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Aloe vera gel as an alternative to antibiotic treatment in buffalo calves conjunctivitis: A feild trial

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Abstract

The interest of medical plant has increased significantly in recent years not only to cure humans but also to cure animal. Among these medicinal plants, *Aloe vera* has been reported to be antibacterial and antifungal and is not associated with any health hazard. This study was conducted in a farm for buffalo's calves at El-Fayoum governorate during the period from March to September, 2019. The infected calves showed all typical clinical symptoms of conjunctivitis at clinical examination. A total of 60 eye swabs collected from diseased calves were subjected to bacteriological and mycological examination and studying the effect of *Aloe vera* gel on the isolated bacterial species in vitro as well as in treatment of infected calves. The isolated bacterial species either pure or mixed media were *staphylococcus aureus* (35%), *Moraxella bovis* (23.3%), *S. epidermidis* (11.7%), *Listeriamonocytogenes* (6.7%), *Pseudomonas aeruginosa* (5%), 3.3% of each *Streptococcus pneumoniae* and *Klebsilla pneumonia* and 1.7%of each *Enterobacter* spp. and *Proteus* spp. while the fungal isolates were *Aspergillus niger* (8.3%),*Candida albicans* (6.7%), *A. flavus* (5%) and 1.7% of each *C. tropicalis, Alternaria* spp. and *Penicillium* spp. Antimicrobial properties of *A.vera* gel was evaluated *invitro* experiments against these species of bacteria and fungi and showed good inhibition activity in a comparison of some traditional antifungal and antibacterial agents. *In vivo* study, infected calves recovery from the conjunctivitis was recorded after 5-7 days for animal treated topically with ciprofloxacin-chloramphenicolas antibacterial in addition to natamycin- fluconazole as antifungal in cases from which fungi isolated showed effective and completed healing occur but some cases specially infected with staphylococcus showed recurrent, while groups of *A.vera* extracts treatment showed complete healing according to type and severity of infection without recurrence. So that, the farm's owner was advised avoiding predisposing factors to the disease as well as using of *A.vera* gels as alternative to antibiotic treatment in eye problems which consider very important in veterinary medicine.

Keywords: *Buffalo's conjunctivitis, antimicrobial potential of Aloe vera, and traditional antibiotics .*

Introduction

After the revolution in the "golden era", when almost all groups of important antibiotics (tetracyclines, cephalosporins, aminoglycosides and macrolides) were discovered and the main problems of chemotherapy were solved in the 1960s, the history repeats itself nowadays and these exciting compounds are in danger of losing their efficacy because of the increase in microbial resistance **(Guschin *et al.*, 2015** and **Mayers *et al.*, 2017)**. Currently, its impact is considerable with treatment failures associated with multidrug-resistant bacteria and it has become a global concern to public health **(Van Duin and David 2016** and **Singh *et al.*, 2018)**.

Pink eye or infectious bovine keratoconjunctivitis (IBK) is a highly contagious and infec-

tious ocular disease of cattle characterized by conjunctivitis and ulcerative keratitis, and is cosmopolitan in distribution **(Alexander, 2010)**. It can be seen any time of the year but mostly in summer and autumn, furthermore, claves are more susceptible when compared to adult cattle **(Kizilkaya *et al.,* 2013** and **Moore, 2017)**. The most common causative agent of IBK is *Moraxella bovis* **(Ahmed, 2019),** moreover, several other infectious agents such as Adenovirus, Mycoplasma, Branhamella (Neisseria), and Listeria have been recovered from the eyes of cattle showing clinical signs like those seen in Moraxella-induced IBK **(Schnee *et al.,* 2015).** Also infection by opportunistic fungi has increased significantly especially *Aspergillus fumigates* and *candida albicans* whichis the important fungi in causing recurrent infections **(Cullen *et al.,* 2017)**. The pathogenesis of the disease is influenced by many factors, such as season, mechanical irritation, host immune response, eyelid pigmentation, and concurrent presence of pathogenic bacteria **(Ward and Powell, 2017)**.

The disease is not fatal and cases of permanent blindness or loss of an eye are rare **(Moore, 2017)**. During the early acute phase of the disease, cases usually respond to treatment with ophthalmic ointments and solutions containing antibiotics. These drugs should be instilled in the conjunctival sacs at frequent intervals, which may be impractical under bad field conditions **(Alexander, 2010)**.

Almost in all pathogenic bacteria, it has been observed that, they are able to obtain the resistance factor to the antimicrobial drugs quickly; therefore, multiple drug resistant bacteria caused the main failure in the treatment of infectious diseases **(Bisht *et al.,* 2009** and **Mill Robertson, 2015)**. So, it is necessary to search and design the alternative approaches to control resistant bacteria. One of the possible strategies is rational localization of bioactive phytochemicals with antibacterial activity **(Ushimaru *et al*., 2007)**.Currently, researchers have investigated plants with extensive variety of secondary compounds that could be a potential source for various antimicrobial agents **(Mahmoud *et al.,* 2004** and **Amer *et al.,* 2007)**. Those plants contain numerous structurally unique bioactive compounds which are decent sources to obtain natural therapeutic agents **(Cowan, 1999)**.

