Chapter 5
Plant Proteolytic Enzymes: Their Role as Natural Pharmacophores

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5.1 Introduction

Proteases or proteinases are enzymes that catalyze cleavage of proteins at peptide bonds generating smaller peptides. Some of them are very specific in their choice of target site while others act rather nonspecifically and hydrolyze the protein substrate if conditions allow into short peptides. They must have appeared early in evolution along with proteins, to keep a balance between synthesis and protein degradation. Their early emergence is confirmed by their ubiquitous presence in most living forms including viruses, plants, and animals.

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M. G. Guevara, G. R. Daleo (eds.), *Biotechnological Applications of Plant Proteolytic Enzymes*, https://doi.org/10.1007/978-3-319-97132-2_5

Proteases involve two groups of enzymes: exoproteases, which cleave the initial or terminal peptide bonds of substrates, designated as amino- or carboxy-peptidases, respectively, and endoproteinases cleaving at inner bonds of the protein.

Classification of proteases is based upon the type of residue relevant at the active site. The hydroxyl group of serine or threonine proteinases and the cysteine group in cysteine proteinases are the nucleophiles during catalysis of these groups of endoproteinases, while activated water is the nucleophile for aspartic-, glutamic-, and metalloproteinases.

As of December 04, 2017, the database (release 12.0) lists 912,290 sequences and provides links to 1022 PDB (Protein Data Bank) (http://www.pdb.org/pdb/home/home.do) entries distributed among 268 families and 62 clans.

The MEROPS database (https://www.ebi.ac.uk/merops/), is a resource for annotation of proteases, their substrates, and their inhibitors. This database is hosted at EMBL-EBI since 2017. PANTHER (*P*rotein *A*nalysis *T*hrough *E*volutionary *R*elationships) (http://www.pantherdb.org/) is another tool that classifies proteases by families and subfamilies, their biochemical function, the biological processes, and metabolic pathways in which participate to facilitate high-throughput analysis.

Most annotated sequences belong to the serine group (36.6%), followed by metallo- (32.6%), cysteine-, (19.5%), aspartic-, (4.9%), threonine- (3.8%), plus minor groups including mixed-type proteases, and unclassified and unknown sequences.

As stated above, proteases like most proteins must have arisen early in evolution since primeval life forms required them for the digestion of food. In fact, a comparison of digestive proteases in evolved species shows similarities with those found in prokaryotic organisms arguing for a common origin.

Once proteins became constituents of cellular structure, they were required to metabolize endogenous proteins. With advances in genomic sequencing, it is now estimated that about 2% of structural genes in an organism code for these enzymes (Barrett and Fred Woessner 2013). For instance, it is estimated the presence in rice (*Oryza sativa*) of >650 genes and in *Arabidopsis thaliana* >800 genes coding for proteases (van der Hoorn 2008). A similar distribution is projected in animal organisms. Despite these impressive figures, the lack of information about their physiological function and their cognate substrates limit our knowledge of these enzymes. A further problem during dissection of a single proteolytic activity is encountered as most proteolytic enzymes act as a group of related members for a reaction(s) to take place, thus coining the concept of "protease web" (Fortelny et al. 2014).

In plants, proteolytic enzymes are found in most cell compartments participating in most stages of plant life, including growth, homeostasis, germination, breakdown of storage proteins during seed germination, organ senescence, and programmed cell death (Huffaker 1990).

An important function is their involvement in the proteasome proteolytic pathway affecting several metabolic processes, such as hormone signaling, cell cycle, embryogenesis, morphogenesis, and plant-environment interactions (van Wijk 2015).

A major source of proteolytic activities is detected in latex producing plants. Laticifers are specialized cells found in 20 plant families from angiosperm orders representing between 15 and 20,000 species (Lewinsohn 1991). The cytoplasm of laticifers stores latex containing defense metabolites which are released in response to physical damage. By analogy, the proteolytic enzymes found in some laticifers resemble the functionality of proteolytic enzymes present in mammalian circulatory system.

In this review, that focuses in the last ten years, we present evidence supporting the role of plant proteolytic enzymes as therapeutic options to treat several symptoms. Two early compilations described the advances in this field in 2008 (Domsalla and Melzig 2008; Salas et al. 2008) and recently, while we were preparing this review a revision covered this subject (Balakireva et al. 2017).

