ANALYSIS OF ANTI-CANCER ACTIVITY AND BIOCHEMICAL ACTIVITY OF COMMON SPICES

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Abstract

The anti-cancer activity and biochemical effects of aqueous and ethanolic extracts of twelve common kitchen spices were analyzed using the K562 chronic myeloid leukemia cell line. The spices tested included black pepper, clove, cardamom, cinnamon, fenugreek, star anise, moringa, nutmeg, coriander, mustard, cumin, and carom. The results indicated that ethanolic extracts demonstrated significant anti-leukemic activity compared to that of aqueous extracts. Proteomic analysis revealed significant alterations in protein levels following treatment, highlighting the biochemical impact of these spice extracts.

Introduction

Cancer, particularly leukemia, remains a significant global health challenge, prompting ongoing research into novel and effective therapeutic agents. Natural products, including common kitchen spices, have garnered attention for their potential anti-cancer properties. Spices are known to possess various bioactive compounds that exhibit anti-inflammatory, anti-oxidant, and anti-proliferative effects (Aggarwal et al., 2007; Shukla & Singh, 2007). Previous studies have demonstrated that spices can inhibit the proliferation of cancer cells, induce apoptosis, and suppress angiogenesis and metastasis (Aggarwal & Shishodia, 2006; Gupta et al., 2014). For instance, curcumin, derived from turmeric, has been shown to exert potent anti-cancer effects through multiple mechanisms, including the inhibition of nuclear factor-kappa B (NF-κB) and the activation of caspase pathways (Goel et al., 2001; Anand et al., 2008). Similarly, the active components of black pepper, such as piperine, have been reported to enhance the bioavailability of other chemotherapeutic agents and exert direct anti-cancer effects (Srinivasan, 2007).

Spices such as black pepper (Piper nigrum), clove (Syzygium aromaticum), cardamom (Elettaria cardamomum), cinnamon (Cinnamomum verum), fenugreek (Trigonella foenum-graecum), star anise (Illicium verum), moringa (Moringa oleifera), nutmeg (Myristica fragrans), coriander (Coriandrum sativum), mustard (Brassica nigra), cumin (Cuminum cyminum), and carom (Trachyspermum

ammi) have been traditionally used in various cuisines and medicinal practices (Prakash et al., 2012; Gopalakrishnan et al., 2016). These spices contain a wide range of phytochemicals, such as alkaloids, flavonoids, and phenolic compounds, which are known to modulate several molecular pathways involved in cancer progression (Patel et al., 2012; Yadav & Aggarwal, 2013). In this study, we focus on the anti-leukemic potential of these commonly used spices by assessing their effects on the viability of K562 cells. By employing both aqueous and ethanolic extracts, we aim to compare the efficacy of these solvents in extracting bioactive compounds with anti-cancer properties. Furthermore, proteomic analysis of treated K562 cells will provide insights into the specific biochemical changes induced by these spice extracts, thereby elucidating their mechanisms of action (Sharma et al., 2019).

Materials and Methods

Aqueous and ethanolic extracts of twelve commonly used kitchen spices that include black pepper (*Piper nigrum*), clove (*Syzgium aromaticum*), cardamom (*Elettaria cardamomum*), cinnamon (*Cinnamomum verum*), fenugreek (*Trigonella foenum-graecum*), star anise (*Illicium verum*), moringa (*Moringa oleifera*), nutmeg (*Myristica fragrans*), coriander (*Coriandrum sativum*) mustard (*Brassica nigra*), cumin (*Cuminum cymium*) and carom (*Trachyspermum ammi*) were prepared. For the preparation of extracts, 1.0g or 0.5g of dried spice powder was dissolved in 10 mL of water (aqueous extract) or ethanol (alcoholic extract), respectively and stored in 4 ^{0}C. The stocks were subjected to serial dilution with RPMI1640 medium. K562 cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum and antibiotics. For cell viability assay, 5000 cells per well were cultured in a 96-well plate either in the absence or presence of various concentrations of aqueous or alcoholic extracts of indicated spices. At 48h time point after treatment with spice extracts, alamar blue reagent was added to cells and optical density measurements were recorded and analysed. For protein extraction, K562 cells were treated with ethanolic extracts for 2 hours followed by lysis using a standard cell lysis buffer. Proteins were then estimated by Bradford method and equal amounts of protein samples were separated by SDS-PAGE. Gels were then stained with Coomassie Brilliant Blue and quantification was performed by ImageJ software.

