, straw and similar materials in the environment (**Akodouch *et al*., 2014** and **Seyedmojtaba *et al.,* 2018)**. Fungal eye infections are extremely rare, but they can be very serious. The most common way for someone to develop a fungal eye infection is as a result of an eye injury, particularly if the injury was caused by plant material such as a stick or a thorn. Many different types of fungi can cause eye infections, such as *Fusarium*, live in the environment and are often associated with plant material **(Thomas and Kaliamurthy, 2013)**.

In addition yeast and bacteria live side by side as commensals in the natural microflora of healthy humans and animals. Since both bacteria and fungi are opportunistic pathogens, it is no surprise that they are frequently isolated from sites of infection. In many cases, it is not always clear which organism is responsible for the initial infection and which organism represents a secondary infection or saprophytic interloper there for a free lunch **(Songer and Post, 2005)**.

As shown in Table (2), positive fungal culture was obtained in ten conjunctival samples (22.22%) and in all ration samples (100%).The most prevalent isolates were *Aspergillus* species (13.332% & 85%) then *Candida* species (8.88% & 35%), followed by the small incidence of *Penicillium* spp., *Alternaria* spp., *Curvularia* spp. and lastly *Rhodotorula rubra*. This agrees with the results of **Turnes and Albuquerque (1984)**. Similar studies also concluded that filamentous fungi are the principal causes of mycotic keratitis in most parts of the world; either *Aspergillus* spp. or *Fusarium* spp. were the most common isolates. Dematiaceous fungi, such as *Curvularia*s pp. and *Bipolaris* spp. are the third most important cause of keratitis in several studies **(Bharathi *et al.,* 2003)**. A study conducted in Saudi Arabia, concluded that the higher prevalence of *Aspergillus* species; among cases of keratitis; could be explained by the fact that its spores could survive the hot dry weather **(Khairallah *et al.,* 1992** and **Eman *et al.,* 2006)**, as is the case in Egypt, contamination of animal rations with different fungal species represented as a source for infection of animals with many diseases due to consumption of these infected rations

Table (2). Incidence of fungal pathogens from examined samples:

Isolated fungi	Eye swab (45)		Ration (20)	
	No.	%	No.	%
No growth	35	77.78	-	-
Aspergillus species	6	13.33	17	85
A. fumigatus	2	4.44	2	10
A. niger	2	4.44	9	45
A. flavus	2	4.44	6	30
***Candida* spp.**	4	8.88	7	35
C. albicans	2	4.44	5	25
C. krusei	1	2.22	1	5
C. tropicalis	1	2.22	1	5
***Penicillium* spp.**	1	2.22	3	15
***Alternaria* spp.**	1	2.22	1	5
***Curvularia* spp.**	1	2.22	1	5
Rhodotorula rubra	1	2.22	1	5

%: was calculated according to the total number of samples of each item (45&20)
No.: Number of positive samples
N.B: Positive fungal culture was (22.22%) in conjunctivitis and (100%) in all culture rations

Concerning the sensitivity of the antibacterial drug, it was found that isolated *Staphylococcus* species were highly resistant. Danofloxacin, lincomycin, enrofloxacin, gentamicin, erythromycin and kanamycin were highly effective whereas amoxicillin/clavulanic acid, ampicillin/sulbactam and oxytetracycline were moderately effective. Most bacteria were resistant to sulpha/trimethoprim, chloramphenicol and cefuroxime. Nearly similar results were obtained where thesensitivity of bacteria to different antibiotics was different **(OkumuÕ *et al.,* 2005)**. Most bacteria were resistant to penicillin G and cefoperazone. While it was found that chloramphenicol was the most effective antibiotic in another study **(Sarma *et al.,* 1989)**. While in the sensitivity of the antifungal drug all isolates showed maximum sensitive to miconazole (MIC) and nystatin (NS), moderate sensitive to amphotericinB (AP)and minimal sensitive to ketoconazole (KT) and itraconazole (IT), whereas resistant to fluconazole (FLU). **Ravi Kumar *et al.* (2010)** recorded that, the maximum sensitivity of *A.flavus* isolates was to 5 flucytosine, ketoconazole and itraconazole and amphotericin B whereas resistant to fluconazole.

