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Original Article

Metabolomics Insight into *PN01* and *SA02*: Anticancer Efficacy and Antidiabetic Enzyme Inhibition

Syeda Tayyaba Asif[1,*], Mohtasheem Ul Hassan[1], Shah Ali Ul Qader[2], Madiha Jamshed[1]

[1]Department of Pharmacognosy, University of Karachi, Karachi, PAKISTAN.
[2]Department of Biochemistry, University of Karachi, Karachi, PAKISTAN.

ABSTRACT

Background: Cancer is a major global health problem, with nearly 10 million deaths recorded in 2020. Approximately 30% of cancers in low- and lower-middle-income nations stem from infectious agents like Human Papillomavirus (HPV). Cervical Cancer (CC03) and HPV is now current issue all over the world especially Pakistani,breast cancer (BC04) and Colorectal Cancer (CRC05) are alarmingly common, while Pancreatic Cancer (PC06) is less frequent but has a high mortality rate. Diabetes also has highest prevalence rate globally, glucoamylase enzyme inhibition has reportedly providing a novel way in management of diabetes. **Objectives:** Regular consumption of spices in an appropriate amount has been linked to reduce a death rate from chronic disorders. **Materials and Methods:** Identification of phytochemicals of commonly used spices named; *Piper nigrum* L., *(PN01)* and *Syzygium aromaticum (SA02)* by GCMS and study their role in multiple cancer. Assay of glucoamylase enzyme inhibitory potential of *PN01* and *SA02* for diabetes management. Therefore, the purpose of the present study is to find out the glucoamylase inhibitory potential of *PN01* and *SA02* at different parameters such as pH, temperature, time interval and days, which was carried out by using glucoxidase kit method. Statistical analysis was done by two-way ANOVA. For this purpose, phosphate buffer solution of *PN01* and *SA02* were used. **Results:** Total 16 bioactive active compounds were found during GC-MS scanning of methanol *PN01* fruits extract wherein major are caryophylline, d-limonene, piperine, α-pinene, copaene and α-caryophylline. Total 14 secondary metabolites identified in *SA02* dried flower buds extract included eugenol (100%), caryophylline (31.39%), and phenol, 2-methoxy-4-(2-propenyl), acetate (23.76%), *PN01* showed maximum glucoamylase inhibition at 60 min, (91.59±0.99), day 3 (89.94±1.23), 37°C temperature (72.71±6.76) and at pH 5 (65.60 ± 4.56), while *SA02* exerted significant glucoamylase inhibition at 37°C (96.05±1.27), 60 min (92.39±2.52) and day 3(91.36±4.39). **Conclusion:** The present study showed that *PN01* and *SA02* phytochemicals identified by GCMS are valuable for multiple cancer treatment. This study also provides the direct evidence that *PN01* and *SA02* extracts have great inhibitory potential of glucoamylase enzyme so; by using these ideal conditions it will be helpful in diabetes and obesity management.

Keywords: Breast Cancer (BC04), Cervical Cancer (CC03), Colorectal Cancer (CRC05), Diabetes, Gas Chromatography Mass Spectrometry (GCMS), Glucoamylase, Human Papillomavirus (HPV), Pancreatic Cancer (PC06), *Piper nigrum* (PN01), *Syzygium aromaticum* (SA02).

Correspondence:
Dr. Syeda Tayyaba Asif
Department of Pharmacognosy,
University of Karachi, KU, 75270, Karachi,
PAKISTAN.
Email: tayyaba8@yahoo.com

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INTRODUCTION

Cancer, a disease marked by the uncontrolled multiplication of cells, is a most prevalent cause of death internationally. Cancer is not a single disease, but a group of over 100 diseases, typically classified by the organ or tissue where the cancer originated. Currently, cancer is treated through a variety of methods, combining conventional approaches like surgery, radiation therapy and chemotherapy with more modern or targeted therapies such as hormone therapy, immunotherapy and nano therapy (Madihalli *et al*., 2022). The global increase in chronic diseases poses a significant challenge and rising health care costs are driving increased research interest. Various food items provide the public with various health benefits due to their secondary metabolites including reduced cancer risk (Christine *et al*., 2008).

In Pakistan, the annual age-standardized CC03 incidence rate is 5.4/100,000 women, with an estimated 4,762 new cases in 2023, and a mortality rate of 3.6/100,000 women from 3,069 deaths in the same year. CC03 is the third most frequently diagnosed cancer among women in Pakistan and the second most common in the 15-44 age groups, with the HPV being the primary cause (Singh *et al*., 2023). A virus infects cervical cells, where it's E6 and E7 proteins block the normal function of p53 and pRB. This allows the cells to grow out of control, leading to abnormal cell

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changes known as dysplasia. Cervical dysplasia can progress slowly over many years into Cervical Intraepithelial Neoplasia (CIN) and eventually invasive CC03 (Haiyan *et al*., 2016). BC04 is the most frequent type of cancer among women, in 103 out of 154 countries, cancer is the leading cause of death. In 2018, a total of 2.1 million new breast cancer cases were diagnosed, which accounted for 2.24% of all new cancer cases. The mortality rate for breast cancer was approximately 15.00% (Bray *et al*., 2018). CRC05's global impact is shifting, while historically concentrated in developed nations, its incidence is growing in developing countries and the overall burden is expected to rise with population aging and the spread of "westernized" lifestyles, such as changes in diet and physical activity (Pasqualino *et al.*, 2016). Worldwide, PC06 ranked as the 12th most frequent cancer which is also responsible for the 7th most cancer deaths (Junjie *et al*., 2021).

Diabetes is chronic metabolic disorder characterized by increased blood sugar level and inadequate insulin release. Type 1 diabetes is due to autoimmune destruction of pancreatic beta cells while type 2 characterized by insulin resistance and progressive beta cell dysfunction. In diabetes, amylase and glucoamylase are responsible for carbohydrate breakdown into simple sugar which then absorbed through wall of small intestine into the blood stream which directly influence in blood sugar level. Inactivation of glucoamylase enzymes contributes to postpartum hyperglycemia in diabetic patients. Therapeutically available antidiabetic medicines exert their medicinal effect through various mechanisms such as elevation of insulin secretion, glucose absorption, and metabolism. Glucoamylase split these smaller sugar particles to release glucose (Whitcomb and Lowe, 2007). α-glucosidase is also a key enzyme; its inhibitors slow down sugar absorption, which decreases postprandial plasma sugar levels and blocks postprandial hyperglycemia (Kumar *et al*., 2011).

Most diabetes and cancer deaths are linked to modifiable lifestyle and environmental factors, such as obesity, poor diet and physical inactivity. Lifestyle and environmental factors including tobacco, obesity, alcohol and low levels of fruits, vegetables and exercise (Mingyang and Edward, 2015). Immune status, smoking habits and number of sexual partners are all factors that can influence the progression of an HPV infection to CC03 (Nainakshi *et al*., 2019). Common CC03 symptoms include vaginal bleeding that's different from normal pattern such as bleeding after sex, between periods or after menopause or changes in vaginal discharge, discomfort in lower back and lower tummy (Emma *et al*., 2012). BC04 symptoms can include a new lump or thickening, alteration in breast structure, nipple discharge, armpit swelling, skin changes like dimpling and redness (Alison *et al*., 2005). CRC05 symptoms included altered bowel habits, rectal bleeding, abdominal pain, weight loss, fatigue and a feeling of incomplete emptying (Margaret *et al*., 2011). PC06 often lacks early symptoms, with signs typically appearing at a more advanced stage including jaundice, abdominal pain, loss of appetite, vomiting, nausea, weight loss and sometimes new onset of type 2 diabetes (Jonathan *et al*., 2020).

