

Effects and antimicrobial properties of freeze-dried colostrums from different species on breast and pancreatic cancer cell lines

Efectos y propiedades antimicrobianas del calostro liofilizado de diferentes especies sobre líneas celulares de cáncer de mama y páncreas

Ali Cingöz*¹ , Aysun Keskin² , Ercan Cacan² 

¹Tokat Gaziosmanpasa University, Department of Food Engineering. Tokat, Türkiye.

²Tokat Gaziosmanpasa University, Department of Molecular Biology and Genetics. Tokat, Türkiye.

*Corresponding author: ali.cingoz@gop.edu.tr

ABSTRACT

Mammalian colostrum (liquid gold) is a commercially valuable and potentially future functional food with high levels of essential nutrients. In this study, colostrums was obtained from Karayaka sheep (*Ovis aries*), Saanen goats (*Capra aegagrus hircus*), Danish red cows (*Bos taurus*), and buffaloes (*Bubalus bubalis*) and determined the effect of these colostrums on MCF-7 breast cancer and AsPC-1 pancreatic cancer cell lines at different doses. In addition, the potential antimicrobial activity of these colostrums against five different bacteria/moulds was determined. For this purpose, antimicrobial activities of lyophilized and dried colostrums were determined by the disc diffusion method, cell viability and cytotoxicity were determined by the MTT test, and migration potentials were determined by wound healing assays. The results showed that these colostrums had similar cytotoxic activities in both AsPC-1 and MCF-7 cancer cell lines, with some minor differences. In addition, in cell migration assays, the MCF-7 cell line treated with cow colostrum showed the most wound closure compared to other colostrums used in the study. We also found that goat and sheep colostrums have a higher antimicrobial effect on *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Aspergillus flavus*, and *Aspergillus niger* than cow and buffalo colostrums. Goat colostrum had the highest antimicrobial effect on *S. aureus*, while buffalo colostrum had the lowest antimicrobial effect on *E. coli*. In conclusion, analyses of the anticancer and antimicrobial activities of colostrum between species shed light on potential health benefits and functional food production.

Key words: AsPC-1; cell migration; MCF-7; wound healing; lyophilisation

RESUMEN

El calostro de mamíferos (también conocido como «oro líquido») es un alimento funcional de gran valor comercial y potencial futuro, ya que contiene altos niveles de nutrientes esenciales. En este estudio, obtuvimos calostro de ovejas Karayaka (*Ovis aries*), cabras Saanen (*Capra aegagrus hircus*), vacas rojas danesas (*Bos taurus*) y búfalos (*Bubalus bubalis*) y analizamos su efecto en las líneas celulares de cáncer de mama MCF-7 y de cáncer de páncreas AsPC-1 a diferentes dosis. Además, se determinó la actividad antimicrobiana potencial de estos calostros contra cinco bacterias/mohos diferentes. La posible actividad antimicrobiana de estos calostros contra cinco bacterias y mohos diferentes. Para ello, se determinó la actividad antimicrobiana de los calostros liofilizados y desecados mediante el método de difusión en disco, y se evaluó la viabilidad celular y la citotoxicidad mediante la prueba MTT, así como los potenciales de migración mediante ensayos de cicatrización de heridas. Los resultados mostraron que estos calostros tenían actividades citotóxicas similares en las líneas celulares de cáncer AsPC-1 y MCF-7, con algunas diferencias menores. Además, en los ensayos de migración celular, la línea celular MCF-7 tratada con calostro de vaca mostró el mayor cierre de heridas en comparación con los demás calostros utilizados en el estudio. También descubrimos que los calostros de cabra y oveja tienen un mayor efecto antimicrobiano sobre *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Aspergillus flavus* y *Aspergillus niger* que los de vaca y búfala. El calostro de cabra tuvo el mayor efecto antimicrobiano sobre *S. aureus*, mientras que el calostro de búfala tuvo el menor efecto antimicrobiano sobre *E. coli*. En conclusión, los análisis de las actividades anticancerígenas y antimicrobianas del calostro entre especies arrojan luz sobre sus posibles beneficios para la salud y la producción de alimentos funcionales.

Palabras clave: AsPC-1; migración celular; MCF-7; cicatrización de heridas; liofilización

INTRODUCTION

After birth, all female mammals secrete a yellowish, thick milk that is rich in nutrients and antibodies and contains everything a newborn baby needs. This fluid, known as colostrum, has been described as 'liquid gold,' 'superfood,' or 'immune milk' [1]. Colostrum is defined as the first natural nutrient secreted by the newborn's mammary glands between 24 and 96 hours (h) after birth. Colostrum, which is very rich in essential nutrients and functional compounds, has an important microflora, including some probiotics. Colostrum contains significant levels of many components with high antimicrobial activity [2]. There are many important components such as monounsaturated and polyunsaturated fatty acids, conjugated linoleic acid, vitamin C, B group vitamins, tocopherol, retinol, cholecalciferol, phylloquinone, macro and micro minerals [3]. Colostrum is used in functional foods and food supplements, especially sports supplements [4], some medicines [5], pharmaceutical creams [6], and infant formulas [7].

The studies have reported antimicrobial effects of compounds such as α -lactalbumin, epidermal growth factor, glycomacropeptides, and glycosaminoglycans [8]. Compared to cow and sheep colostrum, goat colostrum contains higher levels of acidic and neutral oligosaccharides [9]. In a study, it was shown that oligosaccharides produced from bovine colostrum can prevent *Escherichia coli*, *Cronobacter sakazakii*, and *Salmonella enterica* [10]. Lactoferrin is a natural antibiotic that bridges the gap between the innate and adaptive immune systems of mammals with antiviral, antibacterial, and anti-inflammatory effects [11]. Among the compounds found in milk and colostrum, lysozyme reduces the levels of Firmicutes, *Mycobacteriaceae*, *Streptococcaceae*, and *Campylobacter* [12]. Lactalbumin shows antagonistic activity against *E. coli* O127 [13], while lactoferrin shows antimicrobial properties against both Gram-positive and Gram-negative bacteria, including *E. coli* O157:H7 and *Staphylococcus aureus* [14, 15].

