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ANTICANCER ACTIVITY OF HYDRO-ALCOHOLIC EXTRACTS OF FINGER MILLET AGAINST BREAST AND LUNG CANCER CELL LINES

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Abstract
Globally cancer is the most public health concern and a common cause of death among all age groups, even with the availability of chemotherapeutic, radiation and surgical interventions. Phytochemicals are known for their anti-inflammatory, antioxidant and cancer-inhibitory activities. In the present study, GC-MS screening of finger millet extract was done along with impact assessment of methanolic and aqueous methanolic extracts of raw and fermented finger millet grains flour against breast and lung cancer cell lines (MTT assay) at various doses. GC-MS screening revealed the presence of phytosterols. The MTT assay confirmed the anticancer activity of finger millet extracts at 100, 200 and 400 µg/ml in a dose-dependent manner. However, the extract of the fermented samples prepared from aqueous methanol showed the highest cytotoxicity. So, there is a need for HPLC quantification of the screened phytochemicals and dose optimization for safety assessment. Furthermore, fermented samples should also be screened for compound identification and quantification.

Key words: finger millet, fermentation, GC-MS screening, breast cancer, lung cancer,

Introduction

The changing food systems driven by climatic calamities and reduced dietary diversity are increasing the risk of non-communicable diseases i.e., cancer, diabetes, cardiovascular, hepatic and renal disorders. Globally, 20 million people suffered from cancer in the year 2022 according to the estimates of the International Agency for Research on Cancer (IARC). The most frequently diagnosed form was lung cancer with 2.5 million cases (12.4 % of all cancers) followed by breast cancer (11.6 %). Lung cancer was the leading cause of death among all forms with 1.8 million mortalities (Bray et al., 2024). WHO recognized that optimum global public health could only be maintained by preventing the early onset of chronic diseases and ensuring healthier ageing. Epidemiological evidence suggests that despite the advancement, medical science faces the dual challenges of side effects and economic encumbrance of medicines (Thomas et al., 2023).

Hippocrates's ancient quote “Food is medicine and medicine is food” supports the dietary behavior modifications for improving human health. The food industries also focused on dietary diversity and products containing functional ingredients to meet growing global demands (Narchi, 2011). The finger millet (Ragi) is a cereal crop that belongs to the subfamily Chloridoideae of the Poaceae and has been reported to contain numerous phytochemicals including polyphenols, lignans, kaempferol, taxifolin, chlorogenic acid, hydroxybenzoic acid and hydroxycinnamic acid derivatives i.e., ferulic acid, proto catecheuic acid, syringic acid, cinnamic acid and caffeic acid (Sowunmi et al., 2025; Sunil., et al., 2024; Mitharwal et al., 2021). These phytochemicals are potent antioxidants and play anti-proliferative role by scavenging free radicals. Choi and Park, (2015) used ferulic acid (a constituent of finger millet-bound phenolics) against breast cancer cell line while Kawabata et al. (2000) used it against colon cancer in rats and reported a blocking effect on induced carcinogenesis. Few studies were conducted to check the impact of finger millet phytochemicals on cancer cell lines. However, finger millet fiber was extensively explored against colonic cancer and shown to prevent colon cancer (Gupta et al. 2023).

According to mechanistic insights by Hussain et al. (2016), phenolic compounds scavenge the free radicals and reduce cellular ROS thus augmenting anti-inflammatory reactions and apoptosis. Parallel to chemotherapeutic anticancer agents, finger millet phytochemicals induce apoptotic cell death and cancerous cells fail to repair the damage (Pfeffer and Singh, 2018). The characteristic features of apoptotic cells are their shrinkage, DNA breakage and apoptotic bodies' appearance. Several prior studies have demonstrated apoptosis induction by adding phytochemical-rich fractions of millets and cereals (Kuruburu et al., 2022). In a similar study, Narayanan et al. (2024) proposed that finger millet phytochemicals are natural inhibitors of MMP1 which may help in managing oral cancer. However, the molecular mechanism of the anti-proliferative activity of finger millet phytochemicals is still unclear. Nexus to the above, this study aims to evaluate the anticancer activity of hydro-alcoholic extracts of raw and fermented finger millet grains flour on breast cancer (MCF-7) and lung cancer cell line (A549).