The pathogenesis of CC03 is driven by persistent high-risk HPV infection, in which the viral onco-proteins E6 and E7 disrupt the function of the tumor suppressor proteins p53 and pRB which leads to unchecked cell proliferation and the development of abnormal cervical cells (Xulelian *et al*., 2018). BC04 arises from uncontrollable breast cell growth and division, which is triggered by genetic mutations. Oncogenes are mutated versions of normal genes that fuel cell growth. For instance, when the HER2 proto-oncogene is overactive due to mutation, the resulting oncogene (HER2-positive) signals breast cells to divide and multiply uncontrollably (Melisa *et al*., 2023). CRC05 progresses via one of three main molecular pathways including Chromosomal Instability (CIN), Microsatellite Instability (MSI) and the CpG Island Methylator Phenotype (CIMP), each characterized by distinct genetic alterations, leading to a heterogeneous disease with diverse clinical behaviors and treatment responses (Adria *et al*., 2024). PC06 forms tumors when cells in the pancreatic ducts develop genetic mutations that cause uncontrolled growth. Oncogenes activation (like KRAS), tumor suppressor genes inactivation (CDKN2A, TP53 and SMAD4) and signaling pathways dysregulation (like EGFR) are key mechanisms (Thomas *et al*., 2014).

Piper nigrum L., *(PN01)* member of Piperaceae and *Syzygium aromaticum (SA02)* member of Myrtaceae family; are the most commonly used spices, containing several beneficial phytochemicals with biological potential against chronic disorders like diabetes, obesity and multiple cancers (Parthasarathy *et al*., 2008). *Piper nigrum* L., (*PN01)* is routinely used condiments worldwide because of their countless advantages on human health. *PN01* and *SA02* contains alkaloids, flavonoids, glycosides, phenols and terpenes (Luca *et al*., 2023; Rubab *et al*., 2021; Neveu *et al*., 2010; William *et al*., 2004). *PN01* possesses antibacterial, anti-diabetic and anticancer activity (Blessy and Gopinath, 2015). The secondary metabolites of SN02 are medicinally used in many countries as antimicrobial, analgesic, carcino-protective and sedative (Abd *et al*., 2014; Khan and Ahmad, 2012). Phytosterol found naturally in plants have major benefit is to reduce the blood cholesterol level and prevent the human body from coronary cardiac disease (Ostlund, 2002). Piperine, the active component of PN01, significantly reduces cell viability and colony formation in HeLa cells (Buranrat and Benjaporn, 2022).

GCMS spectrometry is a methodological approach which connects the characteristics of GC and MS to recognize the distinct phytochemicals from the extract (Skoog *et al*., 2007). This method offers high reliability and reproducibility, allowing for robust qualitative and quantitative analysis to be performed consistently across different labs and instruments. GC-MS is a well-established analytical tool for identifying unknown

compounds by comparing their mass spectra, generated from unique fragmentation patterns, with reference spectra from spectral libraries. Gas chromatography can isolate vaporous and semi-vaporescent mixtures with significant constancy, however inadequate for their recognition. MS can give almost complete particulars of structure of maximum combination in a manner that they could be truly recognized, but it cannot isolate these immediately (Hussain and Maqbool, 2014).

MATERIALS AND METHODS

Chemicals

All reagents and chemicals were of research grade chemicals and acquired by Sigma Aldrich, Merck. Glucoxidase kit was bought from the Madar Diagnostic which contain reagent with standard solution for testing of glucoamylase enzyme.

Plant material extraction

Piper nigrum dried fruit and *Syzygium aromaticum* dried flower buds were purchased from the herbal market of Karachi, Pakistan. After being authenticated by Prof. Dr. Mohtasheem Ul Hassan, Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan, *P. nigrum* dried fruits and *S. aromaticum* flower buds were cleaned by removal of extraneous particles and converted into powdered form by using a grinder. 10 g of *PN01* and *SA02* powdered spices were soaked into100 mL methanol in separate glass container for 20 days.0.1g/mL *PN01* and *SA02* extracts used for GCMS assay and then stored in airtight glass container and stored at room temperature. While, 20 g *PN01* and *SA02* spices powdered were macerated in 200 mL phosphate buffer solution of neutral pH for three days at room temperature. Phosphate buffer is used instead of solvents because it maintains the optimal physiological pH required for activity and stability of enzyme. These 10% phosphate buffer extracts (0.1 g/mL) were used in enzyme inhibitory assays at different parameters, including time interval, temperature, days, and pH. While, Performance was done in the research laboratory of the Department of Pharmacognosy and the Department of Biochemistry, University of Karachi, Pakistan.

Ethical statement

This article does not use any human participant or animal for secondary metabolites analysis.

Procedure for phytochemical recognition by GC-MS analysis

The compound identification of both spices' methanol extracts was done by observation of their MS division pattern with those saved in the digital library. GC-MS was performed on Agilent equipment 5975 series. Capillary column zebronzebron-5 containing 30 mx320 µmx0.25 µm film central width was utilized. Unadulterated carrier gas helium was employed at 1.5 mL/min flow rate and 44.635 cm/sec average velocity. 50°C was the starting temperature automated to rise to 200°C and later 300°C for 20 min then leave it for 1:1202 min. Leave in solvent for 5 min, and scan time 200 ms. The phytochemical in both extracts were recognize by the retention times of every component, breakage design and MS feature supported by employing National Institute of Standard and Technology (NIST) Liberary. Quardapole detector employed for component recognition (Patil and Jadhay, 2014).

Glucoamylase inhibitory assay

Different parameters for glucoamylase activity in the presence of *PN01* and *SA02* extracts were performed. The glucoxidase kit was used with some modification with glucose as a standard. Various parameters of glucoamylase inhibition were determined by performing the enzyme and extract assay after different time intervals, including 10, 30, 60, 90, and 120 min., pH 5, 6, 7, 8, and 9; days 1, 3, 6, and 9; and temperatures of 27°C and 37°C. Readings were read by using a UV spectrophotometer at 546 nm using glucose as a standard (Kim *et al*., 2022). Standard glucose curve is present in supplementary file. Two-way ANOVA is used for statistical analysis. One glucoamylase unit is defined as "The amount of enzyme that breaks down starch to release1.0 µmol of glucose per minute in a pH 7.0 buffer." 1 unit of plant extract inhibition activity was described as "the amount of inhibitor that cuts glucoamylase activity down by 1 unit."

The % inhibition of glucoamylase was calculated by using the following equation:

$$\text{Test unit (TU)} = \text{At} \times \text{Sc} / \text{As} \times 1 / \text{IU}$$

$$\text{Reducing sugar (mg/mL)} = \text{Ac} \times \text{Sc} / \text{As} \times 1 / \text{IU}$$

$$\text{Residual activity} = \text{Rs} \times 100 / \text{TU}$$

$$\% \text{ inhibition} = 100 - \text{Ra}$$

Where: At = absorbance of test, Ac = absorbance of control, Sc = standard concentration, As = absorbance of standard, IU = inhibitory unit, Rs = reducing sugar, Ra = residual activity, TU = test unit.

RESULTS

GCMS analysis

Structures of phytochemicals recognized by GCMS analysis of *PN01* extract present in spectra's available in supplementary file, which identified a total of 16 bioactive phytochemicals. Phytochemicals identified on GC-MS was based on their chemical structure, chemical formula, molecular weight, peak area, retention time and retention index. The most abundant compounds included caryophylline (100%),

bicyclohexane,4-methylene-1-(1-methylethyl) (62.04%), d-limonene (53.41%), and piperine (24.82%), While a total of 14 phytochemicals were detected from the *SA02* extract present in supplementary file, the chemical composition includes eugenol (100%), caryophylline (31.39%), and phenol, 2-methoxy-4-(2-propenyl), acetate (23.76%), along with smaller amounts of other secondary metabolites.

Glucoamylase

PN01 showed stronger glucoamylase inhibition at 60 min (91.59 ± 0.99), day 3 (89.94 ± 1.23), 37°C temperature (72.71 ± 6.76) and at pH 5 (65.60 ± 4.56) while, *SA02* exerted stronger glucoamylase inhibition at 37°C (96.05 ± 1.27), 60 min (92.39 ± 2.52), pH 7 (35.02 ± 41.51) and day 3(91.36 ± 4.39) (Figures 1-4 and Tables 1-4).