A.vera is one of examples of promising species of medical plants. It was recognized as increasing collagen building, but its antimicrobial effect was not negligible **(Ramasubramanian *et al.,* 2010)**. Mannans, polymannans, anthraquinone c-glycosides, anthrones, anthraquinones, and various lectins are recognized as bioactive compound of *A.vera. Aloe vera* gel consists of 99.3% water with a pH of 4.5. The remaining 0.7% is made up of solids with glucose and mannose constituting for a large part. These sugars together with the enzymes and amino acids in the gel give the special properties as a skin care product **(Belo *et al.,* 2006)**. The gel stimulates cell growth and as such enhances the restoration of damaged skin; it moisturizes the skin because it has a water holding capacity; this moist on the skin and also has a cooling effect **(Meenatshi *et al.,* 2013)**.

A.vera gel is perhaps the most widely recognized herbal remedy in the United State today; it is used to relieve thermal burn, sunburn and promote wound healing **(Foster *et al.,* 2011)**. In addition, it contains over 70 biologically active compounds and research suggests that *A.vera* gel can help stimulate the body's immune system and is claimed to have anti-inflammatory, anti-oxidant, anticancer, healing, anti-aging and anti-diabetic properties **(Kodym *et al.,* 2003)**.

Many studies reported the effective use of this plant when applied topically for the treatment of inflammatory skin disorders and wounds **(Reider *et al.,* 2005** and **Belo *et al.,* 2006)** and works exceptionally well for conjunctivitis specially with good management practices **(Meenatshi *et al.,* 2013)**.The more specific aim of the present study was to assess the effects of topical application of *A.vera* gel for treatment of conjunctivitis in buffalo's calves which consider very important disease in veterinary medicine and detecting of causative bacterial and fungal agents.

Materials and Methods

Animal housing: The studying animals were

kept under poor hygienic conditions in an open area exposed to wind and dust. As shown in photograph (1), they were housed in enclosures made of metal rails and wood. The calves were separated from adults in enclosures made of bushes and dry branches of trees. The animals were provided with minimum shade being exposed to the heat of the sun and direct sunlight (UV light) during most of the day. The floor of the enclosures was not clean with plenty of dung and mud from urine and water flooding from the drinking water troughs. Plenty of flies were seen swarming in the enclosures and around the face of the animals.

Clinical examination of the calves: The surveyed 18 male and 22 female calves were suffering from eye affections in one or both eyes and they were between 3 to6 months in age and 60 to 100 kg in weight. The eyes of each calf were examined for clinical signs of pink eye using a portable light while the calf was restrained in a squeeze chute, and the affected eyes were taking scores according to the severity of the case. Some calves showed copious watery discharge from the affected eye and matting the hair on the lateral aspect of the face (Photo,2). There was severe conjunctivitis and facial edema (Photo,3). Many calves showed keratitis, and white or yellow opacity of the cornea (Photo,4). Flies were seen feeding on eye secretions of some animals.

Collection of samples: According to **Quinn *et al.,* (2011)**sixty conjunctival swabs were collected from the eyes of 40 infected buffalo calves have the signs of the disease in the farm. Samples were taken by entering a sterile cotton swab into the conjunctival sac and then transferred to a sterile test tubes containing sterile trypticase soya broth (TSB) volume of 5 ml, and then the samples transferred to the laboratory as soon as possible for bacteriological and mycological examination.

Isolation and identification of bacteria: The inoculated tubes were incubated aerobically at 37°C for 24 h, then a loopfull from each tube was cultured onto each of the Trypticase soy agar containing 5% blood of sheep, Oxford agar and of MacConkey agar and incubated aerobically at 37 ° C for 24 h, after that it was studying the form of developing colonies that taken from it to Gram stain, subculture of questionable colonies onto different selective media, including Manitol salt agar, Edward agar, MacConkey agar, Trypticase Soya agar (TSA) and blood agar , pure colonies saved on brain heart infusion agar for the purpose of conducting biochemical tests and determine the different types of it **(Richardson *et al.,* 2005** and **Quinn *et al.,* 2011)**.

Isolation and identification of fungi: The collected swabs were prepared and examined for isolation of fungi according to the technique recommended by **ISO (2008)**, after addition of SDA medium containing chloramphenicol antibiotic (0.05 mg /ml media) to inhibit bacterial growth, the plates were left to solidify at room temperature then incubated at 25-27 ^{o}C for 5-7 days. At the end of incubation period, the isolated mold and yeast genera and species were purified by subculture on specific medium of isolation and identified according to the technique recommended by **(Pitt and Hocking, 2009)**.

Antimicrobial sensitivity tests: Bacterial and fungal isolates were inoculated into nutrient broth and incubated at 37 °C for 18 hours for bacteria and at 25°C for 1-3 days for fungi. All these suspensions were diluted with normal saline, and adjusted its turbidity to 1.5×10^{8} CFU/ml using standard McFarland tube number 0.5for used in antimicrobial sensitivity profile according to **Quinn *et al.* (2011)**. Antimicrobial susceptibility testing of each bacterial and fungal isolates to various routinely used antibiotics were determined by disc diffusion technique and using commercially available discs following CLSI guidelines. Sterile swab was used to inoculate the suspension by streaking onto trypticase soy agar media (TSA) for bacteria and sabouraud dextrose agar (SDA) media for fungi. It was then allowed to stay for 3-5 minutes. Sterile forceps was used to place the Oxoid antimicrobial discs on the inoculated plates. Within 30 minutes after applying the disc, the plate was incubated at 37˚C for 18-24h for bacterial while the fungal one at 25°C for 1-3 days. The diameter of each zone of inhibition was measured in millimeter; then compared with Published Limits of **Clinical and Laboratory Standards Institute (CLSI,**

2014).

