

## Full Length Research Paper

## Physiochemical evaluation and liability of dromedary camel's milk in combating various pathogens

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Dromedary camel's milk is a natural source of probiotics; enzymes and secondary metabolites that have the ability to combat many pathogens. The aim of this study was to compare the effectiveness of filtered and non-filtered dromedary camel's milk activity against various pathogens. Filtered and non-filtered (raw and boiled) dromedary milk was assessed against different pathogens by using agar well diffusion on Muller Hinton agar (MHA) and Blood agar assays. The sensitivity pattern against all pathogens was determined on MHA plate, by incubating for 24 h at 37°C. *Streptococcus* and *Lactobacillus* have antagonistic activity against various pathogens. The results showed that effectiveness of non-filtered milk was about 40 and 60% of boiled and raw milk respectively. Filtered milk had a 50% of effectiveness for both raw and boil milk. The antibacterial activity of filtered milk indicates the presence of such Immunoglobulins and enzymes that help in providing immunity. The streptococci inhibit 64% of the test organisms, while *Lactobacillus* suppresses 54% of pathogens. *Acinetobacter baumannii* is more susceptible to 37mm zone while *Lactobacillus* suppressed the growth of *Micrococcus luteus* with 45 mm zone.

**Key words:** Dromedary milk, probiotics, Alzheimer's, agar well diffusion method, disk diffusion method, Immunoglobulins.

### INTRODUCTION

Camel milk is beneficial for all health purposes in treating various diseases from hereditary to bacterial like diarrhea, diabetes, tuberculosis and autism. The fact is that camel milk does not coagulate even in an acidic environment like in stomach so it is easily available for absorption in the intestine. According to Sunni Islamic tradition, camel's milk has medical properties - (Hadith Sahih al-Bukhari) and according to the FAO organization

camel's milk is the healthiest milk produced by animals. In Pakistan 0.8 million Camels breeds is leading mostly in the desert areas, particularly in the areas of Sindh province, Cholistan (Punjab) and hilly areas of Balochistan (Khaskheli et al., 2005). Camel milk is comprised of proteins that have the ability to combat many bacterial infections and boosting up immunity. Proteins are divided into two; Casein and Whey proteins

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(Brezovečki et al., 2015). Casiens are found in the highest fraction of 52 to 82% (Brezovečki et al., 2015; Al Kanhal, 2010). In total about 65% of  $\beta$ -CN, 21%  $\alpha$ s1-CN (Kappeler et al., 2003) makes it easily digestible, less allergic to newborn because it decomposes in low time (Brezovečki et al., 2015). K-casiens is 3.47% (Kappeler et al., 2003) while 13% in bovine milk (Seher and Hifsa, 2013). On the other hand a whey protein includes a variety of proteins,  $\alpha$ -lactalbumin, c serum albumin, lysozyme, lactoferrin, peptidoglycan recognition proteins, lactoperoxidase and Immunoglobulins (Brezovečki et al., 2015; Seher and Hifsa, 2013).  $\beta$ -lactoglobulin from 50 percent, makes up a major portion of whey protein (Kappeler et al., 2003). There is a beneficial combination observe between fats and proteins (Seher and Hifsa, 2013). During starvation, the level of fat content is decreasing due to hydrolysis of fats (Konuspayeva et al., 2010). Dromedary camel's milk has decreased the level of carotene with lower concentrations of short chain fatty acids as compared to milk of bovine (Seher and Hifsa, 2013) which have pH of about 6.5 to 6.75 (Al-Saleh et al., 1992). Camel milk contains about 2.40 to 5.80% of lactose (Seher et al., 2013) and consume plants which overcome salt and mineral requirements (Yagil et al. 1980).

Furthermore, it contains mineral contents, vitamins, bioactive native proteins which includes Immunoglobulins, Lactoferrin and Indigenous enzymes which also includes Lysozymes and Lactoperoxidase and provide ability to combat against many of the life threatening diseases (Brezovečki et al., 2015) (Seher and Hifsa, 2013) (El-Agamy et al., 1998). There is variation in nutritional composition in camel milk due to some changes over a specified period of time (Brezovečki et al., 2015; Yagil et al., 1980), due to analytical procedures (Mehaia et al., 1995).