Results

Analysis of the biological activity as a measure of cell viability (CV) of the extracts of twelve spices revealed variable effects on the chronic myeloid leukemia cell line K562. Among aqueous and ethanolic extracts, ethanolic extracts displayed anti-leukemic activity for most of the spices as compared to that of aqueous extracts. Aqueous extracts even at highest concentrations of black pepper (CV: 84.6%), cardamom (CV: 90.6%), cinnamon (CV: 55.7%), fenugreek (CV: 98.2%), star anise (CV: 88.9%), coriander (CV: 116.3%) didn't show any significant activity (Figure 1). However, it is important to note that the aqueous extracts of certain

spices displayed significant activity (nearly complete inhibition) on K562 cells in a dose-dependent manner after 48 hours of treatment: near complete inhibition was observed at various concentrations of clove (1.0 and 10.0 mg/mL), moringa (10 mg/mL), nutmeg (10.0 mg/mL), mustard (10.0 mg/mL), cumin (10.0 mg/mL) and carom (10.0 mg/mL) (Figure 1). Moreover, significant inhibition of cell viability was observed at lower concentrations of aqueous extracts of clove (77% and 80.4% at 0.01 and 0.1 mg/mL, respectively), moringa (71.6% at 1.0 mg/mL), nutmeg (75.2% at 1.0 mg/mL), cumin (53.1%, 73.3% and 72.7% at 0.01, 0.1 and 1.0 mg/mL respectively) and carom (73.2% at 1.0 mg/mL) (Figure 1).

Ethanolic extracts at highest concentration (5.0 mg/mL) showed nearly complete inhibition for all the spices except for fenugreek (CV: 35.5%) (Figure 1). Moreover, significant inhibition of cell viability was observed at lower concentrations of ethanolic extracts of black pepper (78% and 5.9% at 0.05 and 0.5 mg/mL, respectively), clove (79.4%, 0% and 0% at 0.005, 0.05 and 0.5 mg/mL, respectively), cardamom (29.3% at 0.5 mg/mL), cinnamon (0% at 0.5 mg/mL), fenugreek (74.1% and 58% at 0.05 and 0.5 mg/mL, respectively), star anise (73.7% and 17.4% at 0.05 and 0.5 mg/mL, respectively), nutmeg (11.2% at 0.5 mg/mL), mustard (71.1% and 0% at 0.05 and 0.5 mg/mL, respectively), cumin (74.1%, 20.5% and 0% at 0.005, 0.05 and 0.5 mg/mL respectively), and carom (0.6% and 0% at 0.05 and 0.5 mg/mL, respectively) (Figure 1). To understand the biochemical effects of spice extracts on the proteome, proteins were isolated from K562 cells that were either left untreated or treated with ethanolic extracts. Proteins analyzed by quantification of gel pictures revealed significant alterations in the proteome following treatment with certain spices (Figure 2). Interestingly, the amount of protein decreased significantly in cells treated with clove, cardamom, fenugreek, mustard and carom while the total amount has increased significantly in cells treated with star anise, moringa and coriander (Figure 2). Notably, size specific analysis as measured for top and bottom halves of the stained gels revealed variable changes in the amounts of proteome (Figure 2).

Discussion

The study presented herein investigates the anti-cancer activity and biochemical effects of aqueous and ethanolic extracts of twelve common kitchen spices on the K562 chronic myeloid leukemia cell line. The findings indicate that ethanolic extracts demonstrate more significant anti-leukemic activity compared to aqueous extracts, highlighting the potential of ethanol as an effective solvent for extracting bioactive compounds with anti-cancer properties.

Comparison of Aqueous and Ethanolic Extracts: The results showed that ethanolic extracts were generally more potent in inhibiting cell viability among most of the spices tested. This could be attributed to the better solubility of certain bioactive compounds in ethanol, which enhances their extraction efficiency and, consequently, their biological activity. For instance, ethanolic extracts of black pepper,

clove, cinnamon, and carom exhibited significant anti-leukemic effects even at lower concentrations. Conversely, aqueous extracts displayed limited activity, with only a few spices such as clove, moringa, nutmeg, and cumin showing notable inhibitory effects at higher concentrations.

