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Review

A Review on Purification Methods of Bromelain from Pineapple Stems

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Abstract. Bromelain comprises a group of proteolytic enzymes attained from the tissue of pineapple, *Ananas comosus*, belonging to the Bromeliaceae plant family. Different studies indicated its antithrombotic, fibrinolytic, antimicrobial, anticancer and anti-inflammatory properties. Bromelain has significant applications in clinical, industrial and cosmetic sectors. Therefore, researchers have always explored suitable extraction and purification methods for the final application. The different extraction and purification methods of bromelain identified, compared and evaluated. We have also elaborated the pharmaco-kinetics, clinical applications and potential mechanism of action of bromelain. In this research paper, we also focused on different processing methods of bromelain for different purpose of use.

Keywords: *Ananas comosus*, anti-inflammatory effect, bromelain, extraction, pineapple

Introduction

Bromelain is the general term for some commonly associated proteolytic enzymes or endopeptidases present in the tissue of the Bromeliaceae plant family (Benucci *et al*., 2011). Pineapple, *Ananas comosus*, is the best known plant of this family. Bromelain was first chemically recognized in 1876 (Saengsuk *et al*., 2021; Ramli *et al*., 2018). It was first separated and described in 1891 by the Vicente Marcano, a Venezuelan chemist (Upadhyay *et al*., 2013). The study of bromelain and its isolation had been explored since 1894 (Kritis *et al*., 2020). This plant, in addition, holds a small amount of other proteinases enzymes like ananain, as well as comosain (Chen *et al*., 2020). Bromelain is the major and most broadly explored of these enzymes (Saengsuk *et al*., 2021; Banerjee *et al*., 2020). Physiologically, bromelain is in large quantities found in the stem and fruit of pineapple plants it means the stem includes substantially more bromelain than its fruit. Later studies have shown that, it may also be extracted from the other parts such as root, leaves, peelin limited quantities (Saengsuk *et al*., 2021; Banerjee *et al*., 2020; Upadhyay *et al*., 2013; Babu *et al*., 2008; De León-Rodríguez *et al*., 2008).

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Bromelain isolated from the pineapple fruit is given the EC number EC 3.4.22.33 and is called fruit bromelain (FBM). Similarly bromelain found in the pineapple stem is referred to stem bromelain (SBM) and given a EC number is EC 3.4.22.32 (Golden and Smith-Marshall, 2012). The higher content of SBM guided to the enzyme production commercially and its use as a useful phyto-medical agent (de Lencastre Novaes *et al*., 2016). Researchers have concentrated on the use of pineapple waste mainly as a source of bromelain by isolation (Upadhyay *et al*., 2013). Pineapple waste subsequently as low cost raw material for ethanol processing, phenolic antioxidants, organic acids, biogas and fibre processing by valourization followed by simultaneous saccharification and fermentation (Khedkar *et al*., 2018; Ramli *et al*., 2018; Norziah *et al*., 2014; De León-Rodríguez *et al*., 2008).

Structural chemistry of bromelain. A proteolytic enzyme is the most important ingredient in crude bromelain. It is glycosylated therefore making it a glycoprotein. Stem bromelain is further classified as a cysteine proteinase, belonging to the common group of sulfhydryl proteolytic enzymes. Bromelain is a thiol endopeptidases and thus reported to remain as a combination of substances for e.g. carbohydrates, glucosidase, peroxidases, cellulases, phosphatases, glycoproteins