Methodology

Procurement of raw material

Grains of the brown finger millet were procured from the district Muzaffargarh of South Punjab, Pakistan. The district is 404 feet above sea level, with hot summers and mild winters. The rainfall is ≈ 8.37mm, which is one rainy day/month. The district Muzaffargarh faces severe shortage of water, and tube well water is the main source of irrigation. The lack of transfer of ethnobotanical knowledge from generation to generation and the adoption of cash crops are the major reasons for the less acceptance of finger millet in the region.

Fermentation of finger millet grains flour:

Lyophilized *Lactiplantibacillus plantarum* V299 (0.2 mg) was cultured in 20 mL pre-sterilized falcon tubes containing 10 mL MRS broth, following the method of Lanza et al. (2020). The young

growing cells were inoculated to MRS (DeMann Ragosa Sharpe) agar containing Petri dishes and incubated at 30°C for 24 hours. The fresh colonies were harvested and transferred into 1.5ml falcon tubes and the contents were centrifuged at 13000 rpm. The supernatant was collected in falcon tubes and mixed with 6 mL of sterilized ringer solution. 400 mL distilled water and 100 g of finger millet flour were taken in fermentation jars followed by sterilization and cooling at 30°C. 750µL of *Lactiplantibacillus plantarum* culture was added to each fermentation jar and incubated at 37°C for 48 hours. The samples were dried in a dehydrator at 50°C after fermentation and ground to flour using a laboratory mill (LM-120) Perkin Elmer US and stored at room temperature for further use (Kitum et al., 2020).

Phytochemistry analysis by GC-MS

After preparing finger millet flour extract, the sample was subjected to GC-MS analysis using the procedure outlined by Nsofor et al. (2023). For extract preparation, 10 g of finger millet flour was taken in a 250 mL conical flask containing 100 mL of HPLC-grade acetonitrile. The contents were shaken for 24 hours using an orbital shaker and a stay time of 20 minutes was given to the contents after every 4 hours. Wattman filter paper No. 41 and vacuum-assisted membrane filtration were used to filter the supernatant twice. A rotary evaporator was used to concentrate the filtrate to one-fifth of its volume, the resulting extract was then used for GC-MS analysis after being filtered using a 0.23-micron syringe filter. A capillary column (DB-5ms) was used in the GC-MS. The initial column temperature was at 40°C which was gradually increased to 150°C with an increase in temperature of 100°C per minute. After that, the temperature was increased by 5°C every minute to 230°C and the same was continued until 280°C achieved. Injector port temperature stayed at 280°C while detector temperature was 250°C at the same time with 1ml/minute flow rate. The split ratio was set at 110.1 eV and the ionisation voltage at 70 eV.

Extract preparation for anticancer activity

Methanol and a mixture of aqueous methanol (30:70) were used as solvents to extract the finger millet phytochemicals. Solvent extraction was performed by taking 10 g of sample and 100 mL of solvent in a 250 mL conical flask. The sample and solvent-containing flasks were sonicated for 5 minutes using a sonicator (Elma E30H, California), followed by overnight shaking (HY-4A Laboratory shaker) and again sonicated for 5 minutes. The flasks were kept for 3 hours to sediment the solid contents and the supernatant was filtered using Whaatman filter paper no 41. The solvent from the filtrate was evaporated by a rotary evaporator (RE-2S-VD 1 L Rotovap) until 15 mL was left behind and the contents were stored for further use at 4°C (Meneses et al., 2013).