Preparation of *Aloe vera* gel extracts: The gel portion of the plant was prepared by the method as described by **Olaifa (2017)** as follow: After leaves were collected and washed with water to remove mud and other debris were cut longitudinally using sharp knife, and the inner gel-like pulp in the center of the leaf was separated using large spoonful, then washed with deionized water and homogenized in a home blender, filtrate to separated from its fibers, and used as it is. On the other hand, a part from the last prepared mucilaginous homogenate fresh gel was dried in oven at temperature at 60 °C and further extracted with 95% ethanol, filtrate, and then dried with the help of rotary evaporator at temperature 55°C until the solvent was completely removed. The dried ethanolic extract re-dissolved in its respective solvent (ethanol) for preparing a concentration of 200 µg/ ml; with finally PH of 6.3for using. The remaining dried ethanolic extract was stored dry at -20ºC in deep freezes until used **(Adnan *et al.,* 2015)**.

Antimicrobial susceptibility testing of *A.vera* extract by wells diffusion method: The last prepared bacterial and fungal suspension were streaking onto TSA of plates for bacteria and SDA for fungi, then the plates were left for 5-15 minutes at room temperature to dry. Media were cut into 2 wells (6mm diameter) in each plate by cork borer and 40µl of each *A.vera* fresh gel and ethanolic extract were added. All plates of the tested organisms was then allowed to incubate at 37°C for overnight for bacteria and at 25°C for 1-3 days for fungi. After incubation period, we were noted zone of inhibition of *A.vera* extract on each isolate. The diameters of the zone of inhibitions were measured by measuring scale in millimeter **(Agarry *et al.,* 2005)**, then compared with the results of antimicrobial sensitivity profile against traditional agents.

Experimental design for treatment protocol: A total of 40 buffaloes calves, clinically diagnosed to have infectious keratoconjunctivitis were included in this study of which a single eye was infected in 20 animals and both eyes were infected in the rest. The infected buffalo calves were randomly assigned to 1 of 5 groups at the beginning of the study, and were examined 3 times weekly for 4 weeks. Infected calves in group 1 (no =12) were treated with ciprofloxacin ointment and chloramphenicol eye drop as antibacterial in addition to natamycin-fluconazole as antifungal in cases from which fungi isolated 3-4 times daily for 5 day. Infected calves in group 2 (n = 12) were treated with fresh *A.vera* gel extract as ointment and lotion 3-4 times daily for 5 day. Infected calves in group 3 (n = 12) were treated with ethanolic *A.vera* gel extract 3-4 times daily for 5 day. Group 4 (n = 4) were infected calves not treated and used as control positive for show completed clinical signs (treated later). Group 5 (n = 4) were healthy calves treated with *A.vera* extract to evaluation safety of agent there is no any discomfort or any symptoms on eye. On the other hand the calf's immune status was increased by good level of nutrition, adequate vitamin and trace mineral intake and provide shade and clean water to improving the calf's ability to fight off the disease.

Results

A total of 60 eye swabs were collected from 40 infected buffaloes calves either ina single eye (20) or in both eyes (20); results were illustrated in Tables 1-5 and Photos 1- 4.

Table (1). Isolated bacterial pathogens from culture positive pink eye.

Bacterial species	Pure Growth	Mixed with other bac-teriaor fungi	Total	
			Number	%
Gram-positive:				
S. aureus	18	3	21	35
S. epidermidis	5	2	7	11.7
S. pneumoniae	2	0	2	3.3
L.monocytogenes	3	1	4	6.7
Total	**28**	**6**	**34**	**56.7**
Gram-negative:				
M.bovis.	12	2	14	23.3
P. aeruginosa	2	1	3	5
K. pneumoniae	1	1	2	3.3
Enterobacter spp.	1	0	1	1.7
Proteus spp.	1	0	1	1.6
Total	**17**	**4**	**21**	**35**
Overall total	**45**	**10**	**55**	**91.7**

%: was calculated according to the total number of samples examined (60).

Table (2). Isolated fungal pathogens from culture positive pink eye.

Fungal species	Pure growth	Mixed with other fungi or bacteria	Total	
			Number	%
***Aspergillus* spp.**				
niger	2	3	5	8.3
flavus	1	2	3	5
Total	**3**	**5**	**8**	**13.3**
***Candida* spp.**				
C. albicans	**2**	**2**	**4**	**6.7**
C. tropicalis	**0**	**1**	**1**	**1.7**
Total	**2**	**3**	**5**	**8.3**
***Alternaria* spp.**	**0**	**1**	**1**	**1.7**
***Penicillium* spp.**	**0**	**1**	**1**	**1.7**
Overall total	**5**	**10**	**15**	**25**

%: was calculated according to the total number of samples examined (60).

