Index

Title	Content	Pg. No
	Declaration by Research Scholar-Originality of Research Work	I
	Certificate of Supervisor	II
	Declaration by Research Scholar – Submission of Thesis	III
	Acknowledgement	IV
	Index	V
	List of Figures	XII
	List of Tables	XIV
	Abbreviations	VXI
	Abstract	XX
Chapter: 1	Introduction	
	1.1 Global Burden of Cancer	1
	1.2. Pathogenesis of cancer	3
	1.3. Drugs used in cancer therapy	5
	1.4. Enzymes therapy of cancer	6
	1.5. L-Methionase as an Anti-Cancer Enzyme	7
	1.6. Advantages and Challenges of L-Methionase therapy	8
	1.7. Clinical Studies and Future Developments	10
	1.8. Comparison with Other Enzymes Therapies	11
	1.9 Market value of Anti-cancer enzyme	11
	1.10. Current Status of L-Methionase	12
Chapter: 2	Review of Literature	16
	2.1. Methionine dependency in cancer	16
	2.2 L-Methionase	17

Title	Content	Pg. No
	2.3 Other Anti-Cancer Enzymes	18
	2.4. Mechanism of Action of L-Methionase	20
	2.5 Structure of L-Methionase	21
	2.6. Source of L-Methionase	23
	2.7. Screening of Fungal Isolates for L-Methionase Production	24
	2.8. L-Methionase Activity Assay Methods	25
	2.9. Production of L-Methionase Enzyme	27
	2.10. L-Methionase Enzyme Purification	34
	2.11. L-Methionase Requirements in Cancer Cells	40
	2.12. Utilization of L-Methionase in Cancer Therapy	43
	2.13. Modification of L-Methionase to Reduce Its Side Effects	46
	2.14. Cell Carrier-Based Drug Delivery System	47
	2.15. In Vitro and In Vivo Evaluation of L-Methionase	47
	2.16. Future Prospect For L-Methionase	49
	2.17. Other therapeutic uses of L-Methionase	51
	2.18. Application of L-Methionase in Human Welfare	52
Chapter: 3	Materials and Methodology	
	3.1 Culture media and their component	57
	3.2 Materials	58
	3.3 Instrumentation, Glassware, Plasticware	60
	3.4 Methds of Isolation, Screening and Morphological and Molecular Characterization	63 to 68
	3.5 Optimization of L-Methionase Enzyme	68
	3.7. Purification of L-Methionase Enzyme	76

Content	Pg. No
3.8. Biochemical Characterization L-Methionase Enzyme	82
3.9 Kinetic Analysis of L-Methionase	84
3.10 In Vitro Cytotoxic Assay for Anticancer Activity of Purified L-Methionase	84
3.11 Statistics	88
Results and Discussion	
4.1. Sample Collection and Isolation of Fungi	90
4.2. Screening of Fungal Isolates for L-Methionase Production	95
4.3. Morphological Identification of Fungi	101
4.4. Molecular Identification of L-Methionase Producing Fungi	103
4.5. Optimization of L-Methionase Enzyme	105
4.6. Purification of L-Methionase Enzyme	121
4.7. Biochemical Characterization of L-Methionase	126
4.8. Kinetic study of L-Methionase	131
4.9 In Vitro Anticancer Activity of Purified L-Methionase	133
Summary and Conclusion	140
References	143
Plagiarism Report	181
Publications	
	3.8. Biochemical Characterization L-Methionase Enzyme 3.9 Kinetic Analysis of L-Methionase 3.10 In Vitro Cytotoxic Assay for Anticancer Activity of Purified L-Methionase 3.11 Statistics Results and Discussion 4.1. Sample Collection and Isolation of Fungi 4.2. Screening of Fungal Isolates for L-Methionase Production 4.3. Morphological Identification of Fungi 4.4. Molecular Identification of L-Methionase Producing Fungi 4.5. Optimization of L-Methionase Enzyme 4.6. Purification of L-Methionase Enzyme 4.7. Biochemical Characterization of L-Methionase 4.8. Kinetic study of L-Methionase 5. Summary and Conclusion References Plagiarism Report