Table (3). Antimicrobial sensitivity profile of isolated bacteria:

Isolates number / Antibiotics	*Staphylococcus* spp. (18)	*S. aureus* (10)	*Streptococcus* spp. (18)	*Moraxella bovis* (9)	*E. coli* spp. (11)	*Pseudomonas* spp. (4)	*Corynebacterium* spp. (4)	*K. pneumoniae* (3)
AMC	9 /18	2/10	15/18	7/9	8/11	3/4	4/4	3/3
C	1/18	-	3/18	1/9	4/11	1/4	-	-
CM	11/18	3/10	13/18	8/9	10/11	4/4	3/4	2/3
CXM	-	-	3/18	5/9	6/11	4/4	1/4	2/3
DAN	14/18	5/10	1818	9/9	9/11	4/4	4/4	3/3
E	10/18	3/10	14/18	8/9	9/11	4/4	4/4	2/3
ENR	13/18	4/10	15/18	9/9	9/11	3/4	4/4	3/3
K	10/18	3/10	15/18	9/9	10/11	3/4	3/4	3/3
MY	14/18	4/10	15/18	9/9	9/11	4/4	4/4	3/3
OT	6/18	1/10	12/18	8/9	10/11	1/4	3/4	-
SAM	9/18	2/10	14/18	8/9	8/11	1/4	3/4	2/3
SXT	1/18	-	7/18	2/9	-	1/4	1/4	1/3

Amoxicillin/Clavulanic acid (AMC) 20/10 µg
Chloramphenicol (C) 30µg
Gentamicin (CM) 10µg
Cefuroxime (CXM) 30µg
Danofloxacin (DAN) 30µg
Erythromycin(E) 15µg
Enrofloxacin (ENR) 10µg
kanamycin (K) 30µg
Lincospectin (MY) 10µg
Oxytetracycline (OT) 30µg
Ampicillin/Sulbactam (SAM) 10/10 µ
Sulpha/Trimethoprim (SXT)23.75+1.25µg.

N.B: The rest of the tested isolates from each species are resistant.

Table (4). Antimicrobial sensitivity profile of isolated fungi.

Antifungal Agent	Symbol	(11) *C. albicasns*	(11) *A. niger*	(8) *A. flavus*	(4) *A. fumigatus*
		NO	NO	NO	NO
Miconazole	**(MIC) 10µg**	8/11	11/11	8/8	4/4
Nystatin	**(NS) 50 mg**	9/11	11/11	8/8	4/4
AmphotericinB	**(AP) 10 mg**	6/11	6/11	4/8	0/4
Ketoconazole	**(KT)15 mg**	3/11	4/11	1/8	1/4
Itraconazole	**(IT) 10ml**	2/11	2/11	0/8	0/4
Fluconazole	**(FLU) 25 mg**	1/11	1/11	0/8	0/4

%: was calculated according to the total number of isolated fungi.
N.B: The rest of the tested isolates from each species are resistant.

Over the years, many antimicrobial agents have been used for the control or elimination and treatment of common bacterial and fungal infections in humans and animals. Over time, however, the irrational use of antimicrobial agents has produced strains of multiple antimicrobial resistant **(El-Jakee *et al.,* 2013** and **Singh *et al.,* 2018)**. This situation forced scientists to search for new antimicrobial substances from various sources, like **medicinal plant**s, which are the good sources of novel antimicrobial chemotherapeutic agents. Fortunately, boosting the animal's immune system and treating pink eye is very easy with home remedies and natural cures **(Viegi *et al.,* 2010)**.