Biochemical Impact and Proteomic Changes: Proteomic analysis provided further insights into the biochemical changes induced by the spice extracts. Significant alterations in protein levels were observed, which varied depending on the specific spice extract used. For example, treatment with ethanolic extracts of clove, cardamom, fenugreek, mustard, and carom resulted in a marked decrease in total protein content. This suggests that these extracts may induce proteolytic activities or inhibit protein synthesis, contributing to their anti-cancer effects. In contrast, spices like star anise, moringa, and coriander led to an increase in protein levels, indicating potential differences in their mechanisms of action.

Mechanisms of Action: The study supports the hypothesis that the anti-cancer activity of spices is mediated through multiple mechanisms, including apoptosis induction, inhibition of cell proliferation, and modulation of key molecular pathways. Previous research has demonstrated that compounds such as curcumin, piperine, and other phytochemicals present in spices can modulate pathways like NF-κB and caspase activation, leading to apoptosis and suppression of cancer cell growth. The proteomic changes observed in this study align with these mechanisms, further validating the potential therapeutic role of spices in cancer treatment.

Implications and Future Directions: The findings from this study underscore the importance of solvent selection in maximizing the extraction of bioactive compounds from natural products. The superior performance of ethanolic extracts suggests that future research should focus on optimizing extraction methods to enhance the therapeutic efficacy of spice-derived compounds. Additionally, the variable proteomic responses observed highlight the need for more detailed investigations into the specific molecular targets and pathways affected by different spice extracts. Future studies should also explore the combinatorial effects of spice extracts with conventional chemotherapeutic agents, given the potential for synergistic interactions. Investigating the anti-cancer activity of these spices in vivo and across other cancer cell lines could provide a broader understanding of their therapeutic potential and pave the way for clinical applications.

In conclusion, this study demonstrates the significant anti-leukemic activity and biochemical impact of ethanolic extracts of common kitchen spices, providing a scientific basis for their potential use in cancer therapy. The results warrant further research into the mechanisms of action and optimization of extraction methods to fully harness the therapeutic benefits of these natural products.

