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Original Article

Lepidium sativum extract targets DNA repair pathways in colorectal cancer: In silico ADMET analysis of active compounds

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Abstract

Lepidium sativum has been used traditionally to treat a variety of ailments because of its potential pharmacological properties, however, its impact on the growth and differentiation of colorectal cancer cells is still not well understood. Antiproliferative properties of *L. sativum* leaf extract, was investigated. The MTT assay was performed, followed by the RT2 Profiler PCR Array to analyze the expression of 84 human DNA damage and repair pathway genes in two human colorectal cancer cell lines, HCT116 and SW620, after exposure to L. sativum extracts. The results showed a dose-dependent reduction in cell viability for HCT116 and SW620 cells following treatment with L. sativum extract. PCR array analysis of the 84 DNA repair genes revealed valuable insights into cellular responses and mechanisms behind the non-conventional treatments of cancer cells. RT-PCR analysis confirmed significant modulation of DNA damage response (DDR) genes, including increased expression of apoptosis-related genes DDIT3 and GADD45G, alongside decreased expression of the repair gene MPG. Gas chromatography-mass spectrometry analysis identified 10 compounds with promising pharmacokinetic properties and a favorable ADMET profile, in line with Lipinski's Rule of Five. This study suggests that *L. sativum* extract induces apoptosis in colorectal cancer cells, supporting its potential as a therapeutic agent in cancer treatment.

Keywords: *Lepidium sativum,* Colorectal cancer, RT2 profiler PCR array, HCT116, SW620

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Introduction

Lepidium sativum L., a widely distributed herb used in culinary and traditional medicinal practices, has garnered attention for its diverse biological activities (Hekmatshoar et al., 2022). Research conducted in laboratory and animal models has demonstrated its potential in providing antimicrobial, antidiabetic, antioxidant, antidiarrheal, anticancer, and other health benefits (Painuli et al., 2022). Belonging to the Brassicaceae family with the common name Garden cress, *L. sativum L.* is a widely cultivated edible herb found in many regions across the globe (Manohar et al., 2012). It is an herbaceous annual plant with white flowers and a height ranging from 15 to 45 cm (Aqafarini et al., 2019). Due to its nutritional value, it has been used for various health issues for ages. The seeds are rich in antioxidants like tocopherol, phenols, terpenoids, and vitamin E (Gupta and Gupta, 2023). It also contains a sulfur-containing compound called glucosinolate, known for anti-proliferative activity against certain carcinoma cell lines by inducing mitochondrial-mediated apoptosis through the generation of reactive oxygen species (ROS) (Miękus et al., 2020; Yang et al., 2023)

Cancer is one of the main causes of death all over the world, according to International Agency for Research on Cancer (IARC) reported 20 million new cases of cancer in the year 2022 which can reach upto 35 million by 2050. Agency estimated one in five men or women developing cancer in a lifetime (Jia et al., 2025). Globally 50.2% colorectal cancers are reported from Asia in 2022, with 967 thousand new cases and 462 thousand deaths with higher incidence rates in younges and males. Chemotherapy is used as a primary treatment for cancer but has lot of side effects reliant on the patient and the drugs used (Satiamurthy et al., 2025). Lot of challenges due to side effects of chemotherapy with major health risks, encourages interest in natural therapy remedies (Samuel et al., 2021; Nurgali et al., 2018; Anand et al., 2022). *L. sativum,* used as a natural remedy by ancient civilizations, is emerging with promising results against cancer (Huang et al., 2024). Aqueous extract of *L. sativum* is reported to induce apoptosis in a breast cancer cell line (MCF-7) (Mahassni and Al-Reemi, 2013). *L. sativum* extract extended the lifespan of rodent models of Ehrlich ascites carcinoma (EAC) by reducing liver enzyme and malondialdehyde levels, and increasing glutathione peroxidase activity, indicating antioxidant effects of this herb (El Sayed et al., 2020). Also, methanolic extract of *L. sativum* is reported to trigger apoptosis in human cell lines such as peripheral lymphocytes, colon cancer, and endometrial cancer cell lines, in a dose-dependent manner, with remarkable antioxidant activity due to the presence of high phenolic and flavonoid compounds in the extract (Selek et al., 2018). Its aqueous leaf extracts inhibit the growth of human tongue squamous carcinoma cells by inducing apoptosis and DNA damage by generating reactive oxygen species (ROS) in mitochondria (AlObaidi, 2014; Jahani et al., 2020). Hydroalcoholic extracts of *L. sativum* are also reported with cytotoxicity on HeLa and K562 cell lines, suggesting its potential in cancer treatment (Aslani et al., 2014).

The effect of *L. sativum* on the proliferation and differentiation of colorectal cancer cells is not fully explained. In this study, two human colorectal cell lines (HCT116 and SW620) were treated against *L. sativum* extract and then analyzed through RT2 Profiler PCR Array for 84 genes involved in the human DNA damage and repair pathway.

Material and Methods

Collection of medicinal plants, crude extracts, and fractions preparation

L. sativum seeds were taken from natural farms in southern Saudi Arabia that were not exposed to chemicals or chemical fertilizers. The seeds were dried and ground into a fine powder. This powder was then used to prepare extracts with ethyl acetate. An 80g of seed powdered were extracted in 400 mL of ethyl acetate within a Soxhlet extraction apparatus. Ethyl acetate has ideal solvency properties for many compounds and is safe to handle. After the extraction, the extract centrifuged at 4500 rpm for 10 minutes, then a rotary evaporator was used to concentrate the extract 45°C stored at -84°C until required for further analysis.

Cell culture

The study included two distinct types of human colorectal cancer cell lines: HCT116 and SW620.

MTT assay

In vitro, cytotoxic activity of *L. sativum* extract was conducted using the MTT assay.

Cell viability (%) was calculated using the formula: (O.D of the treated sample) / (O.D of the untreated sample) × 100%.

