

Bromelain: Methods of Extraction, Purification and Therapeutic Applications

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ABSTRACT

Bromelain is a concoction of sulfhydryl proteolytic enzymes. Depending upon the site of extraction it can be regarded as either stem bromelain (SBM) (EC 3.4.22.32) or fruit bromelain (FBM) (EC 3.4.22.33). Bromelain remain enzymatic active over a broad spectrum and endure a range of pH (5.5 to 8.0) and temperature (35.5 to 71 °C). It is one of the extensively investigated proteolytic enzyme owing to its astonishing applications in various industries. This necessitated employing a strategy that result in highest purified bromelain in less steps and lowest cost. Use of modernistic approach such as membrane filtration, reverse micellar systems, aqueous two phase extraction and chromatographic techniques have shown promise in this regard. Besides its industrial applications, bromelain has been widely utilized as a potential phytomedicinal compound. Some of its reported actions include inhibition of platelet aggregation, anti-edematous, anti-thrombotic, anti-inflammatory, modulation of cytokines and immunity, skin debridement and fibrinolytic activity. It also assist digestion, enhance absorption of other drugs and is a potential postoperatively agent that promote wound healing and reduce postsurgical discomfort and swelling.

Key words: Phytomedicinal, Anti-edematous, Fibrinolytic, Anti-thrombotic.

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INTRODUCTION

Bromelain is a chief protease enzymes found in pineapple plant (*Ananascomosus*) (Smith-Marshall and Golden 2012). It has been known chemically since 1876 (Tochi et al. 2008) and was identified for the first time by Marcano in 1891 (Upadhyay et al. 2010). The investigation and isolation of bromelain has been started since 1894 (Neta et al. 2012). Sulfhydryl proteolytic enzymes are the chief constituents of bromelain (Tochi et al.2008; Gautam et al. 2010). Bromelain is abundant in stem and fruit of pineapple plant and it can also be isolated in small amount from pineapple waste such as core, leaves, peel etc. (Hossain et al. 2015). Bromelain present in fruit of pineapple has assigned the EC number EC 3.4.22.33 and is regarded as fruit bromelain (FBM). Likewise bromelain present in the stem of pineapple is called stem bromelain (SBM) and its EC number is EC 3.4.22.32. Stem bromelain possess different biochemical properties and composition as compared to fruit bromelain (Pavan et al. 2012) and contains a variegated blend of different thiol-endopeptidases. Efforts are being made by researchers to achieve highly purified bromelain in less steps and low cost. Modern strategies, such as membrane filtration (Lopes et al. 2009), reverse micellar systems (Upadhyay et al. 2010; Kumar et al. 2011), aqueous two phase extraction (Coelho et al. 2013; Novaes et al. 2013) and chromatographic techniques (Yin et al. 2011) have shown promise in this regard. Pineapple plant also contains minor quantities of other proteinases like ananain and comosain (Nadzirah et al. 2013) but bromelain is regarded as a primary and extensively investigated component (Amini et al. 2016). The reason of being such valuable is due to its miraculous utilization as phytomedical compound (Larocca et al. 2010). Bromelain displays antiedematous, fibrinolytic, anticancer, anti-inflammatory, antibiotic, anticoagulative and antithrombotic functions (Kavitha et al. 2013). It is also a potential postoperatively agent that assists healing and decrease post surgical discomfort and swelling (Graf 2000). Besides clinical approach, bromelain has also employed in various industries including food industries (Maurer 2001), such as breweries (Soares et al. 2012) and flesh processing and tenderization, textile and cosmetic industries (Babu et al. 2008; Ketnawal et al. 2009). With the advent of recombinant DNA technology, scientists and researchers across the globe have been working on recombinant bromelain to achieve exaggerate and novel applications in future (Arshad et al. 2014).

PROPERTIES

Bromelain is actually a group of sulfhydryl proteolytic enzymes (Smith-Marshall and Golden 2012) and encompasses variety of cysteine proteases (Tochi et al. 2008) when extracted from the stem and fruit of pineapple plant (Neta et al. 2012; Pillai et al. 2013). Stem bromelain and fruit bromelain are both single-chain glycosylated enzymes but they possess different characteristics (Barrett et al. 2004). SBM has reduced proteolytic activity and exhibit low specificity for peptide bonds then FBM. Researchers have reported several distinct pH and temperature optima for the activity of bromelain towards its substrates (De Lencastre et al. 2016). The reported optimum pH range for SBM by various researchers is 6-7 and its optimum temperature range is 50-60 °C (Harrachi et al. 1998; Gautam et al. 2010; Xue et al. 2010). While the optimum pH range for FBM is 3-8 and optimum temperature ranges from 37-70 °C (Lopes et al. 2009; Jutamongkon and Charoenrein 2010; Silvestre et al. 2012). Likewise, the molecular weight range for SBM is 26-37kDa (Grzonka et al. 2007; Kumar et al. 2011) and for FBM molecular weight range is 24.5-32kDa (Grzonka et al. 2007; Lopes et al. 2009; Gautam et al. 2010). When bromelain is kept at -20 °C its stability remains intact for a defined time period (Rowan et al. 1990). Amongst other, cysteine is the most efficient compound for the activation of bromelain (Grzonka et al. 2007). SBM is in fact a combination of several thiol endopeptidases (Pavan et al. 2012) and also contains compounds like peroxidases, acid phosphatase, several protease inhibitors glucosidases, cellulases, glycoproteins, carbohydrates and organically intact Ca²⁺ (Maurer 2001; Smith-Marshall and Golden 2012).