One of the important medicinal plants, *A.vera* (*Aloe barbadensis* Miller), is reputed to have medicinal properties. It is a short succulent herb resembling a cactus, with green dagger shaped fleshy, spiny and marginated leaves, and filled with a clear viscous gel that is water-white, totally colorless and odorless, shows no yellow or green coloration from the inner surface of the outer leaf and most used as a treatment. *A.vera* has potent antibacterial, antifungal, and antiviral properties **(Foster, 2011)**. It contains over 70 biologically active compounds and is claimed to have anti-inflammatory, anti-oxidant, immune boosting, anticancer, healing, anti-aging and anti-diabetic properties **(Thiruppathi *et al.,* 2010)**. Aloes, by contrast, is an anthraquinone derivative of the sap of the *Aloe* leaf which has been used for centuries as a purgative **(Gallagher and Gray, 2003** and **Langmead *et al.,* 2004)**. *A.vera*has been evaluated in several different clinical contexts and some promising results have been found for its use in controlling cardiovascular risk factors and diabetes, besides being beneficial in areas of dermatology. One explanatory factor for this is the anti-inflammatory properties of the plant **(Choi and Chung 2003; Tian *et al.,* 2003**and **Davis *et al.,* 2006)**.

The effect of the prepared fresh, aqueous and ethanolic gel extracts of *A.vera* as ***in vitro*** antibacterial and antifungal activities either in a single form or in a combination with the most effective antimicrobial agents resulting from antibiotic resistance profile of the tested isolates against the most predominantly isolated bacteria (*Streptococcus* spp., *S. aureus*, *E. coli* and *M. bovis*) and fungi (*A. niger*, *A. flavus* and *C. albicans*) from conjunctiva and ration's samples corresponding to diseased buffaloes were evaluated according to **Agarry *et al.* (2005)** and **Olaifa (2017)**.

The potency of the extracts against some pathogenic bacterial and fungal isolates was assessed by the presence or absence of inhibition zones and was presented in Table (5) and photos from 3 to 8 respectively. Although all extracts were quite effective against all the tested isolates, there are variations in the diameter of zones of inhibition. As shown in Table (5), *A.vera* fresh gel were used and the results obtained zones of inhibition ranged from 9 mm

to 14 mm against the tested organisms. Greater zones of inhibition ranged from 4 mm to 19 mm were recorded with aqueous extract when the concentration of *Aloe* extracts increased from 25µg/ ml to 200µg/ ml. While remarkable and significant antimicrobial activities were recorded with ethanolic extract even at low concentration (25µg/ml), with zones of inhibition ranged from 9 mm to 27 mm against the same tested organisms. However, it has been reported by many researchers that hydro-alcoholic extract, compared to the aqueous extract, is more effective and has a superior inhibitory influence **(Alizadeh-Behbahani, 2012)**.

A.vera gel is bacteriostatic against *S. aureus*, *S. pyogenes* and also *S.paratyphi*. In an in vitro disc diffusion study by **Suleyman and Sema (2009),** *S. faecalis* and *C. albicans* were cultured to contain 10^8-10^9 CFU ml/1 levels of the organism. A 100% *A.vera* gel obtained from the cold pressed leaves of the plant were used and the results obtained showed significant zones of inhibition of 20mm and 30mm against both these organisms. *A.vera* is also known to be virucidal, especially against the herpes virus.

The results in Table (5) also illustrated that Gram-negative bacteria (*E. coli* and *M. bovis*) show more resistance to the *Aloe* extracts comparatively to Gram-positive bacteria (*Streptococcus* spp. and *S. aureus*). Comparative study on the cell wall structures of bacteria reveals that Gram-positive bacteria have thick peptidoglycan in their cell wall composition while Gram-negative bacteria have only a thin layer of peptidoglycan, but rich in lipoprotein and lipopolysaccharides in their cell structure. Hence, the effects of an antimicrobial agent against Gram-positives bacteria were more tangible than those against the Gram-negatives **(Ghalem and Mohamed, 2008)**.

The control standard traditional antimicrobial had comparatively similar effects to ethanolic *A.vera* gel extract. Where, the inhibitory zone of danofloxacin against tested bacterial isolates (*Streptococcus* spp., *S. aureus*, *E.coli* and *M. bovis*) and miconazole against tested fungal isolates (*A.flavus, A.niger* and *C.albicans* were increased from 22 mm to 30 mm, while the inhibitory zone of ethanolic gel against the same tested bacterial and fungal isolates increased from 10 mm to 27 mm by increasing the concentration from 25µg/ml to 200 µg/ml as shown in Table (5) and photos (3:8). This table also illustrated that, the antimicrobial effect resulting from combination of *A.vera* gel and traditional antibacterial or antifungal not only similar, but also decrease than the effect of ethanolic gel alone beginning with the concentration of 200 µg/ mlof gel, where, the inhibitory zone ranged from 21mm to 27mm, while danofloxacin combined with ethanolic *Aloe* extract at the same concentration against tested bacterial isolates ranged from 20mm to 26mm and with miconazole against tested fungal isolates were increased from 24 mm to 28 mm. Therefore, it can be concluded that these extracts contained compounds that presented an antagonist effect on growth inhibition of tested bacterial or fungal pathogen similar or increased the traditional one.