Table (3). Comparison between antimicrobial activity of commercial antibiotics agents and *A.vera* extracts on the most isolated bacterial species (no: 53).

Bacterial isolates	Number of isolates	Antibiotic agents(Number of sensitive isolates)/ *Aloe vera*								
		OFX (10 µg)	CIP (5 µg)	C (30µg)	AK (30 µg)	CN (10µg)	P (10IU)	AM (10µg)	Aloe as it is	Ethanolic gel
S. aureus	21	17/21	20/21	17/21	20/21	17/21	7/21	12/21	21/21	21/21
M. bovis	14	12/14	13/14	11/14	7/14	4/14	-	13/14	14/14	14/14
S. epidermidis	7	6/7	7/7	5/7	6/7	7/7	3/7	4/7	7/7	7/7
L.monocytogenes	4	3/4	4/4	4/4	1/4	3/4	2/4	¾	4/4	4/4
P. aeruginosa	3	2/3	2/3	2/3	2/3	2/3	1/3	2/3	2/3	3/3
S.pneumoniae	2	2/2	2/2	2/2	1/2	1/2	1/2	1/2	2/2	2/2
K. pneumoniae	2	2/2	2/2	2 /2	2/2	2/2	-	1/2	1/2	2/2
Total	53	44/53 (83.1)	50/53 (94.3)	43/53 (81.1)	39/53 (73.6)	36/53 (67.9)	14/53 (26.4)	36/53 (67.9)	50/53 (94.3)	53/53 (100)

OFX: Oflxacin **AK**: Amikacin **PG**: PencillinG **CH**: Chloramphenicol **CN**: Gentamicin **CIP**: Ciprofloxacin **AM**: Amoxicillin **NO:** Number of sensitive isolates **N.B**: The rest of the tested isolates from each species are resistant

Table (4). Comparison between antimicrobial activity of commercial antibiotics agents and *A.vera* extracts on the most isolated fungal species (12).

Fungal Isolates	Number of isolates	Antifungal Agent (Number of sensitive isolates)/ *Aloe vera*							
		FLU (25 mg)	KT (15 mg)	IT (10ml)	AP (10 mg)	NaT (50 mg)	MIC (10µg)	Aloe as it is	Ethanolic extract
A. niger	5	4/5	3/5	1/5	3/5	5/5	4/5	5/5	5/5
A. flavus	3	2/3	2/3	-	2/3	3/3	3/3	3/3	3/3
C. albicasns	4	3/4	2/4	1/4	3/4	3/4	3/4	3/4	4/4
Total	12	9/12 (75%)	7/12 (58.3)	2/12 (16.7)	8/12 (66.7)	11/12 (91.7)	10/12 (83.3)	11/12 (91.7)	12/12 (100)

MIC: Miconazole **NaT**: Natamycin **AP:** AmphotericinP **KT**: Ketoconazole **IT:** Itraconazol **FLU**:Fluconazole **NO**: Number of sensitive isolates **N.B**: The rest of the tested isolates from each species are resistant**.**

Table (5): Results of treatment protocol in the current study:

Infected-group number	Number of Treated animals	Methods oftreatment			Number ofrecoverd animal							
					Duration by days							
		Method	Treatment	Duration	7		14		21		28	
					No.	%	No.	%	No.	%	No.	%
1	12	Topically	Ciprofloxacin + chloramphenicol **with** natamycin and fluconazole in case of fungal infection as eye drops and ointments	3-4 times daily for 5 day	12	100	11	90.7	10	83.3	9	75
2	12		Fresh gel (gel as it is)		11	90.7	12	100	12	100	12	100
3	12		Ethanolic gel		12	100	12	100	12	100	12	100

Group (4): 4 infected calves not treated to show completed clinical signs (Control positive).
Group (5): 4 healthy calves treated with *A.vera* extract and showed no any adverse effect on eye tissue.
%: Was calculated according to the total number of animals in each group.

Photo. (1): Apparently healthy animal in the farm of the study.