In each well of a 24-well plate, cell suspension (5 X 10^4 cells/mL) was seeded and incubated at 37°C for 24 hours in a 5% CO2 incubator. Various concentrations (50, 125, 250, 500 µg/mL) of *L. sativum* extract were used to test the cytotoxic activity by adding MTT solution and incubated at 37°C for 24 hours and read at 545 nm. Wells containing untreated cells serving as controls and IC50 was determined.

Cell line treatment

The cell lines were treated with the IC50 of *L. sativum* extract. The concentration of the extract was 80 µg/mL for the HCT116 cell line and 98.9 µg/mL for the SW620 cell line.

RNA isolation and assessment of gene expression via RT-qPCR

Total RNA extracted from HCT116 and SW620 cell and used to synthesize cDNA was synthesized using a reverse transcription kit (without RNase inhibitor) from Applied Biosystems, USA and stored at -20°C until further use.

PCR array

The Human DNA Damage Signaling Pathway RT² Profiler™ PCR Array (PAHS-029Z, Qiagen, USA) was employed to screen a panel of 84 genes representative of the human DNA damage and repair pathway by using specific primers and 5 housekeeping genes were used for normalization.

Gene expression

Real-time PCR was used to check the effect of *L. sativum* on expression levels of genes involved in DNA repair signaling pathways. The expression of DNA repair genes was normalized by using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression as an endogenous control.

Gas Chromatography Mass Spectrometry analysis

Gas Chromatography Mass Spectrometry analysis of the *L. sativum* extract was conducted on Thermo GC-Trace Ultra Thermo MS DSQ II.

In silico prediction of toxicological properties

SwissADME and AdmetSAR were used to identified compounds pharmacokinetic properties, or drug-like characteristics.

Statistical analysis

GraphPad Prism® 9.0 and Microsoft Excel® were used for stastistic analysis. The Ct values were processed using the data analysis portal (Geneglobe, Qiagen). These Ct values were then converted to fold changes relative to the untreated control group, and the 2−ΔΔCt method was applied to calculate the fold changes in the treated groups.

Results

Cytotoxic activity of *L. sativum*

The impact of LS on the viability of HCT116 and SW620 cells following a 24-hour treatment is illustrated in Figures 1. It was observed that the anti-proliferative effect of LS on HCT116 cells was more pronounced compared to SW620 cells.

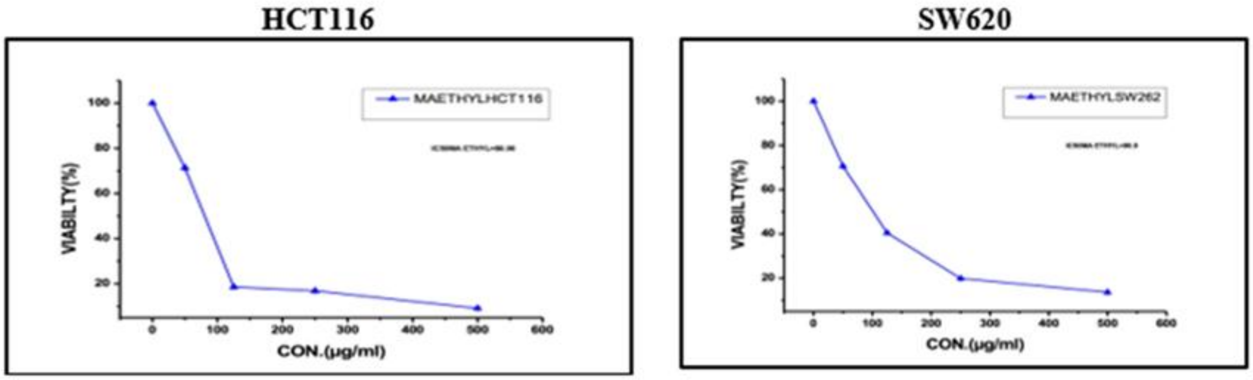
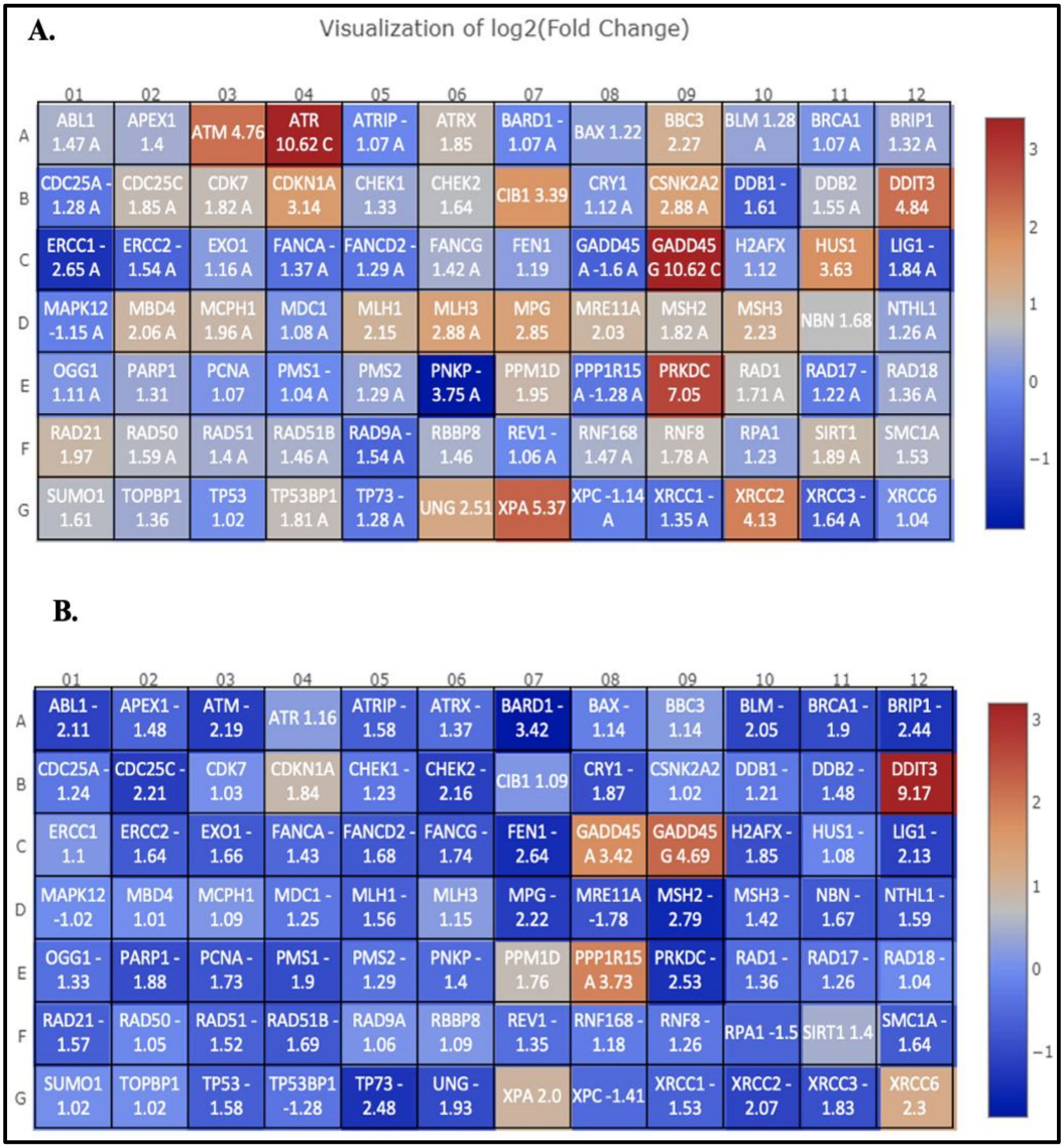