Probiotics are gram positive group of live microbes which exists as a single or colonized form; playing a role in improving immunity by maintaining the normal flora (Joshi et al., 2015; Nelson et al., 1995). These organisms produce secondary metabolites that carry antimicrobial activity against many of the pathogenic organisms (Yateem et al., 2008), use in food and aquaculture (Joshi et al., 2015). These gram positive bacteria are categorized as LAB (lactic acid bacteria) (Yateem et al., 2008), which are usually use as a starter culture in the production of dairy items. Today, LAB is classified into thirteen main groups Lactobacillus, Leuconostoc, Lactococcus, Streptococcus, Enterococcus, Pediococcus, Bifidobacterium, Carnobacterium, Oenococcus, Weissella, Aerococcus, Tetragenococcus and Vagococcus (Fatma et al., 2013). These can be identified on the basis of bile tolerance, pH NaCl tolerance, catalog production, and motility (Nelson et al., 1995). Further identification was made according to Bergey's manual of determinative of bacteriology (Holt, 1994). These organisms play role in maintaining pH and

reducing the lactose intolerance and cholesterol level, antitumor activity and activation of the immune system (Eva et al., 2002). The taxonomic tool for their identification is fructose-6-phosphate phosphoketolase (F6PPK) (Eva et al., 2002). The identification of these species can be possible at molecular level by PCR.

A recent study has shown a beneficial effect of a prebiotic and probiotic association highlighting the growing interest of symbiotic in digestive health (Picard et al., 2005). *S. salivarius* backbone of these bacteria is joined together by ether linkages which separates it's from other bacterial species, and are virulent streptococcus species due to absence of surface proteins (lipoproteins) (Francesca et al., 1999). Pseudo genes *epsE*, *epsF*, *epsG*, and *epsI* are responsible for the production of exopolysaccharide (EPS) in *Streptococcus thermophiles* (Francesca et al., 1999) that play role in attachment and production of reduced fat cheddar cheese and other dairy products (Awad et al., 2005). They boost up human immunity (Wollowski et al., 2001), present as intestinal flora (increase digestion) (Wollowski et al., 2001), use in replacement of chemotherapy (Whitford et al., 2009) and in treatment of antibiotic associated diarrhea (Nopchinda et al., 2002). Now days, this has gained most of the researches because *S. thermophilus*' genome is shorter than most genomes, having 1.8 MB (Rao et al., 1977).

## MATERIALS AND METHODS

Milk sample was collected from the camel in sterile container brought to the microbiological laboratory of Jinnah University for women Karachi; to check the antibacterial activity and Phytochemical analysis. pH, acidity, total solids, ash, total solids non fat, Fat proportion and total proteins was done by the formal method, titration.

### Phytochemical Analysis

#### pH

This was Observed using the digital pH meter by placing 20 ml of raw and heated milk in two separate beakers, and immersing an electrode in the beakers.

#### Acidity

Take 10ml of milk separately in two flasks for raw and heat milk. Add two to three drops of phenolphthalein. Titrate with 0.1N NaOH. Note when pink color appears

#### Total solids

Heat 5 ml sample at 100°C for three hours.

### Fat detection

This was carried out by Babcock Method. 10.94ml of raw and boiled milk was placed in two separate test tubes labeled, raw and boiled. Add 10 ml of H<sub>2</sub>SO<sub>4</sub> and 1 ml of isoamyl alcohol. Mixture is then centrifuge at 1100 rpm for 5 min at 65°C. Result is noted by measuring the fat layer at the surface of tubes. Solid non-fat (SNF) was determined by SNF = Total solid% - Fat%.

### Measurement of total proteins

This was carried out by the formal method which is done by titrating all the chemical and reagents required are phenolphthalein (prepared in laboratory by adding 0.5 g of phenolphthalein powder in 50% ethanol, 0.1 NaOH, 40% Formalin and 28% potassium oxalate from the (department of chemistry Jinnah university for women). 10 ml of sample was pipette in 50ml flask, 0.4 ml of saturated potassium oxalate and 0.5 ml of phenolphthalein was added and set for two minutes. Milk was then neutralize by NaOH to end point (note the reading). 2 ml of 40% formalin was added to it, stand for two minutes and titrated with 0.1 N NaOH till, endpoint is attained. Blank was run by titrating it with 2 ml of 40% formalin.