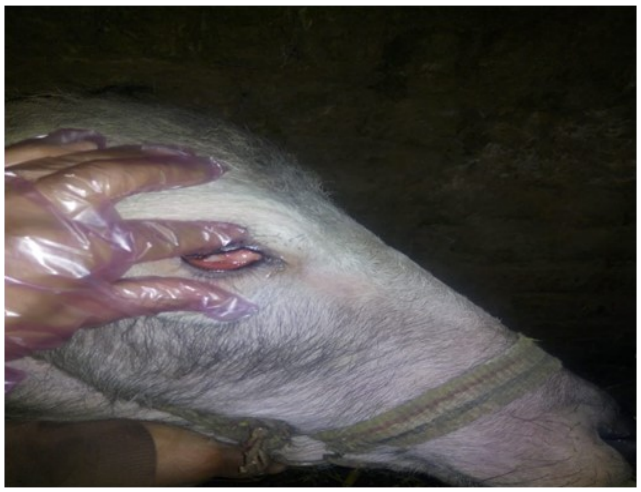
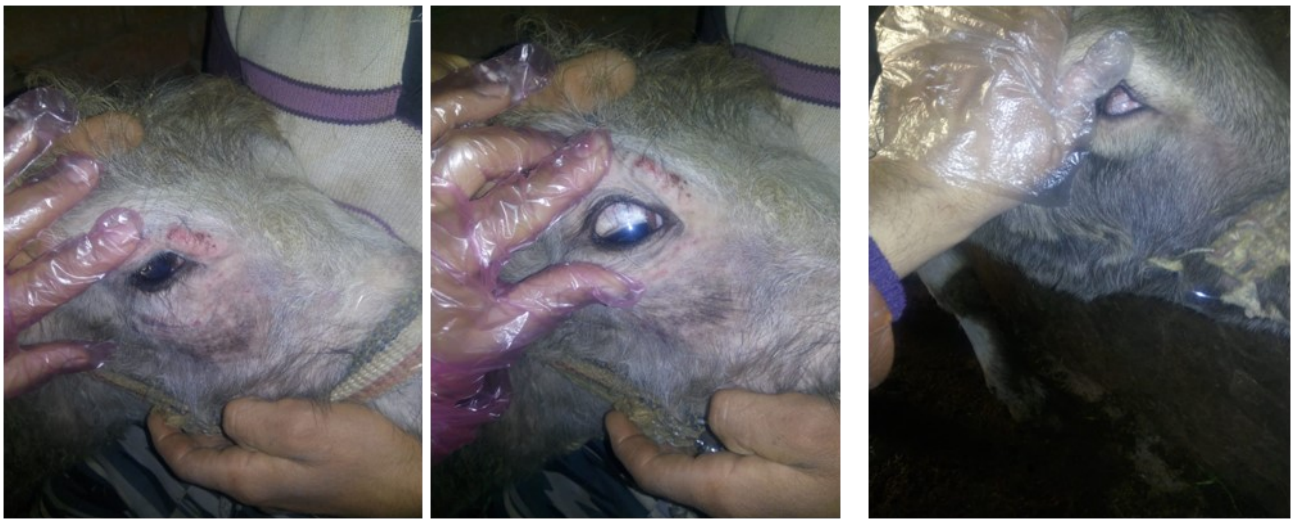
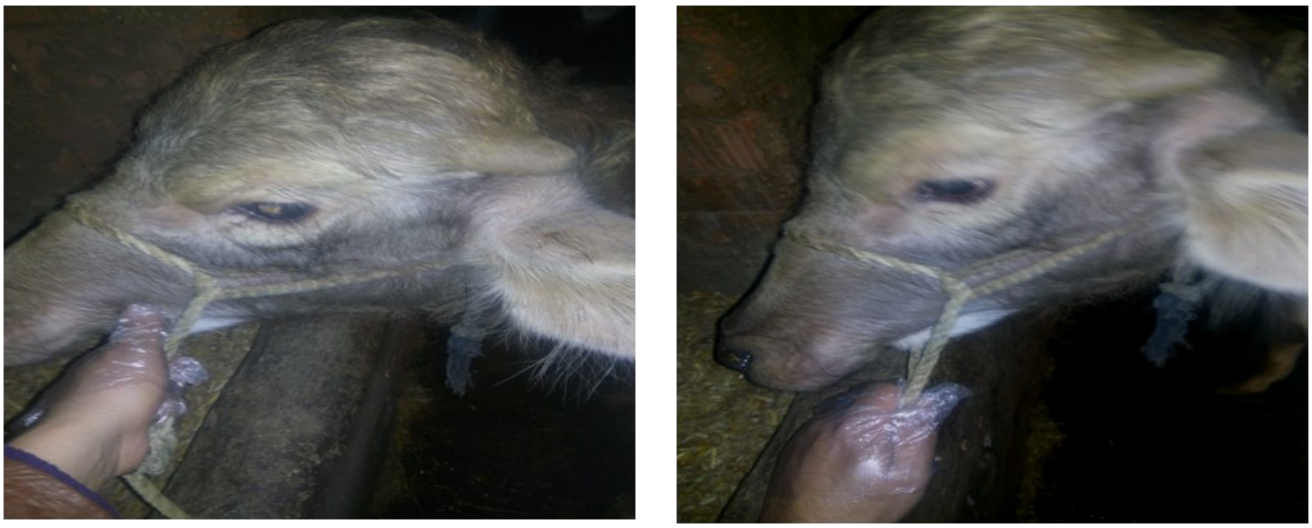


Photo. (2): First degree of conjunctivitis with serous lacrimation



(a) **(b)**

Photo. (3): Inflammation with facial edema (a). Absence of inflammation, redness and complete healing within 5 days of treatment with *A.vera* extract without recurrence (b).



(a) **(b)**

Photo. (4); Third stage of conjunctivitis,**(a):**A calf with edematus medial canthus and yellowish opacity of the cornea. **(b):**After 7 days of treatment with *A.vera* lotion and ointment 3-4 times daily, showed complete healing and remove of inflammation

Discussion

Diseases due to pathogenic bacteria and fungi represent a critical problem to human health and they are one of the main causes of morbidity and mortality worldwide **(world health organization, 1998[**WHO**])**. Resistance to antibiotics and with the toxicity during prolonged treatment with now a day drugs have been the reasons for an extended search for newer drugs to treat opportunistic microbial infections **(Seddik *et al.*, 2010)**. During this process, the investigation of the efficacy of plant-based drugs in traditional medicine has been paid great attention because these drugs elicit few side effects, cheap and easily available **(Chatterjee *et al.*, 2015)**.