Cancer is one of the deadliest diseases, with approximately 20 million cases and 9 million deaths worldwide [16]. According to GLOBOCAN 2022 predictions, breast cancer has the highest incidence of approximately 2.3 million cases, while pancreatic cancer has 495,000 cases. Breast cancer poses a serious risk, with approximately 685,000 deaths, and pancreatic cancer with 466,000 deaths [17]. Colostrum has been used in various cancer studies due to the important functional compounds it contains [18, 19, 20]. The colostrums obtained from different mammalian species have been found to contain similar components, albeit in varying proportions [3]. Lactoferrin, one of the components of colostrum, has been shown to inhibit gastric cancer [21], oesophageal cancer [22], lung cancer [23], colorectal cancer [24], lactoperoxidase, colorectal cancer, liver cancer, breast cancer and prostate cancer [25], conjugated linolenic acid has anti-cancer effects on various cancer cell lines such as ovarian cancer [23], breast cancer and colon cancer [26]. Research suggests that animal colostrum is 100 to 1000 times more potent than human colostrum [27]. Considering the content of colostrum from different sources and its efficacy in cancer, there is still a great need for research on the effect of colostrum on different types of cancer. Due to its high nutrient and bioactive content, colostrum has been used as a medicine to treat infections and heal wounds. The low availability of human colostrum has led researchers to focus on farm animals, and in this context, cow, sheep, goat, and buffalo colostrum have

come to the fore [3]. This study aims to investigate the efficacy of lyophilised and dried colostrum from four different mammals against MCF-7 and AsPC-1 cancer cell lines and five different pathogenic microorganisms. While there are a limited number of studies on MCF-7 cancer in the literature, no study on AsPC-1 cancer cell lines has been identified. In addition, this study aims to contribute to the gap in the literature regarding the antimicrobial properties of colostrum against different bacteria/moulds.

MATERIALS AND METHODS

Material

Colostrums were collected from local farms (Kahramanmaraş, Tokat, Türkiye). Colostrums from a total of 60 animals for each species were pooled after milking and stored frozen (Arçelik, 583630 EB, Türkiye) at (-18°C). All chemicals used were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Merck (Merck KGaA, Darmstadt, Germany).

Lyophilisation

Liquid colostrum samples were frozen (-18°C for 6 h) (Arçelik, 583630 EB, Türkiye) and then freeze-dried under vacuum (0.011 mbar, -75°C for 48 h) with lyophilisator (Christ Gamma, Alpha 2-4 LO+, Germany). The powder was collected when the moisture content was less than 5%, packaged, and stored at -18°C until analysis.

Antimicrobial activity

Aspergillus flavus (ATCC 9170), *Enterococcus faecalis* (ATCC 29212), *E. coli* (ATCC 25922), *Aspergillus niger* (ATCC 6275), and *S. aureus* (ATCC 29213) microorganisms were used in the antimicrobial activity test.

Disc diffusion method

Colonies obtained from 18–24 h fresh cultures of microorganisms (bacteria and moulds) grown on solid media were suspended in saline and diluted to 10^8 cfu·mL⁻¹ by comparison with 0.5 McFarland turbidity tubes. Petri dishes containing MHA were inoculated with 100 μ L bacterial dilutions. The extracts obtained were impregnated on blank discs and the zone diameters were determined at the end of the incubation (37°C, 24 h bacteria, 30°C, 24 h mould) [28].

Determination of minimum inhibitory concentration (MIC)

The macrobroth method described by Oskay *et al.* [29] was used to determine the minimum inhibition concentration (MIC) values of the extracts. From each microbial culture prepared (18 h), 25 μ L (1×10^8 cfu·mL⁻¹) was transferred to 3 mL of MHB and 10 mL of extracts prepared at different dilutions (30 mg colostrum·mL⁻¹ – 0.46 mg colostrum·mL⁻¹). Gentamicin was used as a control.

Cell culture

Breast (MCF-7) and pancreatic (AsPC-1) cancer cell lines were obtained from Tokat Gaziosmanpaşa University Cancer Research Laboratory. The method reported by Berkel & Cacan [30] was

used, and cell counting was performed using the Automated Cell Counter (Thermo Scientific, Countess™ 3, UK).

Cell viability assays

Modifications were made to the method used by Berkel & Cacan [31] to perform MTT assays using the MCF-7 and AsPC-1 cell lines. The cells were grown in a complete medium (RPMI medium, 10% fetal bovine serum, and 1% penicillin/streptomycin) for a period of 24 h at 37°C. Following the incubation (Memmert, IN110, Germany) period, the cells were exposed to varying concentrations of colostrum (0.25–0.00025 mg·mL⁻¹) and incubated (Memmert, IN110, Germany) once more (48 h, 37°C, humidified environment). To assess the potential impact of lyophilised goat, buffalo, sheep and cow colostrums, MTT assays were conducted in accordance with the methodology previously outlined [31, 32]. The percentage inhibition values of colostrums against all cell lines were calculated using the following equation, with the absorbance (PG Instruments, T80+ UV-VIS, UK) values determined between 540 and 570 nm:

$$\text{Cell Survival (\%)} = \left[\frac{(A_t - A_b)}{(A_c - A_b)} \right] \times 100$$

A_t : Absorbance of treated cells

A_b : Absorbance of blank (media) cells

A_c : Absorbance of control (cells)

Statistical analysis

The statistical analysis of the results was conducted using GraphPad Prism 8. The results obtained were expressed as mean ± standard error (SEM). Student's t-test was used to assess the statistical differences between the two groups. The significance level was defined as * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$.

Cell migration assay (Wound healing assay)

Wound healing analysis was performed to determine the effect of goat, buffalo, sheep, and cow colostrum applied on the breast cancer MCF-7 cell line migration. Cells were seeded in a 6-well plate at a density of 400,000 cells per well and were incubated at 37°C at 5% CO₂ to grown up to 80% confluence. Wounds were created by scraping cells with a pipette tip, and non-adherent cells were washed off with a medium. Cells were treated with goat, buffalo, sheep, and cow colostrums at a concentration of 0.0025 mg·mL⁻¹ and monitored at 0, 24, and 48 h after the creation of the wounds. The migration images of treated and untreated control cells were taken under a phase contrast microscope (Olympus, CX-21, China) with a 200× objective. The areas covered by migrating cells were measured with ImageJ. Statistical calculations were tested by One way ANOVA. Significance was accepted at $P < 0.05$ in all cases. The areas (%) occupied by migrating cells were measured with the ImageJ application [33].