Culturing of breast and lung cancer cells

The cryo vials were revived from the liquid nitrogen storage in a flask containing DMEM-HG medium, supplemented with 10% fetal bovine serum (FBS), 100 mg/mL penicillin G (Sigma), and 100 U/mL streptomycin (Sigma). The cultures were maintained in a humidified incubator at 37°C with 5% CO2. Experiments were performed in triplicate. When the cultured cells reached 70-80% confluence they were sub-cultured. For splitting, the cells attached to the flask walls were washed with 1X phosphate-buffered saline (PBS) and incubated with 0.05% trypsin-EDTA until they detached from the flask surface. Detachment was confirmed using an inverted microscope. A few drops of FBS were added to the flask. The cell suspension was then transferred to a 15 ml tube and centrifuged at 2000 rpm for 5 minutes. After centrifugation, the supernatant was removed, and the cell pellet was re-suspended in a 96-well plate to perform MTT (Kaur et al., 2023).

Treatment of cell line with drug

Sample	**Treatment groups**		
Control	-	-	-
Methanolic extract of raw finger millet grains flour (1)	100 µL	200 µL	400 µL
Hydro-alcoholic extracts of raw finger millet grains flour (2)	100 µL	200 µL	400 µL
Methanolic extracts of fermented finger millet flour (3)	100 µL	200 µL	400 µL
Hydro-alcoholic extracts of fermented finger millet flour (4)	100 µL	200 µL	400 µL

The cultured cells were subjected to treatment for 24 hours in a 96-well plate.

MTT assay

MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay was performed to equate proliferative capability and drug toxicity. The monolayer of cells was initially washed with 1x phosphate buffer saline (PBS) (Invitrogen Inc., USA), afterwards wells were stained with 25 µl MTT solution (Invitrogen Inc., USA) for 3h. Cells were de-crystallized by adding DMSO (Invitrogen Inc., USA) and incubated for 24 hours. An ELISA plate reader was used to evaluate absorbance at 570 nm (Chuang et al, 2021).

Statistical analysis

The collected primary data was organized using MS Excel, means and standard deviation were taken using the same software however, the results were compared using statistix 8.1. Factorial design was opted for ANOVA (analysis of variance) to check the differences among control and samples at $p \leq 0.05\%$. furthermore, a Graphpad prism was used for descriptive statistics to check the relation (R square value). The means of the results were compared by Latin square design following the modified method of Navyashree et al. (2022).

Results:

The results of the GC-MS screening of finger millet flour extract showed that 38 peaks were detected unveiling the presence of fatty acid esters, phenols and sterols. Among phyto-sterols, dl-alpha. tocopherol, campesterol, vitamin E, stigmasterol, ginsenol, beta. -sitosterol; thymol, TMS derivative, gamma. -sitosterol, 3,5-proggesterol acetate and fucosterol were common constituents (Table no. 1). These phytochemicals are potential antioxidants and are linked with several health benefits thus making finger millet a health-promoting cereal. Phyto-sterols possess anti-inflammatory and anticancer activity along with supporting immune responses. However, a brief insight suggests that sterols inhibit the production of free radicles that prevent the proliferation of cancer cells and reduce the ageing process.

The MTT assay showed that control group has the highest absorbance, indicating the maximum cell viability or metabolic activity level. This serves as the baseline for comparison with treated groups. Methanolic extract of raw finger millet grains flour (100 µg/ml) showed a slight decrease in absorbance compared to the control but yielded no statistically significant results (Fig. 1). However, an increase in concentration (200 µg/ml) resulted a significant reduction in cell viability (***, $p < 0.001$), showing cytotoxic effects and a further increase in concentration from 200µg/ml to (400 µg/ml) presented a mild decrease in cell viability with a statistical significance (**, $p < 0.01$), indicating some cytotoxicity. When compared to the methanolic extract of raw finger millet grains flour, the hydro-alcoholic extracts of raw finger millet grains flour (B) at a concentration of 100 µg/ml showed reduced absorbance with higher statistical significance (***, $p < 0.001$), indicating greater cytotoxicity than extract (A). The 200 µg/ml of extract B further, showed a slight decline in absorbance with moderate significance (*, $p < 0.05$), and a mild cytotoxicity. The effect extract “B”