EXTRACTION AND PURIFICATION METHODS

Besides its clinical applications bromelain has been subjected to many other industries due to its enormous benefits. Researchers thereby are trying various conventional as well as latest purification techniques to achieve bromelain in highest purified form at reduced cost (Arshad et al. 2014). As compared to fruit, bromelain concentration is high in stem and is thus a cheaply available source of bromelain (Tochi et al. 2008). Other parts of pineapple are also investigated for the presence of bromelain (Ketnawa et al. 2012) including peel, core

and crown etc. Extraction of bromelain from these parts is attractive not only from environmental point of view but also economically (Novaes et al. 2013). Bromelain can be easily extracted from the juice of pineapple by ultrafiltration (Larocca et al. 2010) but still FBM is not commercially available due to being different from SBM (Pavan et al. 2012). The marketable bromelain is mostly extracted from the stem of pineapple through centrifugation, ultrafiltration, lyophilization (Corzo et al. 2011) and two-step Fast Protein Liquid Chromatography (FPLC) (Harrach et al. 1998). Once extracted, the crude mixture containing required enzyme is then

exposed to numerous purification stages to eradicate impurities that may interfere with bromelain to hinder its application and reduce the specific activity of the enzyme (Illanes 2008). Product purity is the key factor which may constitute a large proportion of the total enzyme production cost (Lightfoot 1990). Several conventional isolation and purification techniques are now obsolete because of their low purification potential (Soares et al. 2012). So the extraction and purification strategy (Figure 1) designed should be selective for the desired product, cheap, high yielding and speedy (Gupta et al. 2004).

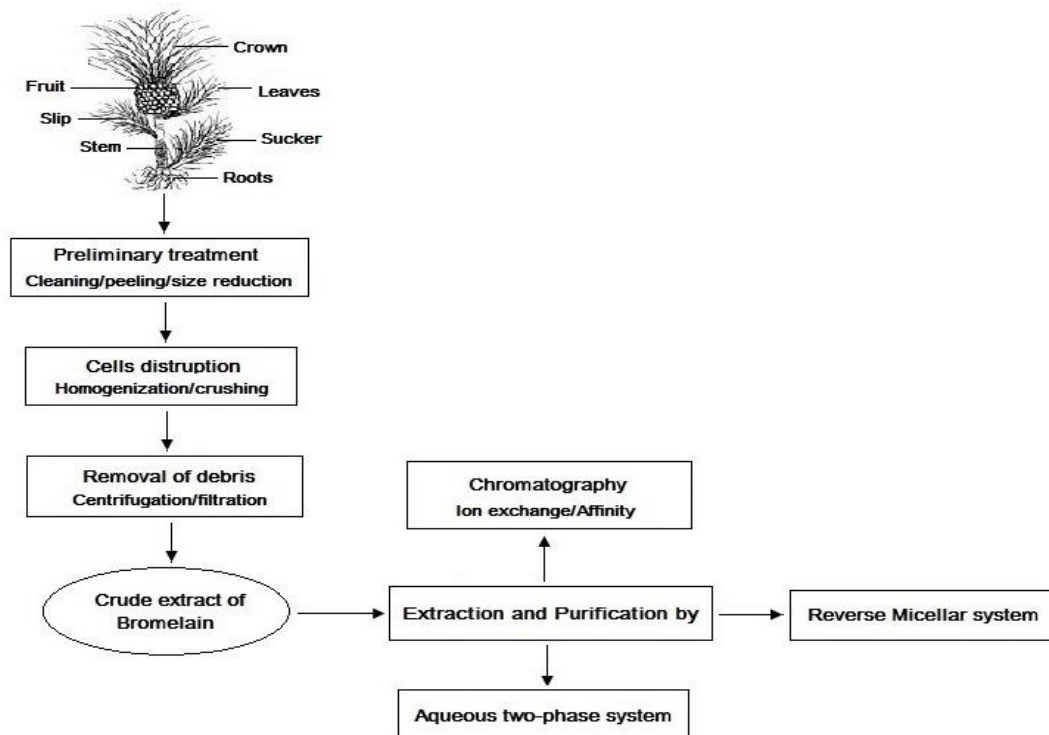


Figure 1 - Overview of extraction and purification of Bromelain.

Due to increased interest of scientists toward bromelain, several new purification techniques have been employed for its extraction and purification (Table no. 1). These include:

- 1) Aqueous two phase systems (Babu et al. 2008; Ferreira et al. 2011; Coelho et al. 2013; Spir et al. 2015).
- 2) Membrane processes (Doko et al. 2005; Lopes et al. 2009)
- 3) Precipitation (Doko et al. 2005; Devakate et al. 2009; Soares et al. 2012).
- 4) Reversed micellar systems (Hemavathi et al. 2007; Navapara et al. 2008; Kumar et al. 2011).

- 5) Different chromatographic techniques (Devakate et al. 2009; Li et al. 2009; Paulo et al. 2012).

