Abstract

Supercritical fluid extraction of bioactive compounds from haritaki (*Terminalia chebula*) using novel pre-treatments and its application in development of functional food

ABSTRACT

Access to healthcare in developing countries has become a major source of worry due to the high costs of prescription drugs, healthcare services, and diagnostics. The tropical regions of the earth are home to *Terminalia chebula Retzius*, which is also known as haritaki in Sanskrit and is a native of South Asia. It is also known as *Chebulae Fructus* (Hezi) in China. Haritaki has been used for therapeutic purposes since ancient times to treat a variety of ailments due to its hypocholesterolemic, anti-inflammatory, anti-allergic, antibacterial, and antioxidant characteristics. These qualities result from the fruit's high concentration of phytochemicals, primarily phenolics such flavonoids, phenol, and vitamin C. Therefore, because haritaki has antioxidative qualities that help guard against oxidative stress, several researchers are interested in using it as a phytotherapy agent. Since haritaki is a seasonal fruit, drying is important as a substitute for other preservation techniques and extends the fruit's shelf life. Additionally, the bioactive components in haritaki may be extracted for potential use in the production of nutraceuticals or functional meals. For the extraction of phenolic mixtures from haritaki, a number of extraction techniques have been used, including subcritical water extraction and reflux framework coupled with water-ethanol and water-propylene glycol. The benefits of using supercritical fluid extraction to save process energy, decrease manpower, and increase shelf life have been highlighted by numerous authors. These techniques have a number of drawbacks, including a labor-intensive procedure, limited yield, toxic solvent residue, and the degradation of chemicals that are sensitive to temperature. The use of the supercritical fluid extraction (SFE) technique can eliminate these drawbacks. The process of creating bioactive chemicals using only technology and benign settings is one that is rapidly advancing.

Thus, the present study was conducted to assess the effect of drying condition on the phytochemicals of haritaki and utilize the novel extraction methods to extract the bioactive compounds. The extracted bioactive compounds are encapsulated to increase the utility in the development of functional food.

To accomplish the goal, the thesis is divided into 8 chapters are as follows:

Chapter 1 presents a brief introduction about various bioactive compounds present in haritaki fruit along with its pharmacological attributes. Also, it further details the various traditional and novel extraction procedures of the bioactive compounds. Further, the chapter covers different biopolymers used in the encapsulation of bioactive compounds for food application in the light of encapsulating techniques. At last, the chapters brief the different functional foods employed for various physiological attributes along with the research gap and justification to conduct the study.

Chapter 2 summarizes the literature review on the great medicinal potential of whole plant and its traditional use to cure a variety of human diseases. The major stress is given on importance of pharmacological compounds, clinical trials, bioactivity, mechanism of action, pharmacotherapeutics, toxicity, standardization, pre-clinical studies, and clinical trials. It further portrays the chemical constituents and bioactive properties of haritaki have been reviewed in detail with a brief description of extraction methods of bioactive compounds and applications.

Chapter 3 investigated the effect of drying temperatures (40-80 °C) on drying kinetics during convective drying of deseeded haritaki fruit. The data from the drying experiments were fitted into seven different drying models and the best model was selected by comparing the coefficient of determination (R^2) and root mean square error (RMSE). Degradation kinetics for vitamin C, antioxidant activity (DPPH radical scavenging activity), total phenol content (TPC) and total flavonoid content (TFC) was evaluated at 60, 70 and 80 °C. Change in colour at these temperatures were also observed. From the results it was found that an increase in the temperature increased the drying rate and the drying time was reduced, and the mathematical model for drying which best fitted the experimental data was 'Approximation of diffusion' model. The degradation of the phytochemicals and change in colour followed the first order kinetics. The rate at which vitamin C degraded was found to be higher than the other components for all temperatures. The rate of change in total colour difference (ΔE^*) decreased with increasing temperature.

Chapter 4 explored the parameters for extraction of phytochemicals from haritaki pulp by means of supercritical fluid extraction were optimized using different approaches and a comparative study was also performed among the optimizing techniques. A central

