Supercritical Fluid Extraction and Ultrasound Assisted Extraction of Phytochemicals from Underutilized Bhimkol (*Musa balbisiana*) Banana Blossom, its Antidiabetic Property and Application

Abstract

This study primarily focuses on the development of phytochemical enriched ready to cook soup mix from bhimkol (*Musa balbisiana*) banana blossom with antidiabetic property. Study comprises extraction of major phytochemicals by using different extraction techniques from bhimkol blossom and optimization by using heuristic functions and their antioxidant properties. The investigations of nutritional components, biochemical, phytochemicals, and volatile phytochemicals were conducted along with study of its antioxidant and antibacterial properties and then nutrition-rich nacho from bhimkol blossom was developed. Major phytochemical extracted were isolated, characterized and then microencapsulated to target its bioactivities in intestinal cells. Microbeads obtained by microencapsulation of major phytochemicals were incorporated in ready to cook soup mix developed from bhimkol blossom powder. Sensory analyses were performed to study the customer demand and market sustainability of the enriched phytochemical ready to cook soup mix. Thorough *in vitro* and *in vivo* investigations of antidiabetic property of bhimkol blossom extract were performed. The importance of this study is mainly to showcase the wide opportunities of underutilized bhimkol banana blossom in the field of food engineering and pharmacological industry.

The organization of thesis is divided into 8 chapters for the consistent presentation of the research work along with the findings.

Chapter 1 comprises the introduction and literature review of the overall present research work. It includes the major detail about bhimkol blossom and its nutritional, phytocomponents, phytochemicals and health-beneficial properties. It also comprises the detail of phytochemicals possessing health beneficial effects, efficient methods of phytochemical extractions, importance of optimization techniques, and its applications as direct incorporation in food model or incorporation of encapsulated phytochemicals in food models. Research gap, the hypothesis of the study and objectives of the present research works are also stated.

Chapter 2 detailed about the optimization of phytochemical extraction from bhimkol blossom by using novel techniques. Three extraction techniques *viz*., conventional, ultrasound-assisted and supercritical fluid extractions (SCFE) were used to study the total polyphenol content (TPC) and antioxidant activity in bhimkol (*Musa balbisiana*) banana blossom. Optimization was carried out to attain major phytochemicals by using response surface methodology-central composite design. Various parts of blossom and whole banana blossom (WB) were investigated for their major phytochemical constituents by conventional method and RP-HPLC (at the optimized condition). The WB revealed higher phytochemical contents than other parts. Both SCFE and UAE were found to be efficient in extraction phytochemicals. The efficiency of SCFE was a bit superior to the UAE and conventional method of phytochemical extraction, but UAE was the most convenient for the operation based on operating cost and maintenance. Highest TPC (2750.37 mg GAE/100g) was obtained in WB by the SCFE method vis-à-vis the antioxidant activity (79.41%) at optimized condition (60^oC, 210 bar, 40 min, and 5 g/min CO_2 flow-rate). RP-HPLC analysis revealed various phytochemicals of different concentrations depending on the extraction method used. The results of the present investigation will be useful in development of functional foods.

Chapter 3 detailed about the investigation of nutrition, phytocomponents, antioxidant activities, antibacterial activities, formulation and optimization of nachos from underutilized bhimkol (*Musa balbisiana*) blossom by metaheuristic approach; optimal mixture designed artificial neural network coupled with particle swarm optimization (ANN-PSO). The overall acceptability obtained from the sensory evaluation was taken as the output layer of the swarm optimization. Investigation showed bhimkol blossom as a good source of nutrition, phytocomponents such as carbohydrate (66.87±0.20%), crude fiber (12.12±0.28%), vitamin E (8.73 mg/g), and fatty acid consisting of monounsaturated (66.67%) and polyunsaturated (33.33%). The whole blossom showed the highest antioxidant at 82.77% in the DPPH assay and major antibacterial activity against *Salmonella typhi* at IC_{50} 25.21 ± 0.14 mg/ml. The optimum set of the parameter from ANN-PSO for best nachos was obtained at overall acceptability 8.9 in epoch 6 and showed high fracturability and crispiness. Principal component analysis among the sensory attributes of commercial, control and bhimkol blossom nachos revealed that the taste and overall acceptability were majorly weighted towards component 1. The least significant difference was found between commercial and bhimkol blossom nachos.