To sum up, the result indicates that the best aloe extract concentration for inhibition of studied pathogens is 200 µg/ ml which is the largest dose used in our study; similar result was reported by **Thiruppathi *et al.* (2010)** who studied the antimicrobial activity of *A.vera* on some pathogenic bacteria and reported that the inhibition zones of *A.vera* extracts were increased by increasing dose.

The mechanism of action of the gel extract on the lysis of pathogenic cells may be due to the pore formation in the cell wall and the leakage of cytoplasmic constituents by the active components such as alkaloids present in the gel extract *A.vera* as revealed by **Shelton (1991)**. There are over 75 known ingredients in the *A.vera* leaf gel and they are all contained in about 1% of the plant, the rest (99%) being water, so they are present only in small amounts. Their action is thought to arise from the synergistic effect of these substances i.e., they can be likened to work together as a team so that the total effect is greater than would be expected from the individual effect of each substance. Thus it was not surprising that *A.vera* leaf gel extract was highly prized just for this reason **(Masoumian and Zandi, 2017)**. Reports suggest that the beneficial effects of *A.vera* gel

are due to its high molecular weight compounds such as polysaccharides **(Shida *et al*., 1985)**, lectin-like proteins **(Grindlay and Reynolds, 1986)** and prostaglandins **(Ali *et al*., 1991)**. Aloe's anti-inflammatory effects may be due to a bradykinin-degrading glycoprotein **(Yagi *et al*., 1987)** and mannose-6-phosphate may have a role in the **wound healing** process **(Davis *et al*., 1994)**. Anthraquinones from various plant extracts have been studied for their possible anti-viral properties **(Sydiskis *et al*., 1991)**.

Ethnomedicinally, the bacteria that are used in the present study for **antimicrobial activity** is known to be associated with the incidence of the gastrointestinal tract, urogenital tract and wound infections. Thus the present study adds credence to the ethno medicinal uses of the plant for the treatment of gastrointestinal disorders **(Foster, 2011)**.

Table (5). Antimicrobial activity of *Aloe vera* gel and traditional antimicrobial agents in a single and combination form against the predominant bacterial and fungal isolates.

Pathogen (1.5×10^8/ml)	Zones of growth inhibition (mm)										
	Aloe vera gel extracts(40µl)								Anti-fungal	Anti-bacterial	Ethanolic Gel (200µg/ml) + Antimicrobial
	Fresh gel	Aqueous gel extracts (µg/ ml)				Ethanolic gel extract (µg/ ml)				Miconazole (MIC)	Danofloxacin (DAN)	
		25	50	100	200	25	50	100	200	(10µg/ ml)	(30µg/ ml)	
Strepto-coccus spp.	12	8	10	12	16	10	15	17	21	-	24	20
S. aureus	13	7	8	10	12	11	16	19	23	-	23	22
E.coli	10	5	10	12	14	10	13	15	22	-	25	23
M. bovis	11	4	7	10	13	16	17	20	21	-	28	26
A. niger	14	8	11	13	15	13	16	19	22	26	-	24
A.flavus	12	9	10	12	15	15	17	19	20	22	-	20
C.albicans	9	7	8	13	19	11	17	22	27	30	-	28

Photo. (2): Antimicrobial activity of *Aloe vera* gel extracts and traditional antibacterial agent against *Streptococcus* species.