DISCUSSION

Currently rate of CC03 is increasing internationally which is hazardous and seeking attention to focus on research studies for a novel drug obtained from natural sources for their treatment. Recently researchers are seeking new digestive enzymes inhibitors from plants to employ against CC03, diabetes and hypertension (Govindappa *et al.*, 2020). Various secondary metabolites were identified in the GC fraction of the *PN01* and *SA02* methanol extracts such as piperine, eugenol, caryophylline, caryophylline oxide, hexadeconic acid, oleic acid etc. which are major antioxidant, anticancer and antihypertensive agents (Ali *et al.*, 2021; Oghogho and Nimenibo, 2019; Sumner, 2000). *PN01* and *SA02* extracts phytochemicals investigation shown phenolic compounds and their derivatives presence showed vital role as anticancer agent (Tariq *et al*, 2017; Adefegha and Oboh 2013).

Eugenol and volatile oils of *SA02* possess anticancer property against HeLa and MCF-7 cell lines for CCO3 and BC04 treatment (Arunava *et al.*, 2018; Parinnesh *et al.*, 2014). Seven

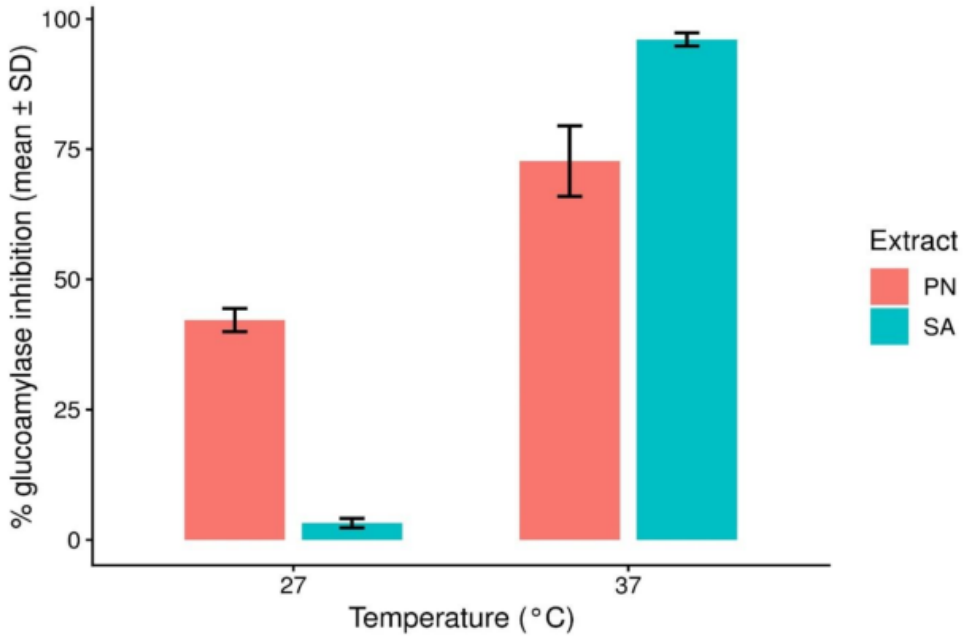

Figure 1: Effect of temperature and extract on glucoamylase.

Table 1: Effect of temperature and plant extract on glucoamylase inhibition.

Temp	Extract	Mean Inhibition	Standard Inhibition	n	Standard Error Inhibition	Mean Standard
27	PN	42.19	2.236	3	1.291	42.19 ± 2.24
27	SA	3.21	0.906	3	0.523	3.21 ± 0.91
37	PN	72.71	6.755	3	3.900	72.71 ± 6.76
37	SA	96.05	1.270	3	0.733	96.05 ± 1.27

For glucoamylase, inhibition by both extracts was strongly temperaturedependent (Table 1). At 27°C, *PN01* and *SA02* inhibited glucoamylase by 42.2 ± 2.2% and 3.2 ± 0.9%, respectively (mean ± SD, *n*=3). At 37°C, inhibition increased to 72.7 ± 6.8% for *PN01* and 96.1 ± 1.3% for *SA02*. Twoway ANOVA revealed highly significant effects of temperature *[p<0.0001]* and extract *[p=0.006]*, as well as a strong temperature × extract interaction *[p<0.0001]*, indicating that SA02 is particularly sensitive to temperature, with very low inhibition at 27°C and nearcomplete inhibition at 37°C.

Table 2: Effect of time and plant extract on glucoamylase inhibition.

Time	Extract	Mean Inhibition	Standard Inhibition	*n*	Standard Error Inhibition	Mean Standard
10	*PN*	28.16	1.76	3	1.02	28.16 ± 1.76
10	*SA*	32.41	29.89	3	17.26	32.41 ± 29.89
30	*PN*	48.00	11.04	3	6.38	48.00 ± 11.04
30	*SA*	44.7	20.91	3	12.07	44.70 ± 20.91
60	*PN*	91.59	0.99	3	0.57	91.59 ± 0.99
60	*SA*	92.39	2.52	3	1.45	92.39 ± 2.52
90	*PN*	90.55	0.39	3	0.22	90.55 ± 0.39
90	*SA*	91.217	0.03	3	0.02	91.22 ± 0.03
120	*PN*	59.343	23.20	3	13.39	59.34 ± 23.20
120	*SA*	61.733	0.06	3	0.03	61.73 ± 0.06

For glucoamylase, both extracts showed a pronounced timedependent increase in inhibition (Table 2). At 10 and 30 min, inhibition by *PN01* was 28.2 ± 1.8% and 48.0 ± 11.0%, and by *SA02* 32.4 ± 29.9% and 44.7 ± 20.9% (mean ± SD, *n*=3). Maximal inhibition was observed at 60–90 min, reaching 91.6 ± 1.0% and 90.6 ± 0.4% for *PN01* and 92.4 ± 2.5% and 91.2 ± 0.0% for *SA02*, respectively, before declining at 120 min (59.3 ± 23.2% and 61.7 ± 0.1%). Twoway ANOVA confirmed a highly significant effect of time on % inhibition *[p<0.001],* whereas the main effect of extract and the time × extract interaction was not significant *[p=0.854; p=0.993].*

amide alkaloids including piperine which is major component of *PN01*, its introduction desensitized CC03 HeLa/PTX cells to paclitaxel, its sensitize mechanism was investigated. Piperine was combined with paclitaxel, the combination treatment promoted cell apoptosis by down regulating phospho-Akt and Mcl-1, this sensitization mechanism was investigated, where piperine showed more significant sensitization effect (Zhoufan *et al*., 2019). BC04 study showed that administering eugenol and piperine directly into the tumor (intratumoral administration) significantly slowed or stopped the growth of TNBC xenografts in mice that lacked a fully functional immune system (Shailima *et al*., 2025; Anna *et al*., 2015). CRC05 studies found that eugenol, volatile oils and piperine reduce β-catenin's movement into the nucleus, providing mechanistic insight into its inhibitory action on the Wnt/β-catenin pathway (Thunyatorn *et al*., 2025; Gracielle *et al*., 2020). Eugenol and piperine can stop the PC06 cells growth by halting their cell cycle progression and inhibiting survival (Hyun *et al*., 2024; Ji *et al*., 2021). α-pinene is monocyclic terpenoid which possess anticancer property by suppressing HeLa cell growth, causing cell cycle arrest in the G0/G1 phase and inducing apoptosis, this observation of pro-apoptotic proteins upregulation and anti-apoptotic protein down regulation revealed that α-pinene activates the intrinsic apoptotic pathway (Xiaosu *et al*., 2022). Eugenol and α-pinene can be a potential therapeutic agent against BC04 because it inhibit BC04 cell growth and proliferation by decreasing miR-21 expression and increasing the tumor suppressor PTEN (Phosphatase and tensin homolog) expression. This modulation of gene expression leads to a reduction in BC04 cell invasion (Ahmad *et al*., 2025; Muhammad *et al*., 2024). α-pinene inhibitory effect on HT-29 colon cancer cell growth stems from reduced cell viability, increased apoptosis and the blocking of the PI3K/AKT pathway (Sara *et al*., 2025). *In vitro* studies of PC06 revealed that α-pinene prevented cell death and decreased cytokine production in pancreatic acinar cells that were artificially damaged by cerulean (Gi *et al*., 2012).