Figure-1. Cell viability colon cancer cell lines following treatment with serial dilution of L. sa-tivum.

RT2 profiler PCR array

After treating with *L. sativum* extract, gene expression analysis revealed notable alterations in DDR gene expression (fold change >2 or <-2) in both the HCT116 and SW620 cell lines. Figure 2 presents the expression profiles of the 84 genes linked to DNA repair pathways in colon cancer cells before and after treatment with *L. sativum* extract at concentrations of 80 µg/ml and 98.9 µg/ml. Heat map analysis, shown in Figure 3, illustrates the differential expression of DDR genes in the two cell lines. The heat maps clearly display varying expression patterns, with red squares representing up-regulated genes, blue squares denoting down-regulated genes, and gray squares indicating genes with no significant change. Notably, certain DNA repair genes were significantly up-regulated in the HCT-116 cells, while the SW620 cells displayed a more diverse range of both up- and down-regulated genes, suggesting a cell line-specific response to L. sativum treatment. To further explore the changes induced by treatment, scatter plot analyses were conducted, as shown in Figure 4. Genes with a greater than two-fold difference in mRNA expression compared to the control were highlighted. In the scatter plots, red circles indicate up-regulated genes, black circles represent down-regulated genes, and green circles show genes with no significant change. The analysis indicated a higher proportion of up-regulated genes in the HCT-116 cells, while the SW620 cells exhibited a more balanced distribution of both increases and decreases in gene expression, reflecting distinct molecular responses between the two cell lines.

Figure-2. The gene expression results revealed differential expression transcript of DDR genes (fold change >2 or <-2) in *the* HCT116 *and* in SW620 cell line.



A.

	01	02	03	04	05	06	07	08	09	10	11	12
A	ABL1 1.47 A	APEX1 1.4	ATM 4.76	ATR 10.62 C	ATRIP -1.07 A	ATRX 1.85	BARD1 -1.07 A	BAX 1.22	BBC3 2.27	BLM 1.28 A	BRCA1 1.07 A	BRIP1 1.32 A
B	CDC25A -1.28 A	CDC25C 1.85 A	CDK7 1.82 A	CDKN1A 3.14	CHEK1 1.33	CHEK2 1.64	CIB1 3.39	CRY1 1.12 A	CSNK2A2 2.88 A	DDB1 -1.61	DDB2 1.55 A	DDIT3 4.84
C	ERCC1 -2.65 A	ERCC2 -1.54 A	EXO1 1.16 A	FANCA -1.37 A	FANCD2 -1.29 A	FANCG 1.42 A	FEN1 1.19	GADD45A -1.6 A	GADD45G 10.62 C	H2AFX 1.12	HUS1 3.63	LIG1 -1.84 A
D	MAPK12 -1.15 A	MBD4 2.06 A	MCPH1 1.96 A	MDC1 1.08 A	MLH1 2.15	MLH3 2.88 A	MPG 2.85	MRE11A 2.03	MSH2 1.82 A	MSH3 2.23	NBN 1.68	NTHL1 1.26 A
E	OGG1 1.11 A	PARP1 1.31	PCNA 1.07	PMS1 -1.04 A	PMS2 1.29 A	PNKP -3.75 A	PPM1D 1.95	PPP1R15A -1.28 A	PRKDC 7.05	RAD1 1.71 A	RAD17 -1.22 A	RAD18 1.36 A
F	RAD21 1.97	RAD50 1.59 A	RAD51 1.4 A	RAD51B 1.46 A	RAD9A -1.54 A	RBBP8 1.46	REV1 -1.06 A	RNF168 1.47 A	RNF8 1.78 A	RPA1 1.23	SIRT1 1.89 A	SMC1A 1.53
G	SUMO1 1.61	TOPBP1 1.36	TP53 1.02	TP53BP1 1.81 A	TP73 -1.28 A	UNG 2.51	XPA 5.37	XPC -1.14 A	XRCC1 -1.35 A	XRCC2 4.13	XRCC3 -1.64 A	XRCC6 1.04

B.