Nutrition rich nachos can be helpful in worldwide implementation in commercially profitable food industries.

Chapter 4 includes the isolation and purification of major phytochemicals by chromatography methods. The preparative obtained by column chromatography and then finally isolated by the RP-HPLC. The yield and purity of isolated quercetin rich fraction were 2.35±0.08 µg/ml and 53.12±0.31%, respectively, which showed similar characteristics to the standard quercetin compound. It also includes its characterization of isolated phytochemical compound by using NMR and FTIR spectroscopies.

Chapter 5 includes the encapsulation of isolated phytochemical rich fraction by using chitosan-alginate polyelectrolyte. This study model an effective encapsulation process of isolated quercetin rich fraction from bhimkol banana (*Musa balbisiana*) Blossom by artificial neural network-ant colony optimization and its characterization. The optimized conditions obtained for encapsulation at epoch 2 with the best validation performance (2.78) were quercetin 0.2%, sodium alginate 4%, chitosan 0.5%, and agitation 300 rpm with 84.54% encapsulation efficiency. A significant positive effect of input layers; sodium alginate and agitation was observed over encapsulation efficiency (output). Encapsulated quercetin showed good pH resistance with 68.27 mg QE/g quercetin release in simulated gastric fluid at 60 min. Non-crystalline behavior of encapsulated quercetin of average particle size diameter 2.71µm with intact morphology has been observed. The overall result demonstrates that it was an effective drug delivery approach of quercetin encapsulation with an appropriate metaheuristic method.

Chapter 6 presents the development of a phytochemical enriched ready-to-cook soup mix from bhimkol blossom. Bhimkol blossom extract at 500 mg/kg body weight of Wister rats was found to be non-toxic during the investigation of *in vivo* acute toxicity. Further, bhimkol blossom extract at 145-250 µg/ml was concluded to be functionally active during cell viability test in THP-1 cells (145 µg/ml IC_{50}). Hence, application of bhimkol blossom powder and isolated phytochemical rich fraction was included at non-toxic level. It includes incorporation of microbeads (encapsulation of isolated phytochemical rich fraction) in ready-to-cook soup mix (RTC-SM) prepared from bhimkol blossom powder. It also includes detail sensory attributes of the developed product analyzed by product component analysis and its antioxidant activity.

Chapter 7 detailed of *in vitro* and *in vivo* antidiabetic study of bhimkol blossom extract was also included. Bhimkol blossom extract showed very good antidiabetic properties by exhibiting inhibition activities over α-amylase, α-glucosidase, and DPP-IV enzymes and by lowering the blood glucose level in diabetic rats. Bhimkol blossom extract also increased glucose uptake in the diabetic L6 cells. The highest uptake of 2_NBDG (5.58±0.01 MFI) was seen at 100 µg/ml of bhimkol blossom extract treatment. A positive effect of bhimkol blossom extract observed over body weight and blood glucose of diabetic rats was illustrated.

Chapter 8 detailed the entire conclusions of the study. It includes the findings of the study along with future scopes. It is concluded that, bhimkol blossom is a nutritional and phytochemical-rich underutilized agricultural by-product with significant antioxidant activities. Ultrasound-assisted extraction was the most convenient technique with good efficiency for the extraction of phytochemicals. Quercetin content was found at highest among other phytochemicals in bhimkol blossom extract. An isolated quercetin-rich fraction was obtained at good purity. By *in vivo* and *in vitro* study of antidiabetic property of bhimkol blossom extract showed very good antidiabetic properties along with increased glucose uptake in the diabetic L6 cells. Encapsulation of isolated quercetin in chitosan-alginate complex showed good drug release properties. Response surface methodology, particle swarm optimization, and ant colony optimization were found to be efficient methods of optimization. Quercetin-enriched ready-to-cook soup mix developed from bhimkol blossom showed high antioxidant activity with significant antidiabetic effects along with other possible health benefits. A detailed in *vitro* / *in vivo* study on the anti-cholesterolemic effect and other health-beneficial properties of bhimkol blossom extract can be carried out in the future.

Keywords: Phytochemicals, Banana blossom, Quercetin, Encapsulation, Antioxidants, Antidiabetic