# Chapter 6

# Fractionation of camel milk to derive bioactive components

# Chapter 6

**Fractionation of camel milk to derive bioactive components.**

## 6.1 Introduction

Camel milk contains numerous proteins such as immunoglobulins, lactoalbumin, lactoperoxidases, lactoferrin, casein, lysozyme, and other proteins. Of the two major whey proteins, α-lactalbumin is the main component in camel milk, while β-lactoglobulin (that is present in milk of other species is deficient in camels. Other whey proteins present in camel milk are serum albumin, lactoferrin, only heavy chain immunoglobulins and peptidoglycan recognition protein. Camel milk contains a number of protective proteins, mainly enzymes that exert antibacterial and immunological properties, viz., lysozyme, lactoferrin, lactoperoxidase and peptidoglycan recognition protein (PGRP) (Dubey et al., 2016; Singh et al., 2017). The significance of these proteins has been highlighted earlier in Table 4 and Table 5.

## 6.2 Outline of work

The present chapter is directed towards purification of the bioactive protein with an anti-cancer property from camel milk. We have begun by SDS-PAGE of the proteins present in the milk, casein and whey (which were fractionated as described earlier in Section 4.2. This was followed by gel filtration chromatography. Whey was also purified with FPLC. SDS-PAGE was used for further characterizing the proteins and obtaining their molecular weight. This Chapter is a continuation of the work done earlier in chapter 5.

## 6.3 Result

### 6.3.1 SDS-PAGE Electrophoresis of camel milk and components

The skimmed camel milk and its casein and whey components have been separated out according to their molecular weights by SDS-PAGE in Figure 13. The molecular weight marker has been shown in lane 1. Skimmed camel milk (lane 4) shows the presence of proteins like: lactoferrin, camel serum albumin, immunoglobulins, 4 types caseins, TRAIL and alphalactalbumin. Lane 2 represents the casein component of camel milk (CMC) which are present between 24 and 36 kDa. The electrophoresis image of casein depicts major protein bands at 35 kDa, 30 kDa, 25 kDa. In it the prominent bands of αS1-casein and αS2-casein at 29 kDa and 36 kDa respectively. Prominent bands of β-casein and *k*-casein are also visible between 24 and 29 kDa. CWP represents camel whey proteins in lane 3. In it a band of α-Lactalbumin is visible at 14.2 kDa, then faint bands of different casein proteins from 24 to 36 kDa (depicting traces of casein protein in the purified whey sample). Protein bands of immunoglobulins are visible from 40 kDa to 60 kDa. Then from 66 kDa to 76 kDa, there are bands of CSA and Lactoferrin. Heavy (50 kDa) and light chain mol. (25 kDa) weight (Omar et al., 2016). Also visible is a very faint band of TRAIL at the molecular weight of about 20 kDa (Mariani and Krammer, 1998; Melendez et al., 2018; Wajant et al., 2001). This study thus elucidates the presence of various bioactive proteins in camel milk and its components. .