was dose-dependent and an increase in concentration (400 µg/ml) showed a drop in absorbance with higher statistical significance (***, $p < 0.001$), presenting a stronger cytotoxicity. The extracts of fermented samples (Fig. 2) showed more cytotoxic effects than raw samples at the same concentration levels as indicated in Fig. 1 & 2. Methanolic extracts of fermented finger millet flour (C) at a concentration of 400µg/ml showed more cytotoxic effects than 200 µg/ml and 100 µg/ml and the same was observed for the hydro-alcoholic extracts of fermented finger millet grains flour extract (D). 400 µg/ml of hydro-alcoholic extract of fermented finger millet grains flour showed a highly significant decrease and was more cytotoxic than all the other samples.

Table No. 1. GC-MS Screening of finger millet flour extract

Sr. No.	Peak No.	Peak Area %	Retention Time	Name of compound	Molecular Formula
	12	4.91	29.454	Vitamin E	C_{29} H_{50} O_2
	21	0.06	34.561	1-cis-Vaccenoylglycerol	C_{21} H_{40} O_4
	22	6.73	35.290	Campesterol	C_{28} H_{48} O
	26	6.29	36.552	Stigmasterol	C_{29} H_{48} O
	30	0.76	38.231	Ginsenol	C_{15} H_{26} O
	31	21.52	38.907	gamma.-Sitosterol	C_{29} H_{50} O
	31	21.52	38.907	beta.-Sitosterol	C_{29} H_{50} O
	32	1.78	39.223	Thymol, TMS derivative	C_{13} H_{22} O Si
	33	1.25	39.443	Fucosterol	C_{29} H_{48} O
	33	1.25	39.443	3,5-Proggesterol acetate	C_{24} H_{34} O_4
	35	0.59	39.913	2-Myristynoyl-glycinamide	C_{16} H_{28} N_2 O_2

Table No. 2. Descriptive statistics of breast cancer cell line (MCF-7)

	Control	A(100µg/ml)	A(200µg/ml)	A(400µg/ml)	B(100µg/ml)	B(200µg/ml)	B(400µg/ml)	C(100µg/ml)	C(200µg/ml)	C(400µg/ml)	D(100µg/ml)	D(200µg/ml)	D(400µg/ml)
Mean	0.9824	0.9717	0.8253	0.7600	0.9179	0.8155	0.7700	0.8599	0.7837	0.6857	0.7070	0.4672	0.3986
Std. Deviation	0.09235	0.03612	0.06329	0.04583	0.04714	0.07237	0.05970	0.02762	0.09397	0.1242	0.06248	0.1076	0.009220
R square													**0.879**

G_0 = Control
G_A = Methanolic extract of raw finger millet grains flour
G_B = Hydro-alcoholic extracts of raw finger millet grains flour
G_C = Methanolic extracts of fermented finger millet flour
G_D = Hydro-alcoholic extracts of fermented finger millet flour

Table No. 3. Descriptive statistics of lung cancer cell line (A549)

	Control	A(100µg/ml)	A(200µg/ml)	A(400µg/ml)	B(100µg/ml)	B(200µg/ml)	B(400µg/ml)	C(100µg/ml)	C(200µg/ml)	C(400µg/ml)	D(100µg/ml)	D(200µg/ml)	D(400µg/ml)
Mean	1.152	1.121	0.8916	0.8214	0.8926	0.8443	0.7765	0.9168	0.8528	0.7932	0.8298	0.6934	0.5868
Std. Deviation	0.05428	0.09660	0.07063	0.05190	0.04967	0.04677	0.06434	0.02219	0.04466	0.09146	0.03133	0.08850	0.06461
R square													**0.858**

G_0 = Control
G_A = Methanolic extract of raw finger millet grains flour

G_B = Hydro-alcoholic extracts of raw finger millet grains flour
G_C = Methanolic extracts of fermented finger millet flour
G_D = Hydro-alcoholic extracts of fermented finger millet flour