RESULTS AND DISCUSSION

In this study, the antimicrobial effects of four different colostrum milks on five different pathogenic microorganisms were investigated (TABLE I). Standard strains of pathogens were used in the study. When the results of the study were analyzed, the highest antimicrobial effect on *S. aureus* was observed in the goat among the colostrum samples. This was followed by sheep, cow, and buffalo

colostrum samples. On the other hand, when the antimicrobial effect on *E. coli* was analyzed, the highest antimicrobial effect was observed in sheep colostrum. This was followed by cow, goat, and buffalo colostrums. Sheep, goat, cow, and buffalo colostrums showed antimicrobial activity against *Enterococcus faecalis*. Goat and sheep colostrums were more effective against *Aspergillus niger* than other colostrums, whereas all colostrums showed similar antimicrobial activity against *Aspergillus flavus*. In goat milk, inactivation on gram positive microorganisms was stronger than gram negative ones. This was thought to be due to the fact that the bioactive compounds contained in goat milk are more active and more effective in the destruction of microorganisms. Many studies show that goat colostrum has antimicrobial activity. In particular, components such as lactoferrin, lactoperoxidase, and lactosidine in goat colostrum have been shown to be effective against pathogenic bacteria such as *E. coli* and *S. aureus* [34, 35]. These components in goat colostrum provide a natural defense mechanism against pathogenic bacteria. The antimicrobial effects of these components may provide a potential area of use in the prevention or treatment of infections.

TABLE I
Antimicrobial properties of colostrums

	Goat	Cow	Sheep	Buffalo
<i>Staphylococcus aureus</i>	20.00 ± 0.71	14.00 ± 0.35	15.00 ± 0.71	13.00 ± 0.71
Gentamicin	25.00	26.00	26.00	25.00
<i>Escherichia coli</i>	14.00 ± 0.35	14.50 ± 0.71	15.00 ± 1.06	10.00 ± 1.24
Gentamicin	21.00	20.00	20.00	21.00
<i>Enterococcus faecalis</i>	16.00 ± 0.71	15.00 ± 0.35	16.50 ± 0.35	14.00 ± 0.71
Gentamicin	24.00	22.00	24.00	22.00
<i>Aspergillus flavus</i>	16.00 ± 0.00	15.00 ± 0.35	16.00 ± 0.35	14.50 ± 0.35
Gentamicin	25.00	25.00	24.00	24.00
<i>Aspergillus niger</i>	18.00 ± 0.71	17.00 ± 0.71	18.50 ± 0.71	16.00 ± 0.35
Gentamicin	24.50	25.00	24.50	25.00

The antimicrobial mechanism in buffalo milk was found to be more effective on *S. aureus*. Studies on the antimicrobial effects of buffalo colostrum are limited, but some studies have shown that buffalo colostrum has similar antimicrobial activity [36]. The antimicrobial components of buffalo colostrum, as in other milk types, may constitute a potential source for the prevention or treatment of bacterial infections. When the inactivation of *E. coli* in cow's milk was compared among other colostrum samples, it was determined that it showed a higher inactivation against *S. aureus*. The effectiveness of this situation on *E. coli*, an indicator of fecal contamination, was found to be significant compared to other pathogenic microorganisms. The antimicrobial effects of bovine colostrum have been widely studied. Components such as lactoferrin, lactosidine, and immunoglobulins in bovine colostrum are known to be effective against pathogens such as *E. coli* and *S. aureus* [37]. These antimicrobial components of cow's colostrum are particularly important in strengthening the immune system of newborn calves and providing protection against infection. In an in vivo study, the antimicrobial effects of bovine colostrum against five

foodborne pathogenic bacterial strains were observed at varying rates [38]. In addition, these components may also have potential benefits for human health. Sheep colostrum samples were found to have the same antimicrobial activity against two pathogens. Fewer studies have been conducted on the antimicrobial effects of sheep colostrum, but the available research suggests that similar antimicrobial components are present in sheep colostrum. Sheep colostrum is thought to have antimicrobial properties similar to other types of milk.

Cytotoxicity effect on cancer cell lines

To examine the cytotoxic properties of different colostrums collected from various farm animals, an MTT assay was performed on the MCF-7 and AsPC-1 cell lines. In addition, cell migration analysis was performed on the MCF-7 cell line to examine the closure of the wounds formed by these natural products and the healing processes of these wounds over time. The results showed that colostrums obtained from goats, buffaloes, sheep, and cows had similar cytotoxic activities on AsPC-1 and MCF-7 cell lines (FIG. 1). For example, cell viability decreases with dose-dependent increase in goat, sheep, buffalo, and cow colostrums, but when applied at a dose of 0.00025 mg·mL⁻¹, cell viability increases compared to other concentrations (FIG.1 A–D). In the MCF-7 cell line, we observed similar results with the AsPC-1 cell line with some differences (FIG. 2). In the MCF-7 cell line, the high dose of 0.25 mg·mL⁻¹ application of goat and cow colostrum increases cell viability compared to the control (FIG. 2A–D), while at other doses cell viability decreases compared to the control (FIG. 2B–C). To increase the accuracy and reliability of MTT results, microscopic examinations of MCF-7 and AsPC-1 cells were performed after 48 h of 0.25, 0.025, and 0.00025 mg·mL⁻¹ colostrum treatment (FIG. 3). The quantitative results obtained in the study support each other with colostrum images.

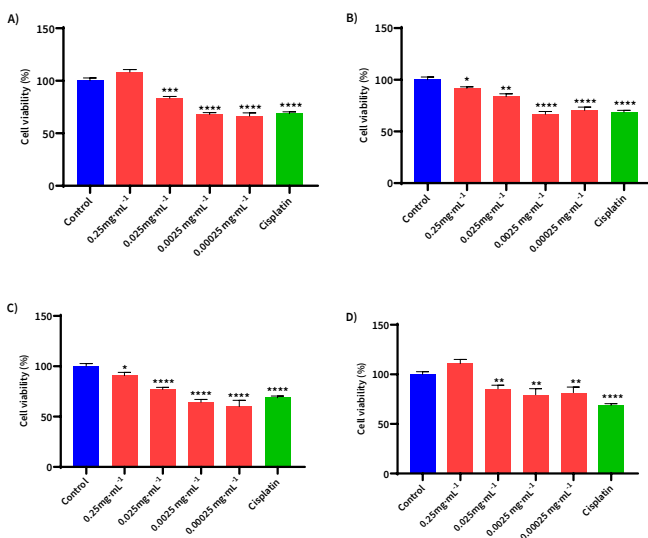


FIGURE 1. Cell viability (%) of AsPC-1 cells following the exposure of various concentrations A) Goat, B) Buffalo, C) Sheep and D) Cow. Experiments were repeated three times. **P*<0.05, ***P*≤0.01, ****P*≤0.001, *****P*≤0.0001