Figure 13: SDS-PAGE of *Camelus dromedarious* milk samples

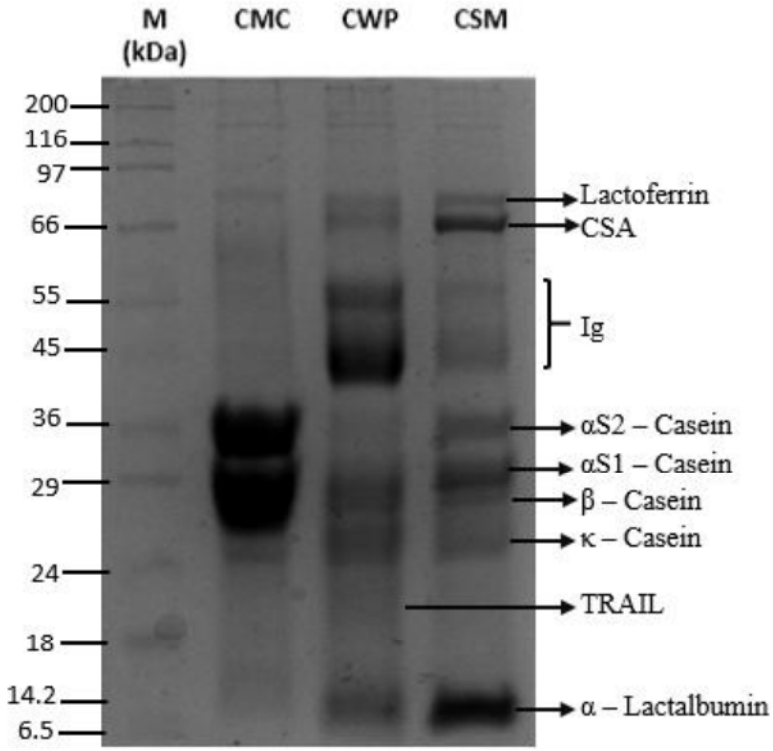



**Figure 13: Protein profile of defatted camel milk, camel milk whey and camel milk casein by SDS-PAGE.**

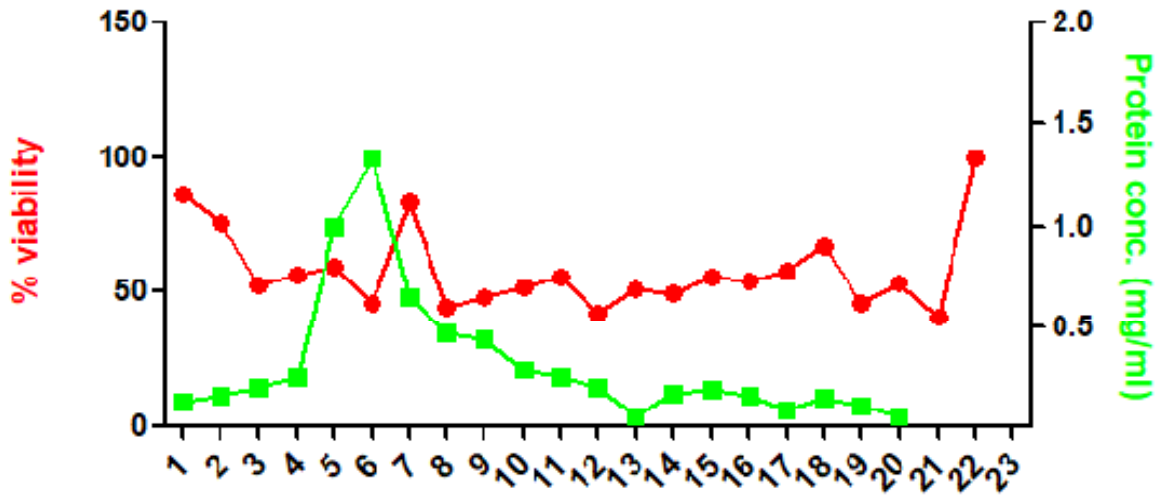
(L to R) Molecular weight marker (M); Camel milk casein (CMC); Camel whey protein (CWP); and Camel skimmed milk (CSM)

**Abbreviations:** M (Molecular weight marker); CMC (Camel milk casein); CWP (Camel whey protein); and CSM (Camel skimmed milk); CSA (Camel serum albumin); Ig (Immunoglobulin)

### 6.3.2 Protein purification by Gel filtration Chromatography

Subsequently, camel whey was subjected to gel filtration column chromatography. It yielded 20 fractions which were quantified for the presence of protein and their cytotoxicity was also estimated. The outcome of the same has been depicted in Figure 14. It can be observed from the given figure that the fraction number 6 had maximum protein content. This was also the fraction

which showed minimal viability or maximum cytotoxicity (about 50%). Fraction number 6 was thus the most significant fraction showing anti-cancer activity. The indication of 21and 22 represent the cytotoxicity of the control drug cisplatin and the untreated cells respectively.




**1 to 20 – whey fractions (obtained from column chromatography)**
**21 – Cisplatin**
**22 – Control**

**Figure 14: Percent cell viability and protein content of fractions separated by gel filtration chromatography**

### 6.3.3 SDS-PAGE of fractions separated by gel filtration chromatography

Subsequently these 20 fractions were also subjected to SDS PAGE to identify the presence of the relevant proteins and to be able to identify our protein of interest in fraction 6. The image of the gel has been shown in Figure 15. The presence of distinct bands can be observed in fractions 4, 6 & 7 in the coomassie blue stained gel at the molecular weight 20 kDa. This indicates that a camel

whey protein of molecular weight 20 kDa may be having an anti-cancer property. This aspect shall be discussed later.

