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Evaluation of antimicrobial effect of *Acacia nilotica* plant extract and selected commercial disinfectants against some pathogens causing mastitis

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ABSTRACT

Objective: This study was conducted to screen the antibacterial activity of *Acacia nilotica* plant extract and four disinfectants (TH_4, Tek-trol, Virkon S and peracetic acid) against pathogens causing mastitis (*E.coli*, *Staphylococcus aureus* and *Streptococcus agalactiae*).

Design: Descriptive study

Animals: Three hundred eighty-two dairy cows

Procedures: Three thousands and seventy seven samples were collected from three dairy farms at Dakahlia province. Samples include animal samples (1528 of both quarter milk and teat skin swabs) and 221 environmental samples include 60 bedding, 60 Milk linear, 36 feed, 36 water, 11 bulk tank milk and 18 workers' hand swabs. All samples were examined bacteriologically for isolation and identification of mastitis causing pathogens (*S. aureus, E. coli* and *St. agalactiae*). Furtherly, minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) tests were used to investigate the antimicrobial activities of selected disinfectants and *Acacia nilotica* extract toward isolated strains.

Results: TH_4 and aqueous *Acacia nilotica* extract were effective against *S. aureus* and *St. agalactiae*, with MIC values as low as 3.13 µg/ml of the original concentrations. Meanwhile, the antimicrobial actions of Tek-trol and *Acacia nilotica* extract toward *E. coli* isolates only reached 12.5 µg/ml. The in-vitro bactericidal effect showed that, MBC values of *Acacia nilotica* plant extract achieved the highest inhibitory concentration up to 25 µg/ml among the tested disinfectants.

Conclusion and clinical relevance: In conclusion, *Acacia nilotica* plant extract have antibacterial activity comparable to commercialdisinfectants for the control of mastitogenic pathogens in dairy farms, providing a promising tool for mastitis control in dairy farms.

Keywords: Antimicrobial activity, Disinfectants, *Acacia nilotica* plant extract, Mastitis causing pathogens.

1. INTRODUCTION

Bovine mastitis is one of the costliest multi-etiological diseases causes devastating consequences in global dairy industry, despite of much research and efforts has been dedicated to control the diseases incidence in dairy herds in the last 7 decades [1]. Recent global estimates revealed that the worldwide annual financial losses of acute and per acute mastitis forms in terms of reduced milk production and inferior milk value, extra labor, higher risk of animals' culling and veterinary cost evaluates by 35 billion US dollars [2].

Bovine mastitis, defined as inflammation of the mammary gland, causes physical, chemical and usually bacteriological changes in milk beside pathological changes in glandular tissues of the udder [3]. According to the severity of the inflammation, mastitis can be categorized in clinical or subclinical forms. Clinical mastitis is diagnosed by visible manifestations such as abnormal milk (changes in color, presence of clots, flakes), abnormal mammary gland (changes in tissue color, swelling) and changes in animal status (body temperature, appetite, and hydration level). On the other hand, detection of subclinical mastitis may be more difficult because of absence of visible clinical signs in the mammary gland and in milk although the fact that it is 15 to 40 times more prevalent than the clinical form [4].

Mastitis is usually caused by more than 137 bacterial pathogens, which can be classified into contagious and environmental. Contagious pathogens as *Staphylococcus aureus* and *Streptococcus agalactiae*, are reside predominantly in the udder and spread during milking while environmental pathogens exists in the cow's environment and usually transferred in any time of cow's life as *Streptococcus uberis, Streptococcus dysgalactiae,* and *Escherichia coli* [5].

Control programs are mainly focused on rapid identification and treatment of clinical mastitis cases, routine whole-herd antimicrobial dry cow therapy. However, awareness of the economic mastitis losses and failure associated with conventional antibacterial therapy is resulting in increasing importance of management and hygiene of the farm in ensuring of udder health [6]. The key elements in the control of mastitis include effective farm management, sound husbandry practices and sanitation, post milking teat dip, treatment of mastitis during non-lactating period, increasing the sanitary measures and culling of chronically infected animals to ensure udder health [7].

