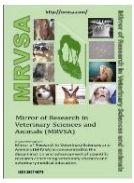
ORIGINAL ARTICLE **Mirror of Research in Veterinary Sciences and Animals MRVSA/ Open Access DOAJ**

Mirror of Research in Veterinary Sciences and Animals (MRVSA)
Journal homepage: http://mrvsa.com/
E-ISSN 2307-8073; 2520-324X (Print)



In vitro Evaluation of Antimicrobial Activities of the Filtrate product of milk of the camel

Rahem. E. AL-Zaiadi [1]

[1] Department of Biology/ College of Pure Sciences \ Al-Muthanna University

ARTICLE INFO
Received: 05.04.2016
Revised: 10. 06.2016
Accepted: 12.06.2016
Publish online: 25.06.2016

***Corresponding author:**
Email address:
rahem.alziadi@yahoo.Com

Abstract

This study was designed to examine the antimicrobial activity of camel's milk filtrate products on the pathogenic bacteria and yeasts in vitro. The milk samples were collected from a 4-5 years she camels (*Camelus dromedaries*) raised in local farm (sample 1) and from Western Desert of Samawa (sample 2). The chemical properties of the milk samples were analysed. The results of the chemical analysis of these samples (sample 1 & 2 respectively), revealed that these samples composed of fat (3.98%, 3.98%), protein (3.64%, 3.12%), lactose (4.62%, 4.84%) and ash (0.68%, 0.80) respectively. The pH of both milk samples was ranging between 6.34 and 6.82. The percentages of acidity as lactic acid were estimated between 0.15% and 0.17% for sample 1 and 2 respectively. *Escherichia coli* O157:H7, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella spp*, *Salmonella typhimiurum*, *Clostridium spp.* and *Candida albicans* were used as pathogenic microorganisms to evaluate the antimicrobial activity of camel's milk filtrate products. Camel's milk filtrate products were revealed different inhibition zone on all pathogenic bacteria and yeasts. The diameters of inhibition zones of sample (1) for both X and 2X concentration were 14,12,11,9,11,11,13,12 and 18,20,16,11,15,14,19,17 mm respectively, and for sample (2) also for X and 2X concentration were 17,15,16,12,11,14,17,16, and 21,20,21,18,19,21,21,20 mm respectively, against *Escherichia coli* O157:H7, *Pseudomonas aeruginosa*, *Enterococcus faecalis* , *Staphylococcus aureus*, *Klebsiella spp*, *Salmonella typhimiurum* , *Clostridium spp. and Candida albicans* respectively. Antimicrobial activity of 2X concentration for desert camel's milk filtrate products was more effective and compared with some antibiotics groups of beta-lactam and amino glycosides. The results showed that the 2X concentration of camel's milk filtrate product was more effective and revealed large inhibition zones. In conclusion, the results of this study showed that Camel' s milk Filtrate product has antimicrobial activity against different pathogenic microorganisms. Moreover, the milk sample collected from the desert camel was more effective than the milk sample collected from camel raised in local farm. In addition, 2X concentration of filtrate products was better than X concentration of both samples, as well as from different other antibacterial..

To cite this article: Rahem. E. AL-Zaiadi. (2016). In vitro Evaluation of Antimicrobial Activities of the Filtrate product of milk of the camel. MRVSA 5 (Special issue) 1st Iraqi colloquium on camel diseases and management. 39-46.
DOI: 10.22428/mrvsa. 2307-8073.2014. 002176.x

Keywords: antimicrobial activity, *Escherichia coli*, camel's milk filtrate products.