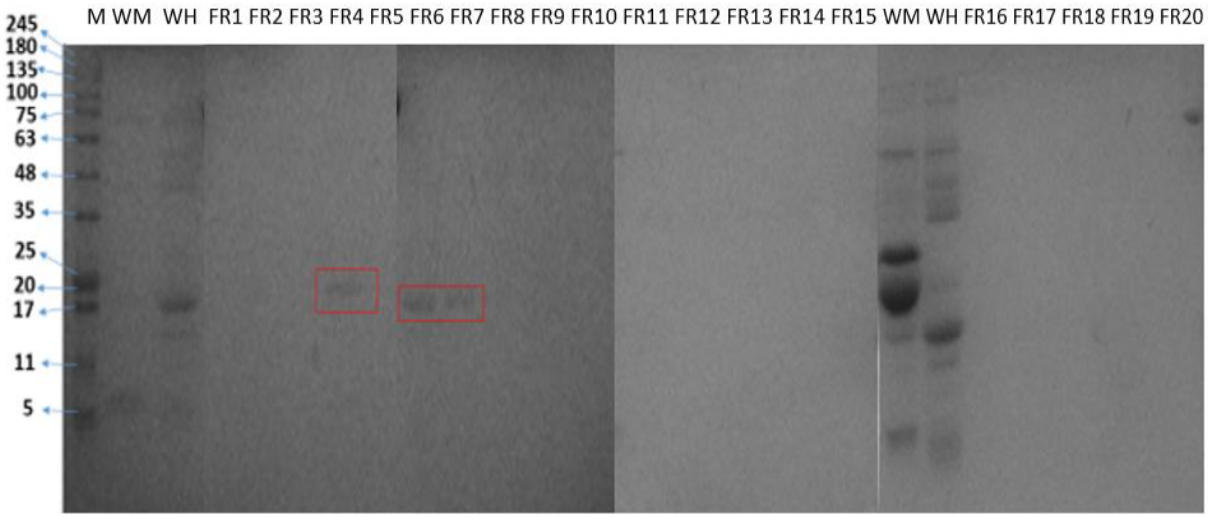



**Figure 15: SDS-PAGE of Fractions obtained from whey using gel filtration column chromatography**

### 6.3.4 Purification of bioactive component from whey by FPLC

The whey fraction was also subjected to FPLC and the fraction thus obtained was subjected to SDS PAGE. The results are shown in Figure 16. The FPLC purified sample also showed the presence of a molecule at the molecular weight of about 20kDa. Thus both these procedures namely FPLC and gel filtration chromatography showed a protein with anti-cancer property at molecular weight 20kDa.

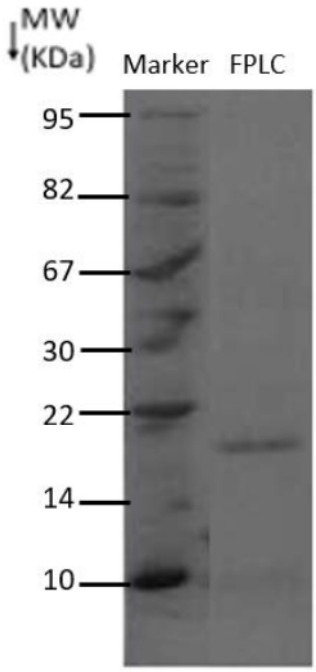



**Figure 16: SDS-PAGE of whey fraction purified by FPLC**

### 6.3.5 LC-MS/MS of whey fraction

For further characterizing the whey components it was subjected to analysis by LC-MS/MS. The results of proteins having score of above 19 have been shown in Table 8. A high score and coverage is an indicator of higher probability of its presence. From here it can be observed that the whey proteins present in intact form included alpha S1 casein, beta casein and lactoferrin. It also showed fragmented forms of kappa casein, Milk fat globule, EGF factor 8, α-lactalbumin and α S1 casein. Amongst the molecules present, lactoferrin and α-lactalbumin are two molecules which are associated with anti-cancer properties. Sequence and structure related studies of α-lactalbumin has been done later, in chapter 7, of this thesis. The presence of the above mentioned proteins is also validated by the SDS-PAGE gels discussed earlier.

**Table 8: Analysis of camel milk whey by LC-MS/MS**

| Accession | Description | Score | Coverage | # PSMs | #AAs | MW [kDa] | calc. pI |
|---|---|---|---|---|---|---|---|
| W0K8B9 | K-casein (Fragment) OS=*Camelus dromedarius* OX=9838 PE=4 SV=1 - [W0K8B9_CAMDR] | 118.52 | 60.13 | 45 | 153 | 17.1 | 8.60 |
| K7DXB9 | Alpha-s1-casein OS=*Camelus dromedarius* OX=9838 GN=CSN1S1 PE=2 SV=1 - [K7DXB9_CAMDR] | 71.97 | 40.99 | 33 | 222 | 25.8 | 5.08 |
| F5BZ34 | Milk fat globule EGF factor 8 (Fragment) OS=*Camelus dromedarius* OX=9838 GN=MFGE-8 PE=2 SV=1 - [F5BZ34_CAMDR] | 67.39 | 37.07 | 30 | 294 | 32.8 | 8.03 |
| M1E4K4 | Beta-casein OS=*Camelus* | 43.36 | 39.22 | 18 | 232 | 26.2 | 5.58 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *dromedarius* OX=9838 GN=CSN2 PE=2 SV=1 - [M1E4K4_CAMDR] | | | | | | |
| W6GH05 | Lactoferrin OS=*Camelus dromedarius* OX=9838 PE=2 SV=1 - [W6GH05_CAMDR] | 28.67 | 12.01 | 13 | 708 | 77.3 | 8.24 |
| A0A2H4WWA5 | A-lactalbumin (Fragment) OS=*Camelus dromedarius* OX=9838 GN=LALBA PE=3 SV=1 - [A0A2H4WWA5_CAMDR] | 24.36 | 42.31 | 12 | 52 | 6.1 | 4.81 |
| K7DXC0 | Alpha-s1-casein (Fragment) OS=*Camelus dromedarius* OX=9838 GN=CSN1S1 PE=4 SV=1 - [K7DXC0_CAMDR] | 19.89 | 48.98 | 7 | 49 | 5.9 | 5.01 |