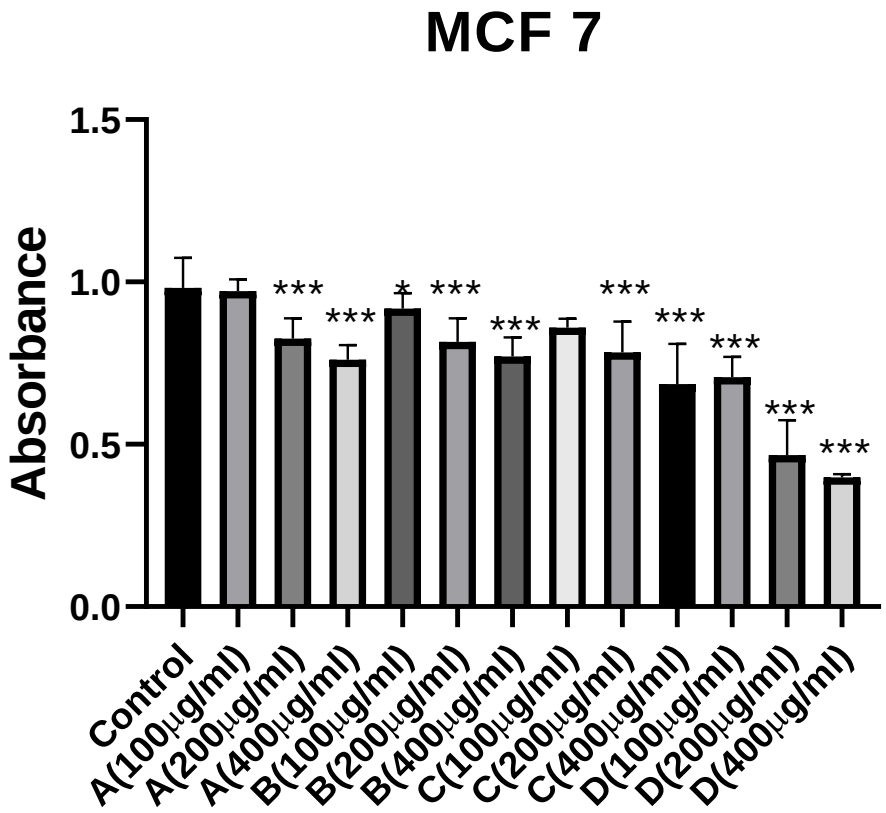

Figure 1: Showing MTT assay to assess the impact of a drug on breast cancer cells. Where *,*** showing the significant difference between control and treated groups ranged from 100µg/ml-400µg/ml leads to significant reductions in cell viability, indicating a dose-dependent cytotoxic effect. The most substantial reduction in cell viability is seen in Treatment Group D (400 µg/ml), with the lowest absorbance and highest statistical significance, suggesting it is the most cytotoxic treatment ($P \leq 0.05$)