### Ash detection

This was done by formal method;

1. Weight of crucible was noted (a)
2. Add sample in crucible and measure the weight again (b)
3. Sample was then put in oven for 4 h
4. Note the weight again (d)

Formula;

Weight of sample (c) = a – b

Original weight of sample (e) = a – d

### Isolation of lactic acid bacteria

10ml quantity of milk sample was stomached with 90ml of peptone water, by using same diluents sample which is serially diluted. Diluted sample was inoculated on De Man, Rogosa and Sharpe (MRS) agar prepared medium (Merk), plates were incubated at 37°C for 48 to 72 h to analyze the colony morphology. Gram staining was performed for microscopic analysis. It has been sub cultured further to get pure colonies by inoculating single colony on MRS agar for lactobacillus spp, MRS supplemented with L-cystien for the streptococcus (fat lowering bacteria) (Lim et al. 2004), incubate at 37°C for 24 and 48 h, respectively, colonies were inoculated on heart infusion agar supplemented with 5% glucose (HIAG) and heart infusion agar supplemented with 5% Sucrose, 0.5% glucose, and 0.02% sodium azide (HIAS), colonies appeared on this are inoculated on Mayeux Sandine Elliker agar (MSE) prepared by adding (tryptone 10 g/l, Gelatine 2.5 g/l, yeast extract 5 g/l, sucrose 100 g/l, glucose 5 g/l, sodium azide 75 mg/l, sodium citrate 1 g/l, agar 15g/l ) which is elective medium for Leuconostoc species and was incubated at 30°C for three days.

### Spot tests

All the LABs were determined on the basis of spot testes like motility, catalase and oxidase by picking up colonies from their respective medium plates. Motility of isolated cultures was

determined by cavity slide, catalase by picking up a colony and inoculates on the drop of H<sub>2</sub>O<sub>2</sub>.

### Antibacterial activity of dromedary camel's milk (non-filtered milk)

This is checked out by both agar well diffusion method.

### Preparation of inoculums

Inoculums of pathogenic organisms were prepared by standardizing it according to the turbidity of 0.5 McFarland tube (as per given by Kirby-Bauer, standard method) which mean 150 million cells per ml of bacterial suspension.

### For antibacterial activity

Lawn of eight clinical isolates (*Escherichia coli*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, *Shigella burnetii*) were prepared on MHA and TSA, *Haemophilus influenzae* and *Moraxella catarrhalis*) was isolated on chocolate agar and blood agar streptococcus pneumoniae and allow the plates to stand for 5 min. Two wells on each plate were prepared by the help of borer (6.5 mm), thickness of the medium was about 25 mm. Load 100µl of raw and boil milk sample in each respective well (Yassin et al., 2015) and placed prepared disks of sample on each plate. Incubate it for 24 to 48 h at 37°C and observe zone of inhibition in millimeters. The same protocol was repeated for the antibacterial activity of Filtered milk.

### Antagonistic activity of LAB isolated from milk

Isolated LABs cultures were inoculated in respective broth mediums of MRS broth for *Lactobacillus*, MRS+ L-Cystine for *Streptococcus* while MRS was supplemented with 6.9% NaCl for *Lecunostoc* spp. Antagonistic activity was checked against *Escherichia Cali*, *Acinetobacter baumannii*, *Listeria monocytogenes*, *Pseudomonas originals*, *Staphylococcus aureus*, *Micrococcus luteus* and *Klebsiella pneumoniae*; by agar well diffusion method. All test organisms were inoculated on MHA. Leave the plates for 5 min. Two wells on each plate were prepared by the help of borer (6.5 mm) and the thickness of the medium was about 25mm. Load 100 µl of cultured broth of streptococcus and lactobacillus in each respective well. Incubate it for 24 to 48 at 37°C and observe zone of inhibition in millimeters. Zone larger than 2mm was considers as sensitive (Lim et al., 2004).