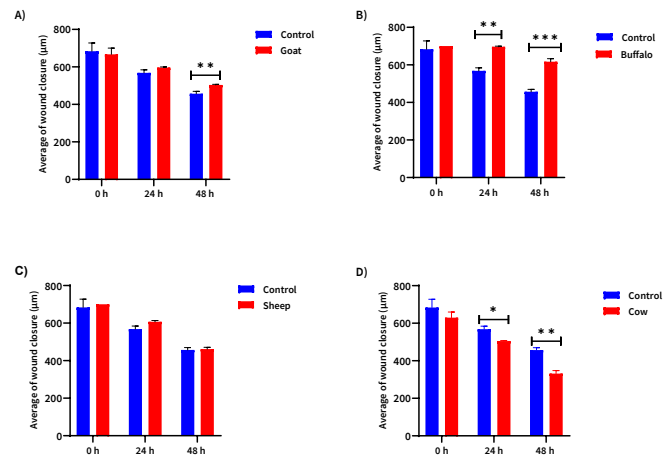


FIGURE 2. Cell viability (%) of MCF-7 cells following the exposure of various concentrations of the colostrums. A) Goat, B) Buffalo, C) Sheep and D) Cow. Experiments were repeated three times. **P*<0.05, ***P*≤0.01, ****P*≤0.001, *****P*≤0.0001

In a study investigating the effects of lyophilised mare and cow colostrum on the human lung cancer cell line A549 and the healthy lung cell line MRC5, it was reported that mare colostrum was the most effective milk type in reducing the A549 lung cancer cell line, and cow colostrum had little effect [39]. Another study reported that cow colostrum has the ability to inhibit the growth of cancer cell lines (MDA-MB-231 and MCF-7) *in vitro* [20]. In a study investigating the effects of donkey milk on cancer (A549) and normal (BEAS-2B) lung cell lines, it was reported to have a higher cytotoxic effect on the A549 tumour cell line [40]. Goat colostrum was found to have strong cytotoxic activity against COLO 320DM cells and COLO 205 cells [41]. In addition it was found that bovine colostrum at a certain concentration caused a 50% reduction in the tumour cell population in the C6 cell line [42]. Bactrian camel (*Camelus bactrianus*) milk and colostrum have been shown to prevent or reduce the growth of cancer cells. Camel colostrum has been reported to prevent the proliferation of colon, human breast (MCF-7), and liver (HepG2) cancer cells [43].

Studies show that lactoferrins in colostrum have beneficial effects on breast cancer cell lines (HS578T, T47D, MDA-MB-231, and MCF-7), lung cancer cell line (A549), and colorectal adenocarcinoma cell line (HT29) are available in the literature [44]. Various components of bovine colostrum have been reported to significantly reduce lung, colon and breast cancer and suppress tumour formation [27]. Lactoferrin, one of the most abundant compounds in colostrum, has been reported to prevent many cancers, such as colon, bladder, tongue, and lung cancers, thereby boosting immunity [45]. Goat colostrum-derived lactoferrin was reported to have dose-dependent anti-proliferative effects in lung, colon, cervical, gastric, and breast cancer cell lines, with the most potent inhibition of cell growth observed in ZR-75-1 breast cancer cells (IC₅₀ = 27.5 µg·mL⁻¹) [46]. Conjugated linolenic acid (CLA) in colostrum has been reported to have anti-tumour properties and CLA has been shown to have anti-carcinogenic properties in the mammary glands of women [47]. Samuel *et al.* [48] reported that extracellular vesicles (EVs) obtained from buffalo and cow milk significantly induced cell death in colon cancer cells (LIM1215) and had direct anti-tumor activity. In addition, polyunsaturated

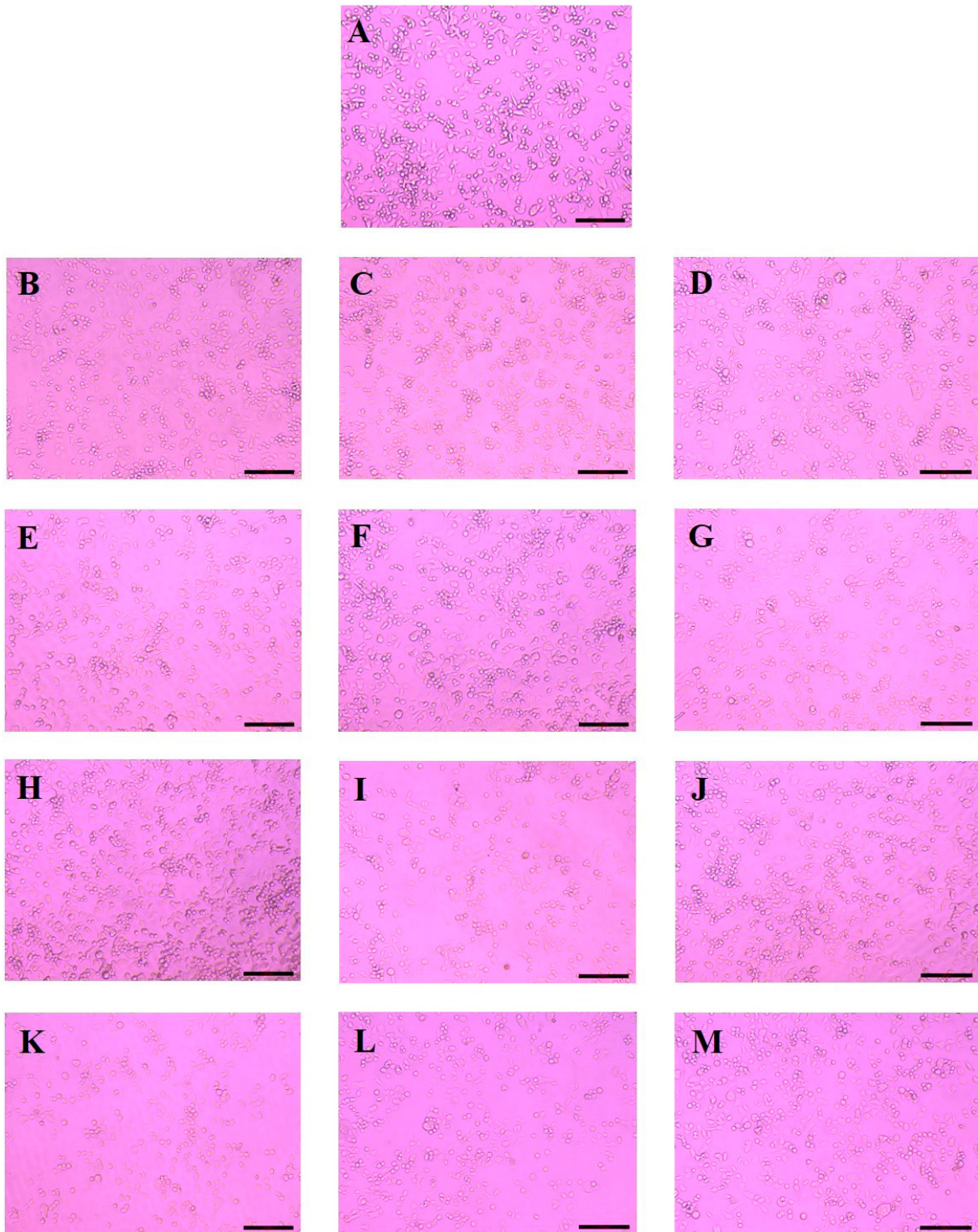


FIGURE 3. Microscopic examination of AsPC-1 cells after 48 h of treatment 0.25, 0.025, 0.0025 mg·mL⁻¹ concentrations of the colostrums. A) Control, B-C-D) Goat, E-F-G) Buffalo, H-I-J) Sheep, K-L-M) Cow (Magnification: 200×)