It is well established that species of Caricaceae family (*C. papaya* and *C. pubescens, syn C. candamarcensis, syn Vasconcellea pubescens,* and *syn V. cundinamarcensis*) have been used ethnopharmacologically to treat digestive disorders and skin fungal ailments by native American populations (Soplin et al. 1995). Similarly, *A. comosus* the source of bromelains was used as medicine by indigenous cultures and in 1957 Heinicke and Gortner (1957) initiated its therapeutic use. In this revision, we mainly focus on the scientific developments within this field covering the last decade. A listing of pharmacological applications discussed here is shown in Table 5.1.

5.2 Proteolytic Enzymes Inflammation and Immunomodulation

Inflammation and immunomodulation are intertwined processes in which the immune response, both innate and adaptive lead to inflammatory outcomes. The plant proteases (papain, bromelain, ficin, etc.) have been used as anti-inflammatory and especially bromelain is used as alternative and/or complementary therapy to glucocorticoids, nonsteroidal antirheumatics, and immunomodulators.

For instance, different doses of the cysteine proteinases from *Bromelia hieronymi* and bromelain used as reference on carrageenan- and serotonin-induced rat paw edema, as well as cotton pellet granuloma model, induced 40–50% inhibition of the inflammatory effect. If the enzyme fractions were treated with E-64 (cysteine proteases inhibitor), the anti-inflammatory effect disappeared, demonstrating that the effect was mediated by cysteinyl enzymes (Errasti et al. 2013). A similar result was observed using the proteolytic fraction of *V. cundinamarcensis* using the rat paw edema model. The anti-inflammatory response was equivalent to the dexamethasone positive control (unpublished data).

The anti-inflammatory action of bromelain has been linked to decreased secretion of pro-inflammatory cytokines and chemokines [granulocyte–macrophage colony-stimulating factor (GM-CSF), IFN-γ, CCL4/macrophage inhibitory protein

Table 5.1 List of plant proteolytic enzymes with pharmacological applications

Protease	Species	Biological activity	Reference
Bromelain, CP	*Ananas comosus*	Dermatology	Ho et al. (2016)
Bromelain, CP	*Ananas comosus*	Chronic rhinosinusitis	Büttner et al. (2013)
Bromelain, CP	*Ananas comosus*	Anti-inflammatory	Shing et al. (2015)
			Amini et al. (2013)
			Secor et al. (2012)
CP	*Bromelia hieronymi*	Anti-inflammatory	Errasti et al. (2013)
Bromelain, CP	*Ananas comosus*	Digestive disorders	Zhou et al. (2017)
			Sahbaz et al. (2015)
Papain, CP	*Carica papaya*	Digestive disorders	Levecke et al. (2014)
			Mansur et al. (2014)
			Luoga et al. (2015)
Bromelain, CP	*Ananas comosus*	Wound healing/ mitogenic activity	Rosenberg et al. (2012)
			Cordts et al. (2016)
			Golezar (2016)
			Wu et al. (2012)
			Aichele et al. (2013)
			Iram et al. (2017)
SP	*Wrightia tinctoria R. Br. (Apocynaceae)*	Wound healing/ mitogenic activity	Yariswamy et al. (2013)
P1G10, CP	*Vasconcellea cundinamarcensis*	Wound healing/ mitogenic activity	Freitas et al. (2017)
			Araujo e Silva et al. (2015)
Papain, CP	*Carica papaya*	Wound healing/ mitogenic activity	Aranya et al. (2012)
			Chen et al. (2017)
			Shoba et al. (2017)
Heynein, CP	*Ervatamia heyneana latex*	Thrombolytic activity	Uday et al. (2017)
SP	*Solanum tuberosum* leaves	Thrombolytic activity	Pepe et al. (2016)
	Euphorbia cf. lactea latex		Siritapetawee et al. (2015)
	Petasites japonicus		Kim et al. (2015)
	Curcuma aromatica Salisb		Shivalingu et al. (2016)
MP-like	*Aster yomena (Kitam.) Honda*	Thrombolytic activity	Choi et al. (2014)
P1G10, CP	*Carica candamarcensis*	Thrombolytic activity	Bilheiro et al. (2013)
CP	*Cnidoscolus urens (L.)* leaves	Thrombolytic activity	de Menezes et al. (2014)

(continued)

Table 5.1 (continued)