Reverse micellar system

Micelle is an aggregate of molecules possessing both polar and non-polar regions. Reverse micelles are thermodynamically stable, minute surfactants that hold water in their interior surrounded by organic phase (Andray et al. 2001). Only protein of interest is entrapped in micelle (Figure 2) whereas other impurities remain in organic phase (Lee and Chong 2011). Reverse micellar system is an encouraging strategy for downstream processing. It is ideal to

extract biomolecules through diluted samples. Reverse micellar system possess higher sample loading capacity, are specific and easy to operate (Chen et al. 2008).

RMS are also being used for the extraction and purification of bromelain from ananas stem and waste. Pineapple core yielded 106% active recovery and 5.2 purification fold (Hebbar et al. 2008). There is also reported use of RMS for extraction of bromelain from pineapple where it yielded 97.56% activity recovery and 4.54 purification fold (Hemavathi et al. 2011). Researchers are implementing various modifications in RMS to upgrade the yield and purification fold. To purify bromelain at larger scale, scale-up studies by phase transfer mode of reverse micellar system are also performed which yielded purification fold of 2.43 with an activity recovery of 81.3% (Hebbar et al. 2011). To uplift the efficacy of process RMS coupled

with ultrafiltration was also studied which resulted in an activity recovery of 95.8 % and purification factor after ultrafiltration of 8.9-fold (Hebbar et al. 2012). The use of affinity based reverse micellar extraction and separation technique to extract and purify bromelain from pineapple waste yielded purification of 12.32 fold with an activity recovery of 185.6 % (Kumar et al. 2011). Moreover the process optimization studies for RMS had been reported (Fileti et al. 2009; Dhaneshwar 2014) and both the batch and continuous extraction of bromelain from pineapple juice by under optimized conditions was also studied (Fileti et al. 2007). A neutral model developed for bromelain extraction from pineapple juice by RMS under optimized conditions gave 4.96 purification factor with productivity of 1.29 ml/min (Fileti et al. 2009).

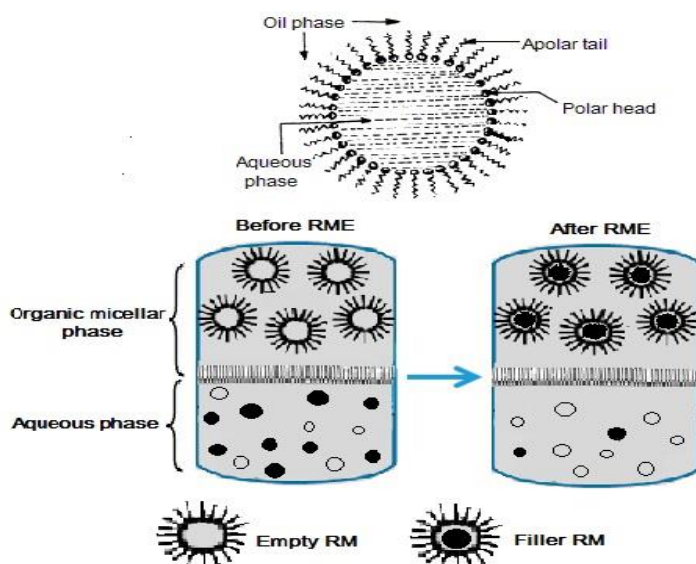


Figure 2 - Stages in working of Reverse Micellar System (RMS).

Aqueous two phase system (ATPS)

The aqueous two-phase systems consists of a polymer and a salt (or two polymers) and have been regarded as extensively used tools for extraction and purification of biomolecules (Rabelo et al. 2004). It is low cost, rapid, possess reusable polymers (Johansson et al. 2011), scalable (Asenjo and Andrews 2011) and can withstand high biomass load as compared to rest of the separation systems (Kaul 2001). ATPS is reported as a challenging technique for the separation of molecules that are hard to be separated through other methods and preferred to be

used in initial stages (Gupta et al. 1999). High active recovery of enzymes during ATPS is due to the presence of PEG which causes alteration in the structure of active sites of the enzyme. Researchers have reported the used ATPS for extraction and purification of bromelain from stem and peel of pineapple (Ferreira et al. 2014).

Extraction and purification of bromelain complexed with polyphenol oxidase through ATPS from the pineapple resulted in 228% active recovery with 4 fold rise in purification (Babu et al. 2008). ATPS has been used to recover bromelain from the peel of

pineapple and gave 113.54% and 206% active recovery with 2.23 and 3.44 purification fold (Ketnawa et al. 2010). In order to optimize the separation of bromelain response surface methodology has been worked in ATPS that yielded 90.33% enzyme and its purification factor was 2.4 (Navapara et al. 2011). When thermodynamic

equilibrium and ATPS were employed together for the purification of bromelain, purification fold came out to be among 25-62 (Ferreira et al. 2011). Moreover, thermoste separation of bromelain resulted in active recovery of 79.5% with 1.25 purification fold (Rabelo et al. 2004).