fatty acids (PUFA), one of the important bioactive compounds in the composition of colostrum, have been reported to have anti-cancer effects [49]. The findings obtained in the study are consistent with the literature. This study fills the gap in the literature in terms of antimicrobial and cancer cell line properties of buffalo and black sheep colostrums.

Cell migration assay (Wound healing assay)

The limited number of studies on colostrum and its products show promise in the prevention of infectious diseases, wound healing therapy and the fight against certain types of cancer. Wound healing assays were performed to determine the effect of goat, buffalo, sheep, and cow colostrums applied at a dose of 0.0025 mg·mL⁻¹ on cell migration in the breast cancer cell line (MCF-7). In cell migration analysis, wound closure was examined by taking images at 0, 24 and 48 h after wound formation (FIGS. 4, 5, 6, 7). At the 0th h of application, cell migration increases and wound distance decreases in goat and cow colostrum compared to the control (FIGS. 4, 7). However, in buffalo and sheep colostrum, cell migration decreases, and wound distance increases compared to the control (FIGS. 5, 6). At the 24th h of application, cell migration

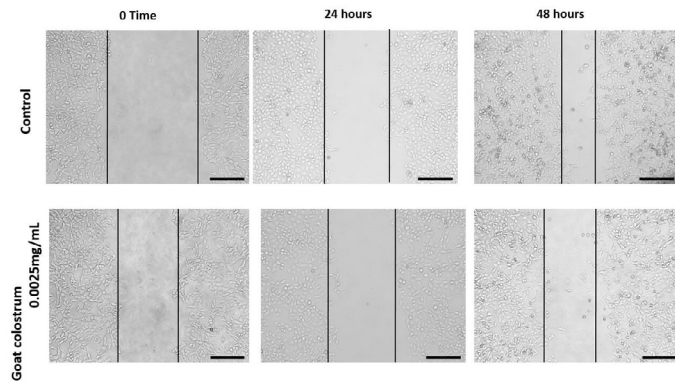


FIGURE 4. Wound assay analysis of MCF-7 cell line treated at 0.0025 mg·mL⁻¹ concentration of goat colostrums at 0, 24, 48 h. The wound assay experiment was created in ImageJ

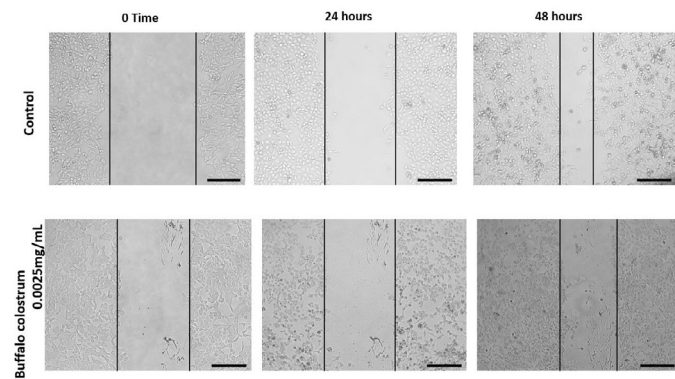


FIGURE 5. Wound assay analysis of MCF-7 cell line treated at 0.0025 mg·mL⁻¹ concentration of buffalo colostrums at 0, 24, 48 h. The wound assay experiment was created in ImageJ

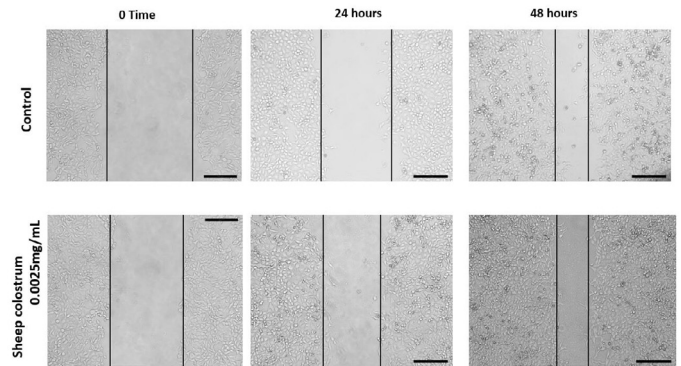


FIGURE 6. Wound assay analysis of MCF-7 cell line treated at 0.0025mg/mL concentration of sheep colostrum at 0, 24, 48 h. The wound assay experiment was analyzed in the image

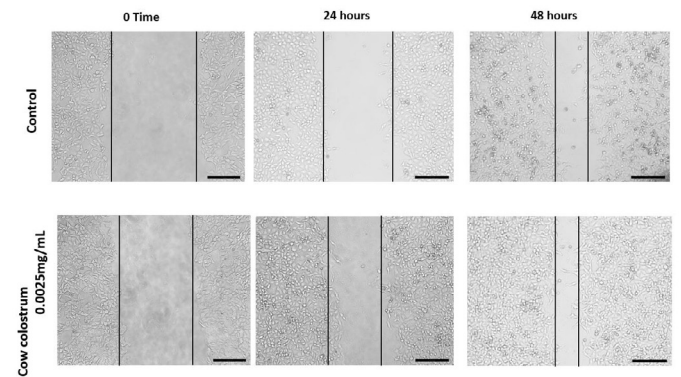


FIGURE 7. Wound assay analysis of MCF-7 cell line treated at 0.0025 mg·mL⁻¹ concentration of cow colostrum at 0, 24, 48 h. The wound assay experiment was created in ImageJ

increases and wound distance decreases in cow colostrum compared to the control (FIG. 7). On the contrary, in buffalo, sheep and goat colostrums, cell migration decreases and wound distance increases compared to the control (FIGS. 4, 5, 6). At the 48th h of the application, while cell migration decreases in the cell line applied with goat and buffalo colostrum compared to the control, it does not change in the sheep colostrum (FIGS. 4, 5, 6).