Introduction

Recently, there are significant increases in the prevalence of resistance to antibiotics in common pathogens of humans and animals worldwide. The increasing morbidity, mortality, and cost of health care are the consequences of the appearance and spread of antibiotic resistance. The major cause for the appearance and spread of antimicrobial resistance has been increasing antimicrobial use that enable the pathogenic microorganisms to modify themselves against the antibiotics. Most Bacteria have developed mechanisms of resistance to all classes of antibiotics available for systemic use in humans and animals. These mechanisms can be divided, by function, into three general groups: (1) inactivation of the antimicrobial, (2) alteration of the site of antibiotic activity, and (3) isolation of the target site from the antibiotic (Neu, 1992; Dixit and Gandhi, 2010; WHO, 2014). The beta-lactam is a broad category of antibiotics which working on inhibition of cell wall by inhibiting of peptidoglycan synthesis. After resistance period, the bacteria often has ability on synthesis of β-lactamase enzyme. This enzyme is able to breaking antibiotic and analyses the lactam ring by breaking the bond between carbon and nitrogen, which suspended the antibiotic and converts it into inactive form (Elander, 2003). The aminoglycosides antibiotics have a direct effect on microbial cell proteins synthesis (Jeffrey Buyten *et al.,* 2005). So resistance to these antibiotics arise by modified enzymes of aminoglycosides which encodes by transfer plasmids (Galimand *et al.,* 2003). All these factors enhance researcher to find alternatives and safe antimicrobial agents from natural sources such as plant or animal products. Review of literature revealed a scarce information regarding the use of milk's camel as antibacterial agent. So, this study was designed to extract camel's milk filtrate products and to investigate its activity as antibacterial agents on the pathogenic bacteria and yeasts in vitro. Moreover, antimicrobial activity of camel's milk filtrate products was compared with antibiotics from betalactam and aminoglycosides categories. Materials and Methods Milk Samples Milk Samples were collected from 4-5 years old she camels that raised in the local farm and also from Western Desert of Samawah in AL- Muthanna governorate. The animals were healthy and free from subclinical mastitis according to the results of California mastitis test (Coles, 1986). The case history of these animals were also collected to be sure that these animals didn’t take antibiotics for a period not less than one month from samples collection. The samples were kept in sterile plastic bottles and transport directly to the laboratory using cooling box. The screening test was done to avoid the impurities. The pasteurization treatment at 72Cºfor 5 second were also conducted. Chemical analysis of milk samples Camel' s milk samples were analyzed by using LAL act scan instrument according to the instructions of Bulgarian Milkotronic company , camel's milk was skimmed using cream Separator (Bulgaria) with speed of 3000 rpm for 30 minutes at the laboratory temperature (Connor, 1995). Camel's milk filtrate product Skim milk was centrifuged (ultra-high centrifugation) using a refrigerated high speed centrifuge (Huttich, Germany) at 14000 rpm for 20 minutes. Later on, the upper part was pulled carefully and gently. This solution was concentrated and called as the filtrate product of milk. The first product (X) was filtrate. The X product was heat at 60 Cº until reaching to its half size (50% of its original size) and called (2X) product.

Pathogenic microorganisms Eight different species of pathogenic bacteria and yeast, were used in this study and kindly provided from the laboratories of bio – technological and food science department / college of Agriculture / Baghdad University. Gram negative bacteria were: *Escherichia coli* O157:H7, *Pseudomonas aeruginosa, Salmonella typhimiurum, Klebsiella spp, Enterococcus faecalis,* while Gram positive bacteria were: *Clostridium spp, Staphylococcus aureus,* as well as *Candida albicans* yeast. All these microorganism are pathogenic for human and animals and also considered as important food pathogens that cause damage for some food (Abu Elnaga *et al.,* 2014). Culture of pathogenic microorganisms isolates All selected microorganisms were cultured according to method described previously by Atlas *et al.,* (1995). The bacteria were cultured on Muller Hinton medium (Oxide), while the yeast was cultured on Sabouraud medium (Oxide). All cultured bacteria were kept at 37 Cº for three hours for bacteria. The yeast culture was incubated at 30 Cº for 24 hours. Growth turbidity was compared with turbidity of the standard McFarland solution by reading optical density using a spectrophotometer on wavelength 450 nm. The dilutions of bacterial cultures density were adjusted to the McFarland with cells number 1.5 x 108 cfu/ml. However, yeast dilutions were adjusted to 4x108 cfu/ml at light intensity 0.98 nm.