Copaene is a tricyclic sesquiterpene found in *PN01* and *SA02,* research work revealed that it possesses antioxidant, anticancer and antigenotoxic activity (Turkez *et al* 2014). This research investigated the anticancer potential of myrcene by evaluating their effects on cell lines (HeLa and HDFa) through cytotoxicity, proliferation, migration and morphology analysis (Luca *et al*., 2023). Studies showed that limonene (96.60%), with minor components (α-pinene, bicyclohexane, β-pinene, β-myrcene, 3-carene and o-cymene) possess the highest inhibitory activity against HepG2, MCF-7 and HeLa cells (Worachot *et al*., 2022). α-pinene, 3-carene, d-limonene and β-myrcene in BC04 showed the significant inactivation of TNFα-induced NF-κB (Jeong *et al*., 2015). α-pinene, α-phellandrene, δ-3-carene and d-limonene revealed cytotoxic activity in lung carcinoma (H460), CC03 (HeLa) and CRC05 (HCT116) under*in vitro* conditions (Emir *et al*., 2024). Beta-caryophyllene triggers apoptosis while suppressing proliferation and metastasis by modulating multiple cellular pathways. The anticancer activity is driven by ROS induction and JAK1/STAT activation. D-limonene exerts its anticancer effects by increasing the expression of autophagy-linked genes, Bax and caspase 3, while decreasing cyclin D1 and Bcl-2 expression. These compounds exhibit synergistic activity when combined with anticancer drugs, suggesting their promise as novel cancer-fighting agents (Gbadebo *et al*., 2025).

Research has shown that *PN01's* β-myrcene is a potent antimicrobial, anti-obesity, and gastroprotective agent (Bonamin *et al*., 2014). Piperine has the potential to treat diabetes through hydrolytic enzymes inhibition (Elisa *et al*., 2021). Caryophylline inhibits alpha glucoamylase, which is found in *PN01* and *SA02*, possess anti-diabetic, and antioxidant property (Oghogho

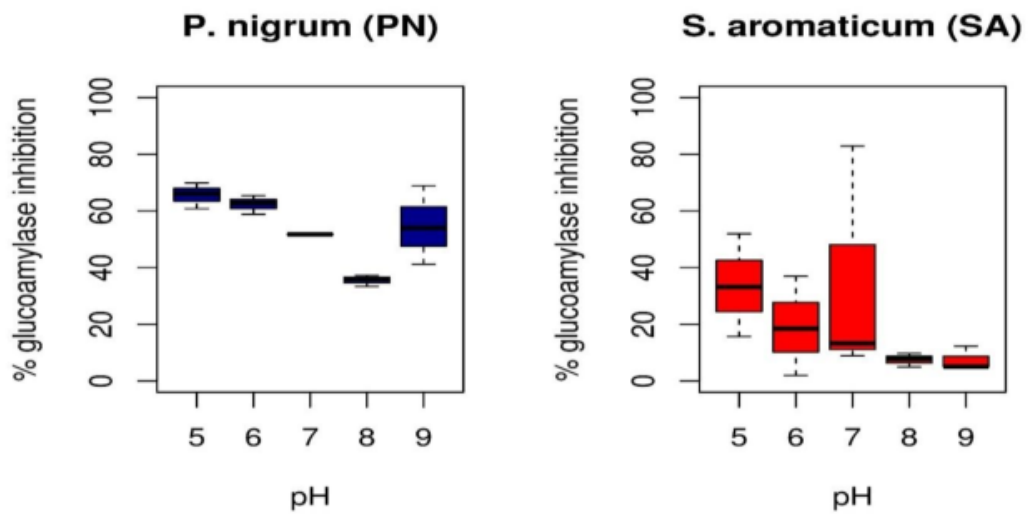

Figure 3: Effect of pH and extract on glucoamylase.

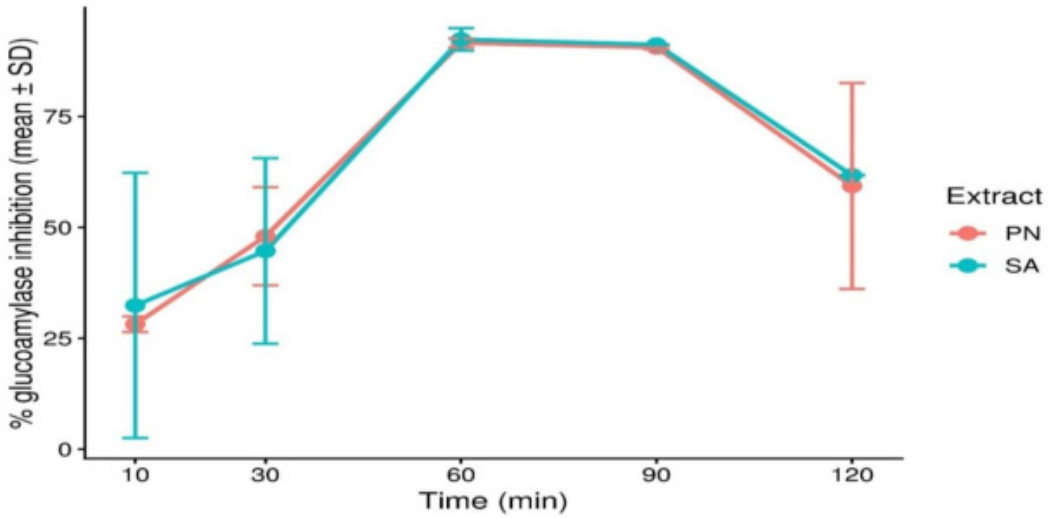

Figure 2: Effect of time and extract on glucoamylase.

Table 3: Effect of pH and plant extract on glucoamylase inhibition.

pH	Extract	Mean Inhibition	Standard Inhibition	*n*	Standard Error Inhibition	Mean Standard
5	*PN*	65.6	4.56	3	2.63	65.60 ± 4.56
5	*SA*	33.60	18.14	3	10.47	33.60 ± 18.14
6	*PN*	62.34	3.30	3	1.90	62.34 ± 3.30
6	*SA*	19.13	17.51	3	10.11	19.13 ± 17.51
7	*PN*	51.74	0.13	3	0.07	51.74 ± 0.13
7	*SA*	35.02	41.51	3	23.97	35.02 ± 41.51
8	*PN*	35.54	2.00	3	1.15	35.54 ± 2.00
8	*SA*	7.45	2.39	3	1.38	7.45 ± 2.39
9	*PN*	54.7	13.86	3	8.00	54.70 ± 13.86
9	*SA*	7.47	4.16	3	2.40	7.47 ± 4.16

For glucoamylase, *PN01* maintained moderate inhibition (35–66%) across pH 5–9, whereas *SA02* showed consistently lower activity (7–34%), with greater variability at some pH values (Table 3). Twoway ANOVA indicated a strong main effect of extract *[p<0.001]*, with *PN01* displaying significantly higher inhibition than *SA02* across the tested pH range. The effects of pH and the pH × extract interaction was not statistically significant *[p=0.053; p=0.507]*.

Table 4: Effect of days and plant extract on αamylase inhibition.