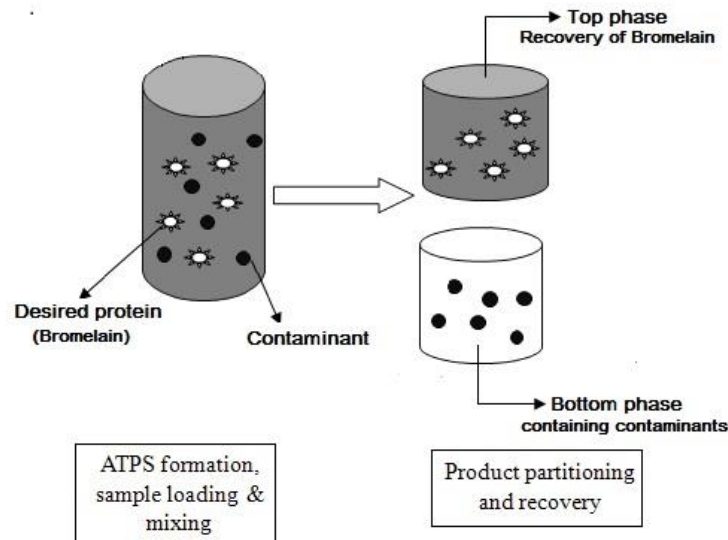


Figure 3 - Extraction and Purification of Bromelain through Aqueous Two Phase System (ATPS).

Chromatographic techniques

Chromatographic techniques significantly conserve the structure of purified protein (Gautam et al. 2010). Several chromatographic techniques such as ion exchange chromatography (Hung et al. 2002; Arumugam and Ponnusami 2013; Iara et al. 2013; Swaroop and Viswanathan 2013), high-speed counter-current chromatography (HSCCC) (Yin et al. 2011), affinity chromatography, gel filtration chromatography and capillary electrochromatography (Cheng and Huang 2004; Babu et al. 2008; Chen et al. 2008) have been employed for the purification of bromelain. All these techniques possess different specifications thus result in different purification efficiency and recovery (Yin et al. 2011). Amongst these, ion exchange chromatography (IEX) is extensively employed for bromelain separation (Devi and Sowmiya, 2014). Ion exchange chromatography is highly specific, scalable, reliable and a cheap purification method (Ng et al. 2009). 10 fold purification of bromelain was achieved using cation exchange chromatography

(Devakate et al. 2009). High-speed counter-current chromatography (HSCCC) has also been used to purify bromelain which generated 3.01g bromelain from 5.00g crude extract (Yin et al. 2011). Immobilized metal affinity membrane (IMAM) has been employed to purify bromelain which resulted in 15.4 fold purification factor with 94.6% recovery (Nie et al. 2008). 87.4% active recovery of bromelain was achieved when Poly Acryl Acid (PAA)-bound iron oxide magnetic nanoparticles were used in order to adsorb bromelain from aqueous solution (Cheng and Huang 2004). In the same context, 13 fold purification factor was achieved when bromelain was adsorbed in expanded bed (Silveira et al. 2009). 3.3 fold enhanced purification of bromelain was achieved by the combinations of precipitation and chromatographic techniques (Devakate et al. 2009).

Purification through membrane filtration

Membrane filtration employs the use of membranes to purify molecules on the basis of size difference. It includes microfiltration and ultrafiltration and is

considered quite useful for enzymes seclusion as well as concentration (Cassano et al. 2003). Membrane filtration is being used for the isolation and purification of bromelain. Simultaneous use of microfiltration and ultrafiltration in order to separate bromelain from pineapple pulp resulted in active recovery of 85% (through microfiltration) and 10 fold concentrated bromelain (through ultrafiltration) (Lopes et al. 2009). When microfiltration, ultrafiltration, ammonium sulfate precipitation and ultracentrifugation were employed in a sequence 98% yield was achieved (Doka et al. 2005).

Membrane filtration in conjugation with other processes results in enhanced purification of bromelain. When stem bromelain was adsorbed on nano-TiO₂ and then passes through ultrafilters, 5.3 purification fold and 64.7% active recovery was achieved (Chao et al. 2009). In a study three methods (Kaolin adsorption, ultrafiltration and tannin precipitation) were performed and compared for bromelain purification. Bromelain with highest proteolytic activity was obtained by ultrafiltration (Li et al. 2009).

Other methods

Various other methods have also been worked to separate bromelain and resulted in enhances active

recovery and purification fold. Ammonium sulfate precipitation is a cheap and extensively used method for protein purification (Saxena et al. 2007). Ammonium sulfate precipitation resulted in 2.81 purification fold of bromelain (Devakate et al. 2009). In a study ethanolic extraction, isoelectric focusing and ammonium sulfate precipitation were compared for the extraction of bromelain. Amongst these three, ethanolic extract resulted in highest bromelain yield (Silvestre et al. 2011). Likewise, a comparison was also made between ethanol, PEG and (NH₄)₂SO₄ to precipitate bromelain in order to recover it from stem, bark and leaves of pineapple plant. The purification factor achieved after using ethanol was 2.07 fold with 30-70% recovery. 20-40% recovery and 4.4 fold purification factor was achieved after precipitation by ammonium sulfate whereas poly (ethylene glycol) failed to precipitate bromelain (Soares et al. 2012). 1400 GDU/ml proteolytic activity with a protein content of 9.33 mg/ml was achieved after using homogenization process for bromelain separation (Loh et al. 2005). The use of recyclable mesoporous silica to adsorb bromelain from pineapple juice resulted in 6.2 fold purification with 97.89% recovery (Arumugam and Ponnusami 2013).