Protease	Species	Biological activity	Reference
CP	*Pseudananas macrodontes* *Bromelia balansae* *Bromelia hieronymi*	Thrombolytic activity	Errasti et al. (2016)
Bromelain, CP	*Ananas comosus*	Antitumoral	Amini et al. (2013)
			Pillai et al. (2013)
			Romano et al. (2014)
			Miranda et al. (2017)
			Bhatnagar et al. (2015)
			Pillai et al. (2013)
P1G10, CP	*Vasconcellea cundinamarcensis*	Antitumoral	Dittz et al. (2015)
SP	*Asian pumpkin*	Bioactive peptides	Dąbrowska et al. (2013)
Papain, CP	*Carica papaya*	Bioactive peptides	Wang et al. (2012)
Papain–Bromelain, CP	*Ananas comosus*/ *Carica papaya*	Bioactive peptides	Gajanan et al. (2016)
Bromelain, CP	*Ananas comosus*	Bioactive peptides	Li-Chan et al. (2012)
CP	Latex *Jacaratia corumbensis*	Bioactive peptides	Arruda et al. (2012)
Bromelain, CP	*Ananas comosus*	Antibacterial	Pu and Tang (2017)
Papain, CP	*Carica papaya*	Antifibrotic	Sahu et al. (2017)
Papain, CP	*Carica papaya*	Drug carrier	Menzel and Bernkop-Schnürch (2018)
Bromelain, CP	*Ananas comosus*	Drug carrier	Parodi et al. (2014)
Papain, CP nanoparticles	*Carica papaya*	Antibacterial	Atacan et al. (2018)
Papain/Bromelain, CP	*Carica papaya/Ananas comosus*	Oral applications	Waleed Majid and Al-Mashhadani (2014)
			Ordesi et al. (2014)
			Divya et al. (2015)
			Mugita et al. (2017)
			Sahana et al. (2016)
			Tadikonda et al. (2017)
			Motta et al. (2014)
			Abdul Khalek et al. (2017)
			Patil et al. (2015)

(continued)

Table 5.1 (continued)

Protease	Species	Biological activity	Reference
Protease inhibitor			
Kunitz-type inhibitor	*Tamarindus indica L.* seeds	Biotechnological health-related application.	Medeiros et al. (2018)
Kunitz family of protease inhibitors	–	Antithrombogenic	Salu et al. (2018)
Trypsin and chymotrypsin inhibitors	*Erythrina velutina* seeds	Gastroprotective antielastase	Oliveira de Lima et al. (2017)
Purified protease inhibitors LC-pi I, II, III, and IV	*Lavatera cashmeriana*	Anticancer activity	Rakashanda et al. (2015)

CP cysteine protease, *SP* serine protease, *MP* metalloprotease

(MIP-1ß)], and TNF by inflamed tissue in inflammatory bowel disease using an in vitro human colon model. Bromelain also enhanced the expression of partly inflammatory cytokines IL-2 and IL-4 and IFN-γ leukocytes (Onken et al. 2008).

Fitzhugh et al. (2008) reported that bromelain decreases neutrophil migration during acute inflammation and specifically removes chemokine receptor CD128 suggesting a reduction in leukocyte binding to blood vessels that consequently impairs cell extravasation. Leukocyte migration is viewed as crucial for the inflammatory response. Furthermore, in some cases bromelain is being used to treat inflammatory symptoms in osteoarthritis or asthma (Brien et al. 2006; Secor et al. 2012).

Instead, it is not clear if papain shares the anti-inflammatory effect attributed to bromelain-like cysteine proteases, as a report shows that this protease activates human mast cells via PAR-2 cell receptors. The induced activation of mast cell is related to release of tryptase and ß-hexosaminidase (Seaf et al. 2016). Also, papain is currently used as a model substance to develop experimental osteoarthritis, lung inflammation, and rhinosinusitis, and all these conditions are associated to inflammatory processes (Patel et al. 2015; Agoro et al. 2016; Tharakan et al. 2018). Also, epicutaneous administration of papain may induce a dysfunction of the skin barrier and increase circulatory IgE and IgG. In this study, the presence of papain in serum is confirmed by identification of papain-specific IGs antibodies (Iida et al. 2014). These data suggest that papain may act as inflammatory protease departing from other cysteine proteases. Meanwhile, topical application of papain to volunteers stung by jellyfish *Chrysaora chinensis* showed pain remission and inhibition of nematocyst discharge (DeClerck et al. 2016) and another report described that papain at doses of 0.325 and 0.75 mg/kg possesses marked anti-inflammatory action against infectious arthritis, like butadion and indomethacin (Rakhimov 2001). Meanwhile, excepting for an early report describing allergic reactions and asthma during occupational exposure to bromelain (Baur and Fruhmann 1979), no additional evidence of inflammatory effect induced by bromelain was described. More recently,

Dutta and Bhattacharyya (2013) rule out the presence of toxic substances in *A. comosus* crown-leaf extracts containing bromelain. It is paradoxical that bromelain is used to treat symptoms, some of which appear to be triggered by papain. Since both papain and bromelain belong to the same group of cysteine proteases (C1A), it is conjectured that unaccounted structural differences must exist between these proteases that justify their different biological role. It is also possible that papain and/or bromelain containing fractions contain contaminating proteolytic isoforms responsible for these "unexpected" effects.