and a number of protease inhibitors (Provin *et al*., 2021; Vicente *et al*., 2016). The activity of all these enzymes depend on the thiol group (SH) of a cysteine residue (Chen *et al*., 2020; Khedkar *et al*., 2018). Bromelain is a glycoprotein which has only a single oligosaccharide moiety per molecule (Taussig and Batkin, 1988). Stem bromelain is composed of a glycosylated, 24.5 kDa single-chain moiety with a pI of 9.55 and A_1%, 280 of 20.1 with seven cysteines and hence most definitely three disulfide bonds (Seguí and Maupoey, 2018; Wan *et al*., 2016; Pavan *et al*., 2012). Stem bromelain has a fairly stable secondary structure and shows activity in the pH range 7-10 with its activity being irreversibly lost beyond pH 10 (Ataide *et al*., 2021). A characteristic molten globule structure of stem bromelain is achieved at alkaline pH and may exhibit a charge heterogeneity which generates numerous chromatographic variants, for unknown reasons, although they are all immunologically similar (Sehirli *et al*., 2021; Bhatnagar *et al*., 2015). Bromelain is reported to be stable for an extended time period if stored under -20 °C (Ramli *et al*., 2018; Rocha and Nerli, 2013).

Comparison of stem bromelain and fruit bromelain. Stem bromelain has higher protease activity compared with bromelain derived from the fruit. Both types of bromelain are single-chain glycosylated monomeric enzymes (Rathnavelu *et al*., 2016). They have dissimilar characteristics. Stem bromelain has lower proteolytic action and its specificity is lower for peptide bonds than fruit bromelain. The reported optimum pH range for stem bromelain has shown by various researchers to be in the range of 6-7 and its most favourable temperature range for showing activity is at 50-60 °C (Arefin *et al*., 2020; 2019). On the other hand, the optimum pH range for fruit bromelain is 3-8, and the best possible temperature ranges from 37-70 °C (Provin *et al*., 2021; Vicente *et al*., 2016). Although both enzymes are monomeric in nature, molecular weight (MW) for stem bromelain is 26-37 kDa and that of fruit bromelain is 24.5-32 kDa. Stem bromelain possesses diverse biochemical characteristics as compared to fruit bromelain and differs in composition with fruit bromelain (Bresolin *et al*., 2013). Stem bromelain contains diverse thiol-endopeptidases (El-Demerdash *et al*., 2020; Kritis *et al*., 2020).

Importance of bromelain. Bromelain has shown to have a variety of beneficial uses as a phytomedical compounds (Khan *et al*., 2021; Sehirli *et al*., 2021). Bromelain has been reported to improve healing after surgery and drugs overdose (Abbas *et al*., 2021; Rathnavelu *et al*., 2016). In addition to its medicinal uses, bromelain has been utilized by many other industries such as the food and brewery industries (Ramli *et al*., 2018) and the clothing, cosmetic and pharmaceutical sectors (Abbas *et al*., 2021; Adu and Mabandla, 2021; Kritis *et al*., 2020). Bromelain has therapeutic benefits in mediating inflammation, allergy and autoimmune disorders (Adu and Mabandla, 2021; Kritis *et al*., 2020; Rathnavelu *et al*., 2016). The available scientific and technological uses of bromelain have opened the doors for better and more profitable markets for pineapple wastes (Provin *et al*., 2021; Banerjee *et al*., 2020; Seguí and Fito Maupoey, 2018). Through applying traditional and new methods, such as controlling pH, hydrophilicity and temperature situation in conjunction with the exclusive properties of bromelain, researchers worldwide have focused their attention on purification strategies (Abbas *et al*., 2021; Bresolin *et al*., 2013; Kumar *et al*., 2011). The quantity of production would vary depending on their anticipated industrial use (De Rezende *et al*., 2021; Khorsandi *et al*., 2021). In recent years, breakthroughs in recombinant DNA technology have enabled potential large-scale recombinant bromelain development and purification for innovative technologies (Rathnavelu *et al*., 2016; Bhatnagar *et al*., 2015).

Isolation and purification methods of bromelain. Bromelain is one of the few plant proteases which can be extracted from a variety of parts of pineapple, such as fruit pulp, stem, peel and the leaves (Batool *et al*., 2021; Banerjee *et al*., 2020; Rico *et al*., 2020; Misran *et al*., 2019; Bhui *et al*., 2009). In this context bromelain concentration is higher in the stem than that in the fruit part. Stem is thus one of the most accessible source of bromelain(Ramli *et al*., 2018; Vicente *et al*., 2016). Rest of the parts are also researched for the existence of bromelain like peel, core and crown, etc. (Adu and Mabandla, 2021; Pavan *et al*., 2012). Researchers strive to search for different more efficient methods to attain extremely pure bromelain in fewer stages and lower costs. Scientists continue to study recombinant DNA technology for bromelain extraction and purification to achieve novel utilization in future (Abreu and Figueiredo, 2019).

