

# ANTIPROLIFERATIVE ACTIVITY OF BUTTERMILK, MILK FAT GLOBULE MEMBRANE AND ISOLATED LIPID FRACTIONS ON TEN HUMAN CANCER CELL LINES

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## Background and Objectives:

Buttermilk, a by-product obtained during the production of butter, contains high amounts of residual milk fat globule membrane (MFGM) which has been assessed for its immunomodulatory, antimicrobial and anticarcinogenic capacity. Besides glycolipids and membrane specific proteins, buttermilk is a major source of milk polar lipids (60-70%), although their proportions vary greatly depending on the milk specie, treatment and the procedure used for their isolation and analysis. The objective of this preliminary study is to evaluate the antiproliferative impact of buttermilk and isolated fractions on ten human cancer cell lines.

## Methods:

Antiproliferative capacity of powder buttermilk (PB) and four isolated fractions as MFGM, total lipids (TL), and neutral (NL) and polar lipids (PL) obtained by SPE fractionation and analyzed by HPLC-ELSD, were assayed on up to ten human cancer cell lines (U251, MCF7, NCI/ADR-RES, 786-0, NCI-H460, PC-3, OVCAR-3, HT29, K-562, HaCat). Concentrations between 1 ng/mL and 100 µg/mL of all buttermilk and isolated samples were tested. Doxorubicin was also included as a positive control at same concentrations.

## Results:

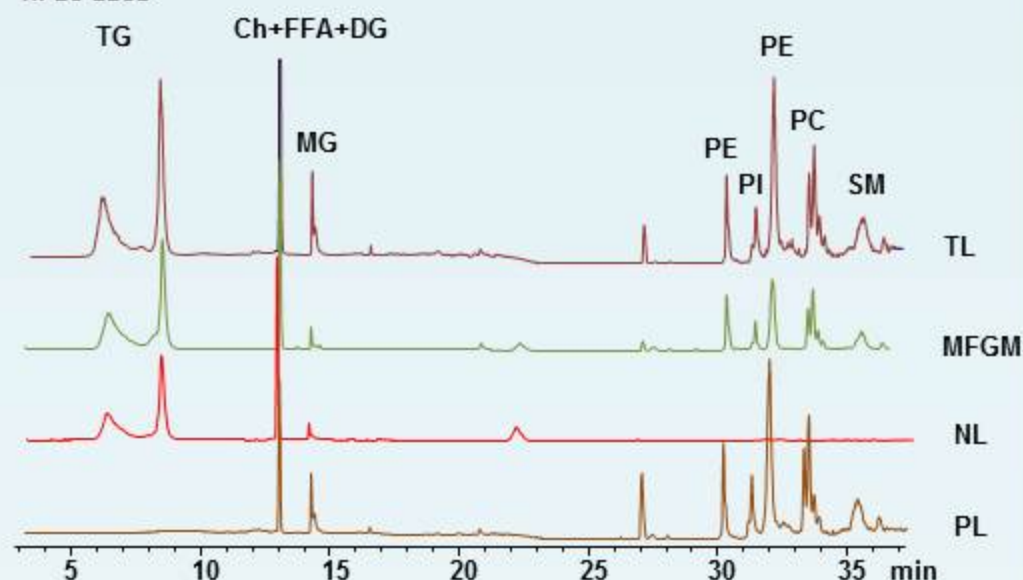
Lipid classes profile and distribution from the samples used in the present work are presented in Table 1 and Figure 1. The obtained data, revealed an increasing amount of the phospholipids percentage, accounted for about 43%, 50% and 85.7% in the TL, MFGM and PL respectively. As expected, the PL fraction resulted in the highest polar lipid concentration. According to the assays carried out with human cancer cell lines, proliferation was not inhibited by buttermilk or the isolated fractions (TL, MFGM, NL and PL). Thus, although slight decrease of human cancer cell lines was observed, a significant antitumor activity effects associated to buttermilk or phospholipids reported elsewhere where not found in this current research. Other authors hypothesize that isolation of lipids using organic solvents may affect bioactivity. Further experiments will be performed using food grade solvents.