A condition known as endometriosis displaying inflammatory symptoms and affecting around 10% of females is apparently caused by estrogen in which endometrium-like tissue grows outside the uterine cavity (Eskenazi and Warner 1997). The disease is accompanied by increased level of inflammatory cytokines IL-1β, IL-6 (Harada et al. 1997), IL-8, TNF-α, and monocyte chemoattractant protein-1 (MCP-1) (Burney and Giudice 2012). Bromelain has been used in combination with N-acetyl cysteine and α-lipoic acid as successful treatment for endometrioses both in vitro and in a rodent model (Agostinis et al. 2015).

5.3 Digestive Disorders

The efficacy of proteinases has been studied at least in three digestive disorders: ulcerative colitis, inflammatory bowel disease, and Crohn's disease. Oral bromelain was initially reported to reduce the severity of colon inflammation in a rodent model (Hale et al. 2005) and fresh pineapple juice decreases inflammation in IL-10-deficient mice with colitis (Hale et al. 2010). Colon biopsies from patients with ulcerative colitis and Crohn's disease had decreased levels of inflammatory cytokines if treated with bromelain (Onken et al. 2008). Meanwhile, bromelain effectively decreases neutrophil migration to sites of acute inflammation and support the specific removal of the CD128 chemokine receptor responsible for activation of IL-8 (Fitzhugh et al. 2008). A recent report suggests participation of TNF-α receptors in the anti-inflammatory effect of bromelain (Zhou et al. 2017). In a related study, papain applied peritoneally or oral bromelain was used to reduce or prevent intraperitoneal adhesions mainly resulting from abdominal surgery (Ochsner and Storck 1936; Sahbaz et al. 2015). However, Stevens (1968) could not confirm the protective effect of papain in an animal model.

Intestinal disorders caused by parasites have been treated with proteolytic plant enzymes. The anthelmintic efficacy of papain and bromelain against rodent cestodes *Hymenolepis diminuta* and *Hymenolepis microstoma* and *Trichuris suis* was demonstrated in vitro and in vivo (Levecke et al. 2014; Mansur et al. 2014; Luoga et al. 2015). In a study analyzing the efficacy of bromelain, actinidin, and papain against *Heligmosomoides bakeri,* papain was more efficacious than bromelain or actinidin as anthelmintic (Luoga et al. 2015).

5.4 Wound Healing and Mitogenic Activity

Initially, the ethnopharmacological properties attributed to plant proteases from the genus Caricaceae were: wound healing of fungal or viral lesions and resolution of digestive problems (Soplin et al. 1995). In most instances, the proteases bromelain and papain, alone or in combination, are nowadays applied in commercial formulations (NexoBrid™ (NXB, Debriding Gel Dressing-DGD, Debrase®) or as isolated active complex fractions in surgical wounds, experimentally induced wounds or burns, as healing enhancers (Singer et al. 2010; Rosenberg et al. 2012; Rosenberg et al. 2014; Cordts et al. 2016; Schulz et al. 2017).

Bromelain has been used to treat symptoms linked to healing: to reduce pain and manage healing after episiotomy (Golezar 2016), improving healing caused by firearm wounds (Wu et al. 2012) and healing of acute crush tendon injury (Aiyegbusi et al. 2010). For a recent review covering the surgical applications of bromelain, we refer to Muhammad and Ahmad (2017). In addition, other proteins like a serine protease from *Wrightia tinctoria* R. Br enhances healing in experimental wound incisions in mice (Yariswamy et al. 2013), and latex of rubber tree *Hevea brasiliensis* increased vascular permeability, angiogenesis, and wound healing in animal model (Mendonça et al. 2010).