Preparation of crude bromelain extract. Marketable and cost effective bromelain is usually extracted from the fruit stem through lyophilization or centrifugation and ultrafiltration method (Chia *et al*., 2019; Chen and

Huang, 2004). In another study, the crude extract was prepared, the peeled stems washed with water to remove soil from the surface of the stems (Yin *et al*., 2011). They were then cut into one-centimeter cube thickness. Then known amounts of waste is crushed along with extraction buffer at 1:1 ratio for 10 min and filtered (Hidayat *et al*., 2018; Côlho *et al*., 2016). The filtrate is then centrifuged at 10,000 g for 15 min and the supernatant, *i.e*., crude enzyme extract is collected. The supernatants are refrigerated at 4 °C after adding 0.05% sodium azide (Yin *et al*., 2011).

***Purification of bromelain from crude extracts*.** Various conventional and latest purification techniques are explored to obtain bromelain of the utmost purified variety at low expenditure (Arshad *et al*., 2014). After extraction, the crude mixture containing the desired enzyme and its isophores are subjected to various purification processes to remove impurities that inhibit bromelain activity and impede its application and final enzyme activity (Gómez-García *et al*., 2021; Norziah *et al*., 2014; Kumar *et al*., 2011). Due to the increased interest in bromelain, researchers have investigated numerous innovative purification methods for its withdrawal and refinement (Banerjee *et al*., 2020; Babu *et al*., 2008). These include precipitation, purification through membrane filtration, aqueous two phase system (ATPS), different chromatographic processes and reverse micellar systems (Chia *et al*., 2019; Wan *et al*., 2016; Vicente *et al*., 2016; Rocha and Nerli, 2013).

***Precipitation*:** Precipitation is considered as most reliable process for the huge amount purification of proteins (Arefin and Amin *et al*., 2020a and b; Naim *et al*., 2015). Precipitation is commonly initiated by the adding organic solvent, a non-ionic polymer, salt, a metal or by altering the pH (Arefin *et al*., 2020a and b). Ammonium sulphate $(NH_4)_2SO_4$ can be used for the purification of bromelain by precipitation method. In this method, the crude plant extract is treated with ammonium sulphate in varying concentrations with mild stirring for 30 min to get 0-20, 20-40, 40-60 and 60-80% saturation. Then the contents are centrifuged at 10,000 rpm for 15 min at 4 °C and the precipitates are obtained and constitute the concentrated bromelain extract (Abreu and Figueiredo, 2019; Chia *et al*., 2019).

***Purification through membrane filtration*.** Membrane filtration is the method for the purification of molecules based on their size variation (Arefin *et al*., 2020a and b). Membrane-based technology is an efficient and sustainable procedure for the production of high quality purified bromelain (Gómez-García *et al*., 2021). Concurrent involvement of micro-filtration and ultrafiltration provides a yield of 85% using microfiltration and 10 times concentrated bromelain by ultrafiltration (Seguí and Maupoey, 2018). Enzymatic pretreatment and diafiltration operation can be used for bromelain production for better flux performance and bromelain separation (Nor *et al*., 2018).

***Aqueous two-phase system (ATPS)*:** Aqueous Double Phase System (ATPS) is an efficient and commercially feasible process for extracting and purifying protein and enzyme mixtures (Rocha and Nerli, 2013). The ATPS consists of either two incompatible polymers (e.g., polyethylene glycol (PEG) and dextran) or one polymer and one salt in an aqueous solution (e.g., PEG and phosphate salt) (Navapara *et al*., 2011). The two phases differentiate as these phase-forming materials are solubilized in an aqueous solution over a critical concentration. This will get rid of unwanted by products at hand in the system which decrease enzyme action (Ketnawa *et al*., 2012). Bromelain was cleaned up by PEO-PPO-PEO and the outcome was the best (Navapara *et al*., 2011) found lower molar masses of PEG showed better performance in the partitioning of bromelain with the help of PEG and $MgSO_4$ magnisium sulphate. This study showed a partition coefficient of 12.6 and a purification factor of 2.4 with a 90% yield.