composite rotatable design (CCRD) was employed with four numerical factors viz. temperature, pressure, time and co-solvent flow rate. The responses were TPC, TFC and antioxidant activity of the extracts. Two different methods viz. response surface methodology (RSM) and artificial neural network (ANN) were used for modelling from the same set of experiments. Subsequently, optimization was carried by three different approaches viz. RSM coupled with numerical optimization by desirability function (RSM-DF), RSM coupled with genetic algorithm (RSM-GA) and ANN coupled with genetic algorithm (ANN-GA). Statistical analyses indicated that the models derived using both the methods i.e., RSM and ANN can be used to predict the response precisely, but RSM (R^2 = 0.9987) method was somewhat found to be superior compared to ANN model (R^2 = 0.9973). When comparing the optimization approaches, it was observed that results obtained from all the approaches were close to each other, but RSM-GA and RSM-DF approaches provided higher values of TPC, TFC and DPPH radical activity compared to ANN-GA approach. The values of TPC (mg GAE/mL), TFC (mg QE/mL) and DPPH (%) for RSM-GA were 428.03, 136.58, 92.63 respectively, for RSM-DF were 432.28, 137.36, 92.54 respectively and for ANN-GA were 414.25, 135.55, 91.32 respectively. From the present study it can be concluded that both RSM and ANN can be used for modelling of the processes with good predictability and optimization can be done using different approaches which will depend upon the specific process or the problem.

In chapter 5, effect of pre-treatments on the extraction of bioactive compounds were observed where ultrasound at 50% amplitude, microwave at 360 W, and enzyme with 400 U result in maximum bioactive properties and has been chosen for combined pre-treatment. In the combined pre-treatment, microwave-assisted ultrasound extraction results in maximum extraction of bioactive compounds among all. TPC, TFC, and DPPH activity were found higher in microwave-assisted ultrasound extraction. From the result, the pattern of bioactive compounds in haritaki pulp according to pre-treatment was UM>MUE>ME>UE. Therefore, we conclude that the combined use of ultrasound and microwave treatments maximizes the extraction of bioactive compounds from the haritaki. The pulp obtained from combined microwave and ultrasound was subjected for SFE extraction and studied for pH and thermal stability where it was seen that, with an increase in pH and temperature, bioactive properties were decreased.

In chapter 6, two different techniques (encapsulator and freeze-drying) were used to encapsulate the bioactive materials of haritaki. Freeze-drying was found an excellent

technique in terms of encapsulation efficiency and yield of bioactive compounds. Thus, selected for further studies. Different combinations of starch:Zein was tried to encapsulate the bioactive compound and based on the yield, encapsulation efficiency, powder density, 100% starch encapsulates presented better results among the combinations. From the SEM study, it was observed that encapsulates had a rough and irregular shape. TGA study revealed that the maximum loss in mass was seen in the 70:30 (starch:zein) combination while the minimum mass loss occurred in 100% zein. From DSC curves, it was noticed that maximum onset point and peak point was presented by 50:50 (starch:zein) encapsulates, while minimum onset point and peak point were depicted by 70:30 (starch:zein) encapsulates. The maximum endpoint was seen in 100% starch encapsulates Whereas 30:70 (starch:zein) encapsulates shown a minimum. FTIR spectra revealed that 100% zein encapsulates had maximum stretching and vibrations in the bond while encapsulates prepared from 100% starch had very minimum stretching and poor intensities in bonds. XRD pattern detailed that, no sharpness in the encapsulates except 50:50 (starch:zein).

Chapter 7 is devoted to developing a functional food where the encapsulated extract obtained from freeze-drying was used in the development of yoghurt. The developed yoghurt was compared with yoghurt developed using encapsulated extract and SFE extract added (guar gum at 0.5 & 1.5%). From the physicochemical studies, in general, it was observed that pH was decreasing with an increase in storage period while acidity was increasing. E5 & E6 had high pH values while C1 & C3 had higher acidity and syneresis value respectively on the 1^{st} day. In the case of syneresis, yoghurt prepared from guar gum resulted in more syneresis and was found to decrease as the storage period increased. Lightness was found to be maximum in C1 throughout the storage period and among the samples. E3 represented the maximum lightness on the 1^{st} and 12^{th} days of storage. On the 24^{th} day of storage, a* value was observed highest in F1. F3 represented the highest ΔE^* on the 1^{st} and 12^{th} days of storage. C3 had a higher value of firmness and adhesiveness than other samples throughout the storage. Sensory analysis was performed for the developed yoghurt and was observed that E1 sample having most acceptability after the C1. In comparison to E1, E3 has the appreciable phytochemical properties observed during storage. Thus, 0.5% guar gum concentration was more acceptable than 1.5%.

Chapter 8 summarises the complete work of drying of haritaki pulp at different temperatures and optimizations with different statistical methods and is fitted with various models to determine the optimum drying temperature with minimum influence of the bioactive compounds. A comparative study was performed between RSM and ANN to optimize the SFE parameters. RSM-DF and RSM-GA gave higher values of the responses as compared to ANN-GA which was the aim of optimization. Effect of various drying techniques and pretreatments were performed to extract the bioactive compounds using SFE. The extracted bioactive compounds were encapsulated using the mixture of starch:zein. The encapsulates were used for the development of functional food to increase the utility of haritaki.

Keywords: Haritaki, bioactive compounds, encapsulates and pre-treatments