List of Figures

Figure No.	Name of Figure	Pg No.
Figure 1.1	Global Cancer Incidence and Mortality by Type (2022, GLOBOCAN)	2
Figure 1.2	Cell Cycle Dysregulation in Cancer Cells	4
Figure 1.3	Mode of Action of L-Methionase Anti-Cancer enzyme	8
Figure 1.4	Compound annual growth rate (CAGR %) for three major anticancer enzymes	12
Figure 2.1	Hydrolytic Reactions Catalyzed by Anti-cancer Enzymes	20
Figure 2.2	Formulation of the reaction for the L-methionase and PLP-catalyzed L-methionine	21
Figure 2.3	Three-dimensional structure of P. putida L-methionase	22
Figure 2.4	Schematic Representation of L-Methionase Activity Assay Methods	27
Figure 2.5	Overview of L-methionase role, DNA methylation, and metabolic reprogramming in cancer cells	42
Figure 2.6	L-Methionase—Setmet combo induces ROS-mediated apoptosis in tumor cells.	44
Figure 2.7	Cancer cell targeting by PS-binding molecules connected to human Annexin-V.	46
Figure 2.8	L-Methionase as a Anti-Cancer Enzyme	53
Figure 3.1	Geographical Locations of Soil Sampling Sites in Gujarat, India	63
Figure 4.1	Morphological Diversity of Fungal Isolates (MF 1–MF 7) from Cotton Field Soils of Morbi	91
Figure 4.2	Morphological Diversity of Fungal Isolates (MF 8–MF 14) from Marine Soils of Porbandar	91
Figure 4.3	Morphological Diversity of Fungal Isolates (MF 15–MF 21) from Marine Soils of Dwarka	92
Figure 4.4	Morphological Diversity of Fungal Isolates (MF 22–MF 28) from Bhagatsingh Garden Soils of Rajkot	92
Figure 4.5	Morphological Diversity of Fungal Isolates (MF 29–MF 35) from Marine Soils of Dandi and Mandavi	93

Figure No.	Name of Figure	Pg No.
Figure 4.6	Morphological Diversity of Fungal Isolates (MF 36–MF 40) from Aji River Soils of Rajkot	93
Figure 4.7	Morphological Diversity of Fungal Isolates (MF 41–MF 44) from Nyari River Soils of Rajkot	94
Figure 4.8	Morphological Diversity of Fungal Isolates (MF 45–MF 50) from Machhu River Soils of Morbi	94
Figure 4.9	Comparative Analysis of L-Methionase Activity, Protein Concentration, and Specific Activity in Selected Fungal Isolates	100
Figure 4.10	Phylogenetic Tree of Fungal Isolate MF 13 Based on ITS Region Sequences Showing Close Relationship with Aspergillus fumigatus	104
Figure 4.11	Optimization of pH on L-Methionase production	106
Figure 4.12	Optimization of temperature on L-Methionase production	106
Figure 4.13	Optimization of substrate concentration on L-Methionase production	107
Figure 4.14	Optimization of Inoculum Size on L-Methionase production	108
Figure 4.15	Optimization of incubation time on L-Methionase production	109
Figure 4.16	optimization of carbon source on L-Methionase production	110
Figure 4.17	optimization of Nitrogen source on L-Methionase production	110
Figure 4.18	Pareto chart of the standardized effects of nine medium factors on L-Methionase production by Aspergillus fumigatus MF13	113
Figure 4.19	(A, B & C) RSM 3D surface plots obtain by design expert 13 representing the effect and relationship between different variables in L-Methionase production A) Temperature vs Yeast extract, B) Temperature vs Dipotassium phosphate C) Yeast extract vs Dipotassium phosphate.	120
Figure 4.20	Chromatogram of L-Methionase Elution Profile from Aspergillus fumigatus MF13 Using Size-Exclusion Chromatography	122
Figure 4.21	SDS-PAGE analysis of L-methionase protein using silver staining (Right) and Coomassie Brilliant Blue staining (Left).	124
Figure 4.22	Effect of pH and pH Stability on the Activity of Purified L- Methionase from Aspergillus fumigatus MF13	127
Figure 4.23	Effect of Temperature on Catalytic Activity and Thermal Stability of Purified L-Methionase from Aspergillus fumigatus MF13	128