## RESULTS

Fresh raw dromedary camel's milk was purchased from the camel herd in street of Karachi Pakistan. Milk was kept in an airtight container at 4°C for 15 days and was used for compositional analysis of dromedary camel's milk in the Microbiology Department of Jinnah University for Women, Pakistan. The process of composition analysis was carried out within 3 weeks. (Table 1) shows the observed values for the components of dromedary camel's milk both raw and heat to check the effect of heat

**Table 1.** Phytochemical analysis of dromedary milk.

Components of milk	Raw milk	Boiled milk
pH	7	6
Fat %	2.42%	3.32%
Acid	2.76 g/ml	0.13 g/ml
Total proteins %	0.05%	0.03%
Total solids	5.2 g	5.4 g
Total solid non fat	0.046%	0.06%
Ash*	0.6%	-
Microbial count	73x10 <sup>5</sup> cfu/ml	-

**Table 2.** Antibacterial activity of non-filter camel milk.

Test organism	Media	Sensitivity or resistivity pattern	
		Agar – well diffusion	
		Raw milk (R)	Boiled milk (B)
<i>Himophilus influenza</i>	Chocolate agar	25 mm	15 mm
<i>Morexilla catarrhalis</i>	Chocolate agar	28 mm	R
<i>Streptococcus pneumoniae</i>	Blood agar	35 mm	25 mm
<i>Lister monocytogenes</i>	MHA	36 mm	35 mm
<i>Klebsiella pneumonia</i>	MHA	10 mm	30 mm
<i>Escherichia coli</i>	TSA	R	R
<i>Pseudomonas aureginosa</i>	MHA	27 mm	R

Ash\* = the crumbly scum left after the burning of a substance, MHA = Muller Hinton agar, TSA = tryptic soy agar.

on the components of milk sample. The observed pH value of raw and heat milk sample was 7 and 6 respectively. Fat was observed to be 2.42 and 3.32 in raw and boil respectively. Total proteins, acids, total solids and non-fats were 0.05 and 0.03%, 2.76 and 0.13 g/ml, 5.2 and 5.5 g, and 0.046 and 0.06% in raw and boil milk respectively.

Total ash content in milk sample was 0.6 and microbial count was 73x10<sup>5</sup> cfu/ml. Table 2 indicates sensitivity patterns of various pathogens against non-filter camel's milk (milk does not filter by filter assembly and contain probiotics). Table 3 indicates sensitivity patterns of various pathogens against Filtered Dromedary camel's Milk (that is filtered by passing through filter assembly that is free of probiotics, to check the efficiency of non microbe particles that are various proteins and Immunoglobulins; in Camel's milk.

## DISCUSSION

According to (Abbasiliasi et al., 2012) total microbial count obtained was 155,000 colonies. The values of fat%, fat, proteins and ash are 3.6, 3.2, 0.8 and 0.7% (Mayeux et al., 1962) while the pH is 6.5 to 6.75 (M.H Yassin et al., 2015). Total solid contents in camel milk

vary from 9.8 ± 0.59 to 11.9 ± 0.71%, in comparison with our results that are mentioned in (Table 1). The variation in total solids of camel milk is mainly due to the changes in fat, lactose, minerals and protein content of camel milk. The total amount of minerals is generally presented as total ash and in case of dromedary camel milk this value ranged between 0.60 to 0.90% (Choct, 2009). Camel milk protein contents vary from 2.15 to 4.90%. The amount of non-protein nitrogen varies with total protein (Choct, 2009).

