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Exploring Antibacterial Activity of Whey Against MDR Human Pathogens

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ABSTRACT

Milk whey is one of the products that can be separated from milk after coagulation by adding rennet or removing acid. Whey is mostly water but also contains lactose, minerals, trace amounts of fat, and non-acidic milk called whey protein. Now- a- days, research had focused the different benefits of whey with respect to industrial products, therapeutics, whey proteins etc. This study is, therefore, aimed at determining the antibacterial properties of whey against MDR human pathogenic bacteria viz., *E.coli, S.aureus, Salmonella, Shigella Pseudomonas, and Klebsiella* strains respectively. Three different whey samples obtained from cow, buffalo and goat milk was used for the present study. The findings suggest that the Goat milk whey was found to be effective against MDR human pathogenic bacteria followed by cow milk and buffalo milk whey. Hence, whey can be utilized for prevention and treatment of human diseases.

Key words: Milk whey, *E.Coli, S.Aureus, Salmonella, Shigella Pseudomonas, and Klebsiella*

INTRODUCTION

Whey is a yellow liquid obtained from the production of fermented dairy products (Minekins et al., 1994). It is one of the products that can be separated from milk after coagulation by adding rennet or removing acid. Whey is mostly water but also contains lactose, minerals, trace amounts of fat, and non-acidic milk called whey protein. Whey has long been considered the best protein supplement for the repair of soft tissue and muscle tissue (Tunick, 2004; Krissansen, 2007).

The food industry uses whey and whey components mainly in food processing. It is based on nutritional value and performance (Foegeding et al., 2008; Tunick, (2008) and immunity (Lappala, 1999); Zemel, 2003; CDRF, 2006). Some of these are immunoglobulins, lactoferrin, lactoperoxidase, glycomacropeptides, bovine serum albumin, α-lactoglobulin and β-lactoglobulin. Whey protein is a mixture of globular proteins; it is of interest due to its effects on human health and is currently being investigated as a way to reduce disease and provide adjunctive treatment for many diseases (Krissansen, 2007).

Bioactive whey components, such as bioactive proteins, provide additional health benefits to consumers and are increasingly used in medicine and nutraceuticals (Foegeding et al., 2002). The protein in milk is an organic combination of amino acids. There are two main types of milk proteins: casein and whey, which form colloidal substances in milk. Substances in the form of alpha lactalbumin, beta lactoglobulin, lactoferrin, serum albumin and lactoperoxidase contained in whey protein play an important role in the prevention of diseases through antibiotic pain and immunity (Ebringer L, El-Fakharany, 2008).).

The whey protein content in mare's milk is higher than in cow's milk. The balance of casein and whey is considered an important factor in determining the level of milk protein allergy in humans, and changes in this balance can reduce milk allergy (El -Agamy E I 2000). Milk proteins, especially whey, are known for their ability to control blood sugar and control body weight by increasing satiety and reducing food intake. (Anderson GH, 2011). Whey protein provides the best absorbable properties as well as essential sulfur-containing chain amino acids such as leucine, isoleucine and valine, which are good for tissue growth and repair, increasing muscle mass and strength. is important (khare *et al*., 2007).

Pure whey carbonation and vegetable soup are successfully produced and sold worldwide. Whey is also used in the production of ethanol, acetic acid, alcohol, bread, latex filling, lactose, beer, chewing gum and caramel (Mann, 1986). The milk is first coagulated by applying rennet or acid, the casein proteins precipitate and the remaining liquid is called whey. Whey is a nutritious food and is considered a good protein because it contains all nine essential amino acids such as tryptophan, leucine, lactose, threonine, valine, lysine, histone amino acid, phenylalanine, isoleucine, methionine and tyrosine. (Power and Daginawala). Casein accounts for approximately 80% of all protein and has been identified as a milk protein that precipitates at pH 4.6. The remaining 20% is whey protein or whey protein that is soluble at this pH. Milk is a good source of bioactive substances that are beneficial to humans (Mils *et al.*, 2011). Whey protein is a mixture of globular proteins; its effects on human health have aroused great interest and are now being investigated as a way to reduce disease and improve the treatment of many diseases (Krissansen,*et al*., 2007). The aim of this study was to explore the antibacterial activity of whey milk against MDR human pathogens.

MATERIAL AND METHODS

The Antimicrobial Activity of Whey obtained from cow milk, goat milk and buffalo milk was conducted at post graduate Department of Microbiology, R. A. College, Washim. Against MDR human pathogens viz., *E.Coli, S.Aureus, Salmonella, Shigella Pseudomonas, and Klebsiella* strains.

Preparation of Whey

The milk was centrifuged at 3,000 × g for 1 h at 4ºC, to produce skim milk (defat). Skim milk that has been obtained is then added HCL 2 N to reach pH 4.6 to separate casein through a centrifugation process at 20,000 × g, 4ºC for 30 min. After the casein is deposited on the base of the tube, acid whey is obtained which is then neutralized to reach pH 6.8 by adding 2N NaOH. Whey neutral was centrifuged in 10000 × g, 30 min at 4ºC (yoshida s.z *et al.,2009 &* kim k.s. *et al.,2009)*

Antibacterial Activity of Whey against MDR human pathogens

Whey samples were collected in sterile test tube. The antibacterial activities of the whey were tested against MDR human pathogens viz., *E.coli*, *S.aureus, Salmonella*, *Shigella*, *Pseudomonas and Klebsiella* provided by Microbiology Research Laboratory, R. A. College, Washim reconfirmed by Kirby-Bauer disc diffusion method. Pathogens were implanted on Muller-Hinton agar media on a plate. Furthermore, sterile round discs (6 mm) were soaked in 100µl of each whey extracted and placed on a plate. Antibiotics were used as positive controls and sterile deionized water as a negative control. The plates were incubated for 24-48 hours at 37° C (Niaz B *et al 2017* & Moradian *et al 2014*). The diameter of the zone of inhibition in the plate was measured and antimicrobial activity was expressed in mm and the results were expressed [15ola paper]. The plain disk soaked in sterile distilled water was used as negative control while, broad spectrum antibiotic viz. tetracycline was used as positive control.