	01	02	03	04	05	06	07	08	09	10	11	12
A	ABL1 -2.11	APEX1 -1.48	ATM -2.19	ATR 1.16	ATRIP -1.58	ATRX -1.37	BARD1 -3.42	BAX -1.14	BBC3 1.14	BLM -2.05	BRCA1 -1.9	BRIP1 -2.44
B	CDC25A -1.24	CDC25C -2.21	CDK7 1.03	CDKN1A 1.84	CHEK1 -1.23	CHEK2 -2.16	CIB1 1.09	CRY1 -1.87	CSNK2A2 1.02	DDB1 -1.21	DDB2 -1.48	DDIT3 9.17
C	ERCC1 1.1	ERCC2 -1.64	EXO1 -1.66	FANCA -1.43	FANCD2 -1.68	FANCG -1.74	FEN1 -2.64	GADD45A 3.42	GADD45G 4.69	H2AFX -1.85	HUS1 -1.08	LIG1 -2.13
D	MAPK12 -1.02	MBD4 1.01	MCPH1 1.09	MDC1 -1.25	MLH1 -1.56	MLH3 1.15	MPG -2.22	MRE11A -1.78	MSH2 -2.79	MSH3 -1.42	NBN -1.67	NTHL1 -1.59
E	OGG1 -1.33	PARP1 -1.88	PCNA -1.73	PMS1 -1.9	PMS2 -1.29	PNKP -1.4	PPM1D 1.76	PPP1R15A 3.73	PRKDC -2.53	RAD1 -1.36	RAD17 -1.26	RAD18 -1.04
F	RAD21 -1.57	RAD50 -1.05	RAD51 -1.52	RAD51B -1.69	RAD9A 1.06	RBBP8 1.09	REV1 -1.35	RNF168 -1.18	RNF8 -1.26	RPA1 -1.5	SIRT1 1.4	SMC1A -1.64
G	SUMO1 1.02	TOPBP1 1.02	TP53 -1.58	TP53BP1 -1.28	TP73 -2.48	UNG -1.93	XPA 2.0	XPC -1.41	XRCC1 -1.53	XRCC2 -2.07	XRCC3 -1.83	XRCC6 2.3

Figure-3. Heat map showing altered expressions of DNA repair genes in A. HCT-116 and B. SW620 cells. Red, gray and blue squares indicate up-regulated genes, non- regulated and down-regulated genes, respectively.

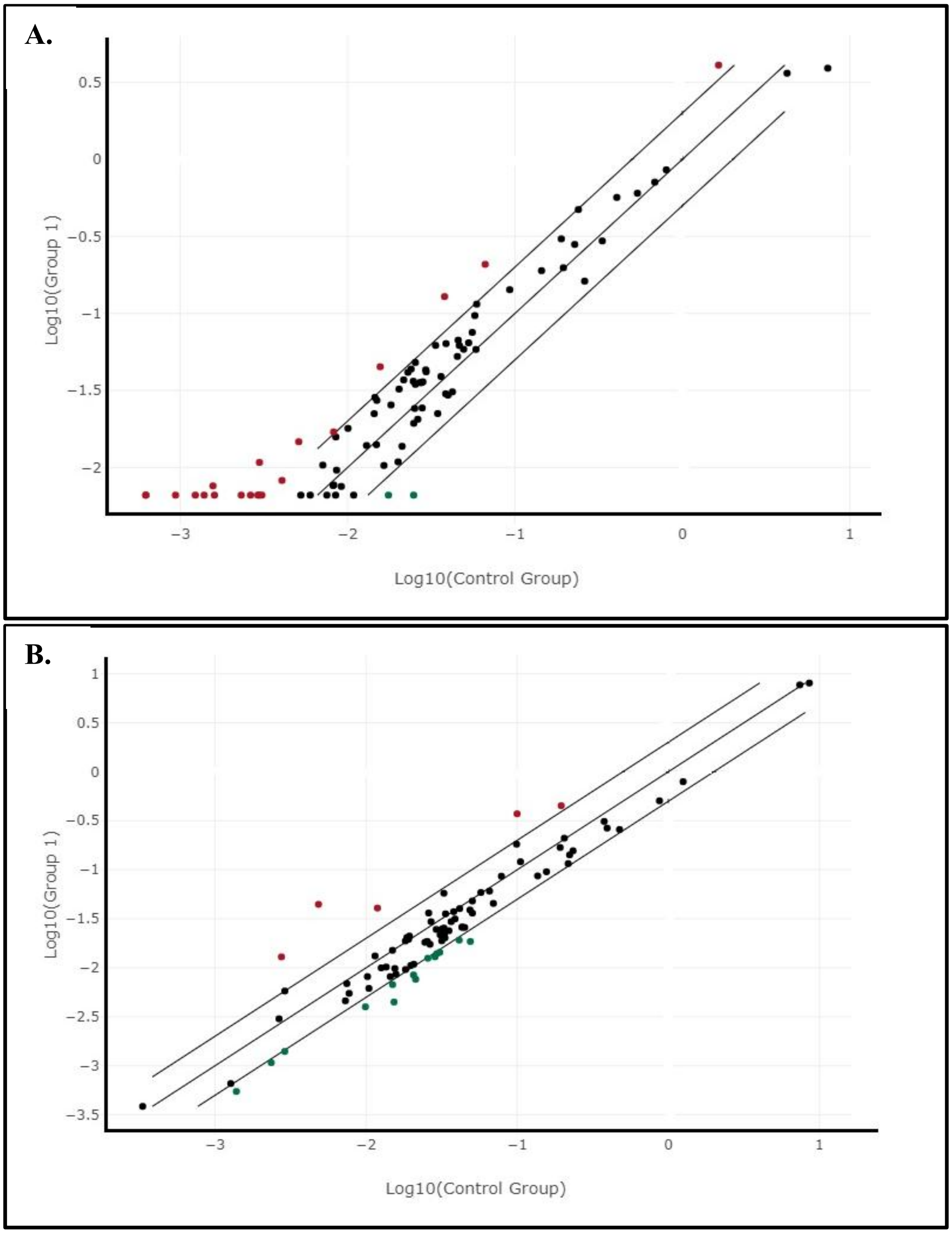


Figure-4. Scatter plot showing genes with > 2-fold difference in mRNA expression in treated A. HCT-116 and B. SW620 cells respectively compared to control. Red, black, and green circles indicate up-regulated genes, down-regulated genes, and non-regulated genes, respectively.