Days	Extract	Mean Inhibition	Standard Inhibition	*n*	Standard Error Inhibition	Mean Standard
1	*PN*	66.73	16.13	3	9.31	66.73 ± 16.13
1	*SA*	87.75	1.77	3	1.02	87.75 ± 1.77
3	*PN*	89.94	1.23	3	0.71	89.94 ± 1.23
3	*SA*	91.36	4.39	3	2.53	91.36 ± 4.39
6	*PN*	82.00	5.70	3	3.29	82.00 ± 5.70
6	*SA*	87.53	1.00	3	0.58	87.53 ± 1.00
9	*PN*	60.59	6.39	3	3.69	60.59 ± 6.39
9	*SA*	87.55	2.84	3	1.64	87.55 ± 2.84

For glucoamylase, storage time and extract type both influenced inhibitory activity (Table 4). *PN01* showed 66.7±16.1% inhibition on day 1, which increased to 89.9 ± 1.2% on day 3, then declined to 82.0 ± 5.7% on day 6 and 60.6 ± 6.4% on day 9 (mean ± SD, *n*=3). In contrast, *SA02* maintained consistently high inhibition throughout storage (87.8 ± 1.8%, 91.4 ± 4.4%, 87.5 ± 1.0% and 87.6 ± 2.8% on days 1, 3, 6 and 9, respectively). Twoway ANOVA revealed significant main effects of days and extract *[p=0.003; p=0.00014]* and a significant Days × Extract interaction *[p=0.013],* indicating that *SA02* retains glucoamylase inhibition more stably over time than *PN01.*

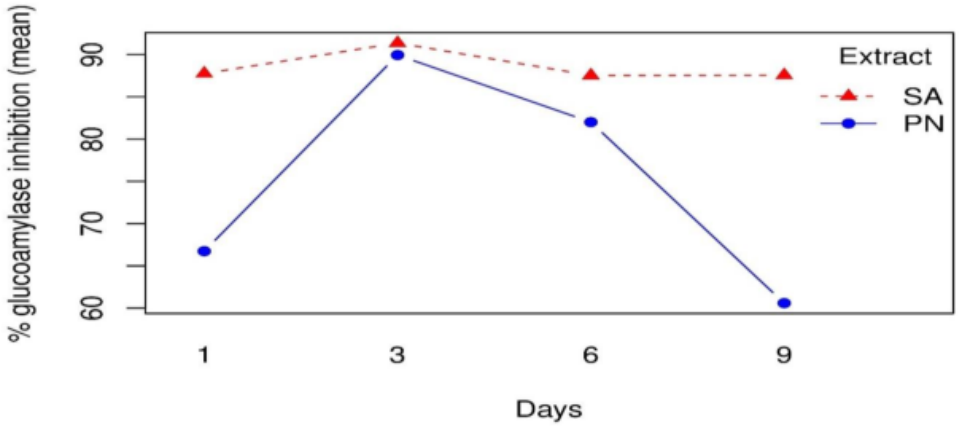

Figure 4: Effect of days and extract on glucoamylase.

and Nimenibo, 2019). According to research, caryophyllene oxide, which is found in *PN01* and *SA02* has antioxidant, anti-inflammatory, and antimicrobial properties. It inhibits α-glucoamylase, which causes lymphoma and neuroblastoma cells to undergo apoptosis (Jaradat *et al.*, 2020). Glucosidase enzymes significantly reduced blood sugar levels in an *in vitro* study of secondary metabolites (Aljarah and Hameed, 2018).

Results of *PN01* and *SA02* showed significant glucoamylase inhibition at various parameters. *SA02* showed the highest glucoamylase inhibition at 37°C temperature (96.05 ± 1.27), on day 3 (91.36 ± 4.39), at 60 min (92.39 ± 2.52), and at pH 7.00 (35.02 ± 41.51). Furthermore, *PN01* exerted maximum glucoamylase inhibition at 60 min (91.59 ± 0.99), day 3.0 (89.94 ± 1.23), 37°C temperature (72.71 ± 6.76), and at pH 5.00 (65.60 ± 4.56). Both plants possess glucoamylase inhibitory potential by hydrophobic and charge interaction with amino acid residue, phenols and flavonoids in *PN01* inhibit glucoamylase, lowering blood glucose levels and aiding in the treatment of diabetes (Elisa *et al.*, 2021; Stephen *et al.*, 2015). By interfering with the active enzyme site and preventing the breakdown of carbohydrates into glucose, eugenol, and polyphenols found in *SA02* inhibit glucoamylase, which lowers postprandial hyperglycemia by delaying the absorption of carbohydrates from the gastrointestinal tract (Stephen and Ganiyu, 2012).

The glucoamylase enzyme present in the intestinal wall has an optimal temperature of the human body, which is around 37.5°C; above this temperature can destroy the enzyme structure, so it gives the best result at human body temperature (Kumar and Satyanaryana, 2009). *PN01* and *SA02* extracts exert hydrolytic enzyme inhibition under varying durations; both extracts exhibited more glucoamylase inhibition at 60 min. Enzyme inhibition can be time dependent; some spices show powerful inhibition initially that decreases gradually, while others may have a delayed but prolonged effect. Both extracts result in glucoamylase enzymes inhibition on all days, but the highest inhibitory potential is achieved on day 3 (*S. aromaticum*) and day 6 (*P. nigrum*), it may be influenced by its concentration or the presence of its chemical constituents. This inhibition aids in postprandial glucose level management and potentially helps in controlling blood sugar level (Elisa *et al.*, 2021; Stephen and Ganiyu, 2012; Sudhir and Mohan, 2002). Both extracts showed maximum glucoamylase inhibition at pH 5 and minimum

inhibition at pH 8 and 9. Maximum glucoamylase inhibition ranges between 4 and 6 pH; extremely high or low pH levels resulted in total loss of activity for most enzymes (Imran *et al*., 2012). Alpha glucoamylase enzyme digests the carbohydrates and postprandial glucose level in diabetic patients, so due to glucoamylase enzyme inhibition, postprandial hyperglycemia can be controlled, and hence the risk of diabetes (Navjot *et al*., 2021).

Both extracts exerted significant glucoamylase enzyme inhibition, but *SA02* has more potential to inhibit glucoamylase than *PN01* due to their high concentration of its phyto-constituents included alkaloids, volatile oil, phenol, and terpenes (monoterpenes, and sesquiterpenes). Especially eugenol, caryophylline and phenol present in *SA02* interact effectively with the hydrolytic enzyme structures to inhibit their activity (Hafizah *et al*., 2016; Mnafgui *et al*., 2013).

CONCLUSION

The present study showed that *PN01* and *SA02* phytochemicals identified by GCMS will be advantageous in different field especially in medical and pharmaceuticals and greatly valuable for treatment of various type of cancer. Secondary metabolites of*PN01* and *SA02* recognized by GCMS analysis, especially piperine, beta-myrcene, 3-carene, copaene, caryophylline and α-pinene have shown promising anticancer effects against CC03, BC04, CRC05, and PC06 cell lines in preclinical studies. Research has demonstrated that secondary metabolites of *PN01* and *SA02* can reduce tumor cell viability, induce apoptosis and inhibit migration in CC03, BC04, CRC05 and PC06. *PN01* and *SA02* exhibited glucoamylase enzymes inhibition, So, their extracts may be accounted for as economic and natural antidiabetic and antiobesity medicine to manage postprandial hyperglycemia by glucoamylase inhibitory potential and their ideal parameters. However, their application in clinical oncology and diabetic centers is limited due to a lack of human studies, bioavailability issues and potential drug interactions.