At the 48th h of the cell line applied with cow colostrum, cell migration increases compared to the control and wound distance decreases (FIG. 7). In the MCF-7 cell line applied with cow colostrum at 0, 24 and 48 h of application, cell migration increases and wound distance decreases compared to the control (FIGS. 7 and 8). The results showed that cow colostrum gave the best response to treatment compared to goat, sheep and buffalo colostrum in the wound healing analysis at 48 h (FIG. 8).

Kim *et al.* [50] reported that extracellular vesicles from bovine colostrum have wound-healing properties and that freeze-dried colostrum retained its original properties and efficacy for wound repair after lyophilisation. In this respect, the results obtained with bovine colostrum in this study are similar to those reported in the literature. The findings on colostrum from other species are new to the literature.

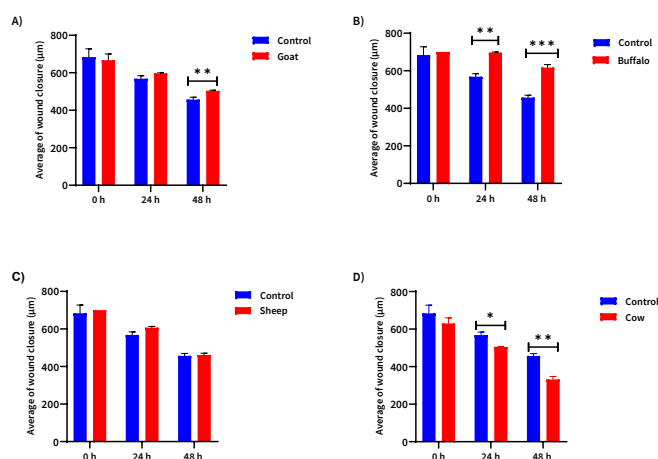


FIGURE 8. The cell line tested was MCF-7, and the wound healing was measured by the ImageJ program. Statistical analysis was conducted by GraphPad. * $P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

CONCLUSION

In the study, colostrums of Karayaka sheep, Saanen goat, Danish red cow, and buffalo were lyophilised and dried, and the antimicrobial and anticancer activities of the colostrums obtained were determined on MCF-7 breast cancer, AsPC-1 pancreatic cancer, and 5 different pathogens. Different colostrums showed similar cytotoxic activities in pancreatic and breast cancer cell lines. Adding colostrum at $0.0025 \text{ mg} \cdot \text{mL}^{-1}$ and below decreased cell viability. In wound closure experiments, cow colostrum was found to be more effective. In addition, goat and sheep colostrums have higher antimicrobial activity against *S. aureus*, *E. coli*, *Enterococcus faecalis*, *Aspergillus flavus*, and *Aspergillus niger* than cow and buffalo colostrums. It was found that goat colostrum had the highest antimicrobial activity against *S. aureus*, and buffalo colostrum had the lowest antimicrobial activity against *E. coli*. In addition, the study adds an important innovation to the literature by determining the effect of colostrum samples from Karayaka sheep, Saanen goats, Danish red cows, and buffalo on pancreatic cancer and the pathogenic microorganism *Aspergillus*. As a result, analysis of the anticancer and antimicrobial activities of colostrum between species sheds light on potential health benefits. This study suggests the use of animal colostrums with anticancer and antimicrobial effects in functional foods or dietary supplements. It is also recommended to investigate that colostrum-enriched products such as bread, biscuits, baby food and pasta for individuals with low immunity.

Conflict of interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

BIBLIOGRAPHIC REFERENCES

- [1] El-Loly MM. Colostrum ingredients, its nutritional and health benefits—an overview. *Clin. Nutr. Open Sci.* [Internet]. 2022; 44:126-143. doi: <https://doi.org/pg6f>
- [2] Ayar A, Sıçramaz H, Çetin İ. The Effect of Bovine Colostrum on the Lactic Flora of Yogurt and Kefir. *JSM Biotechnol. Bioeng.* [Internet]. 2016 [cited 12 Dec. 2024]; 3(4):1063. Available in: <https://goo.su/OHKA>
- [3] Artym J, Zimecki M. Colostrum proteins in protection against therapy-induced injuries in cancer chemo- and radiotherapy: a comprehensive review. *Biomedicines* [Internet]. 2023; 11(1):114. doi: <https://doi.org/pg6g>
- [4] Silva EGDSO, Rangel AHDN, Mürmam L, Bezerra MF, Oliveira JFPD. Bovine colostrum: benefits of its use in human food. *Food Sci. Technol.* [Internet]. 2019; 39(2):355-362. doi: <https://doi.org/gn23x2>
- [5] Anderson RC, Dalziel JE, Haggarty NW, Dunstan KE, Gopal PK, Roy NC. Processed bovine colostrum milk protein concentrate increases the epithelial barrier integrity of Caco-2 cell layers. *J. Dairy Sci.* [Internet]. 2019; 102(12):10772-10778. doi: <https://doi.org/pg6h>
- [6] Reddy RS, Ramachandra CT, Hiregoudar S, Nidoni U, Ram J, Kammar M. Influence of processing conditions on functional and reconstitution properties of milk powder made from Osmanabadi goat milk by spray drying. *Small Rum. Res.* [Internet]. 2014; 119(1-3):130-137. doi: <https://doi.org/f545rg>
- [7] Bagwe-Parab S, Yadav P, Kaur G, Tuli HS, Buttar HS. Therapeutic applications of human and bovine colostrum in the treatment of gastrointestinal diseases and distinctive cancer types: The current evidence. *Front. Pharmacol.* [Internet]. 2020; 11:01100. doi: <https://doi.org/gn23xv>
- [8] Eker F, Akdaşçı E, Duman H, Yalçıntaş YM, Canbolat AA, Kalkan AE, Karev S, Şamec D. Antimicrobial properties of colostrum and milk. *Antibiotics* [Internet]. 2024; 13(3):251. doi: <https://doi.org/pg6j>
- [9] Giorgio D, Di Trana A, Claps S. Oligosaccharides, polyamines and sphingolipids in ruminant milk. *Small Rumin. Res.* [Internet]. 2018; 160:23-30. doi: <https://doi.org/gdbgcx>
- [10] Maldonado-Gomez MX, Lee H, Barile D, Lu M, Hutkins RW. Adherence inhibition of enteric pathogens to epithelial cells by bovine colostrum fractions. *Intern. Dairy J.* [Internet]. 2015; 40:24-32. doi: <https://doi.org/pg6k>
- [11] Kowalczyk P, Kaczyńska K, Kleczkowska P, Bukowska-Oško I, Kramkowski K, Sulejczak D. The lactoferrin phenomenon—a miracle molecule. *Molecules* [Internet]. 2022; 27(9):2941. doi: <https://doi.org/pg6m>
- [12] Maga EA, Desai PT, Weimer BC, Dao N, Kültz D, Murray JD. Consumption of lysozyme-rich milk can alter microbial fecal populations. *App. Environ. Microbiol.* [Internet]. 2012; 78(17):6153-6160. doi: <https://doi.org/f369gd>
- [13] Singh A, Duche RT, Wandhare AG, Sian JK, Singh BP, Sihag MK, Singh KS, Sangwan V, Talan S, Panwar H. Milk-derived antimicrobial peptides: overview, applications, and future perspectives. *Probiotics Antimicrob. Prot.* [Internet]. 2023; 15(1):44-62. doi: <https://doi.org/pg6n>
- [14] Diarra MS, Petitclerc D, Deschênes E, Lessard N, Grondin G, Talbot BG, Lacasse P. Lactoferrin against *Staphylococcus aureus* Mastitis: Lactoferrin alone or in combination with penicillin G on