***Different chromatographic processes*:** Scientists have explored different types of chromatography techniques for bromelain purification. Bromelain yields of 87.4% were obtained when the aqueous bromelain extract solution was passed through poly acryl acid (PAA)-bound ferric oxide magnetic nanoparticles packed column to adsorb bromelain (Chen *et al*., 2020). Immobilized metal affinity membrane (IMAM) was used for bromelain purification which yielded 15.4 times purification factor with 94.6% active yield (Arefin *et al*., 2020a and b). Thus through chromatography, a higher yield is obtained.

Three times purification of bromelain was produced by ion-exchange chromatography than it was found in the process of precipitation with ammonium sulphate (Golden and Smith-Marshall, 2012). Researchers also found 10-fold purification and 84.5% enzyme recovery (Silveira *et al*., 2009) observed that bromelain purification by extended bed adsorption is feasible by using Amberlite IRA 410 as an adsorbent, demonstrating

strong performance for the extract retrieved and a 13 fold purification factor. A higher purification factor resulted in improved flow velocity and other variables such as bed extension, bed voidage, HEPT and axial dispersion. Another study used ion-exchange chromatography with DEAE anion exchanger and compared bromelain concentration and activity in pineapple stem and fruit (Golden and Smith-Marshall, 2012). It was observed in the study that the centrifugal fraction shows greater enzymatic activity than crude extract, the increase was small. The recovered enzyme retained structural integrity according to these findings and displayed greater activity than extraction and centrifugation (Bresolin *et al*., 2013) used this method on DEAE-Sephafore to increase the bromelain specific action. The highest level of action was obtained at pH 7.0 in which the enzyme is most stable. This method yielded 89% enzymatic action up turn and purification factor of 16.93.

***Reverse micellar systems*.** Reverse micellar extraction offer unique features such as large interfacial region, less energy requirement, single-stage and continuous mode processing, low-cost factors and fast scale-up (Kumar *et al*., 2011). The reverse micellar system has also been utilized for the withdrawal and refinement of bromelain from pineapple stem and waste (Chia *et al*., 2019). Moreover, the use of the reverse micellar system (Umesh *et al*., 2008) found a yield of 106% upturn and 5.2 purification fold. Modifications in reverse micellar systems can be done to increase the output and purification fold for larger scale purification, scale-up studies using reverse micellar system gave purification of 2.43 fold with an active recovery of 81.3% (Hebbar *et al*., 2011). RMS if used along with ultrafiltration provides better result. RMS and ultrafiltration together yield an activity recovery of 95.8% but higher purification factor of 8.9-fold (Hebbar *et al*., 2012) which means to yield compound with higher purity rather than RMS alone. The continuous extraction method was preferable to batch extraction method, which may (Kumar *et al*., 2011) used affinity-based reverse micellar extraction and isolation method to isolate and clean bromelain from pineapple stems, with even better result including 12.32 fold cleansing and 185.6% yield.