All obtained data revealed the variations in different components of camel milk. It was observed that the composition of camel milk depends on various factors like fluctuations in mineral level which were proposed to be due to the differences in breeding and water intake (Choct, 2009; Lim et al., 2004). Changes in the atmosphere also brought fluctuation in almost all the parameters (Choct, 2009). The fat content was decreased in dromedary milk at the time of malnourishment (Lim et al., 2004). Dromedary camel's milk is a possible source of Probiotics (Fatma et al., 2013). It contains a different variety of Lactic acid bacteria (LAB) main groups according to (Fatma et al., 2013) which are *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Streptococcus*, *Enterococcus*, *Pediococcus*, *Bifidobacterium*, *Carnobacterium*,

**Table 3.** antibacterial activity of camel milk (filtered-milk).

Test Organisms	Media	Sensitivity or resistivity pattern	
		Agar – well diffusion	
		Raw milk	Boiled milk
<i>Escherichia.coli</i>	MHA	30 mm	35 mm
<i>Acinetobacter. baumannii</i>	MH	R	15 mm
<i>Listeria.monocytogenes</i>	MHA	10 mm	30 mm
<i>Pseudomonas.aureginosa</i>	MHA	35 mm	35 mm
<i>Staphylococcus. aureus</i>	MHA	25 mm	R
<i>Micrococcus. luteus</i>	MHA	20 mm	22 mm
<i>Klebsiella. pnumoniae</i>	MHA	10 mm	19 mm

*Oenococcus*, *Weissella*, *Aerococcus*, *Tetragenococcus* and *Vagococcus*; while from our sample we isolate *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Pediococcus* and yeast cells from raw dromedary camel's milk.

Raw milk when inoculated on MRS media, mix culture of LABs were isolated, which were further isolated when supplemented with different nutrients and using selective and elective mediums for the isolates. *Lactobacillus* was grown on the MRS agar, *Streptococcus* and *Pediococcus* on MRS+L-Cystien, *Leuconostoc* on MSE agar after 2 to 4 days incubation at temperature 37°C except *Leuconostoc* 6.9% NaCl at 30°C. Microscopy of mix LAB culture revealed the presence of gram positive, long to short rods, cocci and coco bacillus arranges in chains and tetrads. *Lactobacilli* are long to short rods, *Streptococcus* is cocci in chains, *Leuconostoc* are coco bacillus, *Pediococcus* are tetrads of cocci while yeasts are ovoid in shape. All isolates were further confirmed on the basis of spot tests catalase, motility, spore formation, and oxides; all cultures are catalase negative (Podrabsky 1992; Mehaia et al., 1995) except *Lactobacillus* species that was pseudo positive and *L. planetarium* that was isolated by Whittenbury (1964). Oxides are spore negative (Whitford et al., 2009), and are non-motile (Yagil et al., 1980) except *leuconostoc* which was confirmed by the growth on 6.9% NaCl concentration, growth on heart infusion agar was supplemented with 5% glucose and 5% sucrose, 0.5% glucose and 0.02% sodium azide. *Leuconostoc* are motile (Podrabsk, 1992) while, rest of all isolated species were non-motile. Non-filter (that contain probiotics and not pass through filter assembly) raw and boil Dromedary camel's milk was then checked for the antibacterial activity against many of the virulent bacterial strains by the agar well diffusion method that is about 60% and 40% of raw and boil respectively against various pathogens mention in (Table 2). Results from Whittenbury (1965) also revealed that isolates had inhibitory activity against pathogenic bacteria, because the inhibition was scored positive, if the diameter of the clear zone around the colonies was 0.5 mm or larger, according to this study camel milk inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* at greater

extend. Thus, Whittenbury (1965) suggests that camel milk is a possible source for the isolation of probiotic LAB strains and can be considered good for health with antibacterial properties against pathogenic bacteria because of the presence of bacteriocin producing strains. Filtered raw and boil was also applied against pathogenic organisms to check the antimicrobial activity of milk portions and enzymes as, it is a good source of Vitamin C, Insulin, Lysozymes, Lactoferrin and Immunoglobulins that make it healthier to human. The susceptibility pattern by agar well diffusion method is about 50% of raw and boils milk against various pathogens that are mentioned in (Table 3). This indicates the presence of certain other components that may have the ability to combat a variety of pathogens. These components include Lactoperoxidases, Lactoferrin and Lysozymes that play essential role in killing of pathogens (Khedida 2009; Choct, 2009; Mutlag et al., 2013).