Studies by our group using P1G10, the proteinase fraction from *V. cundinamarcensis* (equivalent to bromelain or papain) show that its topical application increases healing, in dermabrasion (Lemos et al. 2011), burns (Gomes et al. 2010), and incisional (Freitas et al. 2017) skin models. It also enhances protection and healing of induced gastric ulcers in animal model (Mello et al. 2008; Araujo e Silva et al. 2015). Interestingly, we observed that except for the dermabrasion model, in the other injury models the proteolytic activity is required to achieve efficacy. Also, along with the debriding effect, there is a mitogenic stimulus at the wounding site. The mitogenic property was demonstrated earlier in two of the isoforms (CMS2MS2, CMS2MS3) present in P1G10 fraction (Gomes et al. 2005). We demonstrated that the mitogenic effect found in P1G10 is independent of the proteolytic activity, as CMS2MS2 inhibited with iodoacetamide retained the mitogenic activity (Gomes et al. 2009). Therefore, we proposed that along with the debriding action induced by the proteolytic activity, a mitogenic stimulus triggered by these isoforms is contributing to the healing process (Freitas et al. 2017). Remarkably, almost 50 years before, a study anticipated the mitogenic activity of bromelain, but no further reports confirmed or rebutted this finding (Zetter et al. 1976). Meanwhile, a mitogenic action has been described in thrombin, the serine protease involved in the coagulation cascade, which acts as a mitogen in many cells, following proteolytic cleavage and activation of its cognate PAR receptor (Déry et al. 1998). Also, a cysteine protease in plerocercoid *Spirometra mansonoides* displays growth hormone-like properties (Phares and Kubik 1996). Therefore, involvement of proteases from different sources in proliferative effects is part of the function repertoire. The detailed analysis of structural features of purified proteases unrelated to the canonical proteolytic role is required to identify these novel biological actions.

5.5 Thrombolytic Activity

The thrombolytic role of plant proteases can be examined in two ways; by direct action of proteases within the circulatory network or by indirect action through cleavage of target protein substrates releasing peptides with antithrombotic or prothrombotic action. The first group encompasses two activities; procoagulant and anticoagulant, yet, there is a third group of enzymes which can act both as procoagulant and as anticoagulant depending on the concentration used in the assay.

The thrombolytic effect of plant cysteine proteases has been demonstrated in many cases; heynein—a protease from *Ervatamia heyneana* latex (Uday et al. 2017), a serine-like protease from *Solanum tuberosum* (StSBTc-3) (Pepe et al. 2016), a glycosylated serine protease from *Euphorbia cf. lactea* latex (Siritapetawee et al. 2015), a serine-like protease from *Petasites japonicas* (Kim et al. 2015), a metalloprotease-like enzyme from the edible and medicinal plant *Aster yomena* Kitamura-Honda (Choi et al. 2014), and the proteolytic fraction P1G10 from *V. cundinamarcensis* (Bilheiro et al. 2013). On the other hand, a procoagulant activity was described in a serine protease from *Curcuma aromatica* Salisb (Shivalingu et al. 2016), a cysteine protease in *Cnidoscolus urens* (L.) leaves (de Menezes et al. 2014), and in a thrombin-like activity in latex of *Asclepias curassavica* L. (Shivaprasad et al. 2009). Meanwhile, both prothrombotic and thrombolytic activity have been described in cysteine proteases from *Bromelia balansae, Pseudananas macrodontes*, and *B. hieronymi* (Errasti et al. 2016). In sum, plant proteolytic enzymes can act both as procoagulant and as anticoagulant factors.

5.6 Antitumoral

Proteases from different families and sources have been traditionally used in folk medicine for tumor treatment (Guimarães-Ferreira et al. 2007; Beuth 2008). Cysteine endoproteinases such as bromelain and papain and serine endopeptidases such as trypsin or chymotrypsin, alone or in combination, are some of the proteases with described antitumor activity (Beuth 2008). Despite the studies about their activity in cancer, the underlying mechanism of action is unclear.

Among the proteases with antitumor property, bromelain is the best studied. Its activities include modulation of cell adhesion molecules, reduction of reactive oxygen species (ROS) formation, antiproliferative effect, and induction of apoptosis. Amini et al. (2013) showed that bromelain reduced glycoprotein MUC1 in cells of gastric carcinoma. This adhesion molecule provides invasive, metastatic, and chemoresistant properties to tumor cells. When exposed to bromelain, cells with high level of MUC1 (such as gastric, pancreatic, and breast cells) displayed reduced survival as result of a cascade mediated by ER, EGFR, and PDGFR, as these receptors are stabilized by the external domain of MUC1 (Pillai et al. 2013).

In Caco-2 cells, bromelain also reduced ROS production, which is linked to gastrointestinal tumor development. This activity was observed in a concentration and proteolytically dependent manner, since inhibition of proteolytic activity by iodoacetamide did not decrease ROS production (Romano et al. 2014). Recently, the bromelain antiproliferative effect has been reported in murine melanoma as well as human gastric carcinoma and colon adenocarcinoma cells (Amini et al. 2013; Romano et al. 2014; Miranda et al. 2017).

