# ABSTRACT

L-Methionase has emerged as a potent enzyme with promising applications in cancer therapy due to its ability to selectively deplete methionine an essential amino acid for methionine-dependent tumor cells. This study aimed to isolate and characterize fungal strains capable of producing L-methionase, optimize its production, purify the enzyme, and evaluate its in vitro anticancer potential. Soil samples were collected from diverse ecological regions across Gujarat, India including marine, riverine, and agricultural sites to explore fungal biodiversity. A total of 50 fungal isolates were obtained, and qualitative screening using modified Czapek-Dox agar identified *Aspergillus fumigatus* MF13 as the most potent L-methionase producer. Quantitative assessment through enzyme assay and specific activity estimation further confirmed MF13's enzymatic potential, with a maximum activity of 4.31 U/mL/min and a specific activity of 1.48 U/mg. Molecular identification using ITS sequencing validated MF13's identity as *Aspergillus fumigatus* (GenBank accession: OQ690549). Optimization of enzyme production was achieved using a combination of One-Factor-at-a-Time (OFAT), Plackett-Burman Design (PBD), and Central Composite Design (CCD), culminating in a 2.57 U/mL/min yield under optimal conditions: 30°C, pH 8.0, 2.4 g/L yeast extract, and 1.2 g/L dipotassium phosphate. Purification via cold acetone precipitation and Sephadex G-75 chromatography resulted in a 10.5-fold increase in purity, with a specific activity of 40.0 U/mg and molecular weight of ~45 kDa, as confirmed by SDS-PAGE. Biochemical characterization showed optimal activity at pH 7.5 and 30°C, and notable stability under alkaline and moderate thermal conditions. Enzyme kinetics revealed a Km of 0.674 mM and Vmax of 0.871 U/mL, indicating strong substrate affinity. *In vitro* cytotoxicity assays (MTT) demonstrated dose-dependent anticancer activity of purified L-methionase. HT-29 (colon cancer) cells were highly sensitive ($IC_{50} \approx 175$ µg/mL), while MDA-MB-231 (breast cancer) cells showed resistance ($IC_{50} \approx 390$ µg/mL), suggesting variable methionine dependency. This research highlights *Aspergillus fumigatus* MF13 as a promising source of L-methionase and reinforces the enzyme's potential as a selective anticancer agent. The successful optimization and purification pave the way for further development in therapeutic applications, with future work focusing on overcoming resistance mechanisms and evaluating *in vivo* efficacy.