Table 1. Lipid classes composition (g/100g fat) and phospholipid distribution (g/100g PLS) in the analyzed buttermilk and isolated samples by HPLC-ELSD.

	PB		MFGM		NL		PL	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TG	38.56	2.50	46.23	0.49	71.74	0.09	n.d	-
Ch/DG/FFA	15.30	0.92	11.44	0.08	24.96	0.10	9.25	0.50
MG	3.63	0.58	1.65	0.12	3.30	0.20	5.04	0.35
PLS	42.51	0.99	40.68	0.70	n.d	-	85.71	0.84
PE	8.91	0.60	13.64	0.54	n.d	-	10.62	0.57
PI	8.51	0.90	7.38	0.47	n.d	-	8.69	0.68
PS	36.80	0.20	28.44	0.08	n.d	-	33.35	0.14
PC	27.34	0.26	30.48	0.06	n.d	-	29.43	0.13
SM	18.44	0.35	19.30	0.12	n.d	-	17.91	0.24

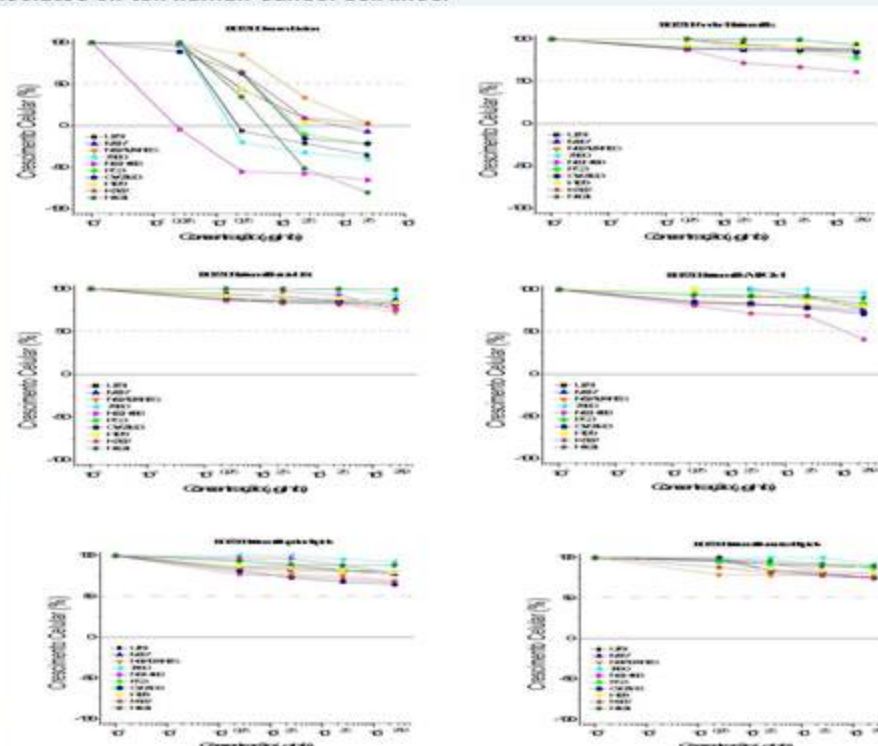
PLS: Total Phospholipids.  
See legend in Figure 1. for abbreviations details.

Figure 1. Chromatographic profile of lipid classes in the analyzed samples by HPLC-ELSD



TG: triglycerides; CH: Cholesterol; DG: diglycerides; FFA: free fatty acids; MG: monoglycerides; PE: phosphatidylethanolamine; PI: -inositol, PS: -serine, PC: -choline, SM: sphingomyelin

Figure 2. Antiproliferative effect of the of buttermilk and different fractions isolates on ten human cancer cell lines.



**Conclusions:** Under the experimental conditions of this preliminary study, none of the buttermilk and lipid isolated fractions assayed, confirmed a significant inhibiting proliferation on the ten human cancer cell lines employed.

**Keywords:** Antiproliferative activity, buttermilk, MFGM, cancer cell lines

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