- bovine polymorphonuclear function and mammary epithelial cells colonisation by *Staphylococcus aureus*. *Vet. Immunol. Immunopathol.* [Internet]. 2003; 95(1-2):33-42. doi: <https://doi.org/bb2mrd>
- [15] Rybarczyk J, Kieckens E, Vanrompay D, Cox E. *In vitro* and *in vivo* studies on the antimicrobial effect of lactoferrin against *Escherichia coli* O157: H7. *Vet. Microbiol.* [Internet]. 2017; 202:23-28. doi: <https://doi.org/gbphqm>
- [16] Menadi S, Kucuk B, Cacan E. Promoter hypomethylation upregulates ANXA2 expression in pancreatic cancer and is associated with poor prognosis. *Biochem. Genet.* [Internet]. 2024;62:2721–2742. doi: <https://doi.org/pg6p>
- [17] Criscitiello C, Corti C. Breast cancer genetics: diagnostics and treatment. *Genes* [Internet]. 2022; 13(9):1593. doi: <https://doi.org/pg6q>
- [18] Miranda C, Igrejas G, Poeta P. Bovine Colostrum: Human and Animal health benefits or route transmission of antibiotic resistance—One Health perspective. *Antibiotics* [Internet]. 2023; 12(7):1156. doi: <https://doi.org/pg6r>
- [19] Lotito D, Pacifico E, Matuozzo S, Musco N, Iommelli P, Zicarelli F, Tudisco R, Infascelli F, Lombardi P. Colostrum composition, characteristics and management for buffalo calves: A review. *Vet. Sci.* [Internet]. 2023; 10(5):358. doi: <https://doi.org/pg6s>
- [20] Sharma A, Shandilya UK, Sodhi M, Mohanty AK, Jain P, Mukesh M. Evaluation of Milk colostrum derived Lactoferrin of Sahiwal (*Bos indicus*) and Karan fries (cross-bred) cows for its anti-cancerous potential. *Int. J. Mol. Sci.* [Internet]. 2019; 20(24):6318. doi: <https://doi.org/pg6t>
- [21] Amiri F, Moradian F, Rafiei A. Anticancer effect of lactoferrin on gastric cancer cell line AGS. *Res. Mol. Med.* [Internet]. 2015; 3(2):11-16. doi: <https://doi.org/pg6w>
- [22] Farziyan MA, Moradian F, Rafiei AR. Anticancer effect of bovine lactoferrin on human esophagus cancer cell line. *Res. Mol. Med.* [Internet]. 2016; 4(1):18-23. doi: <https://doi.org/pg6x>
- [23] Shahzad MM, Felder M, Ludwig K, Van Galder HR, Anderson ML, Kim J, Cook ME, Kapur AK, Patankar MS. Trans10, cis12 conjugated linoleic acid inhibits proliferation and migration of ovarian cancer cells by inducing ER stress, autophagy, and modulation of Src. *PLoS One* [Internet]. 2018; 13(1):e0189524. doi: <https://doi.org/gctt7m>
- [24] Sugihara Y, Zuo X, Takata T, Jin S, Miyauti M, Isikado A, Imanaka H, Tatsuka M, Qi G, Shimamoto F. Inhibition of DMHDSS-induced colorectal cancer by liposomal bovine lactoferrin in rats. *Oncol. Lett.* [Internet]. 2017; 14(5):5688-5694. doi: <https://doi.org/gch2vd>
- [25] Abu-Serie MM, El-Fakharany EM. Efficiency of novel nanocombinations of bovine milk proteins (lactoperoxidase and lactoferrin) for combating different human cancer cell lines. *Sci. Rep.* [Internet]. 2017; 7(1):16769, doi: <https://doi.org/pg6z>
- [26] Niezgodna N, Gliszczynska A, Kempinska K, Wietrzyk J, Wawrzenczyk C. Synthesis and evaluation of the cytotoxic activity of conjugated linoleic acid derivatives (esters, alcohols, and their acetates) toward cancer cell lines. *Eur. J. Lipid Sci. Technol.* [Internet]. 2017; 119(10):1600470. doi: <https://doi.org/pg62>
- [27] Alsayed AR, Hasoun LZ, Khader HA, Basheti IA, Permana AD. Bovine Colostrum Treatment of Specific Cancer Types: Current Evidence and Future Opportunities. *Molecules* [Internet]. 2022; 27(24):8641. doi: <https://doi.org/pg63>
- [28] Ebrahimabadi AH, Djafari-Bidgoli Z, Mazoochi A, Kashi FJ, Batooli H. Essential oils composition, antioxidant and antimicrobial activity of the leaves and flowers of *Chaerophyllum macropodium* Boiss. *Food Control* [Internet]. 2010; 21(8):1173-1178. doi: <https://doi.org/d6w3nn>
- [29] Oskay M, Aktaş K, Sarı D, Azeri C. *Asphodelus aestivus* (Liliaceae)'un antimikrobiyal etkisinin çukur ve disk diffüzyon yöntemiyle karşılaştırmalı olarak belirlenmesi [A comparative study of antimicrobial activity using well and disk diffusion method on *Asphodelus aestivus* (Liliaceae)]. *Ekoloji.* [Internet]. 2007 [cited 12 Dec. 2024]; 16(62):62-65. Turkish. Available in: <https://goo.su/xkc3>
- [30] Berkel C, Cacan E. *In silico* analysis of DYNLL1 expression in ovarian cancer chemoresistance. *Cell Bio. Int.* [Internet]. 2020; 44(8):1598-1605. doi: <https://doi.org/gk58rp>
- [31] Berkel C, Cacan E. Involvement of ATMIN–DYNLL1–MRN axis in the progression and aggressiveness of serous ovarian cancer. *Biochem. Biophys. Res. Commun.* [Internet]. 2021; 570:74–81. doi: <https://doi.org/pg7v>
- [32] Cacan E, Ozmen ZC. Regulation of Fas in response to bortezomib and epirubicin in colorectal cancer cells. *J. Chemother.* [Internet]. 2020; 32(4):193–201. doi: <https://doi.org/pg7t>
- [33] Gülmez Y, Aydın A, Can İ, Tekin Ş, Cacan E. Cellular toxicity and biological activities of honey bee (*Apis mellifera* L.) venom. *Marmara Pharm. J.* [Internet]. 2017; 21(2):251-260. doi: <https://doi.org/pg7w>
- [34] Ariffin SMZ, Hasmadi N, Syawari NM, Sukiman MZ, Ariffin MFT, Hian CM, Ghazali MF. Prevalence and antibiotic susceptibility pattern of *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli* in dairy goats with clinical and subclinical mastitis. *J. Anim. Health Prod.* [Internet]. 2019; 7(1):32-37. doi: <https://doi.org/pg7x>
- [35] Mansor R, Diauddin NS, Syed-Hussain SS, Khalid SF. Antibiotic susceptibility of *Staphylococcus aureus* and *Escherichia coli* isolated from dairy goats in selected farms in Selangor, Malaysia. *J. Vet. Malaysia.* [Internet]. 2019; 31(1):12-16. doi: <https://doi.org/pg7z>
- [36] Bantaw K, Sah SN, Subba Limbu D, Subba P, Ghimire A. Antibiotic resistance patterns of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, *Shigella* and *Vibrio* isolated from chicken, pork, buffalo and goat meat in eastern Nepal. *BMC Res. Notes* [Internet]. 2019; 12(1):766. doi: <https://doi.org/pg72>
- [37] Rana EA, Fazal MA, Alim MA. Frequently used therapeutic antimicrobials and their resistance patterns on *Staphylococcus aureus* and *Escherichia coli* in mastitis affected lactating cows. *Int. J. Vet. Sci. Med.* [Internet]. 2022; 10(1):1-10. doi: <https://doi.org/pg73>
- [38] Shahidi F, Roshanak S, Javadmanesh A, Tabatabaei Yazdi F, Pirkhezranian Z, Azghandi M. Evaluation of antimicrobial properties of bovine lactoferrin against foodborne pathogenic