***Comparison of the processes*.** Based on different published data, we have summarized and compared the advantages and limitations of this process. ATPE showed the highest purification factor of 16.3 (Wu *et al*., 2017), although the active recovery of bromelain was 55.6%. Limitations associated with this method are the high salt concentration, difficult to recover and recycle and low purity of the product (Matagne *et al*., 2017; Yuris and Siow, 2014; Sriwatanapongse *et al*., 2000). Precipitation method had a purification factor of 4.9, and the active recovery of bromelain was 85.97% (Gómez-García *et al*., 2021). Precipitation yields in high precipitant content and becomes inconvenient to separate bromelain. But this is mostly used for commercial applications due to the high active recovery together with low cost (Abbas *et al*., 2021; Banerjee *et al*., 2020). Chromatography is the most costly method for the little separation efficiency, little recovery and little sample loading capability (Arefin *et al*., 2020a and b). Ion exchange chromatography showed a purification factor of 10 and the active recovery of bromelain was 84.5%. The higher purification was obtained by combining IEC and gel filtration chromatography (Arefin *et al*., 2020a and b). Membrane filtration showed a purification factor of 10 and the active recovery of bromelain was 90% (Loon *et al*., 2018; Côlho *et al*., 2016), the advantage of this method is it offers high specificity (Bayat *et al*., 2019), it is more applicable for research purpose (Misran *et al*., 2019; Matagne *et al*., 2017; Yin *et al*., 2011) showed that, HSCCC when combined with RMS was supposed to give the highest purification level. But the cost involved in chromatography is higher than other processes. So, it is better for analytical research purpose.

Pharmacokinetics of bromelain. ***Absorption and bioavailability of bromelain*.** Bromelain is highly absorbed in the body and thus has a high bioavailability (Rathnavelu *et al*., 2016). It can bind to the two anti-proteinases in the blood namely, alpha 2-macroglobulin and alpha1-antichymotrypsin (Neumayer *et al*., 2006). It has been focused that almost 12 g/day of bromelain can be taken with no adverse impacts (Kargutkar and Brijesh, 2018). Bromelain is absorbed across the gut epithelium in its active form and about 40% of total bromelain is absorbed from the intestine (Sehirli *et al*., 2021). Bromelain also has activity in plasma (Adu and Mabandla, 2021).

***Toxicity profile of bromelain*.** Bromelain has been reported to have almost no toxic effects in clinical and preclinical studies. (Taussig and Batkin, 1988) concluded with the results that bromelain in mice, rodents and rabbits has a low toxicity. Preclinical studies also indicated no toxicity in 6 months with a growing intensity of bromelain up to 750 mg/Kg upon daily administration. 1500 mg/Kg/day, if given to rats caused no carcinoma

(Sehirli *et al*., 2021). In the study by Eckert *et al*. (1999), administered bromelain (3000 FIP unit/day) to the human body over a period of 10 days and came up with no note worthy alterations in blood coagulation factors.

Medical use. Bromelain is clinically important as a number of studies suggest El-Demerdash *et al*., 2020; Pavan *et al*., 2012; Chen and Huang, 2004; Ingerslev and Poulsen, 1980), Bromelain improves bioavailability and lowers the adverse impacts of different antibiotics (Bahde *et al*., 2007; Engwerda *et al*., 2001; Eckert *et al*., 1999). Bromelain also functions as an immuno-modulator, being anti-metastatic, anti-edematous, antithrombotic and anti-inflammatory (Abbas *et al*., 2021; Ataide *et al*., 2021; El-Demerdash *et al*., 2020; Vicente *et al*., 2016). The primary therapeutic application for the treatment of burns as infectious showed by (Taussig and Batkin, 1988), in vaccine preparation, antitumor and skin debride. It has been shown that most of the bromelain's biochemical function could not be attributed to a particular proteolytic component and the useful effects of bromelain are possibly related to several things (Bhatnagar *et al*., 2015).

***Impacts of bromelain on blood coagulation*.** Bromelain has been reported to have anticoagulant effects and it is available at an average price of 40-80 US dollar/Kg depending on the source and quality. It affects blood clotting by mounting the fibrinolytic capability of the serum and by inhibiting blood clotting protein, fibrin synthesis. A rat study by (Taussig and Btkin, 1988) showed a dose dependent relationship of its activity on blood clotting. Plasma prekallikrein is a proenzyme. It has to be converted to the kallikrein, the active form of enzyme which helps in coagulation and bradykinin is a compound released in the blood and causes smooth muscle contraction and blood vessels dilation helping in coagulation. Bromelain was reported to decrease prekallikrein (Maurar, 2001).