Antimicrobial activity of camel's milk filtrate products (X and 2X) Well diffusion method was used to study antimicrobial activity of camel's milk filtrate and according to method described by Cleidson *et al.,* (2007). The bacterial and yeast suspension were adjusted to (1.5x 108 and 4x108 respectively) cfu/ml and spread on the surface of nutrient agar and Sabouraud agar respectively. Six milliliters (mm) in diameter wells were done on the surface of the agar by cork piercing. A 50 microliter of camel's milk filtrate products (X and 2X) were placed individually in each wells, while one well were filled with distal water and acted as (control). All plates were incubated at 37 Cº (yeast at 30 Cº) for 16-20 hours. The diameter of the inhibition zone was measured. Two plates were done for each product. Comparison of camel's milk filtrate with some antibiotics Desert camel's milk filtrate (2X) activity were compare with some antibiotics: Beta - lactam group (Amoxicillin AX) and Aminoglycosides group (Tetracycline T, Gentamycin GN, Vancomycin VN). These antibiotics were used at 50mg / 100 ml concentration and added to the wells using the Well diffusion method (Cleidson *et al.,* 2007) that mentioned above. Results and discussion The chemical analysis of the camel's milk collected from sample 1 and 2 revealed the following components: fat (3.98%, 3.98%), protein (3.64%, 3.12%), lactose (4.62%, 4.84%) and ash (0.68%, 0.80) respectively. The geographical location, nutrition conditions and different breeding conditions (desert or local farm camel) , seasons of the year , age , milking stage and number of the births are affected on the stability of camel's milk components (Khaskheli *et al.,* 2005). The pH was ranging between 6.34 and 6.82, and the percentages of acidity as lactic acid were estimated between 0.15% and 0.17% in sample 1 & 2 respectively. Antimicrobial activity of local farms camel's milk filtrate product The diameters of inhibition zones for bacteria and yeast, which were exposed for camel's milk filtrate products from milk samples 1 were (14, 12,11, 9, 11, 11, 13, 12) mm at (X) concentration for *Escherichia coli O157:H7, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Klebsiella spp, Salmonella typhimiurum, Clostridium spp,* and *Candida albicans* yeast respectively . Moreover, the diameters of the inhibition zones at (2X) concentration were (18, 20, 16, 11, 15, 14, 19, 17) mm for *Escherichia coli* O157:H7, *Pseudomonas aeruginosa,*

Staphylococcus aureus, Enterococcus faecalis, Klebsiella spp, Salmonella typhimiurum, Clostridium spp, and *Candida albicans* yeast respectively (Figure. 1). Antimicrobial activity of Desert camel's milk filtrate product

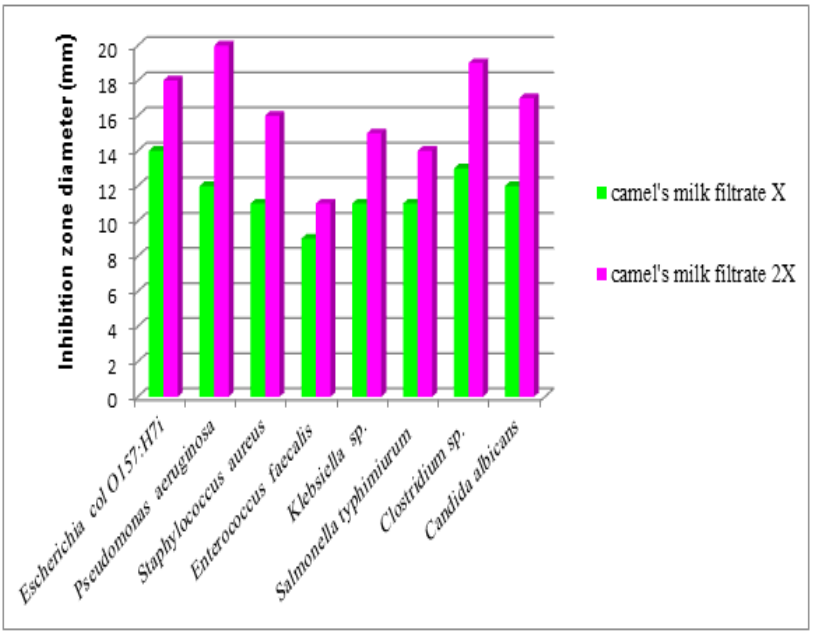


Figure.1: Shows the antimicrobial activity against different pathogenic microbes for camel's milk filtrate product of the local farms she camel.