(a) **(b)** **(c)**

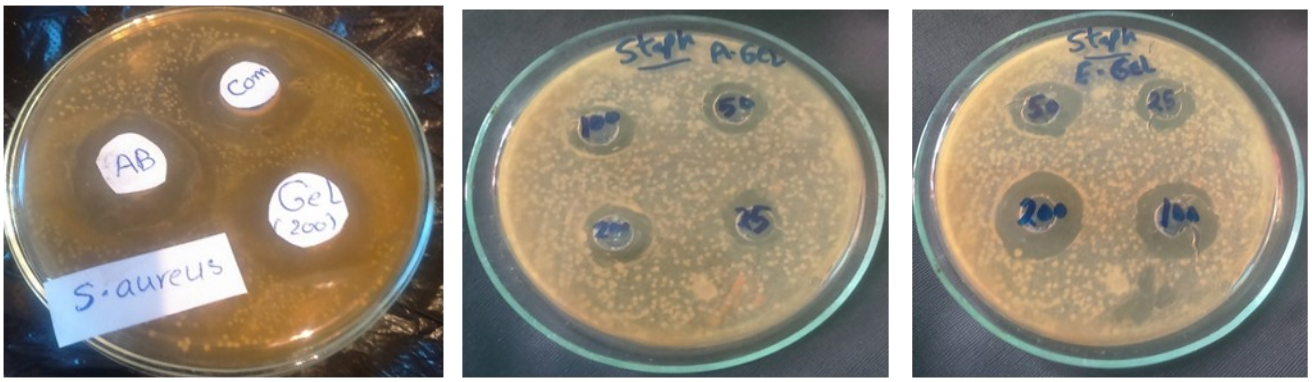
Zone of inhibition of gel danofloxacin and combination of each

Zone of inhibition of aqueous gel extract in the concentration of 25, 50, 100 and 200µg/ ml

Zone of inhibition of ethanolic gel extract in the concentration of 25, 50, 100 and 200µg/ ml

Photo. (3): Antimicrobial activity of *Aloe vera* gel extracts and traditional antibacterial agent against *S. aurus* species.

(a) **(b)** **(c)**



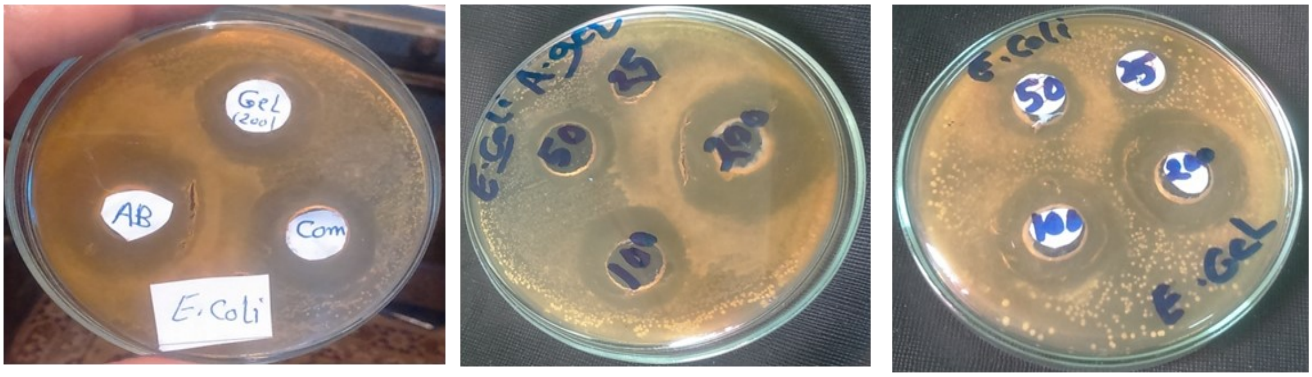
Zone of inhibition of gel, danofloxacin and combination of each

Zone of inhibition of aqueous gel extract in the concentration of 25, 50, 100 and 200µg/ ml

Zone of inhibition of ethanolic gel extract in the concentration of 25, 50, 100 and 200µg/ ml

Photo. (4): Antimicrobial activity of *Aloevera* gel extracts and traditional antibacterial agent against *E. coli* species.