***Effects of bromelain on cardiovascular disease*.** Bromelain is effective in the treatment of cardiovascular diseases and has a lower cost than any cardiovascular drug. According to amazon.com, marketed bromelain supplements of 500 mg capsules of 1200-1800 tablets are available in the price range of 14-30 dollars depending on sources and companies bromelain's anticancer property is mainly attributed to its capability to inhibit blood platelet aggregation. The blood coagulation factors, fibrin and thrombus formation are important in blood coagulation. Fibrinolysis and inhibition of thrombus formation impedes blood clotting in blood. Researchers have reported the fibrinolytic activity as well as inhibition of thrombus development and platelet aggregation lessening activity of bromelain (Pavan *et al*., 2012). Thrombophlebitis is an inflammatory process which helps to a blood clot to form and block one or more veins, specially in legs. Bromelain assists to decrease the potential threats of thrombophlebitis and helps its management. The studies reported bromelain to be effective in curing acute thrombophlebitis by reducing patient walking difficulty and inflammatory symptoms, including skin temperature, tenderness, oedema and discomfort. Bromelain can also inhibit the angina attacks and result in the symptomatic relief in hypertension (Maurer *et al*., 2001). Bromelain has also been reported to protect against ischemic injury (Neumayer *et al*., 2006). It increases blood flow and oxygen transport (Arefin, 2016). In experimental animals, bromelain was found to play an anti-hypertensive role for a prolonged period of administration. Therefore, Bromelain supplements, may reduce risk factors for cardiovascular disease.

***Effect of bromelain on cancer cells*.** Bromelain has been reported in a number of studies to have anticancer effects (Báez *et al*., 2007; Eckert *et al*., 1999). Cell growth and proliferation are usually regulated, and cell cycle disparities can result in abandoned cell growth and transformation into cancer cells. There are various mechanisms inside the cells to defend their DNA from harm from toxins and genomic instability (Arefin, *et al*., 2020a and b; Chobotova *et al*., 2010). Tumor cells when lose check point controls, become responsible for normal cell cycle regulation (Báez *et al*., 2007). Bromelain action in mouse tumor cell lines caused inhibition of cell growth (Juhasz *et al*., 2008). Bromelain therapy greatly reduced the development of Kato-III cell lines in gastric carcinoma (Rathnavelu *et al*., 2016). Bromelain slows down the growth inhibitory response of MCF-7 cells in mammary carcinoma cells, and stimulates the autophagy cycle(Eckert *et al*., 1999). It promotes the monocytic cytotoxicity in women with breast cancer when administered orally.

Five-Flurouracil (5-FU) is drug used to treat cancers of the breast, colon, stomach, rectum and pancreas (de Lencastre *et al*., 2016; Stopper *et al*., 2003). The antitumoral activity of stem bromelain was found to be greater than that of 5-FU, the survival index which was around 263 percent relative to the untreated dose (Ataide *et al*., 2021). When observed with 5-Flurouracil,

bromelain greatly decreased the amount of lung metastases caused by LLC transplants. The bromelain antitumor activity against S-37 and EAT, which are tumor models susceptible to immune system mediators, and the unaffected tumor development in the metastatic model mean that the antimetastatic action comes from a process independent of the main antitumoral effect (Báez *et al*., 2007).

Antimicrobial activity of bromelain. Bromelain inhibits the growth of intestinal bacteria, such as *Vibrio cholera* and *Escherichia coli*. Bromelain can also inhibit enterotoxigenic *Escherichia coli* (ETEC) bacteria. It can protect against *Escherichia coli* based diarrhea. Therefore, bromelain shows potential use as a prophylaxis against ETEC infection (Abbas *et al*., 2021; Zhou *et al*., 2021).

Bromelain has also been reported to exert anti-helminthic activity against *Trichurismuris, Trichoderma viride* and *Heligmosomoides polygyrus*. These are gastrointestinal nematodes. So, it has the capability to counter particular intestinal pathogens. Besides, bromelain has the synergism effect when administered with antibiotics. These two mechanisms can be explored for the benefits of bromelain against specific infections. Further Bromelain has been reported to act as an anti-fungal agent. *Pityriasis lichenoides chronica* is a skin disease. Bromelain has been shown to completely cure the disease caused by *Pityriasis lichenoides*.