- microorganisms in planktonic and biofilm forms (*in vitro*). J. Consum. Prot. Food S. [Internet]. 2020; 15:277-283. doi: <https://doi.org/gn99n5>
- [39] Bursalioglu EO. Effect of cow colostrum, mare milk, and human milk on the viability of lung healthy and cancer cell lines. Iran Red. Crescent Med. J. [Internet]. 2021; 23(5):e409. doi: <https://doi.org/pg74>
- [40] Akca C, Vatan O, Yilmaz D, Huriyet H, Cinkilic N, Cavas T. *In vitro* cytotoxic and genotoxic effects of donkey milk on lung cancer and normal cells lines. Czech J. Food Sci. [Internet]. 2019; 37(1):29-35. doi: <https://doi.org/pg75>
- [41] Balagayathri R, Uma C, Sivagurunathan P, Rao VD, Suman P. Assessment of *In-vitro* Antimicrobial, Antioxidant and anticancer activity of bioactive peptides of the Goat Colostrum. Int. J. Pharma. Res. [Internet]. 2021; 13(3):1694-1708. doi: <https://doi.org/pg8c>
- [42] Tikhonov S, Chernukha I, Dunchenko N. Comparative evaluation antimicrobial and antitumor activities of natural colostrum peptide and its synthesized analogue. Food Sci. App. Biotechnol. [Internet]. 2024; 7(2):333-343. doi: <https://doi.org/pg8d>
- [43] Khan MZ, Xiao J, Ma Y, Ma J, Liu S, Khan A, Khan JM, Cao Z. Research development on anti-microbial and antioxidant properties of camel milk and its role as an anti-cancer and anti-hepatitis agent. Antioxidants. [Internet]. 2021; 10(5):788. doi: <https://doi.org/k7tt>
- [44] Bielecka M, Cichosz G, Czczot H. Antioxidant, antimicrobial and anticarcinogenic activities of bovine milk proteins and their hydrolysates—A review. Int. Dairy J. [Internet]. 2022; 127:105208. doi: <https://doi.org/pg8f>
- [45] Agarwal P, Gupta R. A review on anticancer property of colostrum. Res. Rev. J. Med. Health Sci. 2016 [cited 12 Dec. 2024]; 5(4):1-9. Available in: <https://goo.su/xwjU>
- [46] Kim H, Kim DE, Han G, Lim NR, Kim EH, Jang Y, Cho H, Jang H, Kim KH, Kim SH, Yang Y. Harnessing the natural healing power of colostrum: bovine milk-derived extracellular vesicles from colostrum facilitating the transition from inflammation to tissue regeneration for accelerating cutaneous wound healing. Adv. Healthc. Mater. [Internet]. 2022; 11(6):2102027. doi: <https://doi.org/pg8g>
- [47] Kelley NS, Hubbard NE, Erickson KL. Conjugated linoleic acid isomers and cancer. J. Nutr. [Internet]. 2007; 137(12):2599-2607. doi: <https://doi.org/gfvmts>
- [48] Samuel M, Sanwlani R, Pathan M, Anand S, Johnston EL, Ang CS, Liaskos MK, Mathivanan S. Isolation and Characterization of Cow-, Buffalo-, Sheep- and Goat-Milk-Derived Extracellular Vesicles. Cells [Internet]. 2023; 12(20):2491. doi: <https://doi.org/pg8j>
- [49] Mehra R, Sangwan K, Garhwal R. Composition and Therapeutic Applications of Goat Milk and Colostrum. J. Dairy Sci. Technol. [Internet]. 2021 [cited 12 Dec. 2024]; 10(2):1-7. Available in: <https://goo.su/JmGZEM>
- [50] Kim Y, Kim MJ, Han KS, Imm JY, Oh S, Kim SH. Anticancer activity of lactoferrin isolated from caprine colostrum on human cancer cell lines. Int. J. Dairy Technol. [Internet]. 2009; 62(2):277-281. doi: <https://doi.org/bwj5w>