In B16F10, a highly metastatic murine melanoma cell line, 50 and 25 μg/mL bromelain dose inhibited proliferation at 99.4% and 51.7%, respectively (Miranda et al. 2017). Bromelain also reduced the proliferation in human gastric carcinoma cell lines (KATO-III and MKN45) and in two chemoresistant subpopulations of colon adenocarcinoma (HT29-5M21 and HT29-5F12) with half-maximal inhibitory concentration at 142, 94, 34, and 29 μg/mL, respectively. In MKN45 cells, treatment with bromelain (100 and 200 μg/mL) up to 72 h interrupted Akt signaling pathway (Amini et al. 2013). Both bromelain (3 μg/mL) and iodoacetamide inactivated bromelain (1 μg/mL) reduced proliferation of human colon adenocarcinoma cells (Caco-2) suggesting that proteolytic activity is not involved in antiproliferative effect in this cell line. The downregulation of p-Akt/Akt, ERK, and total expression of p-ERK1/2/, as well as reduction of ROS production was associated to the antiproliferative effect of bromelain (Romano et al. 2014).

The proapoptotic effect of bromelain is largely described in different tumor cell lines. In MCF-7 (human breast carcinoma) cells, bromelain induced autophagy, positively regulated by p38 and JNK but, negatively regulated by ERK1/2 and ensued by apoptosis. This effect is evidenced by chromatin condensation, DNA fragmentation, and nuclear cleavage (Bhui et al. 2010). Likewise, an increase in caspase-9 and caspase-3 activity was observed when GI-101A (human breast carcinoma cells) were exposed to bromelain for 24 h in a dose-dependent manner (5, 10, and 20 μg/mL) achieving 95% of cell death at the highest concentration. The increase in caspase activity was related to an increase of cleaved cytokeratin 18 (CK18), a caspase substrate, and DNA fragmentation (Dhandayuthapani et al. 2012). At 1 and 10 μg/mL, bromelain, but not proteolytically inactive bromelain, increased caspase 3 and 7 in Caco-2 cells. In these cells, the proapoptotic effect of bromelain was not a consequence of its antiproliferative activity, since the protease at 1 μg/mL did not inhibit Caco-2 proliferation (Romano et al. 2014). In gastric cancer cells (MKN45), bromelain reduced levels of Bcl-2, activated the caspase system, and led to an overexpression of cytochrome c which, in association to a reduction in phospho-Akt, contributed to cell death (Amini et al. 2013).

Bromelain also shows a chemopreventive effect in a murine model of colon and skin cancer (Romano et al. 2014; Bhatnagar et al. 2015). In mice colon cancer induced with azoxymethane, intraperitoneal (IP) bromelain reduced crypt foci, polyps, and tumors at 1 mg/kg dose. This dose is 40-fold lower than the documented lethal IP dose of bromelain (Romano et al. 2014). Nanoparticles of bromelain (loaded with lactic-co-glycolic acid) also have chemopreventive action in anthracene-induced skin carcinogenesis murine model. In this model, topical application of formulated nanoparticles containing bromelain delayed onset of

tumorigeneses about 4 weeks, plus mortality rate as well as tumor volume were decreased by 70% and 45%, respectively (Bhatnagar et al. 2015). Compared to the chemotherapeutic effect in animals treated after tumor induction, the chemopreventive activity of bromelain nanoparticles was higher using a 10-fold lower dose than the protocol using free bromelain. Also, bromelain nanoparticles enhanced DNA protection from DMBA-induced damage, as assessed by alkaline unwinding assay and upregulating proapoptotic protein p53 and BAX and downregulating Bcl-2 antiapoptotic protein (Bhatnagar et al. 2015).

Müller et al. (2016) compared the antitumor effects of bromelain and papain in cholangiocarcinoma (CC) cell lines. Both proteases decreased proliferation, invasion, and migration of tumor cells acting downstream of NFkB/AMPK pathway, though bromelain was more effective than papain. Apoptosis was induced after bromelain and papain treatment, attaining 70% and 50% of cell death for bromelain and papain, respectively. Bromelain, but not papain, increased E-cadherin and downregulated N-cadherin in CC cell lines, in a dose- and time-dependent manner, demonstrating an inhibitory effect during the epithelial–mesenchymal transition.