Pre- and post-milking disinfection with an effective product is recognized as the simplest and cheapest method can reduce the incidence of clinical mastitis caused by contagious bacteria by 50% and environmental bacteria by 24% [8]. There are many factors playing an important role in

the success of disinfection process including the selection of suitable disinfectant and application of it.

Disinfectants used as teat dips provides further teat coverage than spraying and so is highly recommended**.** Teat should be fully blanketed to offer adequate disinfection to teat pores and all teat parts which might be in contact with the milking unit. This is obviously essential with pathogens that inhabit teat skin such as *Streptococcus agalactiae* and *Staphylococcus aureus* [9].

Furthermore, mastitis management regularly involves the administration of antibiotics to treat and prevent the disease, which poses severe risks concerning the development of antibiotic resistance [10]. The biocide must be fast in its antimicrobial action, simple in its utilization, cheap, had no bad impact on the food, and degradation into harmless final products to become safety in use [11]. Recently the interest has shifted from the more conventional antibiotic therapies towards the advanced methods as using of plants as new and potential source of antimicrobial agents [12]. **The objectives of this study** were to isolate and characterize predominant bacterial causes of mastitis in dairy herds and to evaluate the antibacterial efficacy of commercially used disinfectants and *Acacia nilotica* plant extract as antibiotic alternative for the prevention and control of mastitis in dairy herds.

2. MATERIALS AND METHODS

2.1. Study area and period

This study was carried out in three private dairy farms with different levels of biosecurity in three districts namely Danabik village, Shawa village and Gamasa city; all located at Dakahliya province, Egypt during the period from July 2019 till January 2020.

2.2. Sample size

Purposive sampling technique was applied to all available dairy cows in the study area. A total of 3326 samples collected from three selected dairy farms were chosen conveniently relying on dairy cow availability.

2.3. Samples collection

Samples were collected from the cows and their surrounding environment in both examined farms. Cows' samples include individual quarter milk samples & teat apex swabs from all milking cows. While, environmental samples involved bedding, feed, water, milk linear swabs, and bulk tank milk (BTM), in addition, hand swabs from milk workers were collected under strict aseptic conditions then labeled and transported as soon as possible to the laboratory of Department of Hygiene and Zoonosis, Mansoura University in ice-cooled containers for further processing and microbiological examination. All procedures were done under the guidelines of Ethical committee of Faculty of Veterinary Medicine, Mansoura University.

2.3. Animal sampling

2.3.1. Quarters milk samples

A total of 1528 quarters milk samples from apparent health, sub-clinical and clinically mastitic cows were collected before milking aseptically using disposable latex gloves that were changed between each animal. After washing and drying of cow's udders and teats, each teat end was disinfected with cotton swabs soaked in 70% ethanol. The first few streams of milk were thrown away then 10 ml of milk from each functioning udder quarter was collected into a sterile test tube and labeled with cow number, farm name and date then transported on ice to the laboratory for microbiological examination [13]**.**

2.3.2. Teat skin swabs

A total of 1528 teat skin swabs (1 swab/teat of 382 cows) were collected aseptically from the same cows and quarters using disposable latex gloves that were changed between each animal as method described by [14]**.**

2.3.3. Environment sampling

A total of 36 bedding samples were collected manually from different sites of yards particularly from a wetted area with high moisture and organic matter load at depth of 5 cm in a sterile glass bottle, according to [15].

Feed (n=36)

Approximately 500 grams of feed were taken along the entire length of a bunk or feeder by using worn sterile gloves. The collected feed samples were placed into a plastic bag and transported to the laboratory in an icebox for further processing [16].

Water

Water samples (n=36) from cattle water troughs and buckets were collected aseptically in sterilized bottles and transported to the laboratory in an icebox for further processing [17].

2.3.4. Milk linear swabs

A total of 60 milk linear samples were collected from internal surfaces of teat cups after the cleaning process of milking machines, and before the sanitization step by using sterile moistened cotton swabs with Tryptic soya broth (TSB) [18].