Following treatment with *L. sativum*, the expression levels of six transcripts were found to be elevated in HCT116 cells: ATM by 4.76-fold, DDIT3 by 4.84-fold, GADD45G by 10.62-fold, MPG by 2.85-fold, PRKDC by 7.75-fold, and XRCC2 by 4.13-fold. Conversely, in SW620 cells, DDIT3 was upregulated by 9.17-fold and GADD45G by 4.69-fold, while ATM, MPG, PRKDC, and XRCC2 were downregulated by -2.19-fold, -2.22-fold, -2.53-fold, and -2.07-fold respectively. Overall, there were six upregulated genes observed in the HCT116 cell line, while in the SW620 cell line, two genes were downregulated and four genes were upregulated as shown in Table 1.

Table-1. Changes in gene expression levels were observed in both cell lines following 24 hours of treatment with *L. sativum* extract, as indicated by fold change analysis.

Cell Lines	Symbol	Refseq	Fold Change Regulation
HCT116	**ATM**	NM_000051	4.76
SW620			-2.19
HCT116	**DDIT3**	NM_004083	4.84
SW620			9.17
HCT116	**GADD45G**	NM_006705	10.62
SW620			4.69
HCT116	**MPG**	NM_002434	2.85
SW620			-2.22
HCT116	**PRKDC**	NM_002434	7.05
SW620			-2.53
HCT116	**XRCC2**	NM_005431	4.13
SW620			-2.07

To validate the observed upregulation and downregulation identified through PCR array analysis, we conducted RT-PCR assays to measure the mRNA levels of the six genes associated with DNA Damage and Repair pathways in both HCT-116 and SW620 cell lines. Specific primers listed in Table 2 were utilized for this purpose. Additionally, we examined the expression of the human GAPDH gene from the same RNA samples to facilitate quantification and normalization procedures.

Table-2. List of Primers.

Genes	Forward primer	Reverse primer
ATM	5`-TGCGTGGCTAACGGAGAAAA	5`-ATCACTGTCACTGCACTCGG
DDIT3	5`-TCCAACTGCAGAGATGGCAG	5`-TCCTCCTCTTCCTCCTGAGC
GADD45G	5`-ACTAGCTGCTGGTTGATCGC	5`-TCCACGTTCAAGACTTTGGCT
MPG	5`-TCGAGTGTGTCAGGGTGTTT	5`-CTAGCTGGTCGCTGCTTCTT
PRKDC	5`-CAAGCGCATCATCATCCGTG	5`-GTAAGCCGCCTTCTCCTCTT
XRCC2	5`-TGGATAGACCGCGTCAATGG	5`-TCCACATCACACAGTCGTCG