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ABBREVIATIONS

ANOVA: Analysis of Variance; **BAX Bcl2:** Antagonist X Protein, Bcl-2 B-cell Lymphoma 2; **BC04:** Breast Cancer; **CC03:** Cervical Cancer; **CDKN2A and SMAD4:** Tumor Suppression Genes; **CIMP CpG:** Island Methylator Phenotype; **CIN:** Chromosomal Instability; **CRC 05:** Colorectal Cancer; **Cyclin 1:** Cell Cycle Regulatory Protein; **EGFR:** Epidermal Growth Factor Receptor; **GCMS:** Gas Chromatography Mass Spectroscopy; **G0:** Quiescent Phase; **G1:** Grdae 1 Cancer; **HDFa:** Human Dermal Fibroblast; **HeLa:** Henrietta Lacks; **HCT116:** Human Colorectal Cancer Cell Lines; **HepG2:** Liver Cancer Cell Line; **HPV:** Human Papillomavirus; **JAK1:** Janus Kinase 1; **KRAS:** Kirsten Rat Sarcoma Gene; **MCF-7:** Michigan Cancer Foundation 7; **MS:** Mass Spectrometry; **MSI:** Microsatellite Instability; **NIST:** National Institute of Standard and Technology; **NF-Kb:** Nuclear Factor κB; **PC06:** Pancreatic cancer; **PN01:** *Piper nigru;* **PI3K/AKT:** Phosphoinositide 3 kinase/Akt: **p53:** Tumor Protein 35; **Prb:** Retinoblastoma protein; **PTX:** Paclitaxel; **ROS:** Reactive Oxygen Species; **SA02:** *Syzygium aromaticum*; **STAT:** Single Transducers and Activators of Transcription Proteins; **TNBC:** Triple Negative Breast Cancer; **TNFα:** Tumor Necrosis Factor-α; **TP53:** Tumor Protein 53; **At:** Absorbance of test; **Ac:** Absorbance of Control; **IU:** Inhibitory Unit; **Rs:** Reducing Sugar; **Ra:** Residual Activity; **TU:** Test Unit; **Sc:** Standard Concentration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Syeda Tayyaba Asif conducted the whole experiments. Madiha Jamshed carried out calculations. Mohtasheem Ul Hassan and Shah Ali Ul Qader analyzed the data and helped in manuscript preparation.

SUMMARY

Optimizing the glucoamylase inhibitory potential of *PN01* and *SA02* extracts alongside phytochemical profiling offers a promising strategy for managing chronic disorders.

REFERENCES

Abd E A, M., Mesallamy, A., Gerby, M. E., and Awad, A. (2014). Anti-tumor, antioxidant and the phenolic constituents of clove flower buds. *J Microb Biochem Technol*. *10, s8-s007*. http://dx.doi.org/10.4172/1948-5948.S8-007.

Adefegha S. A. and Oboh G. (2013). Phytochemistry and mode of action of some tropical spices in the management of type-2 diabetes and hypertension. *African Journal of Pharmacy and Pharmacology.* 7(7), *332-346*. DOI: 10.5897/AJPP12.014.

Adria Canellas-S., Elena S, Eduard B. (2024). Mechanism of metastatic colorectal cancer. *Nature reviews gastroenterology & hepatology*. *21, 609-625*. https://doi.org/10.1038/s41575-024-00934-z.

Ahmad M.N., Muhammad T.S., Aimen, M., Waqas, A.K., Muhammad I., *et al.* (2025). Eugenol: An insight into the anticancer perspective and pharmacological aspects. *Food Science & Nutrition.* 13(8), *e70727*. https://doi.org/10.1002/fsn3.70727.

Ali, T.Z., Tala, A.Z., & Khalid, R.H. (2021). Anticancer properties of Eugenol; A review. *Molecules.* 26(23), *7407*. https://doi.org/10.3390/molecules26237407.

Alison B., Amanda R., Caroline B., Myra H. (2005). Understanding why women delay in seeking help for breast cancer symptoms. *Journal of Psychosomatic Research*. *58(4), 321-326*. https://doi.org/10.1016/j.jpsychores.2004.10.007.

Aljarah, A. K. & Hameed, I. H. (2018). *In vitro* anti-diabetic properties of Methanolic extract of *Thymus vulgaris* using α-glucosidase and α-amylase inhibition assay and determination of its bioactive chemical compounds. *Indian Journal of Public Health Research and Development*. *9(3), 388-392*. DOI Number: 10.5958/0976-5506.2018.00241.3.

Anna L. G., Caroyln D. D, Kimberly M. S., Laurence M., Henry A., *et al.* (2015). Piperine inhibits the growth and motility of triple-negative breast cancer cells. *Cancer letters*. *357(1),129-140*. https://doi.org/10.1016/j.canlet.2014.11.017

Arunava, D., Harshadha, K., Dhinesh K. S., Hari, R. K., Bindhu J., (2018). Evaluation of therapeutic potential of eugenol- a natural derivative of *Syzygium aromaticum* on cervical cancer. *Asian Pac J Cancer Pre. 19(7),1977-1985*. https://doi.org/10.22034/APJCP.2018.19.7.1977

Blessy, S. & Gopinath, P. (2015). Antibacterial Activity of Ethanolic Extract of Black Pepper against Clinical Isolates of *Staphylococcus*. *Journal of Pharmaceutical Sciences and*

Research. *7(11); 1015*. Antibacterial Activity of Ethanolic Extract of Black Pepper Against Clinical Isolates of Staphylococcus - ProQuest

Bonamin, F., Mores T. M., Dos Santos, R.C., Kushima, H., Faria, F.M., *et al.* (2014). The effect of a minor constituent of essential oil from *Citrus aurantium*: The role of β-myrecene in preventing peptic ulcer disease. *Chemico-biological interactions*. *212,* 11-19. http://dx.doi.org/10.1016/j.cbi.2014.01.009

Bray F, Ferlay J, Soerjomataram I, Siegel R, Torre L, Jemal A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA*: a cancer journal for clinicians. 68(6), 394-424. doi: 10.3322/caac.21492.

Buranrat, Benjaporn. (2022). Proliferative inhibition, Apoptotic induction. Migratory suppression of *Piper nigrum* extract on HeLa cervical cell line. *Indian Journal of pharmaceutical sciences*. *84(5)*, *1210*. doi. 10.36468/pharmaceutical-sciences.1016

Chirstine M. Kaefer, John A. Milner. (2008). The role of herbs and spices in cancer prevention. *The journal of Nutritional Biochemistry*. 19(6),347-361.https://doi.org/10.1016/j.jnutbio.2007.11.003.

Emma L. L, Alice E. S, Jane L, Debble R-A., JO W. (2012). What do British women know about cervical cancer symptoms and risk factors? *European Journal of Cancer*. *48(16),3001-3008*. https://doi.org/10.1016/j.ejca.2012.05.004.

Emir H., Lamija K., Suada C., Merima I., Lejla M., et al. *(*2014). Anticancer potential of pink pepper fruit essential oil: study on human cell lines of lung cancer carcinoma (H460), cervical adenocarcinoma (HeLa) and colorectal carcinoma (HCT116). *Technologica acta*. *17(2), 43-49*. DOI: 10.51558/2232-7568.2024.17.2.43

Elisa, M-B., Genesis, V. B-C, Alejandro, H- M., Vianey, D., Rocio, T-P, *et al.* (2021). *In vitro* alpha amylase and alpha glucosidase enzyme inhibition andantioxidant activity by capsaicin and piperine from *Capsicum chinense* and *Piper nigrum* fruits. *Journal of environmental science and health*. *56(3), 282-291*. https://doi.org/10.1080/03601234.2020.1869477

Gbadebo, Ololade S., Oke, Elizabeth D., Ajibuwa, Felix A. (2025). Anticancer properties of beta-caryophylline and d-lemonene terpenes: A review. *Asian Pacific Journal of Tropical Biomedicine.15(4), p 129-140*. DOI: 10.4103/apjtb.apjtb_7_25

Gi-Sang B, Kyoung-Chel P, Sun Bok C, IL-JOO J, MEE-OK C, et al. *(*2012). Protective effects of alpha pinene in mice with cerulean induced acute pancreatitis. *Life Sciences*. *91(17-18), 866-871*. *http://www.sciencedirect.com/science/article/pii/S00243 20512004870*

Govindappa M., Lavanya M., Aishwarya P., Pai Kavya, Lunked P, Hemashekhar B., *et al.* (2020). Synthesis and characterization of endophytic fungi, *Closporium perangustum* mediated silver nanoparticles and their antioxidant, anticancer and nano-toxicological study. *BioNanoScience*. *10(4), 928-941*. https://doi.org/10.1007/s12668-020-00719-z.