2.3.5. Bulk Tank Milk (n=11)

Milk samples (100 mL) were aseptically collected from bulk tanks and individually packed in sterile cups and

transported in an ice container to the laboratory for bacterial identification [8].

2.3.6. Worker hand swabs

Hand swabs from workers (n=18) were collected during working. A sterile cotton-tipped swabs previously dipped with TSB were rolled over the palm of hands, fingertips, nails, and area between fingers of hands. All swabs directly inserted in sterile plastic containers filled with 5 ml of TSB [19].

2.4. Detection of mastitis in dairy animals

Clinical examination for all sampled cows in both farms were recorded followed by screening of udders by California mastitis test (CMT) for detection of sub-clinically mastitic animals then culturing of milk samples from apparently normal and mastitic quarters as technique employed by [20].

2.5. Isolation and identification of mastitis causing pathogens

Milk samples were pre-incubated aerobically at 37°C for 24 hours, then centrifuged at 3000 rpm for 20 minutes, to discard cream and supernatant and obtain sediment for streaking onto the surface of isolation media. Moreover, one gram of each bedding and feed samples, after thorough mixing was weighted and triturated well in a sterile mortar with 99 ml of sterile TSB then aseptically strained through sterile gauze; the filtrate was collected in a sterile flask and incubated at 37°C for 24 hours. Likewise, each sterile cotton collected swab was incubated in nutrient broth aerobically at 37°C for 24 hours for bacteriological examination [21].

A loopful from the pre-enriched culture from each incubated sample was streaked directly onto Baird-Parker agar, Edward's selective media and MacConkey's agar (Oxoid, UK) as selective media for isolation of *S. aureus, St. agalactiae & E. coli,* respectively. The inoculated plates were then incubated aerobically at 37°C for 24-48 hours. The typical colonies were picked up and sub-cultured on nutrient agar slants and incubated aerobically at 37 °C for 24–48 hours to get a pure culture [22].

2.6. Identification of bacterial isolates

Smears from suspected pure colonies of each bacterial isolate were stained with Gram's stain as described by [23]**.** Smears examined microscopically to observe the morphology, arrangement and staining reaction. Gram-positive bacteria appeared cocci in shape with pale to dark purple color while Gram-negative bacteria appeared rod with pale to dark red color.

The suspected isolates were subjected to the biochemical tests as mentioned by [20]. *Staphylococcus aureus* isolates were identified by biochemical tests including catalase production, mannitol fermentation, coagulase test and haemolytic activities [24]**.** The characteristics of purified colonies of suspected *Streptococcus* were identified using the following tests: Catalase, Oxidase test, CAMP test (Christie-Atkins-Munch-Peterson) and Aesculin hydrolysis, as methods previously adopted by [24]**.** While, *E. coli* isolates were characterized biochemically using the following tests: Urea hydrolysis test, Oxidase test, Indol, Methyl-red, Voges-Proskauer, Citrate utilization as methods previously adopted by [25,26]**.**

2.7. In vitro sensitivity of bacterial isolates to certain antimicrobials (commercially used disinfectants and Aqueous *Acacia nilotica* extract*)*

Four commercial types of disinfectants commonly used as teat dips in veterinary practice were diluted to desired concentration using sterile distilled water according to the manufacturer's recommendation including Tek-Trol (1/256) (Antec international TD, UK), TH4+ (0.5%) (SoGeVal, France), Virkon S (1%) (Antec international TD, UK) and peracetic acid. Aqueous *Acacia nilotica* extract was kindly provided from the Department of Plant Chemistry, Faculty of Agricultural, Mansoura University **(Annex 1)**.

Before the preparation of bacterial inoculum, a 0.5 McFarland standard was prepared as methods described by [27]. Then 4-5 well isolated and identified bacterial colonies from an overnight agar culture were inoculated into TSB and incubated at 35-37°C/24h. The broth culture turbidity was adjusted with a sterile broth to get turbidity equivalent to that of 0.5 McFarland standard according to [28]. The final inoculum density of this suspension was about $1x10^4$ CFU/ml.