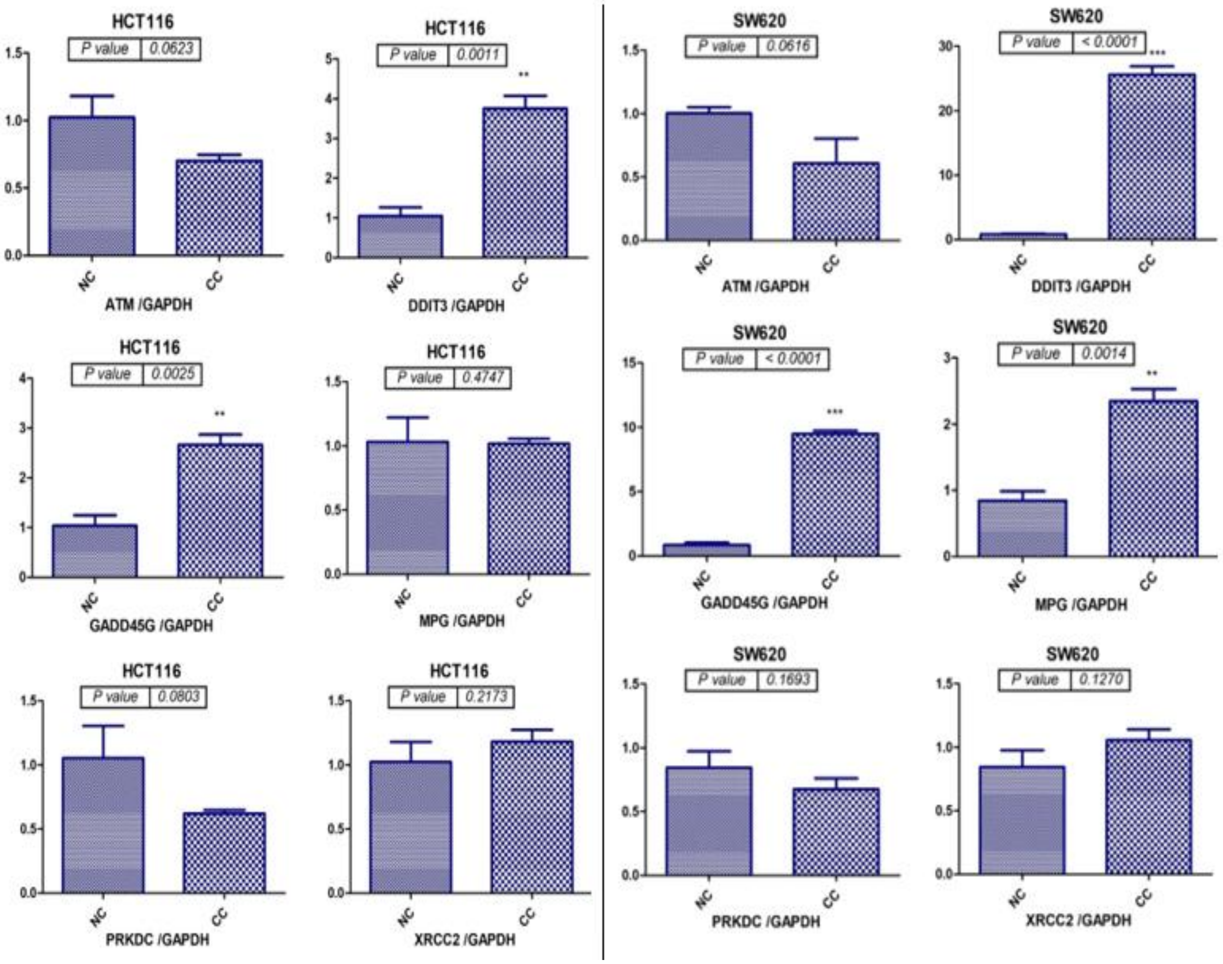


Figure-5. The mean fold change differences between the selected genes and GAPDH.

After normalization, significant differences in gene expression compared to GAPDH were observed in the HCT-116 cell line for DDIT3 and GADD45G. In the SW620 cell line, significant differences were noted in DDIT3, GADD45G, and MPG expression between treated and untreated cells.

Gas Chromatography Mass Spectrometry analysis

The GC-MS analysis of L. sativum identified nine distinct chemical constituents, as illustrated in Figure 6. The retention times provided insight into the structural and chemical nature of these compounds, which belonged to various chemical categories, detailed in Table 3.

Table-3. GC–MS profiling of *Lepidium Sativum* Extract.

Name	**R. Time**	**Area**	**Peak Width 50% (min)**	**Quality**	**Mol Weight (amu)**
Benzyl nitrile	9.829	93556	0.306	35	117.058
Benzene, (isothiocyanatomethyl)-	12.888	2184838	0.838	83	149.03
Hexadecanoic acid, methyl ester	19.056	84005	0.288	93	270.256
Octadecanoic acid	19.538	295159	0.844	64	284.272
9-Octadecenoic acid (Z)-, methyl ester	20.739	322772	0.288	94	296.272
Oleic Acid	21.201	889643	0.488	62	282.256
cis-9-Hexadecenoic acid	23.091	100418	0.438	64	254.225
8-Hexadecenal, 14-methyl-, (Z)-	24.498	67560	0.319	74	252.245
cis-13-Octadecenoic acid, methyl ester	28.114	126765	0.188	58	296.272

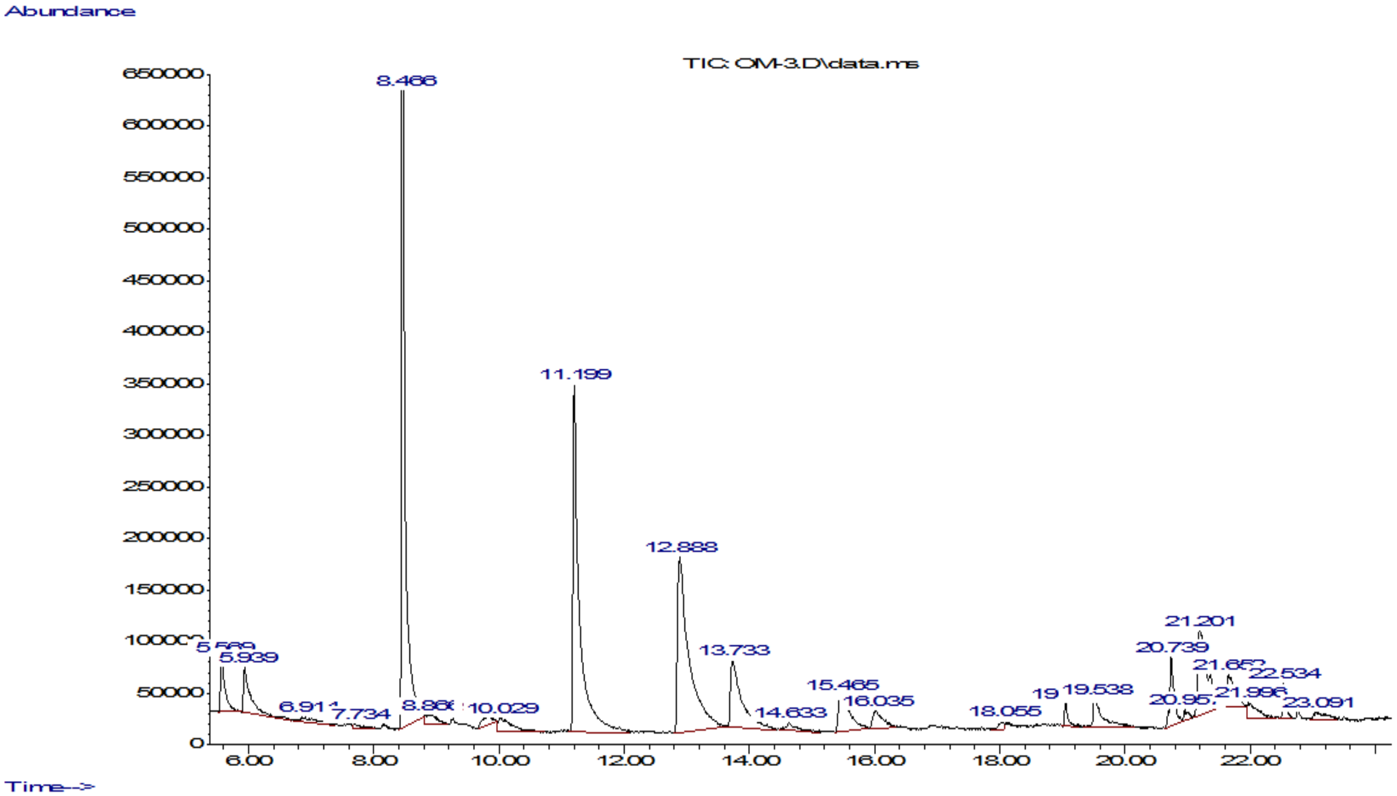


Figure-6. The GC–MS profile of Lepidium Sativum Extract.

