The research work comprises of a detailed and systematic phytochemical and pharmacological evaluation of various extracts, fractions and phytoconstituents of *Allium Sativum* Linn (Family: Liliaceae; Garlic) for antidiabetic (antihyperglycemic) activity.

**Preparation of extract/s and fraction/s**

Three varieties garlic were chosen for the study viz, Multiple clove garlic (G1), Single clove garlic (G2) and Himalayan garlic (G3). Procured plant materials were authenticated by botanist from Botanical Survey of India, Pune and the samples were deposited for future use (Specimen Number: ACPL1N, ACPL2 and NID-31 respectively). Methanol extracts of all three variety of fresh garlic cloves and peels were prepared (Garlic Bulb: ME-GB1, ME-GB2 and ME-GB3; Garlic Peel: ME-GP1, ME-GP2 and ME-GP3). Butanol fraction (BF-GB1, BF-GB2 and BF-GB3) was obtained from methanol extract of garlic bulb. Methanol extract of Himalayan garlic peel was further fractionated with acetone and methanol (AF-GP3 and MF-GP3).

**Phytochemical investigation of extract/s and fraction/s**

Preliminary phytochemical screening of the garlic extracts and fractions revealed the presence of organo sulfur compounds (cysteine derivatives), phenolics, flavonoids and saponins especially of furostanol saponins. The amount of total phenolics and flavonoids in himalayan garlic peel (ME-GP3) was found to be higher than garlic bulb extract and fraction. Its acetone fraction (AF-GP3) contains highest amounts of total phenolics and flavonoids among all the extracts and fractions. The saponins found in extracts and fractions of garlic bulb/peels were of haemolytic in nature and concentrations which causes 50 % haemolysis (HD50) was determined. Himalayan garlic bulb (ME-GB3) contains higher amount of saponins and its butanol fraction was rich in saponins. Even thiosulfinates of garlic also contributed to haemolytic activity. TLC profiles and HPTLC finger prints were developed for all the extracts and fractions.

**Antioxidant studies on extract/s and fraction/s**

The crude extract/s and fraction/s exhibited antioxidant activity and the results were comparable to standard antioxidants, ascorbic acid (for DPPH assay and TBARS assay) and rutin (for total reducing power).
DPPH assay: The methanol extracts of all garlic peel showed very good DPPH scavenging activity compared to all garlic bulb extracts and fractions. Among the garlic peel extracts, Himalayan variety and its acetone fraction (ME-GP3 & AF-GP3) exhibited strong radical scavenging activity.

TBARS assay: The methanol extracts of garlic peel showed very good antilipid peroxidation activity compared to all garlic extracts and fractions. Among the garlic peel extracts, Himalayan variety and its acetone fraction (ME-GP3 & AF-GP3) exhibited stronger antioxidant activity.

In vitro antioxidant activity by methaemoglobin assay: The dose dependent increase in the antioxidant activity was observed in human haemolysate treated with all the three extracts/ fractions. The methanol extract of Himalayan garlic peel (ME-GP3) showed potent activity with IC₅₀ value of 2.31 µg/ml.

Total reducing power: All the eleven extracts/fractions showed concentration dependent increase in the total reducing power. The methanol extract of Himalayan garlic peel and its acetone fraction (ME-GP3 & AF-GP3) showed strong reducing potential among all the extracts which definitely contributed to its antioxidant activity.

Acute toxicity studies of garlic extracts/fractions

Based on the antioxidant and acute toxicity study, three active extracts/fractions {methanol extract and butanol fraction of single bulb garlic (ME-GB2 & BF-GB2) and methanol extract of garlic peel (ME-GP3)} have been selected for evaluation of anti diabetic study.

Acute toxicity studies of the three selected active extracts/fractions have shown that the extract/s and fraction were safe upto 2000 mg/kg and are categorized under category 5 of GSH as per OECD guidelines 423.

Evaluation of Antidiabetic activity of active extract/s and fraction/s by in vivo studies

Diabetic model 1: The above mentioned three active extracts/fractions have been evaluated for Antidiabetic activity in STZ-Nicotinamide induced model of type 2 diabetes in rats. All the extracts showed good antidiabetic activity, however garlic extract and garlic peel extract (ME-GB2 & ME-GP3) exhibited promising activity.

Diabetic model 2: Based on the above study, two bioactive extracts (ME-GB2 & ME-GP3) were selected for detailed evaluation antidiabetic activity by neonatal STZ
induced model of type 2 diabetes in rats. Methanol extract of garlic bulb (250 mg/kg) showed excellent antihyperglycemic activity as compared to methanol extract of garlic peel (50 mg/kg) in reducing fasting blood glucose levels. The results also revealed the insulin sensitizing action of extracts in overcoming insulin resistance. Statistical significant decrease in serum insulin and glycated haemoglobin levels were also observed in garlic bulb treated animals. ME-GP3 showed better antihyperlipidemic activity since the lipid profile viz, triglycerides, total cholesterol, LDL-c and VLDL-c levels were reduced statistically at p<0.05.

**Isolation of constituents from Bioactive extracts:**

Three compounds (F1, F2 and F3) have been isolated from bioactive extract (ME-GP3) by preparative TLC and compound F1 was obtained with highest purity. All the three constituents are strong scavengers of DPPH radical. HPLC and HPTLC studies were carried out in order to confirm the purity. These compounds were subjected to various spectral studies like UV, LC-MS, IR and NMR for its characterization. The isolated compound F1 likely a phenyl propanoid compound that could be having the basic structure of guaiacyl glycerol-β-ferulic acid ether with less than one -CH₂- in the side chain. The isolated compounds F2 and F3 could not be elucidated with the available data.

**In vitro antidiabetic studies of active fraction:**

All the three isolated antioxidant constituents were found to be present in the acetone fraction (AF-GP3) of bioactive extract (ME-GP3). Acetone fraction was subjected to *in vitro* antidiabetic target studies viz, α-glucosidase (AGI), Dipeptidyl peptidase IV (DPP IV) and Protein Tyrosine Phosphatase 1B (PTP1B). The acetone fraction exhibited parital inhibition on α-glucosidase and PTP1B and least inhibition of DPP IV.