According to the World Health Organization, 80% of the world population still relies mainly

on plant drugs**.** Among these medicinal plants, *Aloe vera* has been reported to be antibacterial and antifungal and is not associated with any health hazard **(Masoumian and Zandi, 2017)**. *A.vera* got importance because of its curative and therapeutic properties. **Woźniak *et al.,* (2012)** found that, *A.vera* extracts can be used on human and animals corneal cells safely. It may be used in eye drops to treat inflammation and other ailments of external parts of the eye.

It is difficult for any spray or ointment to remain in the eye long enough to eliminate the bacteria (as tears wash them away) unless treatments are repeated multiple times daily, so application is generally required three to four times daily to be effective. It is also important to continue the treatment in both eyes even for a few days after the symptoms are alleviated to prevent the recurring **(Kaithwas *et al.,* 2008)**. Therefore, the present study was envisaged to evaluate the antimicrobial activity of *A.vera* plant against pathogenic bacteria and fungi in treatment of eye infected calf.

Clinical observations revealed severe conjunctivitis, corneal edema, whitish yellow opacity on center of cornea and the affected eyes were either unilateral or bilateral and there is no change in appetite or defecation. Our observation in accordance with **Abdullah *et al.* (2013)** who concluded that, conjunctivitis, lacrimation, blepharospasm and different stages of corneal opacity and ulceration are few clinical manifestations of pinkeye. **Faez *et al.*(2017)** added that, as the disease progresses without treatment, ocular discomfort and visual disturbance may lead to in appetence or inability to locate food. These will then consequently affect the body condition score resulting in emaciation.

There are several pathogens associated with severe conjunctivitis and edema of the cornea in cattle **(Moore, 2017** and **Ahmed *et al.,* 2019)**. Tables (1) cleared that; bacterial isolation ratio was 100% as bacteria isolated from all examined swabs (60). The total bacterial isolates were (55/60, 91.7%), where the bacterial species either pure or mixed media were *S.aureus* (35%), *M.bovis* (23.3%), *S. epidermidis* (11.7%), *L.monocytogenes* (6.7%), *P.aeruginosa* (5%), 3.3% of each *S. pneumoniae* and *K. pneumoniae* and1.7%of each *Enterobacter* spp. and *Proteus* spp. These finding was correlate with **Zafer *et al.* (2005)** and **Adnan *et al.* (2015)** who isolated different types of bacteria from the infected eyes of cow's contagious keratitis and conjunctivitis and these bacteria were *S.aureus, M.bovis, Proteus* spp. *Pseudomonas* spp. and *Pasturella* spp.

The isolates of bacteria *Staphylococcus* spp. which accounted for 46.7% in this study as these bacteria are part of the bacterial environment for humans and animals worldwide. *M. bovis* which comensals bacteria located on the mucous membranes of humans and mammals and considers conjunctiva, nose and pharynx the storage places for these it, this bacteria do not live long outside the body of the host and directly transmitted by flies and insects. *Enterobacter* and *Proteus* species are endemic mostly gastrointestinal tract of humans and animals, and some types exist on other parts of the body and on plants, in soil and live in saprophytes or commensals or pathogenic to humans and animals. The virulence strains these bacteria secrete hemolysin, contain cilia and secrete Cytotoxin which breaks lipopolysaccharide of neutrophils **(Quinn *et al.,* 2011)**.

The results in Table (2) also showed that, fungal isolation ratio was (15/60, 25%) from all examined swabs for 4 different species of fungi. The results of our study agreed with **Samuelson *et al.* (2010**), where the isolated fungal species were *A.niger* (8.3%), *C. albicans* (6.7%), *A. flavus* (5%) and 1.7% of each *C. tropicalis, Alternaria* spp.and *Penicillium* spp. Similar to what has been isolated in this study. **Sgorbini *et al.* (2010)** demonstrated that, fungi found in the conjunctival sac of cows might represent transient seeding from the environment, as suspected in other species. Also showed that, although various fungal agents can be recovered from the conjunctiva, fungal conjunctivitis is rarely observed clinically. In comparison to fungal keratitis, relatively few organisms have been implicated in fungal conjunctivitis. *Candida* spp. can cause conjunctivitis after topical corticosteroid and antibacterial therapy to an inflamed eye. Internationally, *Aspergillus* species is the most common isolate

in cases of fungal keratitis worldwide, followed by fusarium and penicillium **(Mian *et al.*, 2016)**.