Table 1 - Some modern techniques for the extraction and purification of Bromelain

Purification techniques	Types	Yield (%)	Purification fold/factor	Reference
Aqueous two-phase system	PEG/K ₂ SO ₄ aqueous two-phase system	228	4.0	Babu et al. 2008
	PEG/poly(acrylic acid) aqueous two-phase system	335.27	25.78	Novaes et al. 2013
	PEG/(NH ₄) ₂ SO ₄ unconventional aqueous two-phase system	-	11.80	Coelho et al. 2013
	PEG/MgSO ₄ aqueous two phase system	113.54	2.23	Ketnawa et al. 2010
	PEG/MgSO ₄ aqueous two phase system	108.45	-	Katnawa et al. 2009
	PEG/MgSO ₄ aqueous two phase system	206	3.44	Ketnawa et al. 2011a
	Block copolymers aqueous two phase system	79.5	1.25	Rabelo et al. 2004
	PEG/MgSO ₄ aqueous two phase system	-	25-62	Ferreira et al. 2011
	Four various aqueous two phase system	-	-	Ketnawa et al. 2011b

Filtration	Microfiltration & ultrafiltration	85-100	10	Lopes et al. 2009
	Microfiltration, ammonium sulfate precipitation, ultracentrifugation	50	-	Doka et al. 2005
	Nano-TiO ₂ , ultrafiltration	64.75	5.3	Chao et al. 2009
Reverse micellar systems	Reverse micelle systems	85	4	Chaurasiya et al. 2015
	Reverse micelle systems	102	5.2	Hebbar et al. 2008
	Reverse micelle systems	97.56	4.54	Hemavathi et al. 2007
	Optimized reverse micelle systems	89.6	2.8	Navapara et al. 2011
	Affinity-based reverse micelle system	185.6	12.32	Kumar et al. 2011
Chromatography	High speed counter-current chromatography, reverse micelle system	-	-	Yin et al. 2011
	Immobilized metal affinity membrane	94.6	15.4	Nie et al. 2008
	Precipitation, ion exchange chromatography	-	3.3	Devakate et al. 2009
	Cation exchange chromatography	-	10	Nadzirah et al. 2013

APPLICATIONS OF BROMELAIN

Bromelain has been known for its vast commercial applications. It is being used in food and beverage industries (Neta et al. 2012), in meat tenderization, in cosmetic industries (Orsini 2006) as well as in textile industries (Arshad et al. 2014). Besides its industrial applications, bromelain possess multiple therapeutic actions (Summarized in Table no. 2). Some of its reported actions include inhibition of platelet aggregation, anti-edema (MacKay et al. 2003), anti-thrombotic and fibrinolytic activity (Errasti et al. 2016), anti-inflammatory action (Brien et al. 2004), anti-tumor action (Dhandayuthapani et al. 2012; Pillai et al. 2013), modulation of cytokines and immunity, skin debridement properties (Hu et al. 2011; Rosenberg et al. 2012), enhanced absorption of other drugs (Orsini 2006), mucolytic properties, digestive assistance, enhanced wound healing (Taussig et al. 1988), cardiovascular and circulatory improvement (Kavitha et al. 2013). Some of its therapeutic applications are briefly discussed below.

Anti-inflammatory agent

Inflammation is the body's attempt of self-protection which takes into account the discharge of various factors that causes the distention of capillary and enhances their permeability, as a result

of which the tissues appear to be inflated and inflamed (Jaber 2002). Mostly NSAIDS (non-steroidal anti-inflammatory drugs) are prescribed to ward off the classical signs of inflammation (Calor, dolor, rubor and tumor) (Charles et al. 2011), however these drugs severely injures GIT (Gastrointestinal tract) and possess numerous hazardous after effects (Walker et al. 2002) so, as an alternative bromelain has been demonstrated as an anti-inflammatory agent and is in practice for acute inflammation and several related conditions (Hale et al. 2005a). Anti-inflammatory action of bromelain is mediated by retarding the formation of pro-inflammatory prostaglandins (Graf 2000), reducing the cell surface receptors such as hyaluronan receptor CD44 and plasma fibrinogen levels (Yuan et al. 2006). It also reduces the level of bradykinin, CD4+ T lymphocytes (Manhart et al. 2002) and enhances the activity of serum fibrinolytic thus induces the production of proinflammatory mediators and anti-inflammatory prostaglandins as a result of which the distention and permeability of capillaries is reduced (Darshan and Doreswamy 2004; Hale et al. 2005; Tochi et al. 2008). Figure no. 4 displays how bromelain respond to certain mediators of acute inflammation.