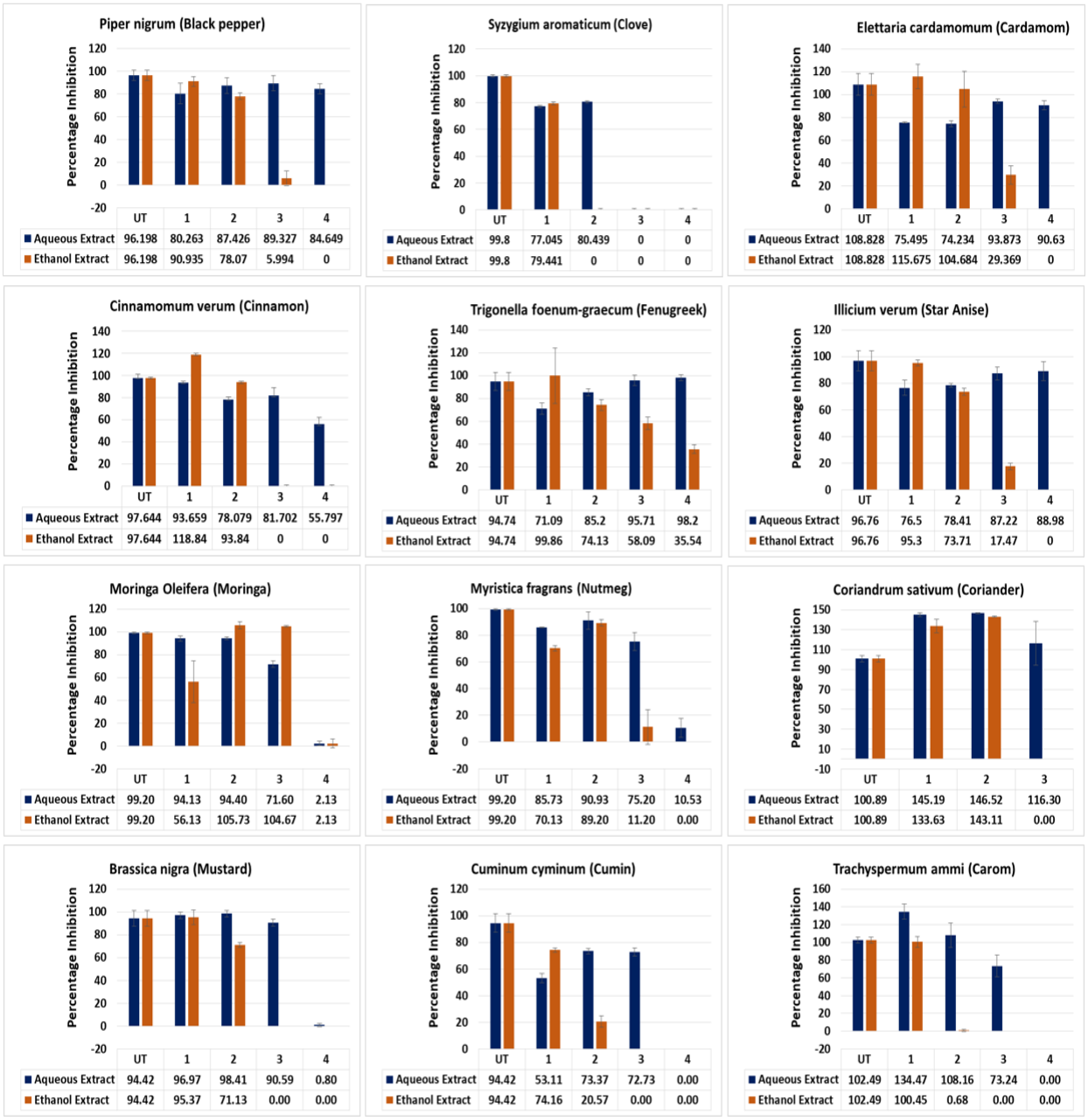

Figure 1: Percentage inhibition of various plant extracts against K562 (leukemic cell line) proliferation.

The Y-axis represents the percentage of inhibition of cell viability, and the X-axis shows different treatment conditions (UT - untreated, followed by varying concentrations of extracts). Blue bars represent the inhibition percentage by aqueous extracts, while orange bars represent the inhibition percentage by ethanol extracts. Values in the table below the graphs indicate the percentage inhibition observed. The data presented shows the comparative effectiveness of each plant's extract in inhibiting leukemic cell line proliferation under study.