Minimal inhibition concentration (MIC) and minimum bactericidal concentration (MBC) tests were applied to evaluate the sensitivity for each bacterial isolate to tested disinfectants by using the broth dilution method as technique recommended by [29]. Briefly, in 96–well plates, 50 µL of Mueller–Hinton broth (MHB) media was added to each well from 2 to 12. Then 100 µL of diluted tested disinfectant was added in the first well in each raw of plates, which furtherly two-fold serial dilutions were made by transferring 50 µL from first well to 11^{th} well. Meanwhile, 12^{th} well was antimicrobial free well served as growth control. Each test and growth control wells were inoculated with 50 µL of a prepared bacterial inoculum (10^4 CFU/ml).

After well mixing, the inoculated 96-well microtitration plates were incubated at 37°C for 24-48 hours. The plate was

examined for bacterial growth, which observed as turbidity using a spectrophotometer at 600 nm. The least disinfectant concentration where no turbidity was recorded as the MIC value (mg/ml). Then 100 µL from wells that showed no visible bacterial growth was spread on the surface of non-selective agar plates (Mueller Hinton agar plates- MHA) and were incubated for 24 hours at 37°C, in order to determine the least disinfectant concentration killed 99.9% of the final bacterial inoculum, which was recorded as the MBC value (µg/ml).

3. Results

3.1. Phenotypic characterization of mastitis causing bacteria isolated from the examined dairy farms

Table 1. Phenotypic characterization results of mastitis causing bacteria isolated from the examined dairy farms.

Test	Positive *S. aureus*		Positive *St. agalactiae*		Positive *E. coli*	
	Animal samples (n=3056)	Farm samples (n=221)	Animal samples (n=3056)	Farm samples (n=221)	Animal samples (n=3056)	Farm samples (n=221)
Gram staining	210	130	113	40	340	200
Shape	196	127	95	30	328	200
Motility	150	115	89	29	311	183
Urease	152	112	----	----	270	165
Citrate	149	109	----	----	262	144
Coagulase	147	103	89	27	----	----
Catalase	147	103	85	27	----	----
Oxidase	147	103	85	22	----	----
Kligler's iron agar (KIA)	----	----	----	----	251	144
Lysine iron agar (LIA)	----	----	----	----	251	144
Haemolysis	----	----	85	22	----	----
Gelatin Hydrolysis	147	103	-----	----	----	----
Christie,Atkins,Munch-Petersen (CAMP)	-----	----	85	22	----	----
Total	147 (4.8%)	103 (46.6%)	85 (2.8%)	22 (9.95%)	251 (8.2%)	144 (65.2%)

Table 2 showed phenotypic characterization results of mastitis causing pathogens from animal and farm sources. Microscopical examination showed typical gram +ve, non-motile cocci *S. aureus* bacteria and upon biochemical testing, 147 and 103 of *S. aureus* isolates among animal and farm samples, respectively gave positive results for Urease, Citrate, Coagulase, Catalase and gelatin hydrolysis, and negative for oxidase tests. Meanwhile, *St. agalactiae* isolates showed lower positive levels by phenotypic characterization, where 85 and 22 isolates from animal and farm samples, respectively were gram +ve, non-motile cocci, negative for urease, coagulase, catalase and oxidase, while positive for hemolysis and CAMP tests. Among all examined *E. coli* isolates from both sources, 251 and 144 of it were negative motile rods with clear negative results for urease, citrate and coagulase and positive for Kligler Iron Agar (KIA) and Lysine iron agar (LIA) tests.

On the level of animal samples, the percentages of *E. coli*, *S. aureus* and *St. agalactiae* isolates were 8.2, 4.8 and 2.7%, respectively. Meanwhile from environmental samples the percentages were 65.2, 46.6 and 9.95%, respectively.