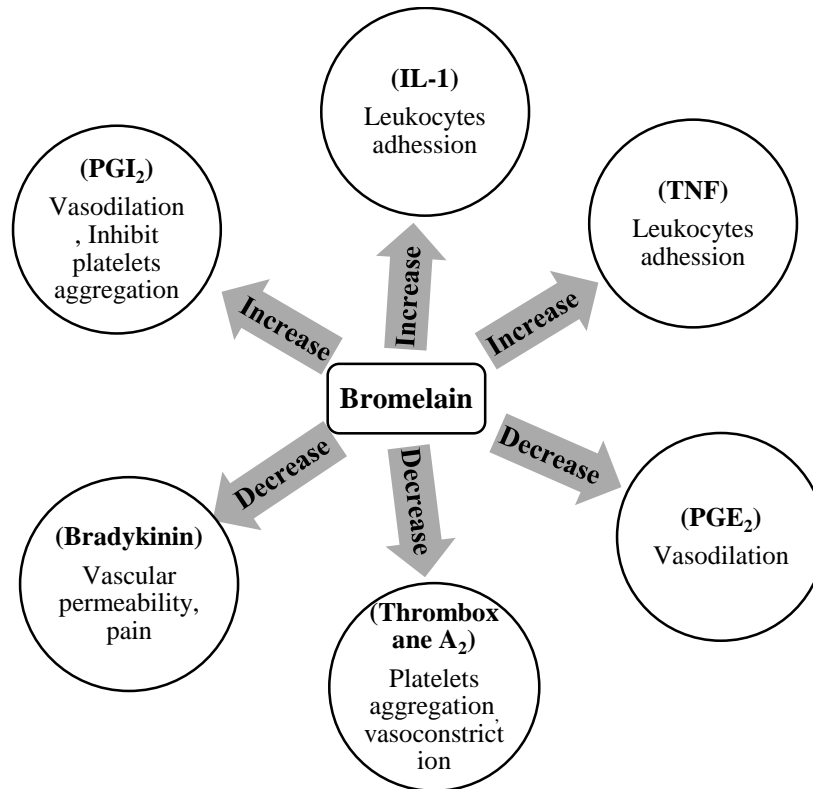


Figure 4: Effect of Bromelain on certain mediators of acute inflammation

Rhinitis and rhinosinusitis

Rhinosinusitis results in inflammation of nasal cavity and paranasal sinuses (Guo et al. 2006) whereas chronic rhinosinusitis (CRS) is more persistent and long-term inflammation which causes disruption of mucus membranes (Mehdi et al. 2014). Bromelain is an effective mucolytic agent and is being efficiently used in rhinitis, rhinosinusitis as well as in chronic rhinosinusitis (Guo et al. 2006; Buttner et al. 2013). It decreases the formation of pro-inflammatory prostaglandin and reduces swelling in nasal passages (Helms and Miller 2006). Bromelain also decrease mucus production and aids its drainage (Tochi et al. 2008). Beneficial effects of bromelain have been viewed in allergic rhinitis as well where it reduces edema and inflammation (Thor and Kelly 2000).

Arthritis

NSAIDS are commonly prescribed in arthritis (Henriksson et al. 2014). Bromelain can be used as harmless alternative and researchers have found that bromelain exhibits promising efficacy in arthritis (Osteoarthritis and rheumatoid arthritis) (Pavan et al. 2012). Osteoarthritis, the major form of arthritis, is a leading cause of disability these days. Bromelain is a potent analgesic and displays a direct effect on certain mediators of pain like bradykinin (Brien et al.

2004). Recently it has been found that bromelain, when used in conjugation with other nutraceuticals such as turmeric, results in enhanced efficacy in the treatment of degenerative joint pain diseases (Conrozier et al. 2014).

Colonic inflammation

Bromelain notably reduce the harshness of colonic inflammation and when taken orally, it significantly reduces the severity of ulcerative colitis (Hale et al. 2005). The chief anti-inflammatory mechanism of bromelain comes out to be proteolytic in nature by which it eradicates cell surface receptors involved in leukocyte defection and activation (Hale et al. 2010). Bromelain also vary the emission of certain chemokines and thus reduces the prevalence and sternness of spontaneous colitis. Due to these researchers regard bromelain as a novel treatment for inflammatory bowel disease (Onkan et al. 2008).

Anti-cancer agent

In healthy cells, autophagy occurs naturally whereas in tumor cells, this process is deactivated. Cancerous cells metastasize through circulation and transport system to nearby tissues. In many types of cancer p53 gene, which induces apoptosis, is inactivated thus apoptotic cell death is evaded in such cancerous cells (Baez et al. 2007). Researchers have recognized

numerous anti-cancer mechanisms of bromelain such as switching off the essential gene signal NF-kappaB, suppression of Cox-2 expression, upregulation of p53 and Bax and by initiation of autophagy (Tochi et al. 2008; Bhui et al. 2010). Bromelain exhibits antimetastatic activity, stimulates several caspases and promotes apoptosis. Moreover the use of bromelain in cancer lessens tumor size and results in reduced damage to healthy cells and possesses less after effects as compared to chemotherapy (Bhui et al. 2009; Amini et al. 2016). Bromelain remarkably results in tumor regression for certain cell lines. These include P-388 leukemia, sarcoma (S-37), A-431 epidermoid carcinoma, A-375 melanoma, lewis lung cancer (Batkin et al., 1988) and ADC-755 breast cancer (Bhui et al. 2011; Pavan et al. 2012). Furthermore, the use of bromelain in conjugation with chemotherapeutic medicine improves the efficiency of these drugs to certain extent (Pillai et al. 2013; Amini et al. 2014).

Breast Cancer

Bromelain, a pharmacologically active compound, is a potent anti-tumorigenic agent (Neta et al. 2012). In mammary carcinoma cells bromelain affects MCF-7 cells by slowing down their growth inhibitory response and activate the process of autophagy. Furthermore, bromelain promotes natural cell death (apoptosis) in cancerous cells (Bhui et al. 2010). When taken orally, it also encourages the scarce monocytic cytotoxicity in breast cancer patients (Eckert et al. 1999). Mainly in mammary cancer cells increased dosage of bromelain promotes the process of natural cell death (apoptosis) (Dhandayuthapani et al. 2012).