Tables (3) recorded the susceptibility profiles of bacterial isolates recovered, most bacterial isolates showed high rate of susceptibility to ciprofloxacin (94.3%), ofloxacin (83.1%), chlormphenicol (81.1%) and amikacin (73.6%); moderate susceptible to gentamycin and amoxicilin (67.9 each) and less sensitive to penicilin (26.4%). Interestingly, one isolate of *P. aeruginosa* was resistant to all the tested antibacterials. Similar records were documented by **Kaliamurthy *et al.* (2005)** and **Abu Samra *et al.* (2016)** with slight variation. **Mandell *et al.* (2010**) noted a lot of studies to resistance of bacterial isolated from the healthy and infected eyes to many antibiotics known-Tables (4) illustrated that, the susceptibility profiles of fungal isolates recovered, most fungal isolates showed high rate of susceptibility to natamycin (91.7%), miconazole (83.3) and fluconazole (75%); moderate susceptible to Ketoconazole and amphotericinB (66.7 each) and less sensitive to itraconazole (16.7%).our study antifungal drugs sensitivity in accordance with **Raksha and Gurjeet (2014)** who clearedthat, *Aspergillus niger* showed maximum sensitive to natamycin (NaT, 100%); moderate sensitive to amphotericin-B (AP, 50%); and minimal sensitive to ketoconazole (KT, 33%), Itrconazole (IT, 17%) but not accordance in Fluconazole (FLU,0 %).

This study supports the use of antibacterial ciprofloxacin and chloramphenicol and antifungal natamycin and fluconazole as drugs of choice for topical treatment of keratoconjunctivitis. Our suggestion agrees with **Murat *et al.* (2006)** who reported that, ciprofloxacin, a derivative of quinolone carboxylic acid, has greater activity in vitro. It has high lipid solubility and low ionic partitioning, suggesting that it may be extensively distributed to ocular tissues and may be beneficial in the treatment of pink eye. And agree also with **Abu Samra *et al.* (2016)** who concluded that, chloramphenicol is a well known broad spectrum antibiotic with specific therapeutic activity against Gram-positive and Gram-negative bacteria, rickettsiae, chlamydiae and anaplasmae, so it is used for the treatment of a wide variety of eye infections . Interestingly, our findings go hand in hand with **Gilger (2011)** who detected that, Natamycin is a topical antifungal medication that works well for fungal infections involving the outer layer of the eye, particularly those caused by fungi such as *Aspergillus* and *Fusarium*. However, infections that are more severe may require treatment with fluconazole by mouthor injected directly into the eye.

The majority of medicinal plant species are rich in biomolecule contents which possess antibacterial properties and are not associated with any health hazard **(Masoumian and Zandi, 2017)**. Among these medicinal plants, *A.vera* has been used for therapeutic purposes from ancient times, numerous cosmetics and medicinal products are made from it **(Thiruppathi *et al.*, 2010)**. *A.vera* is known to promote healing due to the presence of active components such as anthraquinones and chromones **(Pandey and Mishra, 2009)**. *A.vera* is antimicrobial and anti-inflammatory, particularly effective as it activates the macrophages which fight bacterial infection while at the same time it results in increasing circulation to the area which finally is responsible for accelerated healing **(Schulz *et al.*, 2004** and **Bozzi *et al.*, 2007)**. Wounds treated with *A.vera* heal faster than other wounds not so treated as it contains not only vitamins E and C, as well as zinc, but also polysaccharides which reduce inflammation and stimulate fibroblast and epidermal growth and therefore the repair process **(Faez *et al.*, 2017)**.

Tables (3) and (4) also recorded *in vitro* the antimicrobial activities of *A. vera* fresh gel and ethanolic extract against isolated Gram positive and Gram negative bacteria as well as fungi, and also compared with 7 standard antibacterial and 6 antifungal used. It found activity of *A.vera* extract healing of eye infected with *S. aureus* and other bacteria more active from all antibiotics used, that may return to extract have multi-vitamins 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 agents pos-

sess inhibitory effect on fungi, bacteria and viruses **(Barandozi and Nejatzadeh, 2013** and **Adnan *et al.*, 2015)**.

Our findings also recorded that, *A.vera* dried gel ethanolic extract have shown tremendous inhibitory effects than fresh gel against bacterial (94.3% &100%) and fungal isolates (91.7&100%) respectively. **Cowan *et al.* (1999)** reveals that, biologically active components against microorganisms identified from plants are aromatic or saturated organic compounds and mostly obtained through initial ethanol or methanol extraction. This argument explains the higher activity for ethanol extract than *A.vera* gel in the study. These records go hand in hand with **Mian *et al.*, (2016)** who found that, the highest activity was shown by dried gel ethanol extract dissolved in dimethyl *formamide* (DMF). This might be possible that in water the biologically active components were not completely active, where in DMF certain components were completely active showing higher biological activities. Our findings are also in commitment with those of **Ke-Qiang and Bruggen (2001)** who recorded maximum *B. subtilis* growth inhibition with ethanol extracts. **Lawrence *et al.* (2009)** also found that, ethanol extract had higher inhibitory effect than methanol as ethanol was efficient in extracting the biologically active components which show lethal affects against *B.cereus.*

Early detection of animals with the first clinical signs (tearing, squinting, and blinking as shown in Photo. 2), identifying the causative pathogens, prompt the effective treatment which based on the time taken for complete recovery with no recurrence, temporary isolation and preventive treatment of animals newly introduced to the herd and good management practices are essential to reducing spread and limiting damage to the eye **(Faez *et al.*, 2017** and **Ahmed, 2019)**. So from the first day of the study and after ensured that the lesions are not due to foreign bodies or parasites, in addition to these rules our treatment also based on, separation of infected animals, affected animals provided with shade and gloves and protective clothing should be worn and then disinfected between animals when affected individuals are being handled.

