ABSTRACT

The crassulacean herb *Bryophyllum pinnatum* finds extensive worldwide application in traditional medicine. Inspite of extensive studies on biochemical characterization including CAM metabolism and numerous therapeutic potentials revealed so far, a little information is available on the bioactive phytoconstituents of this herb. Thus, the research emphasis has been given to study extensively some of the bioactive potential of the leaf extract of *B. pinnatum*

To enrich the bioactive principles during extraction, optimization of the process was carried out using response surface methodology (RSM). The process resulted in a 2 fold increase in total phenolic content where the use of solvent could be reduced up to 37 %. Statistical analyses (R^2, *p*, *t* and *F* values) and contour plots reiterated the efficacy of the developed model. Validation of the RSM model for extraction of TPC was confirmed by HPLC analysis.

Methanolic, aqueous and aqueous-methanolic extracts of *B. pinnatum* leaves were screened comparatively for their biological activities *viz.* antioxidant, antityrosinase, antiglycation, antiinflammatory and antidiabetic potential by *in vivo* and *in vitro* means. Aqueous-methanolic extract was an efficient antioxidant principle in addition to tyrosinase and glycation inhibitory properties, as shown by the IC_{50} values when compared to authentic standards. Antiinflammatory activity screened in mice model rendered the aqueous-methanolic extract (200 mg/kg BW) as most efficient. Antihyperglycemic potential screened in alloxanised mice revealed efficiency of all three *B. pinnatum* extracts (400 mg/kg BW) as potential antidiabetic formulations. A correlation between the extracts and their biological activities was evident.

Biochemical analyses of leaves showed its richness in macro- and micro-nutrients, polyphenols, saponins, flavonoids and glycosides. Extracts were chromatographed in silica and LH-20 matrices and further characterized by TLC, HPLC, ^{1}H- and ^{13}C- NMR, LC- and GC- MS/MS leading to the identification of gallic, ferulic, sinapinic and caffeic acids. Pharmacodynamic investigation conducted using HSA and dietary phenolic acids (FA) of *B. pinnatum* involved in molecular interactions investigated by fluorescence, CD and FTIR studies. Thermodynamic variations monitored by ITC revealed weak interaction (van der Waals and hydrogen bond) between the macromolecule and ligand, while a negative free energy indicated a thermodynamically favorable interaction facilitating slow drug release. The interaction pattern of HSA-FA docked posed was also hypothesized/ supported by molecular docking studies. Microscopic studies were performed to localize the various phytochemicals in *B. pinnatum* leaf tissue.

Keywords: *Bryophyllum pinnatum*; Optimization; Response surface methodology; Antioxidant; Antityrosinase; Antiglycation; Isothermal titration calorimetry; Human serum albumin; Ferulic acid; Molecular docking.