Result and Discussion

The results of the study showed that whey indicates the presence of effective antibacterial activity, which confirms its use against infection. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the disc. Disc diffusion method produces recordable results for all the three type of whey samples against the MDR human pathogens viz., *E.coli, S.aureus, Salmonella*, *Shigella, Pseudomonas and Klebsiella.*

The results are presented in table 1. The Buffalo milk whey showed antimicrobial activity against most of the test pathogens. In case of buffalo milk whey, the maximum zone of inhibition was found against *Shigella* (14 mm) followed by *E.coli* (13mm), *Salmonella* (13 mm) , *S. aureus* (12 mm) and *Klebsiella (*12mm)However,in case of *Pseudomonas* no zone of inhibition was observed.

The Cow milk whey showed antimicrobial activity against most of the test pathogens. In case of Cow milk whey, the maximum zone of inhibition was found against *Klebsiella (* 15 mm) followed by *Shigella* (14 mm), *E.coli* (14 mm), *S. aureus* (13 mm), *Pseudomonas* (13 mm) and *Salmonella* (12mm).

The Goat milk whey showed antimicrobial activity against most of the test pathogens. In case of Cow milk whey, the maximum zone of inhibition was found against *Shigella* (18 mm) followed by *S. aureus* (17 mm), *Salmonella* (16 mm) *Klebsiella (*14 mm), *E.coli* (14 mm) and *Pseudomonas* (11 mm). Goat milk whey was found to be effective against MDR human pathogenic bacteria. Followed by cow milk and buffalo milk.

Finally the Average Zone of inhibition given by MDR human pathogenic bacteria against Goat milk whey was found to be (15 mm) followed by Cow milk whey (13.5 mm). Least zone of inhibition was given by Buffalo milk whey (10.6 mm) as compare to positive control i.e. Ciprofloxacin (30.67mm.). However, ciprofloxacin was highly purified and used in the concentration of 10mcg. On the other hand, milk whey was used in its original crude form.

Our findings are in accordance with the findings given by other researchers working on the same line of research. The inhibitory role of Lactobacillus against pathogenic bacteria can be attributed to the production of antibacterial compounds like organic acids, hydrogen peroxide and bacteriocins (Soccol et al., 2010) which, however, is directly dependent on the concentration of antimicrobial compound in CFS (Dasari et.al.,2014)

Conclusion

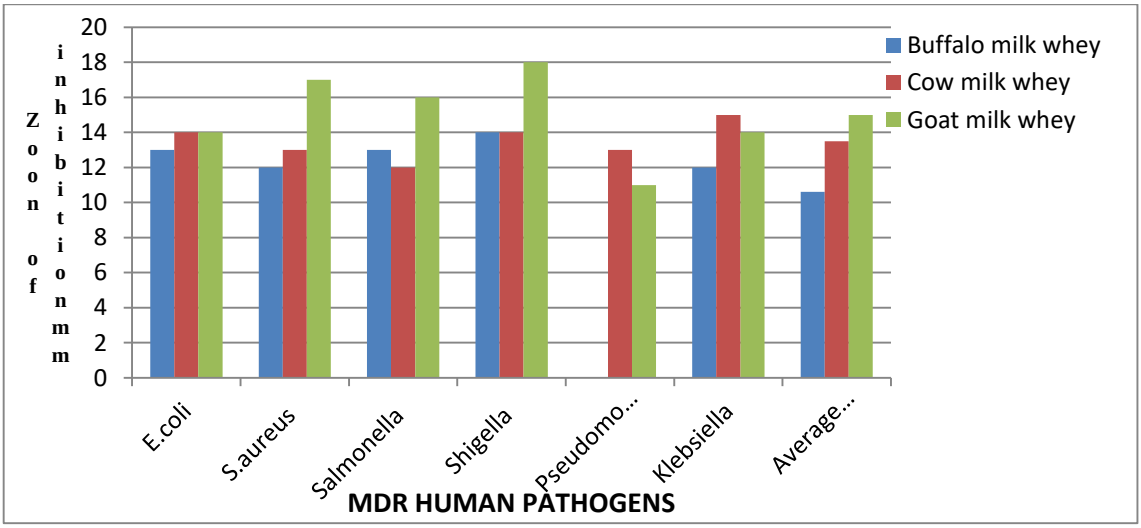
The result of our study indicates that milk whey products contain beneficial probiotics which revealed antibacterial activity against the selected MDR human pathogenic isolates. Studies need to be carried out enlightening the phenotypic and genotypic characteristics of milk whey, in order to clarify their role in human health.

Table 1: Antimicrobial activity of different whey samples against MDR human pathogens

MDR human pathogens	**Buffalo milk whey**	**Cow milk whey**	**Goat milk whey**	**Positive control Ciprofloxacin (10mcg)**
	Zone of inhibition in mm			
E.coli	13	14	14	30
S.aureus	12	13	17	34
Salmonella	13	12	16	29
Shigella	14	14	18	30
Pseudomonas	NZ	13	11	29
Klebsiella	12	15	14	32
Average Zone (mm)	**10.6**	**13.5**	**15.0**	**30.67**

NZ: No zone of inhibition

Graph 1: Antimicrobial activity of different whey samples against MDR human pathogens



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