(a) **(b)** **(c)**



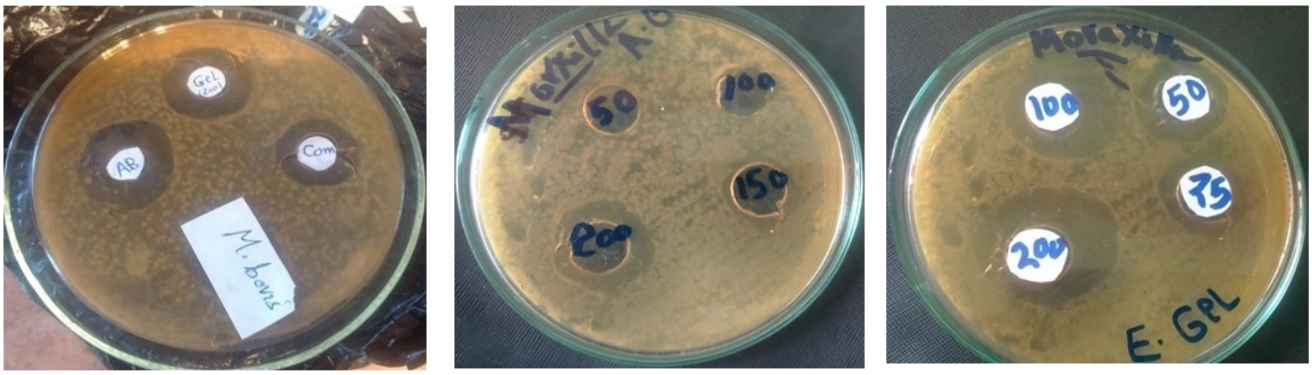
Zone of inhibition of gel, danofloxacin and combination of each

Zone of inhibition of aqueous gel extract in the concentration of 25, 50, 100 and 200µg/ ml

Zone of inhibition of ethanolic gel extract in the concentration of 25, 50, 100 and 200µg/ ml

Photo. (5): Antimicrobial activity of *Aloe vera* gel extracts and traditional antibacterial agent against *M. bovis* species.

(a) **(b)** **(c)**



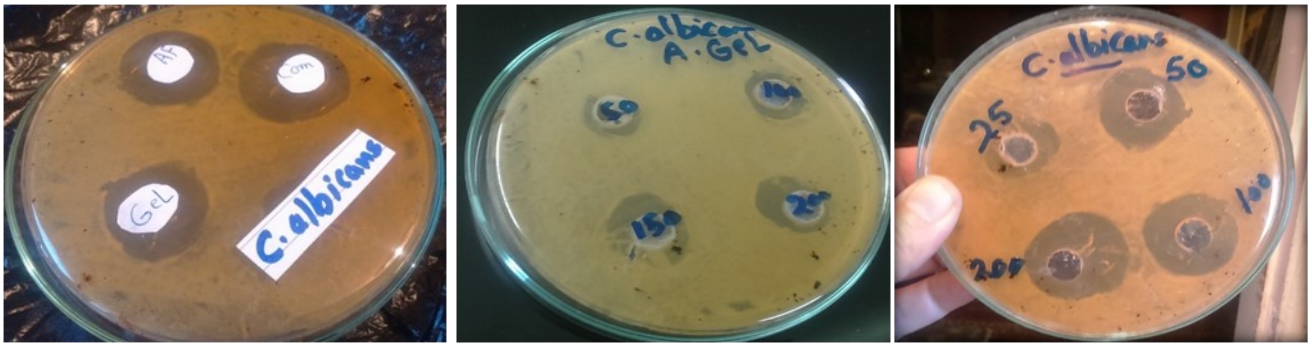
Zone of inhibition of gel, danofloxacin and combination of each

Zone of inhibition of aqueous gel extract in the concentration of 25, 50, 100 and 200µg/ ml

Zone of inhibition of ethanolic gel extract in the concentration of 25, 50, 100 and 200µg/ ml

Photo. (6): Antimicrobial activity of *Aloe vera* gel extracts and traditional antifungal agent against *C. albicans* species.

