CONCLUSIONS

From the present study, the significant conclusions could be drawn as:

The microorganisms which are efficient in uricase production were screened by means of primary screening on uric acid containing agar plates. The selected uricase producers as *Pseudomonas aeruginosa Ph3, Pseudomonas aeruginosa 5Y2* and *Bacillus cereus GHMS* were then used to produce uricase enzyme in the nutrient broth medium containing uric acid as an inducer uric acid by optimizing pH, temperature, agitation speed, uric acid concentration and found to produce uricase enzyme 13.4, 17.7 and 19.4 U/mL respectively.

Due to the increased uricase production, the *Bacillus cereus GHMS* strain was selected for further uricase production. The uricase produced from *Bacillus cereus GHMS* was found to be inducer dependent in which uric acid was more effective among the purine metabolites such as the adenine, guanine, and xanthine.

The production of uricase enzyme was increased upto 31.3 U/mL to the addition of 1 % Polyvinyl Alcohol (PVA) which is known to decrease the surface tension.

So in order to further enhance the uricase production, nutrient media was optimized using Taguchi DOE methodology with L18 trials.

The Physico-chemical properties of the crude uricase were studied by optimization of pH and temperature. The optimum activity by crude uricase was found at pH 7.5 and temperature 30^0C.

The optimized medium was further validated and scaled up in 7.5 L bioreactor under controlled conditions and obtained 39.7 U/mL of uricase crude extract.

The obtained 200 mL of crude extract was purified by means of anion-exchange chromatography using DEAE-Sepharose as a matrix and size-exclusion chromatography using Superdex-75 pg as a matrix with a final specific activity of 87 U/mg.

The purity at each step was checked by SDS-PAGE analysis and final purified molecular weight of uricase was calculated as 34.5 kDa. The purified SDS gel band was trypsinised using in-gel trypsinization protocol and characterized by MALDI-TOF by which molecular weight of uricase was obtained as 35 kDa which was comparable with the SDS-PAGE analysis containing 301 amino acids sequence.

Using deduced amino acid sequence the structural and functional characterization was studied. The properties of purified uricase was analyzed thoroughly by optimizing pH and temperature which found to be 8.0 and 30^0C respectively, however before purification, the optimum pH for uricase activity was 7.5.

The efficacy of purified uricase was studied in uricase deficient hyperuricemic Swiss albino mice. The 5 µg of uricase dose was found to be significant at 6 h of administration by intraperitoneal rout which decreases serum uric acid level significantly.

The immunological properties of the purified *Bc*-uricase was studied by measuring the total immunoglobulin G content in the serum after administration of uricase and observed that there were no any significant alteration in the total IgG content and

hence the purified uricase was non-immunogenic in nature may be applied in the treatment of hyperuricemia and gout in humans.