In studies using the proteolytic fraction P1G10, from *V. cundinamarcensis* we confirmed the antitumoral effect. Mice bearing B16F1, a low metastasizing melanoma, subcutaneous injection of 5 mg/kg of P1G10 reduced ~70% the tumor mass and survival rate increased ~97% compared to the control. In tumor, P1G10 reduced hemoglobin and vascular endothelial growth factor (VEGF), resulting in antiangiogenic effect, and increased N-acetylglucosaminidase (NAG) which was linked to macrophage activation. P1G10 also induced around 60% of DNA fragmentation in B16F1 cells after 24 h of exposure (50 μg/mL) leading to apoptosis, as pretreatment with the pan-caspase inhibitor (ZVAD) abolishes this effect. A cell rounding and reduced ability to adhere to ECM components was initially observed after 15 min exposure to P1G10 (Dittz et al. 2015).

In clinical trials, there are evidences that proteases improve cancer treatment as a complementary systemic enzyme therapy. Stage II clinical studies demonstrate that systemic enzyme therapy (trypsin, chymotrypsin, and papain) decrease tumor—and/or side effects—therapy induced in plasmocytoma, and breast and colorectal cancer patients (Beuth 2008). These findings motivate further studies to unravel the mechanism underlying the antitumoral effect of plant proteases.

5.7 Production of Bioactive Peptides

Enzymatic hydrolysis to produce bioactive peptides with nutraceutical activity is an area of intense research. Use of plant proteases for production of bioactive peptides from food proteins is being investigated. Papain, bromelain, ficin, or pumpkin serine protease have been used separately or in combination with other proteolytic enzymes to produce antihypertensive peptides inhibiting the angiotensin-converting enzyme (Tavares et al. 2011), peptides with antithrombotic activity whose efficacy was like aspirin (Shimizu et al. 2009), peptides with antioxidant activity (Wang

et al. 2012; Gajanan et al. 2016), peptides that inhibit dipeptidyl-aminopeptidase IV and a-glucosidase during diabetes (Li-Chan et al. 2012; Lacroix and Li-Chan 2012; Nongonierma and FitzGerald 2014), peptides with hypolipidemic hypocholesterolemic activity (Morimatsu et al. 1996), antimicrobial peptides (Salampessy et al. 2010; Arruda et al. 2012; Dąbrowska et al. 2013), and immunomodulating peptides (Kong et al. 2008) are described. We refer here to a recent report reviewing the production of bioactive peptides (Mazorra-Manzano et al. 2017).

5.8 Other Applications

Without doubt, the applications of proteolytic enzymes are not restricted to the uses described above. New promising applications appear in the literature and are mentioned now.

Liposomes or nanoparticles to deliver proteolytic enzymes or products obtained by hydrolysis with plant proteases have been recently developed and described here: fabrication of core-shell nanofibers for controlled delivery of bromelain and salvianolic acid B for skin regeneration used in wound therapeutics (Shoba et al. 2017) magnetic nanoparticles containing papain as antibacterial (Atacan et al. 2018), papain-containing liposomes for treatment of skin fibrosis resulting from second degree burn (Sahu et al. 2017), a liposome for skin application of papain on hypertrophic scar (Chen et al. 2017), liposomes containing papain hydrolyzed bioactive peptides with antioxidative and ACE-inhibitory properties, from bean seeds protein (Chay et al. 2015), a bromelain hydrolyzed antibacterial liposomal peptide from rice bran protein against *Listeria monocytogenes* (Pu and Tang 2017), antihypertensive biopeptides from stone fish (*Actinopyga lecanora*) protein hydrolyzed with bromelain and stabilized by encapsulation in chitosan nanoparticles (Auwal et al. 2017), nanoparticles as well as self-emulsifying drug delivery systems displaying papain or bromelain to cleave mucin (Menzel and Bernkop-Schnürch 2018), encapsulated gold nanoparticles containing bromelain, cisplatin, and doxorubicin for treatment of osteosarcoma (Iram et al. 2017), bromelain hybrid nanoparticles on lactobionic acid conjugated to chitosan in an antitumoral study (Wei et al. 2017), nanofibers for delivery of bromelain and salvianolic acid B during skin regeneration after wounding (Shoba et al. 2017), bromelain-functionalized lipid-core nanocapsules to investigate their effect against human breast cancer cells (Oliveira et al. 2017), hyaluronic acid nanoparticles to enhance targeted delivery of bromelain in Ehrlich's ascites carcinoma (Bhatnagar et al. 2016), katira gum nanoparticles to enhance the anti-inflammatory effect of bromelain (Bernela et al. 2016), to enhance the therapeutic effect of the antibiotic levofloxacin (Bagga et al. 2016), as nanoparticles to protect against 7,12-dimethylbenz[a]anthracene-induced skin carcinogenesis (Bhatnagar et al. 2015), as oral anticancer treatment formulated as nanoparticles (Bhatnagar et al. 2014), to enhance diffusion of silica nanoparticles at the tumor

extracellular matrix (Parodi et al. 2014), and both papain and bromelain to decrease the toxicity of elastic niosomes (a microsphere used for drug delivery) and to increase the activity of metalloprotease-2 (Manosroi et al. 2012).