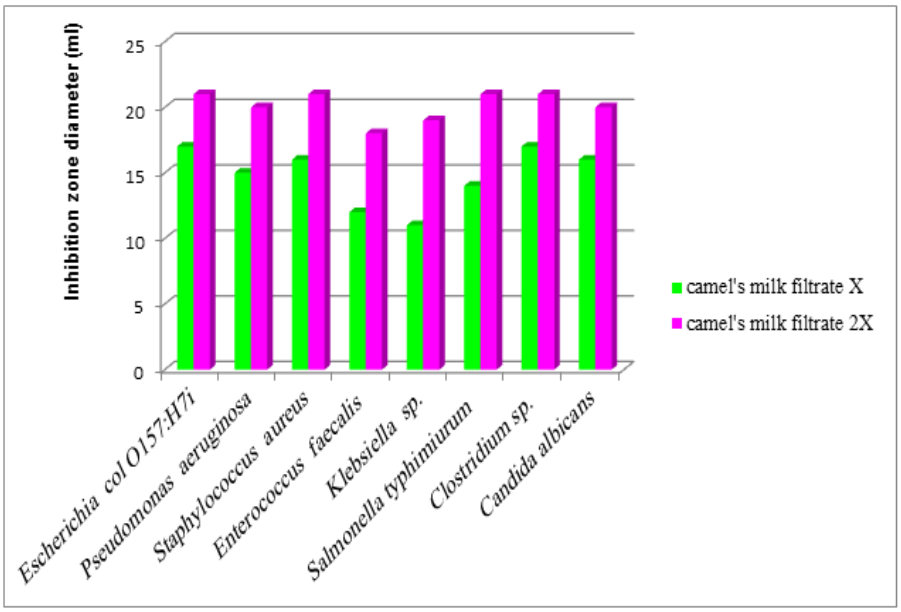


Figure .2: Shows the antimicrobial activity against different pathogenic microbes for camel's milk filtrate product of the desert she camel.

The diameters of inhibition zones for bacteria and yeast, which were exposed for camel's milk filtrate products from milk samples 2, were (17, 15, 16, 12, 11, 14, 17, 16) mm at (X) concentration for *Escherichia coli O157:H7, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Klebsiella spp, Salmonella typhimiurum, Clostridium spp,* and *Candida albicans* yeast respectively . Moreover, the diameters of the inhibition zones at (2X) concentration were (21, 20, 21, 18, 19, 21, 21, 20) mm for *Escherichia coli O157:H7, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Klebsiella spp, Salmonella typhimiurum, Clostridium spp,* and *Candida albicans* yeast respectively (Figure 2).

The results of this study revealed the efficiency of camel's milk filtrates products that extracted from collected milk from both local (sample 1) and desert (sample 2) she camel. The filtrate products revealed clear inhibition zones of the growth of both gram positive and negative tested pathogenic bacteria and yeasts. This results are compatible with previous studies (Agrawal *et al*., 2003), who proved that camel's milk have inhibition effects against gram negative and positive bacteria because it possess of high concentration of inhibitory substances such as peptide like insulin. This substances appeared to have a considerable role in the inhibition of microorganisms such as *Escherichia coli, Listeria monocytogenes, Staphylococcus aureus* and *Salmonella typhimurium*. Moreover, other inhibition factors such as Lysozyme, Hydrogen peroxide, lactoferrin, lactoperoxidase and immune proteins contribute also in inhibition action (Benkerroum *et al.*, 2004). Kappler, (1998) approved that camel's milk is rich with peptide (PGRP) peptidoglycan recognition protein, which is a highly effective against pathogenic bacteria because its ability to conjugate to the bacterial cell wall.