Gracielle C. De A, Luiz F. S. O, Danilo P, Harold H. F, Ricardo M. K, et al. *(*2020). Piperine suppresses the Wnt/β-catenin pathway and has anticancer effects on colorectal cancer cells. *Scientific reports*. 10(1),11681. https://doi.org/10.1038/s41598-020-68574-2.

Hafizah, U. T., Raja, A.S., Aisha, A., & Shazia, A. (2016). Chemical Composition and Antidiabetic Activity of Essential Oils Obtained from Two Spices (*Syzygium aromaticum* and*Cuminum cyminum*). *International Journal of food properties*. *9(10), 2156-2164*. https://doi.org/10.1080/10942912.2015.1110166.

Haiyan Z, Hui L, Wenwen Z, Zhaojun S, Xiaoli H & Xueqiuong Z. (2016). Molecular mechanisms of cisplatin resistance in cervical cancer. *National center for biotechnology information.10.* 1885-1895 *(2016)*. https://doi.org/10.2147/DDDT.S106412

Hussain, S. Z. & Maqbool, K. (2014). GC-MS Principle, Technique and its application in Food Science. *International Journal of Current Science*. *13: 116-126*. http://www.currentsciencejournal.info/issuespdf/Syed%2014.pdf.

Hyun, H.K., Suk, Y. L., & Dae, H.L. (2024). Apoptosis of pancreatic cancer cells after co-treatment with eugenol and tumor necrosis factor-related apoptosis-inducing ligand. *Cancers. 16(17),3092*. https://doi.org/10.3390/cancers16173092.

Imran, M, Asad, JM, Gulfraz, M, Qureshi, R, Gul, H, et al. *(*2012). Glucoamylase production from *Aspergillus niger* by using solid state fermentation process. *Pakistan Journal of Botany*. *44(6), 2103-2110*. https://www.academia.edu/download/79065662/44.pdf.

Jaradat, N., Maharik, N. A., Abdallah, S., Shawahna, R., Mousa, A., et al. *(*2020). "*Nepeta curviflora* essential oil: Phytochemical composition, antioxidant, anti-proliferative and anti-migratory efficacy against cervical cancer cells, and α-glucosidase, α-amylase and porcine pancreatic lipase inhibitory activities." *Industrial Crops and Products*. *158, 112946*. https://doi.org/10.1016/j.indcrop.2020.112946

Jeong-H, L, Kicheol L, DA H. L, Soon Young S., Yeonjoong Y., Young H. L. (2015). Anti-invasive effect of beta-myrecene, a component of the essential oil from *Pinus koraiensis* cones, in metastatic MDA-MB-231 human breast cancer cells. *Scientific reports*. *58(4),563-569*. https://doi.org/10.1007/s13765-015-0081-3

JI H. J, JAE-H, R, HWA J, L. (2021). *In vitro* inhibition of *Piper nigrum* and piperine on growth, migration and invasion of PANC- 1human pancreatic cancer cells. *Sage journal reviewer gateway,* 16(11), 1934578X211057694. https://doi.org/10.1177/1934578X211057694.

Jonathan D., MD, Rishisurana, MD, Juan W V., MD, Rachna T S., MD. (2020). Pancreatic cancer. *The Lancet*. *395(10242), 2008-2020*. DOI: 10.1016/S0140-6736(25)00261-2

Junjie H., Veeleah L., Chun H. N., Lin Z, Jinqiu Y., Xiang Q. L., Kelvin N, et al. *(*2021). Worldwide burden of, risk factors for and trends in pancreatic cancer. *Gastronenterology*. *160(3), 744-75*. https://doi.org/10.1053/j.gastro.2020.10.007

Khan, M. S. A. & Ahmad, I. (2012). Biofilm inhibition by *Cymbopogon citratus* and *Syzygium aromaticum* essential oils in the strains of *Candida albicans*. *Journal of Ethnopharmacology*, *140(2), 416-423*. https://doi.org/10.1016/j.jep.2012.01.045

Kim R.H., Antonisamy P., Kim S.Y., Lee G., Ham D. H., Kown B. K. (2022). Inhibitory effect of *Amomum villosum* water extracts on alpha glucosidase activity. *Physiological and molecular plant pathology, Elesvier*. *117. 101779*. https://doi.org/10.1016/j.pmpp.2021.101779.

Kumar, S., Narwal, S., Kumar, V., & Prakash, O. (2011). A-glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacognosy reviews*. *5 (9), 19*. doi:10.4103/0973-7847.79096

Kumar, P., & Satyanaryana, T. (2009). Microbial glucoamylases: characteristics and application. *Crit Rev Biotechnol*. *29(3), 225-55*. https://doi.org/10.1080/07388550903136076

Luca P., Francesca V., Christian B., Cecilia O., Romana F., et al. *(*2023). Myrecene: A natural compound showing anticancer activity in HeLa cells. *Molecules*. *28(18), 6728*. https://doi.org/10.3390/molecules28186728.

Luca S. V., Kittl T. & Minceva M. (2023). Supercritical CO2 extraction of spices: A systematic study with focus on terpenes and piperamides from black pepper (*Piper nigrum* L.,). *Food Chemistry*. *406, 135090*. https://doi.org/10.1016/j.foodchem.2022.135090

MADIHALLI S. C, ABHIJIT D, MALLAPPA K. S. (2022). Introduction to cancer and treatment approaches. *Paclitaxel*. *1-27*. https://doi.org/10.1016/B978-0-323-90951-8.00010-2

Margaret A, Tom G, Richard D N, Peter R, William H. (2011). The diagnostic value of symptoms for colorectal cancer in primary care: a systematic review. *British Journal of General Practice*. *61(586),* e231-e243. https://doi.org/10.3399/bjgp11X572427.

Melisa B. Nicoud, Monica A. Taquez D, Ignacio O, Helena A. S, Karina F, Vanina A. Medina. (2023). Histaminergic system as a promising target for breast cancer treatment. *Scientific report: Springer nature link*.*385-414*. https://doi.org/10.1007/16833_2022_104.

Mingyang Song & Edward L. Giovannucci. (2015). Cancer risk: Many factors contribute. *Science*. *347(6223), 728-729*. https://doi.org/10.1126/science.aaa6094.

Mnafgui, K., Kaanich, F., Derbali, A., Hamden, K., Derbali, F., Slama, S., Elfeki, A. (2013). Inhibition of key enzymes related to diabetes and hypertension by Eugenol *in vitro* and in alloxan-induced diabetic rats. *Archives of Physiology and Biochemistry*. *119(5), 225–233*. https://doi.org/10.3109/13813455.2013.822521

Muhammad Y, Mahdi A, Shana A, Parisa K, Elham G, Ghasem F G B, Fatemeh G, Mojgan A. (2024). Therapeutic potential of alpha pinene in breast cancer: targeting miR-21 and PTEN gene expression. *Galen Medical Jounal*. *13*. e3613. https://doi.org/10.31661/gmj.v13i.3613

Nainakshi, K., Nadiya, K., Sukhpal, K., Sandhya, G. (2019). Risks factors of cervical cancer: a case-control study. *Asian-Pacific Journal of oncology nursing*. *6(3),308-314*. https://doi.org/10.4103/apjon.apjon_73_18

Navjot, K., Vanktesh, K, Surendra, K, Nayak, P. W, Paranjit, K, Sanjeev K. S. (2021). Alpha-amylase as molecular target for treatment of diabetes mellitus: A comprehensive review. *Chemical biology and drug design*. *Wiley*. *98(4), 539-560*. https://doi.org/10.1111/cbdd.13909.