Application of bromelain in debridement burns. Quick de-bridement and/or elimination of eschar is an important step in the treatment of intense partial and full-thickness burns (Pavan *et al*., 2012). It aims at reducing wound bioburden and enables early healing of wounds by careful care or skin grafting (Hirche *et al*., 2017; Rathnavelu *et al*., 2016). Effective removal of the eschar within 72 h is considered to boost the outcome of burn wound treatment (Rathnavelu *et al*., 2016). When 35 percent bromelain in a lipid base used as a cream, helps in necrotic tissue debridement and accelerates healing due to the presence of escharase in bromelain (Rosenberg *et al*., 2012). It cannot hydrolyse regular protein sub-strates or glycosaminoglycan multiple substrates (Pavan *et al*., 2012). As bromelain facilitates the debridement mechanism and provides better and faster healing and efficient re-epithelialization, scientists suggest bromelain for treating postoperative wounds and relieving discomfort and swelling, between others (Arefin *et al*., 2020a and b).

Anti-inflammatory activity of bromelain. Though bromelain has many therapeutic activities, the most prominent effect is its anti-inflammatory type action. Bromelain was prescribed as an adjunctive treatment strategy for chronic inflammatory, malignant and autoimmune disorders and reported to increase the treatment efficiency of the diseases (Grover and Samson, 2016). Mast cell proteases helps in the treatment of asthma and allergic disorders by inhibiting the concerned proteins and globulins (Rathnavelu *et al*., 2016). Bromelain, as a protease, is assumed to produce similar effects like other proteases in the treatment of asthma and allergies (Secor *et al*., 2005). Bromelain, in conjunction with the quick reaction to cellular stress, effectively stimulates the stronger immune system. Bromelain decreases the release interleukins and tumor nercosis factors (Neumayer *et al*., 2006). Bromelain, when taken orally, has been shown to induce both analgesic and anti-inflammatory results in rheumatoid arthritis. It triggers expression of TGF-β, one of the main inflammatory regulators in patients with osteomyelo fibrosis and rheumatoid arthritis. There are many studies that reported the immuno-modulatory effect of bromelain (Rathnavelu *et al*., 2016; Stopper *et al*., 2003) found in their research that bromelain in inflammatory bowel disease would decrease the expression of INF-ÿ and TNF-α. Bromelain administration prior to surgery will promote early recovery from discomfort from pain and postoperative surgery. Its analgesic properties mean that in women with episiotomy, bromelain can be useful in reducing swelling, bleeding, and discomfort (Ohsaki *et al*., 2021; Naim *et al*., 2015). Bromelain has been reported to be beneficial for sinusitis and it can be used for the treatment (Arefin *et al*., 2015; Maim *et al*., 2015). Sinusitis patients reported complete relief from breathing complications and inflammation of the nasal mucosa (Sharma and Chaudhary, 2021). So, bromelain has a wide range of action against inflammation.

Conclusion

Owing to its remarkable uses in numerous fields, bromelain is one of the thoroughly researched proteolytic enzymes. Researchers have long studied to employ strategies to yield the most purified bromelain in fewer stages efficiently. Different traditional and new purification approaches as well as their combinations, are being explored and proved to be effective in this regard. Ion exchange chromatography is an expensive method, precipitation, and aqueous two-phase extraction involve

high salt concentration streams. Our research indicates that bromelain is a safe and versatile therapeutic agent and is being used for many therapeutic purposes e.g., bronchitis, sinusitis, arthritis and inflammation. It has also been reported to have anticancer antimicrobial effect. After oral administration, bromelain is finely absorbed in the body even after prolonged use, it does not have major side effect. All these pieces of evidence reviewed in this article propose that bromelain is a potential and effective in treatment of many diseases.

Conflict of Interest. The authors declare that they have no conflict of interest.

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