Previous study approved that the antibacterial activities of camel's milk was more than the activities of other single immune proteins and peptides due to the synergistic effect of proteins and peptides that present naturally in the milk. Moreover, milk Lactoferricins, Casocidin-I and Isracidin have also antibacterial activities because they are conjugated and release the liposaccharide molecules that located in outer cell membrane of the gram negative bacteria, and this mechanism is similar to lactoferrin molecules (Clare & Swaisgood, 2000).

Desert camel's milk filtrate product revealed high inhibition effects in compare with camel's milk filtrate product that extracted from local farm raised camel. This difference occurred due to the nature and quality of the desert pasture. Previous study approved the effectiveness of desert plants (pasture) that contain amino acids, non-protein nitrogenous materials, proteins and inorganic elements, in addition, to the effect of the high salinity of the desert plants (Shehabi *et al*., 2004).

Results of the comparative study of antimicrobial activity of desert camel's milk filtrate product (2X) with some antibiotics

Results of this comparative study presented in tables (1). This study revealed that the pathogenic microorganisms have clear resistance for some antibiotic, in spite of the high concentration that has been used. However, the desert camel's milk filtrate product (2X) revealed clear variable inhibition zones on the tested microorganisms. The results of the sensitivity test revealed the resistance of the following bacteria for different antibiotics:

Escherichia coli O157:H7 (Amoxicillin (AX) and Vancomycin (VN)), *Pseudomonas aeruginosa* (Gentamycin (GN) and Vancomycin (VN)) , *Staphylococcus aureus* (Amoxicillin (AX)) , *Enterococcus faecalis* (Gentamycin (GN) and Vancomycin (VN)), *Klebsiella spp.* (Gentamycin (GN)) , *Salmonella typhimiurum and Clostridium spp.* (Tetracycline (T)), while *Candida albicans* showed resistance to Amoxicillin ,Vancomycin, Gentamycin and Tetracycline.

The development of bacterial resistant are one of the important problems that face the health sector since long time ago. Resistance of bacteria to antimicrobials drugs emerges through one of the following ways: natural resistance in certain types of bacteria; genetic mutation; or by one species acquiring resistance from another (General Background, 2015). Resistance can develop spontaneously due to accidental mutations; or more commonly following gradual build up over time, and because of mistreatment of antibiotics or antimicrobials (About Antimicrobial Resistance, 2015). Resistant microbes are increasingly difficult to treat, requiring alternative medications or higher doses—which may be more costly or more toxic. Pathogenic bacteria resistant to multiple antimicrobials are called multidrug resistant (MDR); or sometimes superbugs (Antibiotic Resistance Questions & Answers, 2013). Antimicrobial resistance is on the rise with millions of deaths every year (World Health Organization, 2014). A few infections are now completely untreatable due to resistance (Tricia *et al.,* 2006). So, looking for alternative antimicrobial derivatives is the ideal choice for treatment of resistance microorganisms. The results of this study is approved the use of camel's milk filtrate product as alternative derivatives to inhibit the growth of pathogenic microorganism in vitro.

Table.1: Shows antimicrobial activity of the camel's milk filtrate product (desert she camel) (2X) in compare to antibiotics.

Pathogenic isolates	Inhibition zone diameter mm				
	Desert Camel's Milk Filtrate 2X	Antibiotics			
		VN	T	GN	AX
Escherichia coli O157:H7	21	-	11	16	-
Pseudomonas aeruginosa	20	-	19	-	12
Staphylococcus aureus	21	18	14	15	-
Enterococcus faecalis	18	-	16	-	10
Klebsiella sp.	19	15	14	-	12
Salmonella typhimiurum	21	-	14	12	14
Clostridium sp.	21	18	-	14	16
Candida albicans	17	-	-	-	-

*each number represent rate of two repeated
*- no inhibition

In conclusion, the results of this study showed that camel's milk filtrate product has antimicrobial activity against different pathogenic microorganisms. Moreover, the milk sample collected from the desert camel were more effective than the milk sample collected from the camel that raised in local farm. In addition, 2X concentration of filtrate products were better than X concentration for both samples.

References

Abu Elnaga Riham, Hedia H, Nagwa S, and Mona S. (2014) Bacterial aspect of Food Poisoning. Life Sci J. 11(3):290-298]. http://www.lifesciencesite.com.