Neveu, V., Perez-Jimenez, J., Vos, F., Crespy, V., Chaffaut, D., *et al.* (2010). Phenol-Explorer: An online comprehensive database on polyphenol contents in foods. *Database.* 2010, *bap024*. https://doi.org/10.1093/database/bap024

Oghogho, O. & Nimenibo-UADIA R. (2019). "Phytochemical screening, GC-MS analysis and *in vitro* inhibition of alpha-amylase and alpha-glucosidase activities by methanol extract of *Psidium guajava* leaves and fractions." *Journal of Pharmacognosy and Phytochemistry*. 8(5), 634-640. 8_4_759_102-libre.pdf

Ostlund, R. E. JR. (2002). Phytosterols in human nutrition. *Annual review of nutrition*. *22(1), 533-549*. https://doi.org/10.1146/annurev.nutr.22.020702.075220

Parthasarathy, V. A., B. Chempakam & T. J. Zachariah. (2008). Chemistry of spices. Cabi. https://doi.org/10.1079/9781845934057.0000

Parvinnesh S. K., Raden, M. F., Ferry F. S., Dimas E. L., Rizky, A. (2014). Anticancer potential of *Syzygium aromaticum* in MCF-7 humancancer cell lines. *Pharmacognosy Res*. 6(4);*350-354*. https://doi.org/10.4103/0974-8490.138291

Pasqualino F., Gabriele C., Macro G., Felice P., Raffaele E. M. P, Francesco C. (2016). Worldwide burden of colorectal cancer: a review. *Updates in Surgery*. *68(1), 7-11*. https://doi.org/10.1007/s13304-016-0359-y.

Patil, A. & V. Jadhav. (2014). GC-MS analysis of bioactive components from methanol leaf extract of Toddalia *asiatica* (L.). *Int. Pharm Sci Rev Res*. *29(1), 18-20*. Microsoft Word - 04

Rubab, A. K., Sivakumari, K., Rajesh, S., Mangaladevi, S. & Kayalvizhi, S. (2021). Phytochemical profiling of selected spices (*Ferula asafetida, Piper nigrum, Elettaria cardamomum, Cinnamomum aromaticum* and *Syzygium aromaticum*) extract. *International Journal of Zoology and Applied Biosciences*. *6, (1); 35-45*. https://doi.org/10.5281/zenodo.

Sara, S., Yasaman, Z., Elham, H., Reza, S., Seyedeh, M. (2025). Exploring the effects of alpha-pinene on apoptosis induction in human colon cancer cells via the PI3K/AKT signaling pathway: an *in vitro* study. *Springer nature link*. *26(1), 27*. https://doi.org/10.1186/s43042-025-00656-0.

Shailima, R., Mugahed A.A., Tae, H.O, & Baji, S. (2025). A review of six bioactive compounds from preclinical studies as potential breast cancer inhibitors. *Molecular Biology Research*. 52(1), *203*. https://doi.org/10.1007/s11033-025-10300-0.

Singh, D, Vignat, J, Loraenzoni, V, Eslahi M, Ginsburg, O, Lauby-Secretan, B, *et al.* (2023). Global estimates of incidence and mortality of cervical cancer in 2020: a baseline analysis of the WHO Global Cervical Cancer Elimination Initiative. *Lancet Global Health.11(2), e197-206*. https://doi.org/10.1016/

Skoog, D. A., Holler F. J. & Crouch S. R. (2007). Instrumental analysis. Brooks/ Cole, Cengage Learning Belmont. Microsoft Word - Vassar main doc.doc

Stephen, A. A., & Ganiyu, O. (2012). *In vitro* inhibition activity of polyphenol- rich extracts from *Syzygium aromaticum* (L.) Merr. & Perry (Clove) buds against carbohydrate hydrolyzing enzymes linked to type 2 diabetes and Fe2+ - induced lipid peroxidation in rat pancreas. *Asian Pacific journal of Tropical Biomedicine. 2(10), 774-781*. https://doi.org/10.1016/S2221-1691(12)60228-7.

Stephen, A. A., Ganiyu, O., Sunday I. O., & Kolawole, O. 2015. Alteration of starch hydrolyzing enzyme inhibitory properties, antioxidant activities, and phenolic profile of clove buds (*Syzygium aromaticum* L.) by cooking duration. *Food Science and Nutrition. 4(2), 250-260*. https://doi.org/10.1002/fsn3.284.

Sudhir, R., & Mohan, V. (2002). Postprandial hyperglycemia in patients with Type 2 diabetes mellitus. *Treat Endocrinology. 1(2), 105–116*. https://doi.org/10.2165/00024677-200201020-00004.

SUMNER, J. (2000). The natural history of medicinal plants, *Timber press*. cabidigitalli brary.org/doi/full/10.5555/20003017295

Tariq, A., Sadia, S., Pan, K., Ullah, I., Mussarat, S., et al. *(*2017). "A systematic review on ethnomedicines of anti-cancer plants." *Phytotherapy Research. 31(2), 202-264*. https://doi.org/10.1002/ptr.5751.

Thomas K, Christine S, Munish A, Edwin C. Thrower. (2014). Risk factors for pancreatic cancer: underlying mechanisms and potential targets. *Frontiers in Physiology. 4-2013*. https://doi.org/10.3389/fphys.2013.00415.

Thunyatorn, Y., Weerakitt., Sunee, C., Sumet, K., Ngampuk T., *et al.* (2025). The effect of ethanolic extracts from *S.aromaticum* and *S.nervosum* on antiproliferative activity and apoptosis in HCT116 and HT-29 cells. *Int J. Mol. Sci.* 26(14); *6826*. https://doi.org/10.3390/ijms26146826.

Turkez, H., Togar B., Tatar A., Geyikoglu F. & Hacimuftuoglu A. (2014). Cytotoxic and cytogenetic effects of α-copaene on rat neuron and N2a neuroblastoma cell lines. *Biologia. 69(7), 936-942*. https://doi.org/10.2478/s11756-014-0393-5

William R. J., Spencer J. P. & Rice-Evans C. (2004). Flavonoids: antioxidants or signaling molecules? *Free radical biology and medicine. 36, (7), 838-849*. https://doi.org/10.1016/j.freeradbiomed.2004.01.001.

Whitcomb, D. C. & Lowe, M.E. (2007). Human pancreatic digestive enzyme. *Digestive diseases and sciences. 52(1),* 1-17.https://doi.org/10.1007/s10620-006-9589-z

Worachot, S., Thipphiya, K., Benjaporn, B., Teeraporn, K., Nyuk, L, M., Vijitra, L. (2022). Cytotoxicity and antiproliferative activity of essential oils from lemon, wild orange and petitgrain against MCF-7, HepG2 and HeLa cancer cells. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 50(3), 12713*. https://doi.org/10.15835/nbha50312713.

Xiaosu, H., Pei Y., Wenqing, D., Chang, Z., Qiuxiang, X., et al. *(*2022). α–Pinene inhibits the growth of cervical cancer cells through its proapoptotic activity by regulating the miR-34a-5p/Bcl-2 signaling axis. *Drug development research*. 83(8), 1766- 1776. https://doi.org/10.1002/ddr.21994.

Xuelian, W, Xiumin H, Youzhong, Z. (2018). Involvement of Human Papilloma viruses in cervical cancer. *Frontiers in Microbiology. 9.* https://doi.org/10.3389/fmicb.2018.02896

Zhoufan X, Yulu, W., Jie, X, Jiachuan, L., & Jianqing, Y. (2019). Alkaloids from *Piper nigrum* Synergistically Enhanced the Effect of Paclitaxel against Paclitaxel-Resistant Cervical Cancer Cells through the downregulation of Mcl-1. *Journal of Agriculture and Food Chemistry.* 6(18), *5159-5168*. https://doi.org/10.1021/acs.jafc.9b01320

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