3.2. MIC & MBC of some disinfectants and Acacia nilotica plant extract toward mastitis causing pathogens

Table 2. Minimal inhibition concentration (MIC) of tested antimicrobials (disinfectants and plant extract) (mg/mL) against *S. aureus.*

wells	Conc. (µg/ml)	*S. aureus* (10^4 CFU/ml)				
		Tek-Trol	TH_4	Virkon S	Peracetic acid	*Acacia nilotica* extract
1	**100**	-	-	-	-	-
2	**50**	-	-	-	-	-
3	**25**	-	-	-	-	-
4	**12.5**	+	-	+	+	-
5	**6.25**	+	-	+	+	-
6	**3.13**	+	-	+	+	+
7	**1.56**	+	+	+	+	+
8	**0.78**	+	+	+	+	+
9	**0.39**	+	+	+	+	+
10	**0.20**	+	+	+	+	+
11	**0.10**	+	+	+	+	+
12	-	+	+	+	+	+

(-) No microbial growth; (+) showed microbial growth; Well (1) Positive antimicrobial control; Well (12) Negative antimicrobial control

The antibacterial effect of four commercially disinfectants and *Acacia nilotica* plant extract against *S. aureus* isolates were determined through evaluation of MIC and summarized in table 2. The MIC ranges of tek-trol, Virkon s and peracetic acid were varied from 100-25 µg/ml, whereas *Acacia nilotica* plant extract had lower MIC ranges (100-6.25 µg/ml), meanwhile TH_4 had the lowest MIC range among all tested antimicrobials varied between (100-3.13 µg/ml). These findings indicate that all *S. aureus* isolates were susceptible to all tested antimicrobials even at low concentrations up to 3.13 µg/ml.

Table 3. Minimal inhibition concentration (MIC) of tested antimicrobials disinfectants and plant extracts) (mg/mL) against *Streptococcus agalactiae.*

wells	Conc. (µg/ml)	*Streptococcus agalactiae* (10^4 CFU/ml)				
		Tek-Trol	TH_4	Virkon S	Peracetic acid	*Acacia nilotica* extract
1	100	-	-	-	-	-
2	50	-	-	-	-	-
3	25	-	-	-	-	-
4	12.5	-	-	+	+	-
5	6.25	-	-	+	+	-
6	3.13	+	-	+	+	+
7	1.56	+	+	+	+	+
8	0.78	+	+	+	+	+
9	0.39	+	+	+	+	+
10	0.20	+	+	+	+	+
11	0.10	+	+	+	+	+
12	-	+	+	+	+	+

(-) No microbial growth; (+) showed microbial growth; Well (1) Positive antimicrobial control; Well (12) Negative antimicrobial control.

Table 4. Minimal inhibition concentration (MIC) of tested antimicrobials (disinfectants and plant extracts) (mg/mL) against *E. coli*.

wells	Conc. (µg/ml)	*E. coli* (10^4 CFU/ml)				
		Tek-Trol	TH_4	Virkon S	Peracetic acid	*Acacia nilotica* extract
1	100	-	-	-	-	-
2	50	-	-	-	-	-
3	25	-	-	-	-	-
4	12.5	-	+	+	+	-
5	6.25	+	+	+	+	+
6	3.13	+	+	+	+	+
7	1.56	+	+	+	+	+
8	0.78	+	+	+	+	+
9	0.39	+	+	+	+	+
10	0.20	+	+	+	+	+
11	0.10	+	+	+	+	+
12	-	+	+	+	+	+

The MIC for *St. agalactiae* against the tested antimicrobials is listed in Table 3. TH_4 had the lowest MIC (100-3.13 µg/ml) followed by tek-trol and *Acacia nilotica* plant extract (100-6.25 µg/ml), whereas virkon s and peracetic acid had higher MIC (100-25 µg/ml) to inhibit bacterial growth.

Table 5. Minimal bactericidal concentration (MBC) results of tested antimicrobials (disinfectants and plants) against mastitis causing bacteria (*S. aureus*, *Streptococcus agalactiae* and *E. coli* from the examined dairy farms.

Mastitis causing pathogens	MBC (µg/ml))				
	Tek-Trol	TH_4	Virkon S	peracetic acid	*Acacia nilotica* extract
S. aureus	100	100	100	100	100
	50	50	50	50	50
	25	-	-	25	25
Streptococcus agalactiae	100	100	100	100	100
	-	50	50	50	50
	-	-	-	25	-
E. coli	100	100	100	100	100
	50	50	50	50	50
	-	25	-	-	25

The antimicrobial activities of evaluated disinfectants and *Acacia nilotica* plant extract towards *E. coli* isolates were illustrated in Table 4. Generally, the results showed that the MIC of these substances could be arranged as the following: tek-trol & *Acacia nilotica* plant extract (12.5 µg/ml) > TH_4, virkon s and peracetic acid (25 µg/ml).