Figure No.	Name of Figure	Pg No.
Figure 4.24	Influence of Metal Ions at 1 mM and 5 mM on the Specific Activity of Purified L-Methionase from Aspergillus fumigatus MF13	130
Figure 4.25	Michaelis-Menten Kinetics of L-Methionase Produced by Aspergillus fumigatus MF13	131
Figure 4.23	Effect of L-Methionase on HT-29 Cell Viability (%) at Vari Concentrations (25, 50, 100, 200, and 400 µg/mL) as Determined MTT Assay	134
Figure 4.24	Morphological changes in HT-29 cell lines treated with test compounds	135
Figure 4.21	Effect of L-Methionase on MDA-MB-231 Cell Viability (%) at Vari Concentrations as Determined by MTT Assay	138

List of Tables

Table No.	Name of Table	Pg No.
Table 1.1	Methionine Dependency and Therapeutic Potential of L-Methionase	3
T 11 13	Across Various Cancer Types	
Table 1.2	Differences Between Normal and Cancer Cells	5
Table 1.3	Classification of Chemotherapeutic Agents with Mechanisms and Representative Drugs	5
Table 1.4	Advantages vs Challenges of L-Methionase Therapy	9
Table 1.5	Comparison of other Enzymes	11
Table 1.6	Market Value of Anti-Cancer Enzymes	12
Table 2.1	Sources of L-Methionase Enzyme	24
Table 2.2	Summary of L-Methionase Purification Steps and Conditions	35
Table 2.3	Biochemical Characterization of L-Methionase	40
Table 3.1	Source of Fungal isolates	63
Table 3.2	Experimental Variables at Two Levels for L-Methionase Production	75
	Using Plackett-Burman Design	
Table 3.3	FPLC Operational Parameters for Size-Exclusion Chromatography of	79
	L-Methionase	
Table 4.1	Quantitative assay of L-Methionase by Rapid Plate Method	96
Table 4.2	Zone of Diameter of Positive Fungal Isolates	97
Table 4.3	Quantitative Estimation of L-Methionase Activity, Protein	99
	Concentration, and Specific Activity in Selected Fungal Isolates	
Table 4.4	Morphological Characterization of positive fungal isolates	102
Table 4.5	Placket Burman design for optimization of parameters influencing L-	112
	Methionase production	
Table 4.6	ANOVA for the experimental result of Placket Burman experimental	113
	design	
Table 4.7	CCD for optimization experiment for L-methionase by A. fumigatus	117
T 11 40	MF 13.	110
Table 4.8	Analysis of ANOVA and significance level of the response surface of	118
Table 4.9	the full quadratic model for the L-methionase production Purification summary of L-Methionase enzyme	126
1 avic 4.7	1 in greation summary of L-memionase enzyme	140
Table 4.11	Cell Viability of HT-29 Cells Treated with L-Methionase	133
Table 4.12	Cell Viability of MDA-MB Cells Treated with LMethionase	138

List of Abbreviations

ALL: Acute Lymphoblastic Leukemia

ANOVA: Analysis of Variance

APS: Ammonium Persulfate

ATF-MGL-FP: Amino-Terminal Fragment-Methionine Gamma-Lyase-Fusion Protein

ATCC: American Type Culture Collection

BCR-ABL: Breakpoint Cluster Region-Abelson Murine Leukemia Viral Oncogene Homolog