(a) **(b)** **(c)**



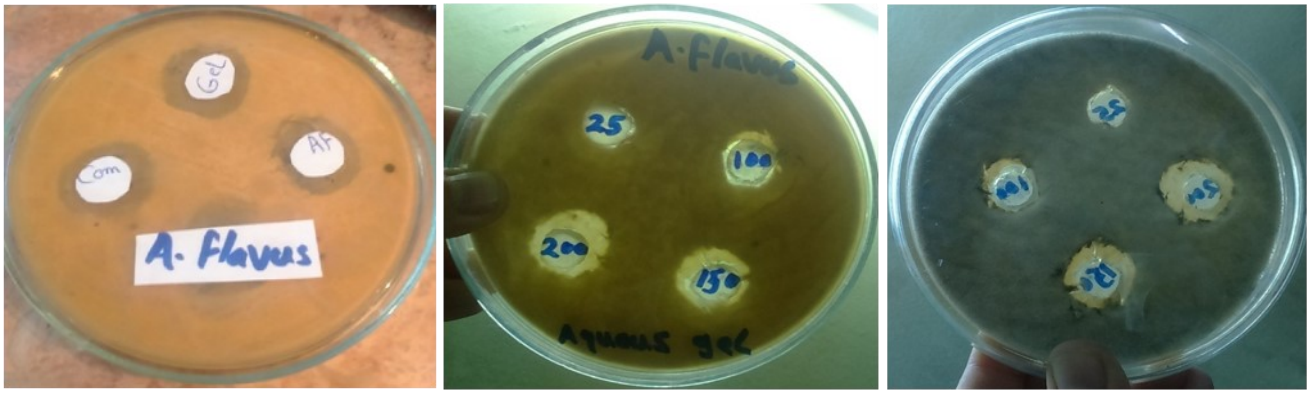
Zone of inhibition of gel, miconazole and combination of each

Zone of inhibition of aqueous gel extract in the concentration of 25, 50, 100 and 200µg/ ml

Zone of inhibition of ethanolic gel extract in the concentration of 25, 50, 100 and 200µg/ ml

Photo. (7): Antimicrobial activity of *Aloe vera* gel extracts traditional antifungal agent against *A. flavus*.

(a) **(b)** **(c)**



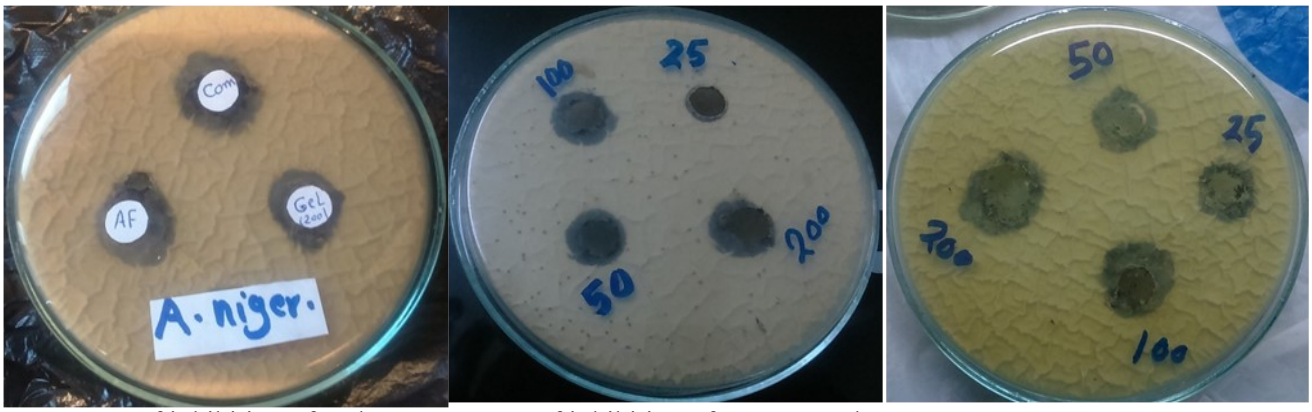
Zone of inhibition of gel, miconazolea nd combination of each

Zone of inhibition of aqueous gel extract in the concentration of 25, 50, 100 and 200µg/ ml

Zone of inhibition of ethanolic gel extract in the concentration of 25, 50, 100 and 200µg/ ml

Photo. (8): Antimicrobial activity of *Aloe vera* gel extracts traditional antifungal agent against *A. niger.*