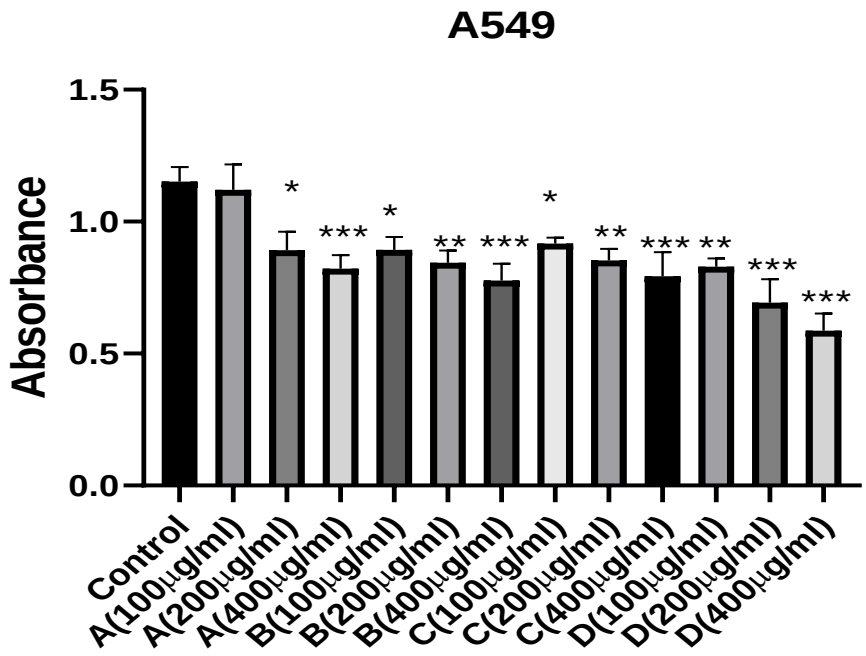

Figure 2: Showing MTT assay to assess the impact of the drug on Lung cancer cells. Where *,**,*** showing the significant difference between control and treated groups ranged from 100ug/ml-400ug/ml leads to significant reductions in cell viability, indicating a dose-dependent cytotoxic effect. The most substantial reduction in cell viability is seen in Treatment D (400 µg/ml), with the lowest absorbance and highest statistical significance, suggesting it is the most cytotoxic treatment ($P \leq 0.05$)

Discussion

The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay provided insightful results on lung and breast cancer cell lines and divulged that methanolic and hydro-alcoholic extracts of raw and fermented finger millet grains flour showed cytotoxic effects at 100, 200 and 400 µg/ml, as confirmed by changes in the metabolic activity and viability of the cells. The highest absorbance was shown by the control group serving as a baseline for the comparative evaluation of the cytotoxic effects of different treatments on cell viability. Methanolic and hydro-alcoholic extracts of raw and fermented finger millet flour presented dose-dependent cytotoxicity with maximum reduction in cell viability at the concentration of 400 µg/ml. However, non-significant results were observed when lower doses (100 µg/ml) of the methanolic extracts of raw finger millet flour were tested against liver and breast cancer cell lines. But increased higher dose (200 µg/ml) of the same sample reduced cell viability showing significant cytotoxicity (***, $p < 0.001$). The dose-depended relationship indicates that higher concentrations may surpass the threshold levels of cytotoxicity. A similar trend was observed for the hydro-alcoholic extracts of raw finger millet grains flour (G_B), with mild cytotoxicity at a concentration of 100 µg/ml ($p < 0.01$) and markedly reduced cell viability at 200 µg/ml and showed dose-dependent cytotoxicity.

The results of methanolic and hydro-alcoholic extracts of fermented finger millet flour were significant ($p < 0.05$) even at lower doses i.e., 100 µg/ml. Nonetheless, higher doses (200 and 400 µg/ml) showed surprising results against cell viability, highlighting an impactful cytotoxic effect on A549 cells. Again this dose-dependent relationship of methanolic and hydro-alcoholic extracts was prominent on breast and lung cancer cell lines. The increased cytotoxic effect of extracts prepared from fermented samples was an insight into further investigation (GC-MS screening and HPLC-based quantification of bioactive compounds). Among all samples, hydro-alcoholic extracts of fermented (G_C) finger millet flour showed a dramatic decline in cell viability (***, $p < 0.001$).

Comparative to all other tested samples, the 200 µg/ml of "G_C" exhibited the highest cytotoxicity with the lowest absorbance thus inhibiting cell viability. The results align with the previous findings and demonstrate the cytotoxic potential of similar compounds on cell lines, further supporting the notion that increasing drug concentrations induces stronger cytotoxic responses. The dose-dependent relationship is consistent with pharmacological principles, thus validating the notion that increased exposure to a potent cytotoxic drug inhibits cell viability and proliferation. There is only one study by Kuruburu et al. (2022) on the anticancer activity of finger millet-free and bound phenolics against colorectal and breast cancer ascribing it to tannins and phenolic compounds. However, numerous scientists explored the anticancer potential of different phytochemicals and linked it with cellular toxicity and apoptosis.