In silico evaluation of pharmacokinetics and toxicity for compounds in *Lepidium sativum* extract

The pharmacokinetic properties of selected compounds from *Lepidium sativum* extract were analyzed using Lipinski's Rule of Five, with data retrieved via the SwissADME online platform. The results, summarized in Table 4, showed that none of the compounds breached more than one of the established criteria, indicating promising drug-like characteristics and good oral bioavailability. Furthermore, toxicological assessments as shown Table 5.

Table-4. Physicochemical properties of the identified compounds from *Lepidium Sativum* for good oral bioavailability.

Compound	**Lipinski Rules**					**Lipinski's Violations**
	MWg/mol	HBA	HBD	Log P	MR	
	≤500	≤10	≤5	≤5	40–130	
Benzyl nitrile	117.15	1	0	1.75	35.96	0
Benzene, (isothiocyanatomethyl)-	149.21	1	0	2.91	45.38	0
Hexadecanoic acid, methyl ester	270.45	2	0	5.54	85.12	1
Octadecanoic acid	284.48	2	1	5.93	90.41	1
9-Octadecenoic acid (Z)-, methyl ester	296.49	2	0	5.92	94.26	1
Oleic Acid	282.46	2	1	5.65	89.94	1
cis-9-Hexadecenoic acid	254.41	2	1	4.94	80.32	0
8-Hexadecenal, 14-methyl-, (Z)-	252.44	1	0	5.33	83.56	1
cis-13-Octadecenoic acid, methyl ester	296.49	2	0	5.9	94.26	1

MW= molecular weight; HBA= hydrogen bond acceptor; HBD= hydrogen bond donor; Log P= lipophilicity; MR= molar refractivity.

Table-5. Toxicological of Compounds Identified in *Lepidium sativum*.

Variable	**Compound Formula**										
		C_8H_7N	**C_8H_7NS**	**$C_{17}H_{34}O_2$**	**$C_{18}H_{36}O_2$**	**$C_{19}H_{36}O_2$**	**$C_{18}H_{34}O_2$**	**$C_{16}H_{30}O_2$**	**$C_{17}H_{32}O$**		
Absorption											
Blood-Brain Barrier		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		
Human Intestinal Absorption		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		
Caco-2 Permeability		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		
P-glycoprotein Substrate		NS	NS	NS	NS	NS	NS	NS	NS		
P-glycoprotein Inhibitor		NI	NI	NI	NI	NI	NI	NI	NI		
	Distribution										
Subcellular localization		ly	ly	mt	mt	pm	pm	pm	pm		
	Metabolism										
CYP450 2C9 Substrate		NS	NS	NS	NS	NS	NS	NS	NS		
CYP450 2D6 Substrate		NS	NS	NS	NS	NS	NS	NS	NS		
CYP450 3A4 Substrate		NS	NS	NS	NS	NS	NS	NS	NS		
CYP450 1A2 Inhibitor		I	I	NI	I	I	I	I	I		
CYP450 2C9 Inhibitor		NI	NI	NI	NI	NI	NI	NI	NI		
CYP450 2D6 Inhibitor		NI	NI	NI	NI	NI	NI	NI	NI		
CYP450 2C19 Inhibitor		NI	NI	NI	NI	NI	NI	NI	NI		
CYP450 3A4 Inhibitor		NI	NI	NI	NI	NI	NI	NI	NI		
	Toxicity										
AMES Toxicity		NAT	NAT	NAT	NAT	NAT	NAT	NAT	NAT		
Carcinogens		NC	NC	C	NC	C	NC	NC	C		
Acute Oral		II	II	III	IV	III	IV	IV	III		
Rat Acute		2.6059	2.6127	1.4915	1.3275	1.7357	1.3991	1.3991	1.7295		

mt= Mitochondria; ly= Lysosome; pm=plasma membrane; NS= Non-substrate; S=Substrate; I=Inhibitor; NS=Non-inhibitor; NAT= Non AMES toxic; C= Carcinogens; Noncarcinogenic=NC I =LD50 ≤ 50 mg/kg; II =50 mg/kg < LD50 < 500 mg/kg; III =500 mg/kg < LD50 < 5000 mg/kg; IV=5000 mg/kg < LD50.

Discussion

Recent studies have confirmed the significant impact of natural products derived from medicinal plants, fungi, and dietary sources in the treatment of cancers (Ali Abdalla et al., 2022; Talib et al., 2020). Over the past few decades, there have been notable advancements in the synthesis and application of chemotherapeutic drugs. However, despite their efficacy, the use of these drugs is hindered by toxicities and the development of drug resistance (Wang et al., 2019; Zhong et al., 2021). There is an urgency in finding treatments with reduced side effects compared to conventional therapies.

In the present study, two colorectal cancer cell lines with varying genetic backgrounds regarding the p53 state were chosen to serve as in vitro models of colorectal cancer. Initially, cells were tested for cellular viability against *L. sativum* extract, which exhibits dose-dependent cytotoxic effects in both cell lines (Figures 1&2). *L. sativum* is rich in polyphenols, flavonoids, and imidazole alkaloids, which are known to induce apoptosis (Doke and Manisha, 2014; Wadhwa et al., 2012; Behrouzian et al., 2014). The anticancer activity of *L. sativum* on human tongue squamous carcinoma (CAL-27) was reported by AlObaidi (2014) in a dose-dependent manner, with doses of 70, 100, and 150 µg/ml, which is in agreement with our results on colorectal cell suggesting same trend of anticancer effects of *L. sativum* on other types of cancer cells. Mahassni and Al-Reemi (2013) reported necrosis and apoptosis verified through morphological changes induced by *L. sativum* extract in the human breast cancer cell line MCF-7, highlighting the anticancer activity of *L. sativum* in the present study through increased apoptosis rates as observed by screening 84 DNA repair genes using PCR array analysis.