The MBC values of tested disinfectants and *Acacia nilotica* plant extract against mastitis isolated pathogens (*S. aureus, St. agalactiae* and *E. coli*) were shown in Table 5. Tek-trol, peractic acid and *Acacia nilotica* plant extract had higher bactericidal effect (25 µg/ml) toward *S. aureus* than virkon s and peracetic acid (50 µg/ml). Whereas, peracetic acid had the maximum inhibition effect at 25 µg/ml concentration when compared to the other evaluated antimicrobials against *St. agalactiae*. For *E. coli*, MBC values showed higher bactericidal effects with TH_4 and *Acacia nilotica* plant extract 25 µg/ml when compared with the others, which achieved MBC up to 50 µg/ml.

4. DISCUSSION

Bovine mastitis is a multifactorial disease caused mainly by poor hygiene related to various biological causes involving bacteria, viruses, fungi and etc. Bacteria are classified as the major etiological agents for mastitis, comprising *Staphylococcus aureus, Escherichia coli, Streptococcus agalactiae, Corynebacterium bovis, Pseudomonas aeruginosa,* and *Bacillus cereus* [30-32].

Mastitis is deemed as the most crucial danger to the dairy industry since it leads to a significant decline in animal health and milk production, eventually causing massive economic losses [32]. It is considered as one of the more costly diseases in dairy farms which associated with many losses including discarded milk, increased number of culled cows, cost of antibiotic treatment and reduced milk quality and price [33]**.** Therefore, it was interesting to carry out this investigation to find out the percentage of some mastitic pathogens including *S. aureus, St agalactiae* and *E. coli* in dairy farms and to establish a practical approach for prevention and control of mastitis in dairy herds.

Results of our study revealed that *E. coli* was the most prevalent isolated organism from both animals and environmental samples followed by *S. aureus* and *St. agalactiae*. This convection was previously cited by many authors as [34-36] who reported that *E. coli* was a major environmental opportunistic pathogen involved in acute bovine mastitis with a usually fast recovery rate, yet, in extreme cases induced sepsis concurrent with fever. Infrequently, *E. coli* caused a subclinical and persistent infection. Meanwhile, [37] found that the major causative agents isolated from mastitic dairy cows in Assiut Governorate, Egypt were *S. aureus, St agalactiae* and *E. coli* with prevalence 52.5, 31.25 and 16.25%, respectively.

The highest isolation rates of mastitis causative agents from environmental samples than animal samples could be attributed to absence of disinfection and improper washing of udder before milking so these bacteria can be transmitted during milking from infected animal to another healthy one in absence of regular cleansing and disinfection of teat cups, this results are in harmony with that detected by [38,39] who observed that the percentage of *St. agalactia* infection was lower (3%) in herds where teat cups were cleaned with water and detergent after each milking compared with herds where teat cups were cleaned only with water (18%) but when cleaned with water and detergent twice a week (27%). This might be the practical hygienic procedures such as disinfection of teat cups, regular removal of manure, selection of hygienic water source and antisepsis of milker's hands before milking process reduce the level of contamination with bacterial pathogens, consequently, reduce the prevalence of mastitis.