(a) **(b)** **(c)**



Zone of inhibition of gel, miconazole and combination of each

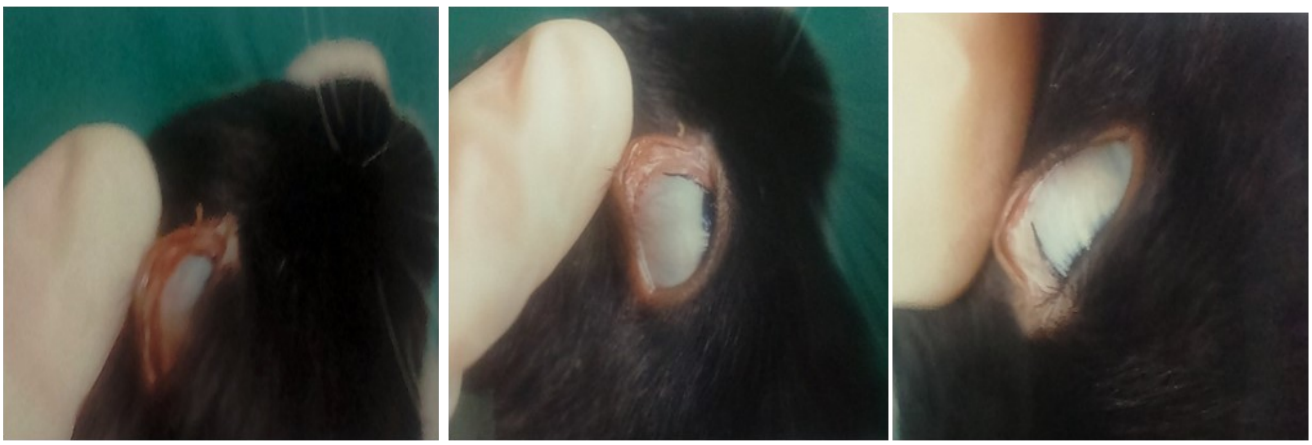
Zone of inhibition of aqueous gel extract in the concentration of 25, 50, 100 and 200µg/ ml

Zone of inhibition of ethanolic gel extract in the concentration of 25, 50, 100 and 200µg/ ml

The production of experimental keratoconjunctivitis for identification and demonstrating the virulence were made up according to **Rrichard (2018)**. The test is performed in a rabbit with an average weight of about 1-1.5 kg by installation a life bacterial suspension (10^8/ml) into the conjunctiva. Purulent conjunctivitis develops within 24- 48h, when the infected rabbit was treated with fresh *A.vera* extract showed healing within 5 days as showed in Photo (9). **Braude *et al.*(1981)** recorded that, bacterial suspension provokes characteristic conjunctivitis among infected rabbit, this usually heals spontaneously and the animal rarely dies. Furthermore, **parker and collier (1990)** observed that, the installation of a pure culture of *S. aureus* into the conjunctiva of the rabbit gives rise to severe conjunctivitis within 24h followed by keratitis and the animal itself rarely dies. Components in *A.vera* gel, such as aloin and emodin, have antibacterial and antiviral properties. Some other important *A.vera* benefits are its ability to reduce inflammation and speed up healing. *A.vera*h as been shown to exhibit some wound healing effects, including the encouragement of granulation tissue, **(Thiruppathi *et al.*, 2010)**.

Photo. (9): Rabbit showing ocular lesion and conjunctivitis after experimental infection.

(a) **(b)** **(c)**



(A): Sever conjunctivitis appears within 36 h

(B) Rabbit showing complete recovery after 5 days of treating

(C): Control non-infected eye (saline installation).

It can be concluded that the *A.vera* gel extract was more efficient against all types of tested microbes even at low concentrations. In this research work, it was observed that, plants showed remarkable activity against tested gram -negative and gram-positive bacteria as well as fungi referred to the presence of some bioactive components especially in crude extracts of *A.vera* gel. From this study, it can also be concluded that, the process of extraction for a particular compound is dependent on the solubility of the component in the solvent (water or organic solvent), and the inhibitory activities were found to be dose-dependent. The data expected promising results for using *A.vera* gel extracts as an alternative herbal antimicrobial agent replacing synthetic one to prevent and treat some infectious diseases. Our further studies will be worked at using of *A.vera* gel extract in treatment of pink eye infected animals.

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