Topical administration of antimicrobial formulations has been recommended as a potentially cost-effective and less labor-intensive method for treatment of eye problems **(Ahmed, 2019)**.

The results in Table (5) suggested that, *invivo*ciprofloxacin ointment and chloramphenicol eye drops treatment in addition to natamycin-fluconazole as antifungal in cases from which fungi isolated were able to cure pink eye and completed healing occur but some cases specially infected with staphylococcus showed recurrent (30%) during 21 days after completed healing). This result was agreement with **Naglic *et al.* (2000)** who concluded that, ciprofloxacin ointment for 3-5 days in addition to chlormphenicol eye drops was very efficacious in treating caprine and bovine infectious kerato conjunctivitis. **Adnan *et al.* (2015)** confirmed that, ciprofloxacin ointment with chloramphenicol eye drop treated infected buffaloes calves was showed effective and completed healing occur but some cases specially infected with staphylococcus showed recurrent.

On the other hand *A.vera* fresh gel and ethanolic extract showed slightly higher efficacy than ciprofloxacin ointment+ chloramphenicol eye drops, where all infected buffalo calves showed complete recovery and 100% healing to the extract and healing period was also record as 5-7 day according to the severity of infection without recurrence as shown in Table (5) and Photos(3) and (4). It is very important to say that, the healthy group of buffalo calves treated with *A.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. This result go hand in hand with several studies **(Lawless and Allan, 2000; Lawrence *et al.*, 2009**and **Mhya and Mankili, 2015**).The potential to develop antimicrobial compounds from higher plants appears rewarding as it will propel to the expansion of a phytomedicine to turn against multidrug resistant microbes as confirmed by **Agarry *et al.* (2005)**; **Ilaiyaraja *et al.* (2010)** and **Mehrotra *et al.* (2010)**.

Biological activities of *A.vera* are due to the presence of some important polysaccharides present in the gel, but it is very difficult to attribute the therapeutic properties to an individual polysaccharide component **(Masoumian and Zandi, 2017)**. The composition and biological activities of *A.vera* gel vary with type of genotype, seasonal variation, type of separation and extraction method **(Hamman and Viljoen, 2008)**. 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 **(Prajapati *et al.,* 2011;Barandozi and Nejatzadeh, 2013** and **Adnan *et al.,* 2015)**.

A.vera contains 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 **(Habeeb *et al.,* 2007** and **Chatterjee *et al.,* 2015)**. Moreover contain Polysaccharides and phenol reported refer direct bacterial activity through the stimulation of phagocytic leucocytes that killed bacteria. Modern studies showed that *A.vera* gel healing ulcer induced by *Helicobacter pylori* through direct effect as antibacterial and healing properties, there for healing activity 100% was demonstrated in our study due to inhibition of bacterial and fungal that it was proven during in vitro study to reach high percent of sensitivity of %100 **(Esua andRauwald, 2006)** by disk diffusion **(Agarry *et al.,* 2005** and **Rakshaand Gurjeet, 2014)** as shown in Table (3) and (4).

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 loose 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 **(Ingrida *et al.,* 2014).** Our study showed that, PH of *A.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 *A.vera* contain vitamin C act as co-factor of collagen synthesis that reduce ulceration, eye treated with extract showed healing with short period less than 7 day due to emollient nature with increase corneal, conjunctive epithelial and keratocyte proliferation that aid to complete re-epithelization, so the main cause of Keratoconjunctivitis 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 **(Havens *et al.,* 2009).**

A.vera gel showed greater all these result may be return to *A.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 of salicylic acid **(Esua and Rauwald, 2006** and **Surjushe *et al.,* 2008). Choche and shendersK (2014)** said that, the main causes of healing *in vivo* may be explain as due to extract contain many vitamins such as A, C and E that responsible of antioxidant activity and immune modulation activity of extract.

Conclusion: Pinkeye is a complicated, multifaceted disease. It is said that "an ounce of prevention is worth a pound of cure," The best plan is to reduce or remove as many risk factors as possible in order to keep the eyes healthy and better equipped to fend off disease. Prevention is based on maximizing herd immune status by good level of nutrition, adequate vitamin and trace mineral intake, parasite control, and basic biosecurity practices; maintaining as irritant-free environment with good face fly control, mow tall grass, provide shade and clean water, and reduce sources of stress such as overcrowding and overgrazing; and minimizing the presence of pathogens by immediate detection and isolation of affected animals followed by effective and long acting drug will speed healing time. The uses of medicinal plant as *A.vera* is a very useful alternative method for treating eye infection, as it could inhibit the growth of studied pathogens. The obtained results demonstrate that this plant extract could represent a new source of potent anti-microbial, nontoxic, less expen-

sive than the allopathic drugs. This possibility referred to the presence of some bioactive components in crude extracts of aloe vera gel due to which it has showed strong antibacterial -antifungal effects. The data showed promising results in case of *A.vera* gel extracts as an alternate herbal antimicrobial agent replacing synthetic one to prevent and treat some infectious diseases

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