In a study on the anticancer activity of campesterol, Majumder et al. (2024) concluded that phytosterols bind estrogen receptor alpha (ERα) thus treating breast cancer. The mechanistic insight on anticancer activity of stigmasterol by Zhang et al. (2022) revealed that stigmasterol promotes apoptosis, inhibits cell proliferation and induces autophagy in cancer cells by regulating the PI3K/Akt signalling pathway and generates mitochondrial reactive oxygen species mainly affecting cyclin proteins and CKD (cyclin-dependent kinase). Furthermore, phytosterols are involved in chromatic dissolution, DNA fragmentation and mitochondrial membrane suppression thus resulting in crescent-shaped nuclei (Hanif et al., 2023). These mechanistic insights support the results and validate that finger millet phytochemicals act to prevent breast and lung cancer.

Conclusion

In conclusion, the GC-MS screening of finger flour extracts highlighted the presence of variety of phytochemicals particularly phytosterols i.e., tocopherol, 1-cis-Vaccenoylglycerol, Campesterol, Stigmasterol, Ginsenol, gamma.-Sitosterol, beta.-Sitosterol, Thymol, TMS derivative, Fucosterol, 3,5-Proggesterol acetate and 2-Myristynoyl-glycinamide. However, these phyto-constituents of finger millet must be quantified further to evaluate their anticancer potential. MTT assay results were highly significant for methanolic and aqueous-methanol extracts but the fermented samples unveiled greater anticancer activity against lung and breast cancer cell lines so, fermented samples should be evaluated further to investigate the potent compounds. Further research should explore the mechanisms of action behind these effects and evaluate the selective toxicity of these compounds compared to non-cancerous cells to assess their potential as cancer therapeutics.

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Author's Contributions

The writers of the original manuscript, Muhammad Amir, Prof. Dr. Saeed Akhtar and Dr. Tariq Ismail were responsible for conceptualization, evaluating, and editing. Wisha Saeed and Tahir Maqbool handled the formal analysis, research, funding procurement, reviewing, and editing. Resources, Syed Zeeshan Haider Naqvi and data curation and oversight, Dr. Muhammad Naeem Zubairi