About Antimicrobial Resistance. (2015). www.cdc.gov.

Agrawal R P, Swami S C, Beniwal R, Kochar D K, Sahani M S, Tuteja F C & Ghouri S K. (2003). Effect of camel milk on glycemic control, risk factors and diabetes quality of life in type-1 diabetes: a randomised prospective controlled study. Journal Camel Practice Research. 10: 45–50.

Antibiotic Resistance Questions & Answers. (2013). Get Smart: Know When Antibiotics Work. Centers for Disease Control and Prevention, USA. 30 June 2009.

Atlas R M, Brown A E & Parks L C. (1995). Laboratory Manual of Experimental Microbiology – Mosby Company – Year book, Inc, St. Louis.

Clare D A & Swaisgood H E. (2000). Bioactive Milk Peptides: A Prospectus. Journal Dairy Science, 83:1187–1195.

Cleidson V, Simone M, Elza F and ArturSmânia J. (7002). Screening Methods to Determine Antibacterial Activity of Natural Products. Brazilian Journal of Microbiology 38:369-380ISSN 1517-8382.

Coles E H. (1986). Veterinary clinical pathology, 4th. ed. Canada, W.B Saunders Company, pp. 448-462

Dixit K and Gandhi D N. (2010). Biotherapeutic Properties of Probiotic Yeast *Saccharomyces* Species in Fermented Dairy Foods. Available from URL: http://www.dairyscience.info /probiotics/105-biotherapeutic-probiotic yeast. Accessed 16 Apr 2010.

Elander RP. (2003). "Industrial production of beta-lactam antibiotics". Applied microbiology and biotechnology 61 (5–6): 385–392. Doi: 10.1007/s 00253-003-1274-y. PMID 12679848

Galimand M, Courvalin P, Lambert T. (2003). Plasmid-Mediated High Level resistance to amingly-coside in enterobacteriaceae due to 16sr RNA methylation. Antimicrob. Agents chem. 47: 2565-2571**.**

General Background (2015). About Antibiotic Resistance. www.tufts.edu. Retrieved -10-30.

Jeffrey Buyten, Francis M, Quinn B and Matthew R. (2005) Antibiotics. Grand Rounds Presentation, UTMB, Dept. of Otolaryngology

Kappeler S R. (1998). Compositional and structural analysis of camel milk proteins with emphasis on protective proteins, Ph.D. Thesis, Swiss Federal Institute of Technology, Zurich, Switzerland.

Khaskheli M, Arain MA, Chaudhry S, Soomro AH & Qureshi TA. (2005). Physico-chemical quality of camel milk. Journal of Agriculture and Social Sciences, 2: 164-166.

Kourelis A, Kotzamanidis C, Litopoulou-Tzanetaki E, Scouras Z G, Tzanetakis N and Yiangou M. (2010). Preliminary Probiotic Selection of Dairy and Human Yeast Strains. J Biol Res Thessaloniki 13:93–104.

Neu HC. (1992). The crisis in antibiotic resistance. Science. 257:1064-1073

O'Connor CB. (1995). Rural Dairy Technology ILRI Training Manual 1 International Livestock Research Institute, Addis Ababa, Ethiopia.

Shehabi A A, Mahafzah A M. and Al-Khalili K Z. (2004). Antimicrobial resistance and plasmid profiles of urinary *Escherichia coli* isolates from Jordanian patients. Eastern Mediterranean Health Journal 10(3): 322–328.

Shoeib A A and Ba-hatheq A M. (2007). Effect of Camel's Urine on Pathogenic *P. aerginosa* and *E. coli* Isolates, towards its Maintains to Their Antibiotic (s) Resistance and the Presence of Plasmid. Saudi J. Biol. Sci. 14 (2): 177-184. (Text in Arabic).

Tricia D M, Wayne M, Paul DB. (2006). Antimicrobial Resistance of *Escherichia coli* isolates from broiler chickens and humans. BMC Veterinary Research. 2:7.

World Health Organization. (2014). Antimicrobial Resistance: Global Report on Surveillance 2014.