**Abbreviation:** PSM (Peptide spectrum match); AA (Amino acid); MW (Molecular weight)

LC-MS/MS determination of a sample facilitates the absolute or relative abundance of individual proteins. Protein Score is the sum of the ion scores of all peptides that were identified. Coverage is the percentage of the protein sequence covered by identified peptides. A peptide-spectrum match (PSM) scoring function assigns a numerical value to a peptide-spectrum pair (P,S) expressing the likelihood that the fragmentation of a peptide with sequence P is recorded in the experimental mass spectrum S. The number of PSM's is the total number of identified peptide spectra matched for the protein.

**6.4 Discussion**

This chapter relates to the study on purification of bioactive component from camel whey by gel filtration chromatography and FPLC. The presence of a bioactive compound of molecular weight 20kDa was found. Literature was searched to seek a suitable milk protein of about 20kDa. Further it should also have the capability to induce anti-cancer ability. TNF-related apoptosis inducing ligand (TRAIL) is one such molecule and has been shown to be present in camel milk whey. The present study points in this direction but the validation or proof would definitely require further studies Furthermore, the PAGE shows the presence of some additional proteins which also have been earlier discussed.

Studies have shown high levels of TRAIL in human milk and colostrum. Its presence has been implicated for anti-cancer property in human milk. TRAIL is a cytokine that is produced and secreted by most normal tissue cells. It causes apoptosis primarily in tumor cells by binding to certain death receptors. TRAIL and its receptors have been used as the targets of several anti-cancer therapeutics. TNF family members, the ligand TRAIL is primarily expressed as a type 2 transmembrane protein which can be processed by proteases to release the soluble form (Aggarwal, 2003).

The ability of mammalian milk and its fractions to kill cancers have been recently studied by a few authors (Shariatikia et al., 2017). They have studied the effect of milk, casein and whey proteins derived from many different mammals on MCF7. Their results showed that mare, donkey, cow and camel milks, casein and whey proteins have potent cytotoxic activity against MCF7 cells in a dose dependent manner while sheep and goat milks and their proteins did not reveal any cytotoxic activity. Our study also exemplifies the ability of camel milk and whey to be cytotoxic to the cervical cancer cell line, HeLa. Further we have purified the whey proteins to get the cytotoxic factor, possibly TRAIL.

LCMS/MS analysis was conducted to better understand the components of camel milk. Numerous proteins such as alpha S1 casein, beta casein and lactoferrin were identified. It also showed fragmented forms of kappa casein, Milk fat globule EGF factor 8, α-lactalbumin and alpha S1 casein. Amongst the molecules present lactoferrin and α-lactalbumin are two molecules which are associated with anti-cancer properties.

It is a commonly expected notion that proteins / polypeptides are enzymatically broken down completely to amino acids before their assimilation. It has been observed that there exist cellular mechanisms by which proteins/large polypeptides maybe carried across the cell intact. These molecules cross the basolateral border by co-transporters and facilitated transporters. Some large peptides or proteins can be carried across the cell by transcytosis. This is particularly true in infants where the intestinal tract is not completely developed and the pH of stomach is not as low as adults. It is the mechanism whereby the immunoglobulins in maternal milk along with all its immunological properties is transferred to the child.

## 6.5 Conclusion

In this chapter the camel milk whey has been fractionated and the bioactivity of these fractions has been studied. Of the 20 fractions that were obtained fraction 6 showed maximum protein concentration and maximum cytotoxic potential. The molecular weight of protein from this fraction was about 20kDa. A similar molecular weight fraction was also obtained by FPLC. Literature suggest TRAIL to be a possible anti-cancer molecule of similar molecular weight to be present in various secretions including milk. The present study also indicated the possibility of presence of TRAIL in the fraction purified from camel milk. Further studies are required for its better validation.