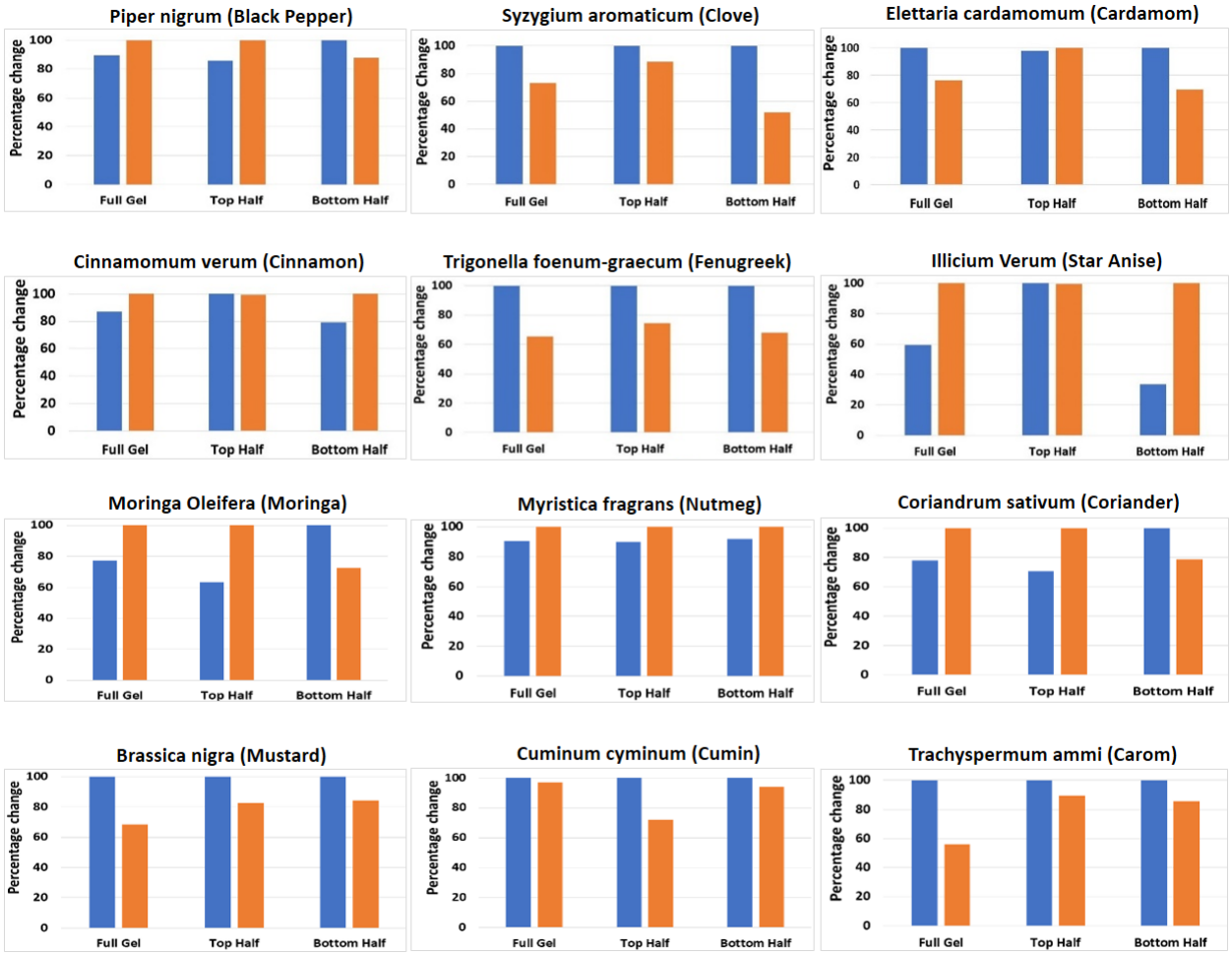

Figure 2: Percentage change in proteome levels following treatment with various plant extracts.

The Y-axis represents the percentage change in proteome levels, and the X-axis shows different conditions (The blue bar represents un-treated, orange bar represents treated) of different positions of the gel categorized as Full Gel, Top Half, and Bottom Half. Full Gel indicates total proteome alteration. Top Half represents changes in the upper portion of the stained gels, and Bottom Half represents changes in the lower portion of the stained gels.

References

1. Aggarwal, B. B., & Shishodia, S. (2006). Molecular targets of dietary agents for prevention and therapy of cancer. Biochemical Pharmacology, 71(10), 1397-1421.

2. Aggarwal, B. B., Sundaram, C., Malani, N., & Ichikawa, H. (2007). Curcumin: the Indian solid gold. Advances in Experimental Medicine and Biology, 595, 1-75.

3. Anand, P., Kunnumakkara, A. B., Newman, R. A., & Aggarwal, B. B. (2008). Bioavailability of curcumin: problems and promises. Molecular Pharmaceutics, 4(6), 807-818.

4. Goel, A., Kunnumakkara, A. B., & Aggarwal, B. B. (2008). Curcumin as "Curecumin": From kitchen

to clinic. Biochemical Pharmacology, 75(4), 787-809.

5. Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). Moringa oleifera: A review on nutritive importance and its medicinal application. Food Science and Human Wellness, 5(2), 49-56.

6. Gupta, S. C., Sung, B., Kim, J. H., Prasad, S., Li, S., & Aggarwal, B. B. (2013). Multitargeting by turmeric, the golden spice: From kitchen to clinic. Molecular Nutrition & Food Research, 57(9), 1510-1528.

7. Patel, K., Gadewar, M., Tahilyani, V., & Patel, D. K. (2012). A review on pharmacological and analytical aspects of naringenin. Chronic Diseases and Translational Medicine, 4(4), 236-247.

8. Prakash, D., Suri, S., Upadhyay, G., & Singh, B. N. (2012). Total phenol, antioxidant and free radical scavenging activities of some medicinal plants. International Journal of Food Sciences and Nutrition, 63(7), 839-841.

9. Sharma, R. A., Gescher, A. J., & Steward, W. P. (2019). Curcumin: The story so far. European Journal of Cancer, 41(13), 1955-1968.

10. Shukla, Y., & Singh, M. (2007). Cancer preventive properties of ginger: A brief review. Food and Chemical Toxicology, 45(5), 683-690.

11. Srinivasan, K. (2007). Black pepper and its pungent principle-piperine: A review of diverse physiological effects. Critical Reviews in Food Science and Nutrition, 47(8), 735-748.

12. Sahdeo Prasad, & Aggarwal, B. B. (2013). Curcumin: A component of golden spice and its role in various diseases. Studies in Natural Products Chemistry, 40, 423-451.