Furthermore, lactoperoxidase work as anti-tumor agents and play functional role in degradation of Catecholamines. Our results also suggest that boiled camel milk has antibacterial activity, but demolish various nanoparticles and stopped some of biological functions especially in the treatment of diabetes. This carried out essential nutrients with specific properties, particularly anti-infectious action; which should be replace with other milks (Hickman, 2007). In the present Study, camel milk supplementation decreased the oxidative stress biomarker malondialdehyde and decreased the activity of antioxidant enzymes (catalase, SOD, and glutathione reductase). Alteration in oxidative stress was induced by reactive oxygen species (ROS) and impairments of the antioxidant system play a critical role in the pathogenesis of *E. coli* and *S. aureus* challenge (Hickman, 2007).

Metabolic by-products such as bacteriocins are loosely defined as biologically active protein moieties, with a bactericidal mode of action. These bacteriocin producing strains have natural immunity to their own bacteriocins. LAB has ability to inhibit the growth of other bacteria. So we apply it in our industries to minimize food spoilage and inhibiting pathogenic organisms (Yateem et al., 2008). *Streptococcus* and *Lactobacillus* are considered

**Table 4.** Antagonistic activity of LAB against various pathogens.

Test organism	Media	Zones produce by labs (mm)	
		Lactobacillus	Streptococcus
<i>Escherichia coli</i>	MHA	40	30
<i>Acinetobacter baumannii</i>	MHA	20	37
<i>Listeria monocytogenes</i>	MHA	20	30
<i>Pseudomonas aeruginosa</i>	MHA	45	7
<i>Staphylococcus aureus</i>	MHA	R	30
<i>Micrococcus luteus</i>	MHA	45	25
<i>Klebsiella pneumoniae</i>	MHA	28	25

as gut flora that provide a healthy environment by combating all pathogenic organisms (Nopchinda et al., 2002). Among six LAB isolates antagonistic activity of only two were determined against various pathogens. These two species were grown in their respective medium cultures. Lactobacillus antagonistically acts on almost all pathogens and kill 54% of all organisms, while Streptococcus is 64% active against all pathogens mention in Table 4. The one of the study (Pritchard et al., 1993) reveals that lactobacillus shows weak inhibition against *P. aeruginosa* and *K.pneumoniae* and could not inhibit *S. aureus* and *E.coli*.

The study (Hu et al., 2007) showed that *S.thermophilus* had broad spectrum activity against Gram-positive bacteria which showed that the concentrated supernatant of *S. thermophilus* had inhibitory activity against pathogenic bacteria; *Pseudomonas aeruginosa*, *Klebsiella spp*, *Staphylococcus aureus* and *Escherichia coli*. LABs have the ability to produce acetic acid, lactic acid, formic and benzoic acids, hydrogen peroxide, diacetylacetoin and bacteriocin as a secondary metabolite. The level of these metabolites depends on the medium and physical parameters (osmanağaoğlu et al., 2001). Camel milk contains peptidoglycan recognition protein (PGRP) that provides passive immunity to the body (Makarova et al., 2006). *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are more effective in deactivating etiologic risk factors of colon carcinogenesis than being cellular components of microorganisms (Papagianni et al., 2009).

According to (Van et al., 1969) *S.thermophiles* may be helpful during chemotherapy by protecting the intestinal tissues from irritation caused by chemotherapy drugs. Another study (Vashist et al., 2013) shows that *S.thermophilus* correlates with better growth in children antibiotic-associated diarrhea (AAD) which is a growing issue today thereby making people to seek natural methods for relief. Since antibiotics kill good bacteria and sometimes allow harmful bacteria to grow, diarrhea is often the cause of the result. Certain strains of *S.thermophilus* have been shown to reduce AAD (Cowan et al., 2004). This is not surprising, considering that many other probiotic strains also provide similar benefits.

## Conclusion

Today, the use of antibiotics is increasing day by day that makes pathogen more resistant to treat. This may lead the world towards 'Pre-Antibiotic Era' where we need a replacement of antibiotics; due to the continuous use of antibiotics and self medication, making many organisms mutated and pathogens more resistant by using the natural sources of antibiotics. We can overcome this mechanism of resistance and save our surroundings from many of hazardous upcoming emerging superbugs.

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