Declaration of Conflicting Interests

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References

1. Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., & Jemal, A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 74(3), 229-263.
2. Thomas, S. A., Browning, C. J., Charchar, F. J., Klein, B., Ory, M. G., Bowden-Jones, H., & Chamberlain, S. R. (2023). Transforming global approaches to chronic disease prevention and management across the lifespan: integrating genomics, behavior change, and digital health solutions. Frontiers in public health, 11, 1248254.
3. Narchi, N.E. (2011). One knowledge, two conduits: the social, demographic, and toxicological factors that govern seri ethnomedicine. The University of Georgia. PhD dissertation. https://getd.libs.uga.edu/pdfs/narchi_nemer_e_201105_phd.pdf
4. Choi YE, Park E. (2015). Ferulic acid in combination with PARP inhibitor sensitizes breast cancer cells as chemotherapeutic strategy. Biochem Biophys Res Commun. 13;458(3):520-524.
5. Kawabata, K., Yamamoto, T., Hara, A., Shimizu, M., Yamada, Y., Matsunaga, K., Tanaka, T., & Mori, H. (2000). Modifying effects of ferulic acid on azoxymethane-induced colon carcinogenesis in F344 rats. Cancer letters, 157(1), 15–21.
6. Gupta, M., Asfaha, D. M., & Ponnaiah, G. (2023). Millets: A Nutritional Powerhouse With Anti-cancer Potential. Cureus, 15(10), e47769.
7. Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M. C., & Rahu, N. (2016). Oxidative Stress and Inflammation: What Polyphenols Can Do for Us?. Oxidative medicine and cellular longevity, 2016, 7432797.
8. Nsofor, W.N. Nwaoguikpe, R.N. Ujowundu, F.N. Keke, C.O. Uba, M.T.U. Edom, C.V. 2023. Phytochemical, GC-MS, FTIR and Amino acid profile of methanol extract of Tetrapleura tetraptera fruit. J. Drug Deliv. Ther. 13(2), 61-69.
9. Pfeffer, C. M., & Singh, A. T. K. (2018). Apoptosis: A Target for Anticancer Therapy. International journal of molecular sciences, 19(2), 448.
10. Sathiya Narayanan, S., Kanishka, R., Sowmiyan, M. N., Nithish, V., Kumar, V. R., Mary, A. V., & Kesavan, R. (2024). Targeting MMP1 in Oral Cancer: In silico analysis of phytochemicals from finger millet as potential inhibitors.
11. Kitum, V. C., Kinyanjui, P. K., Mathara, J. M., & Sila, D. N. (2020). Effect of Lb. plantarum BFE 5092 fermentation on antinutrient and oligosaccharide composition of whole red haricot bean (Phaseolus vulgaris L). International Journal of Food Science, 2020, 1-8.
12. Meneses, N. G., Martins, S., Teixeira, J. A., & Mussatto, S. I. (2013). Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains. Separation and purification technology, 108, 152-158.
13. Chuang, W. Y., Lin, L. J., Hsieh, Y. C., Chang, S. C., & Lee, T. T. (2021). Effects of Saccharomyces cerevisiae and phytase co-fermentation of wheat bran on growth, antioxidation, immunity and intestinal morphology in broilers. Animal bioscience, 34(7), 1157.
14. Kaur, G., Bhadada, S. K., Sachdeva, N., Saikia, U. N., Dahiya, D., Seth, S., Raik, S., Behera, A., & Rao, S. D. (2023). Establishment and characterization of long-term human primary parathyroid tumor subclones derived from Indian PHPT. 3 Biotech, 13(5), 161. https://doi.org/10.1007/s13205-023-03540-z
15. Navyashree, N., Sengar, A. S., Sunil, C. K., & Venkatachalapathy, N. (2022). White Finger Millet (KMR-340): A comparative study to determine the effect of processing and their characterisation. Food Chemistry, 374, 131665.
16. Sowunmi, L. I., Babalola, Y. O., & Ogunbamowo, P. O. (2025). The Cultivation, Nutritional Polyphenolic Contents, Bioavailability, and Health Benefits of Finger Millet (Eleusine coracana (L.) Gaertn.): An Updated Review. Food Security and Nutrition, 97-116.

17. Sunil, C. K., Gowda, N. N., Nayak, N., & Rawson, A. (2024). Unveiling the effect of processing on bioactive compounds in millets: Implications for health benefits and risks. Process Biochemistry.
18. Mitharwal, S., Kumar, S., & Chauhan, K. (2021). Nutritional, polyphenolic composition and in vitro digestibility of finger millet (Eleusine coracana L.) with its potential food applications: a review. Food Bioscience, 44, 101382.
19. Kuruburu, M. G., Bovilla, V. R., Naaz, R., Leihang, Z., & Madhunapantula, S. V. (2022). Variations in the anticancer activity of free and bound phenolics of finger millet (Eleusine coracana (L) Gaertn; variety KMR-301) seeds. Phytomedicine Plus, 2(2), 100276.
20. Zhang, X., Wang, J., Zhu, L., Wang, X., Meng, F., Xia, L., & Zhang, H. (2022). Advances in Stigmasterol on its anti-tumor effect and mechanism of action. Frontiers in oncology, 12, 1101289.
21. Majumder, R., Banerjee, S., Mandal, M., Patra, S., Das, S., & Mandal, M. (2024). A Virtual Drug Discovery Screening Illuminates Campesterol as a Potent Estrogen Receptor Alpha Inhibitor in Breast Cancer. Journal of Medicinal Chemistry.
22. Hanif, A., Ibrahim, A. H., Ismail, S., Al-Rawi, S. S., Ahmad, J. N., Hameed, M., ... & Tanwir, S. (2023). Cytotoxicity against A549 human lung cancer cell line via the mitochondrial membrane potential and nuclear condensation effects of nepeta paulsenii briq., a perennial herb. Molecules, 28(6), 2812.