BLAST: Basic Local Alignment Search Tool

B.O.D.: Biochemical Oxygen Demand

BSA: Bovine Serum Albumin

BCNU: Bis-Chloroethylnitrosourea

CAGR: Compound Annual Growth Rate

CCD: Central Composite Design

CDKN2A: Cyclin-Dependent Kinase Inhibitor 2A

CV: Column Volume

DEAE: Diethylaminoethyl

DMEM: Dulbecco's Modified Eagle Medium

dNTPs: Deoxynucleotide Triphosphates

DMSO: Dimethyl Sulfoxide

DNA: Deoxyribonucleic Acid

DNMT: DNA Methyltransferase

DTT: Dithiothreitol

DTNB: 5,5'-Dithiobis-(2-nitrobenzoic acid)

EDTA: Ethylenediaminetetraacetic Acid

FBS: Fetal Bovine Serum

FDA: Food and Drug Administration

FP: Fusion Protein

FPLC: Fast Protein Liquid Chromatography

5-FU: 5-Fluorouracil

GEMM: Genetically Engineered Mouse Model

GLOBOCAN: Global Cancer Observatory

HCC: Hepatocellular Carcinoma

Hcy: Homocysteine

HCT-116: Human Colon Tumor-116 (Colon Cancer Cell Line)

HepG2: Hepatoma G2 (Liver Cancer Cell Line)

HIV: Human Immunodeficiency Virus

IC₅₀: Half-Maximal Inhibitory Concentration

IGF2: Insulin-Like Growth Factor 2

ITS: Internal Transcribed Spacer

LPCB: Lactophenol Cotton Blue

MBP: Methyl-Binding Protein

MBTH: 3-Methyl-2-benzothiazolonehydrazone

MCF-7: Michigan Cancer Foundation-7 (Breast Cancer Cell Line)

MgCl₂: Magnesium Chloride

MGL: Methionine Gamma-Lyase

miRNA: MicroRNA

MMP: Matrix Metalloproteinase

MRI: Magnetic Resonance Imaging

MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide

NADH: Nicotinamide Adenine Dinucleotide

NaCl: Sodium Chloride

NaOH: Sodium Hydroxide

NCCS: National Centre for Cell Science

NHL: Non-Hodgkin Lymphoma

OFAT: One-Factor-At-a-Time

PBD: Plackett-Burman Design

PBS: Phosphate-Buffered Saline

PCR: Polymerase Chain Reaction

PDA: Potato Dextrose Agar

PDX: Patient-Derived Xenograft

PEGylation: Polyethylene Glycol Conjugation

PET: Positron Emission Tomography

PI: Propidium Iodide

PLP: Pyridoxal 5'-Phosphate

PMSF: Phenylmethylsulfonyl Fluoride

PpLM: Pseudomonas putida L-Methionase

PS: Phosphatidylserine

PSA: Prostate-Specific Antigen

qPCR: Quantitative Polymerase Chain Reaction

RASSF1: Ras Association Domain Family Member 1

RB1: Retinoblastoma 1

RLGS: Restriction Landmark Genomic Scanning

ROS: Reactive Oxygen Species

RPMI-1640: Roswell Park Memorial Institute-1640

rMETase: Recombinant Methioninase

RSM: Response Surface Methodology

SAM: S-Adenosylmethionine

SD: Standard Deviation

SDS: Sodium Dodecyl Sulfate

SDS-PAGE: Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

SEC: Size-Exclusion Chromatography

SeMet: Selenomethionine

SGLT: Sodium-Glucose Linked Transporter

SRB: Sulforhodamine B

TEMED: N,N,N',N'-Tetramethylethylenediamine

tHCY: Total Homocysteine

TIMP: Tissue Inhibitor of Metalloproteinase

TNB: 2-Nitro-5-Thiobenzoate

Tris-HCl: Tris-Hydrochloride

TUNEL: Terminal deoxynucleotidyl transferase dUTP Nick End Labeling

U/mL: Units per Milliliter

UHRF1: Ubiquitin-like with PHD and RING Finger Domains 1

uPA: Urokinase Plasminogen Activator

Vmax: Maximum Velocity

v/v: Volume per Volume

WHO: World Health Organization

μg/mL: Micrograms per Milliliter

μL: Microliter

μm: Micrometer

Mm: Millimolar