Epidermoid carcinoma and Melanoma

The efficacy of bromelain against particular melanoma and epidermoid carcinoma cell lines was checked where bromelain successfully exhibited anti-cancer activity. Bromelain not only minimized their proliferation and reduced the expression of Cox-2 gene (Bhui et al. 2012) but also induced apoptosis and suppressed lung metastasis of melanoma cells (Carla et al. 2007). CD44 are the surface proteins present on human leukemia Molt 4/8 cells and are involved in cancer matastasis. Bromelain efficiently reduced the volume of this protein in leukemia and melanoma cells (Harrach et al. 1994).

Inhibitor of thrombus formation

Platelets aggression and thrombus formation are the leading factors of cardiac discomfort (Bousser 2013).

Akt (a protein kinase) controls numerous cellular processes and is a key player in cardiovascular diseases. It is activated upon phosphorylation (Chaanine and Hajjar 2011). Apoptosis is a chief stimulator of several cardiovascular disorders (Maulik and Das 1994). Upon its activation, Akt inactivates several proapoptotic genes that causes cessation of apoptosis to some extent resulting in cardioprotection (Shiraishi et al. 2004). Bromelain is an effective cardioprotective agent. It possesses antithrombotic and anticoagulant activities and reduces platelet aggregation. It also prohibits the attachment of platelets to endothelial (Metzig et al. 1999). Bromelain results in enhanced phosphorylation of Akt which causes inhibition of apoptotic cell death (Juhasz et al. 2008). Bromelain inhibits clumping of platelets and also put a stop to thrombus formation (Metzag et al. 1999; Bhattacharia 2008). It has the ability to reduce angina and also possess antihypertensive action (Maurer 2001).

Angina Pectoris and Thrombophlebitis

Bromelain has been effective in the treatment of cardio vascular diseases (Tochi et al.2008). Bromelain promotes the disruption of thrombus, reduces the platelets clumping, blood viscosity and thus reduces the harshness of angina pectoris (Metzik et al. 1999;Maurier 2001) and transient ischemic attack (TIA). Bromelain also helps to reduce the potent risks of thrombophlebitis and aids its treatment (Pavan et al. 2012). Bromelain is also recommended for the treatment of acute thrombophlebitis (Ley et al. 2011).

Burns Debriding agent

A dry Scab formed on the skin especially after burn is known as eschar which may result in prolonged recovery and also enhance the chance to develop infection (Singer et al. 2010b). Debridement refers to the elimination of dead, damaged, or infected tissue from the site of eschar to improve the healing potential of the remaining healthy tissues (Rosenberg et al. 2012). Debriding the burn eschar surgically is quite challenging and can cause many difficulties (Pavan et al. 2012). On the other hand; enzymatic debridement can minimize such complications and is a safe alternative (Krieger et al. 2012). Amongst these, bromelain is regarded as a potential candidate (Ahle and Hamlet 1987; Maurer 2001). Topical bromelain is being used these days in debriding necrotic tissues (Hu et al. 2011; Wu et al. 2012). Bromelain encompasses a fraction of Escharase which is a main debriding agent (Pavan et al. 2012).

Bromelain is quite specific in its action. It selectively acts upon the effected tissues without harming the normal ones (Rosenberg et al. 2012). Bromelain simplifies the debridement process and offers improved and accelerated healing and quick reepithelialization (Singer et al. 2010a; Hu et al. 2011; Rosenberg et al. 2012). Researchers have found bromelain to be useful in healing post-surgical wounds and aid to lessen their aching and swelling etc (Graf 2000; MacKay and Miller 2003).

Asthma

Asthma refers to the inflammation of air passages resulting in swollen or inflamed airways. Inflammation in asthma is caused mainly due to amplification of eosinophils and T lymphocytes level in the bronchial mucosa and broncho-alveolar lavage (BAL) fluid (Leblond et al. 2005). Bromelain is an efficient therapeutic agent and is being used successfully in allergic airway disease (AAD) (Secor et al. 2012). Bromelain treats asthma by decreasing the level of total BAL leukocytes (eosinophils and lymphocytes) and cellular infiltrates (Secor et al. 2005) and also notably lessens BAL CD4+, CD8+ T CD4+ and CD25+T cells (Jaber 2002; Darshan and Doreswamy 2004). It also reduces interleukins IL-4, IL-12, IL-13, IL-17 and IFN- α in the serum as well

as changes the proportion of CD4+/CD8+ (Secor et al. 2005a; 2007b; 2012c; 2013d).

Blood Coagulation and Fibrinolysis

Fibrinolysis is a process to prevent fibrin clot from growing and allows the body to clear fragments of clots safely (Maus and Hajjar 2005). Bromelain is regarded as an effective fibrinolytic agent and prevents blood from coagulation (Taussig and Batkin 1988) by exaggerating the transformation of plasminogen to plasma which in turn inhibit fibrin (a protein involved in blood clotting) synthesis (Bhattacharia 2008). It also decreases the proportion of fibrinogen in serum, represses ADP induced platelet aggregation and delay both prothrombin time (PT) and activated partial thromboplastin time (APTT) (Kaur et al. 2014; Errasti et al. 2016). Both extrinsic and intrinsic pathway results in the formation of fibrin (as shown in Figure no. 5). However bromelain limits its formation by reducing some of the intermediates of clotting cascades (factor X and prothrombin) and increase fibrinolysis. It also decreases prekallikrein and thus inhibits the generation of bradykinin at the site of inflammation. This results in reduction of pain and edema as well as increases circulation at the site of injury.