DNA repair was observed through elevated DDIT3 and GADD45G levels post treatment in both cells. DDIT3 and GADD45G are linked with endoplasmic reticulum stress and promote apoptosis and GADD45G alone is a negative regulator of cell growth at DNA damage checkpoints (Siwecka et al., 2019). The increased expression suggested a beneficial effect of *L. sativum* extract on cell viability and proliferation, through DNA-damaging agents. Downregulation of MPG was observed in SW620 cells on *L. sativum* treatment, indicating apoptosis rather than facilitating DNA repair in cancer cells. The MPG gene is expressed in the endoplasmic reticulum and mitochondria and initiates short and long patch base excision repair (BER) for deamination and alkylation in DNA (Wallace et al., 2012).

Upregulation of GADD45G leads to apoptosis and growth arrest in Acute Myeloid Leukemia cells following chemotherapeutic treatment by impairing homologous recombination DNA repair, increasing DNA damage, and inducing apoptosis in cancer cells while leaving normal cells unaffected (Guo et al., 2021). Additionally, another study by Liebermann and Hoffman (2008) validated the involvement of GADD45 proteins, including GADD45A, GADD45B, and GADD45G, in stress signaling responses to various physiological and environmental stressors, including oncogenic stress (Liebermann and Hoffman, 2008). These proteins can induce cell cycle arrest and promote survival, senescence, DNA repair, and apoptosis. Furthermore, Lin et al. (2020) demonstrated that both DDIT3 and GADD45G were significantly increased in cancer cell lines and gastric cancer tissues compared to normal cell lines and healthy tissues. The overexpression of DDIT3 was found to enhance colony-forming ability, gastric cancer cell proliferation, sphere formation, and cancer stem cell stemness. The increased expression of DDIT3 and GADD45G, which are involved in the apoptosis process, was observed while the expression of MPG, responsible for repair mechanisms, decreased significantly, confirming that *L. sativum* extract induces apoptosis in cancer cells.

GC-MS analysis revealed the presence of compounds known for noteworthy anticancer activities. Isothiocyanatomethyl-Benzene is reported to inhibit cancer cell growth and stop carcinogenesis in HeLa, MCF-7, and MDA-MB-231 cell lines through caspase-3 apoptotic activation. On the other hand, isothiocyanatomethyl-Benzene shows low cytotoxicity against peripheral blood mononuclear cells, emphasizing its use as a selective anticancer agent (Paul et al., 2019). Hexadecanoic acid is known for colon cancer suppression; it can activate caspase 3 to induce pro-apoptotic effects in human oral squamous cell carcinoma (hOSCC) models and inhibit cancer cell proliferation (Budi et al., 2022). Octadecanoic acid is cytotoxic against MOLT-4 leukemia cells and can interact with DNA topoisomerase I to induce apoptosis in murine models (Reza et al., 2021). Moreover, oleic acid (methyl oleate) is reported to suppress expression levels of HER2 (erbB-2), a key oncogene involved in tumorigenesis and metastasis, inhibiting cell proliferation in cancer cells. It can also affect intracellular calcium signaling related to cell proliferation and increase intracellular reactive oxygen species (ROS) levels and caspase-3 activity to induce pro-apoptotic effects in cancer cells (Jiang et al., 2017).

These compounds were further analyzed for ADME properties, detailing their safety profiles. According to Lipinski's Rule of Five, which is commonly used to assess the drug-likeness of orally administered compounds, the ideal criteria include: (i) a molecular weight of 500 Da or less; (ii) no more than 5 hydrogen bond donors; (iii) no more than 10 hydrogen bond acceptors; (iv) a logP value of 5 or below; and (v) a molar refractivity within the range of 40 to 130. Compounds meeting these benchmarks are typically considered to have favorable oral bioavailability (Bhat and Chatterjee, 2021). In this study, all tested compounds satisfied Lipinski's guidelines.

For toxicity assessment, the Ames test predictions indicated that the compounds were non-mutagenic and displayed low acute toxicity levels. Nonetheless, toxicological screening revealed that a few compounds may possess mild carcinogenic potential. Using the AdmetSAR tool, predicted carcinogenicity scores were found to be relatively low, with values such as 0.5347 for hexadecanoic acid methyl ester, 0.5217 for 9-octadecenoic acid (Z)-, methyl ester, and 0.6164 for 8-hexadecenal, 14-methyl-, (Z), indicating a low risk of carcinogenic effects (Asano et al., 2023; Ibrahim et al., 2021; Bhat and Chatterjee, 2021).

Conclusions

The anticancer potential of *L. sativum* extract in colorectal cancer cell lines showed dose-dependent cytotoxicity, induced apoptosis, and altered key gene expressions linked to DNA damage and repair. Bioactive compounds identified via GC-MS exhibited favorable drug-like properties and low toxicity supporting their therapeutic relevance.

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Contribution of Authors

AlDaihan S & Alanazi M: Contributed to conceptualization, resources, visualization, supervision and project administration.

Bin Watyan MA, AlAmri A, Alghamedi SO & Shaik J: Contributed to research methodology.

Shaik J, Bhat RS, AlZahrani M: Contributed to software analysis.

Bin Watyan MA, AlAmri A & Alghamedi SO: Contributed in method validation.

Bin Watyan MA, AlAmri A, Alghamedi SO & Khayyat AI: Contributed to formal analysis.

Bhat RS, AlZahrani M & Shaik J: Helped in investigation.

Bin Watyan MA, AlAmri A, Alghamedi SO, Shaik J & Bhat RS: Helped in data curation.

Bhat RS: Contributed in writing—original draft preparation.

Bin Watyan MA, AlDaihan S & Bhat RS: Helped in writing—review and editing.

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