The continuous and misuse of antibiotics in the treatment of mastitis pathogens and other diseases in both humans and animals has led to developing multidrug resistant organisms, so there is a great need for an alternative. The alternative must be cheap, sustainable and friendly to the environment [40]. Antimicrobials obtained from plants have much therapeutic potential and are effective in the treatment of infectious diseases in animals as well as humans. They may also simultaneously mitigate many of the side effects that are associated with synthetic antimicrobials [41]**.** In our study, the bactericidal activity of *Acacia nilotica* plant extract was compared with four tested disinfectants (Tek-Trol, TH_4+, Virkon S and peracetic acid) against mastitogenic pathogens including *E. coli*, *S. aureus and St. agalactiae*. The results revealed that *Acacia nilotica* plant extract has good antibacterial activity against isolated pathogens. Previous studies by [42] reported that acetone extracts of *A. nilotica* had antibacterial activity with MIC of 6.25mg/ml and 12.5mg/ml against S. aureus (HM626197), for leaves and bark respectively. This is in harmony with [43] who reported that plant extracts with MIC values less than 1.0mg/ml were considered to have good antibacterial activity and those with MIC values less than 0.1 mg/ml were regarded as having significant activity [44]**.**

On the other hand, the inappropriate choice or insufficient low concentrations of disinfectants from the most important factors lead to failure of environmental disinfection process besides the developing of resistant bacterial isolates to the most of disinfectants. It necessitates routine in vitro evaluation to the efficacy of disinfectants against bacterial isolates from dairy herds to determine the most effective concentrations. Our results indicated that TH4+ is the most effective disinfectant followed by Tek-Trol, Virkon S and peracetic acid against *E. coli*, *S. aureus and St. agalactiae*. These results in accordance with [45] **who** revealed that TH4 was the most powerful disinfectant because of synergism between four quaternary ammonium and glutaraldehyde as an acid solution. Fazlara and Ekhtelat [46] calculated the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of benzalkonium chloride (one of QACs) against *S. aureus* and *E. coli* as 40 mg/L and 45 mg/L, sequentially. Meanwhile, [47] found that Virkon S had a superior bactericidal effect more than TH4 against *E. coli* isolated from the environment, which was nearly 70 % and 50 %, respectively.

Conclusion

Bacterial infections are main causes of mastitis in dairy farms, where *E. coli, S. aureus* and *St. agalactiae* are major pathogens found in animal and environmental samples. Control program of mastitis relies on adoption of hygienic measures, antibiotic treatment and disinfection strategies. There is a growing concern about finding alternatives for antibiotics used for controlling of these pathogens like plant extracts. This study confirms the effectiveness of aqueous *Acacia nilotica* plant extract as bactericidal agent against isolated key pathogens. The safety of plant extracts is a worldwide concern, and so further studies must be

conducted to clearly distinguish their biological effects. Additionally, Tek-trol and TH_4 as commonly used disinfectants in dairy farms achieved the lowest MIC and MBC values among all tested disinfectants.

Conflict of Interest

There is no conflict of interest in the current research work.

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Research ethics committee permission

The Ethical approval was obtained from a guidance of Research, Publication and Ethics of the Faculty of Veterinary Medicine, Mansoura University, Egypt, which complies with all relevant Egyptian legislations on research and publications.

Authors' contribution

F.A.E, and M.M.E. involved in the conception of the idea, methodology design, laboratory work, performed data analysis, interpretation, manuscript preparation for publication and final revision. N.M.O. participated in the sampling, the laboratory work and data analysis. M.A.M. contributed her scientific advice. All authors read and approved the final manuscript. The funders had no role in data collection and analysis, decision to publish, or preparation of the manuscript.

Annex 1. Antimicrobials used (class, trade name, active ingredients & recommended concentration).

Class of disinfectant	Trade name	Active ingredients	Recommended concentration
Phenol	Tek-Trol	Ortho-phenylphenol (12%) Ortho-benzyl-parachlorophenol (10%) para-tertiary-amylphenol (4%)	1/256
Quaternary ammonium compound and glutaraldehyde	TH_4	Glutaraldehyde (7%); alkyl (C12, 67%; C14, 25%; C16, 7%; C18, 1%) Dimethyl benzyl ammonium chloride (26%)	0.5%
Oxidizing agent	Virkon-S	Potassium peroxymonosulfate (21%); sodium chloride (1.5%)	1%
Peracetic acid	H_2O_2	Acetic acid and hydrogen peroxide	2%
Acacia nilotica	Egyptian thorn	Saponins, anthraquinones, tannins, flavonoids, terpenoids, alkaloids and glycosides	10mg/ml

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