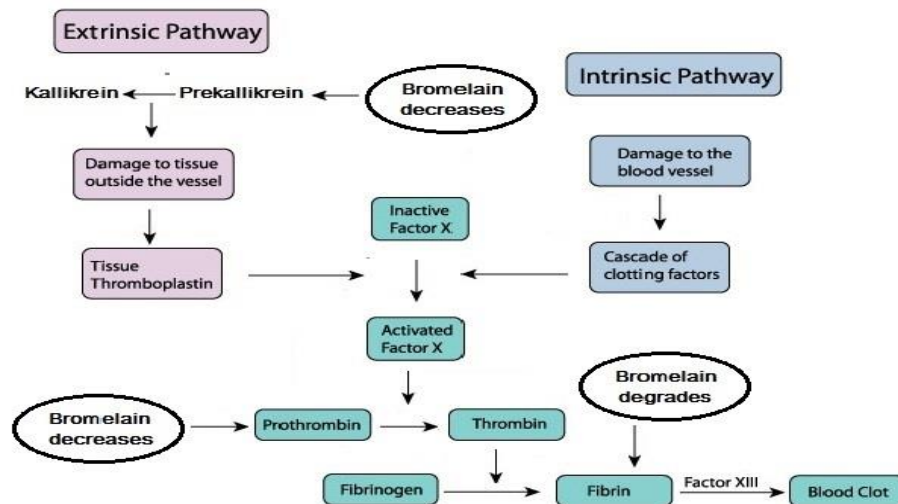


Figure 5: Pathways through which Bromelain inhibits blood clotting.

Dermatological disorders

Pityriasislichenoideschronica (PLC)

Pityriasislichenoides is a rare cutaneous disorder and is characterized by the development of multiple, scaly, erythematous to brown papules on the trunk and extremities (Someshwar and Udare 2012). Bromelain has been investigated for use in PLC.

After treatment all the victims of PLC were fully recovered with zero side effects which is a virtue of its immuno-modulatory and antiviral trait (Massimiliano et al. 2007).

Scleroderma

It is a disease characterized by progressive skin hardening and induration that is caused by abnormal growth of connective tissues (Gaby 2006). Bromelain has also shown promise in treating scleroderma (Gaby 2006). (Gabrielli et al. 2009).

Table no. 2: Summary of clinical applications of Bromelain.

	Disorders	Observed effects	References
Anti-Inflammatory agent	Asthma	Change CD4+ to CD8+ T lymphocyte ratio and decreases AAD (Allergic airway disease).	Jaber et al. 2002; Secor et al. 2008
	Chronic Rhinosinusitis	Retards formation of pro-inflammatory prostaglandin resulting in fast recovery.	Bakhshae et al. 2014
	Colonic inflammation	Decreased the occurrence and severity of spontaneous colitis	Darshan; Doreswamy 2004
	Osteoarthritis	Reduces in joint tenderness, pain and swelling	Klein 2006; Walker et al. 2002
	Rheumatoid arthritis	Reduce joint stiffness	Maurer 2001
	Soft tissue injuries	Have high wound healing capacity	Baumann et al. 2007; Lemay et al. 2004
Anti-tumor agent	Breast cancer	Decrease tumor size and cause apoptosis	Baez et al. 2007
	Leukemia	Causes tumor regression	Maurer 2001; Pavan et al. 2012
	Lung carcinoma	Bromelain possesses antimetastatic and anticoagulant functions	QIMR 2005
	Ovarian cancer	Decrease tumor growth and invasive potential	Maurer 2001
	Melanoma	Inhibits nuclear factor-kappaB (NF-kb), a protein involved in cancer	Kalra et al. 2007
Improvement of Gastrointestinal Tract related discomforts	Postoperative gastrointestinal dysmotility (ileus)	Recovers abdominal distention and increase water content of fecal pellets	Wen et al. 2006
	Constipation	Improves stool release in patients	Wen et al. 2006
	Exocrine pancreas insufficiency	Improves digestion	Knill-Jones et al. 1970
	Nausea, vomiting, diarrhea	Neutralizes the effects of intestinal pathogens that causes NVD	Pavan et al. 2012
Inhibition of thrombus formation	Angina pectoris	Bromelain stops aggregation of platelets and causes blood thinning	Taussig and Nieper 1979
	Transient ischemic attacks	Reduces its severity	Taussig and Nieper 1979
	Thrombophlebitis	Treats thrombophlebitis	Kelly 1996
	Thrombosis	Break down cholesterol plaques	Kelly 1996
Treatment of Dermatological disorders	Burns/Eschar	Topical bromelain results in complete debridement	Rosenberg et al. 2004
	Wrinkles	Conditions skin	Reddy et al. 2013
	Pityriasis lichenoides chronica	Complete clinical recovery	Massimiliano et al. 2007
	Scleroderma	Resolves lesions	Gaby 2006

CONCLUSION

Bromelain is a concoction of several thiol endopeptidases. It is distinguished as stem bromelain and fruit bromelain depending upon the site of extraction. It is one of the extensively investigated proteolytic enzyme owing to its astonishing applications in various industries. This necessitates to employ a strategy that result in highest purified bromelain in less steps and lowest cost. Use of modernistic approach such as membrane filtration, reverse micellar systems, aqueous two phase extraction and chromatographic techniques have shown promise in this regard.

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