5.9 Protease Inhibitors

Protease inhibitors from *Tamarindus indica* L. seeds have been described with the ability to reduce levels of plasmatic leptin (Medeiros et al. 2018), two proteins from *Delonix regia* and *Acacia schweinfurthii*, of the Kunitz protease family, inhibited blood coagulation, platelet aggregation, and thrombus formation (Salu et al. 2018), two proteins fractions with gastroprotective and antielastase properties were described in *Erythrina velutina* seeds (Oliveira de Lima et al. 2017), protease inhibitors in *Lavatera cashmeriana* preventing human lung cancer cell proliferation *in vitro* (Rakashanda et al. 2015) and lupin seeds peptides inhibitors of metalloproteinases (Carrilho et al. 2009). In addition to these plant proteins, many nonprotein plant metabolites have been isolated and display inhibitory properties. Their analysis is not included here.

5.10 Oral Applications

Following an initial account describing the application of papain to remove dental decayed tissue (Bussadori et al. 2005), more than 40 reports confirm the initial finding. Several commercial preparations containing proteases, such as Papacarie® and Carie Care™: are now available and used alone or in combination, as alternatives to mechanical drilling for caries (Divya et al. 2015; Sahana et al. 2016).

It is claimed that Papacarie® does not adversely affect the microleakage of composite restorations and provides a suitable surface for bonding like conventional tooth drilling (Hafez et al. 2017), it offers a less painful alternative among children for caries removal, Carie Care™ a papain-containing formulation demonstrates antimicrobial action against *A. actinomycetemcomitans*, a major periodontal disease causing pathogen (Kush et al. 2015), local application of actinidin or papain prevents or reduces dental plaque formation and reduces oral biofilm on the tongue in elder subjects in vivo and in vitro (Mugita et al. 2017) and three randomized controlled trials; one using papain, bromelain and Miswak (teeth cleaning twig made from the *Salvadora persica*) containing dentifrice to combat dental plaque formation and gingivitis in human subjects (Tadikonda et al. 2017); the other two controlled clinical trials evaluating the long-term chemo-mechanical removal of caries and pain using Papacarie® confirming the absence of pain during the intervention and the preservation of intact dental tissue (Motta et al. 2014; Abdul Khalek et al. 2017).

5.11 Final Statement

The number of therapeutic applications involving plant proteolytic enzymes increased significantly within the last 10 years. Reports on oral applications involving Papacarie® and Carie Care™ experienced a significant increase suggesting a possible future commercial use. On the other hand, reports highlighting the use of bromelain to treat several conditions are on the rise. Treatment of digestive disorders, as anti-inflammatory and as immunomodulator, deserves attention for their link with cancer. A possible drawback is that most studies involve a proteolytic fraction composed of isoforms with diverse properties. Assessment of the biological properties of each isoform is necessary to precisely adjust formulations with the appropriate combination of isoforms to treat specific conditions.

References

Abdul Khalek A, Elkateb MA, Abdel Aziz WE, El Tantawi M (2017) Effect of papacarie and alternative restorative treatment on pain reaction during caries removal among children: a randomized controlled clinical trial. J Clin Pediatr Dent 41:219–224. https://doi.org/10.17796/1053-4628-41.3.219

Agoro R, Piotet-Morin J, Palomo J, Michaudel C, Vigne S, Maillet I, Chenuet P, Guillou N, Le Bérichel J, Kisielow M, Flodby P, Borok Z, Crandall ED, Le Bert M, Quesniaux V, Muller M, Di Padova F, Ryffel B, Gabay C, Couturier-Maillard A (2016) IL-1R1-MyD88 axis elicits papain-induced lung inflammation. Eur J Immunol 46:2531–2541. https://doi.org/10.1002/eji.201646366

Agostinis C, Zorzet S, De Leo R, Zauli G, De Seta F, Bulla R (2015) The combination of N-acetyl cysteine, alpha-lipoic acid, and bromelain shows high anti-inflammatory properties in novel in vivo and in vitro models of endometriosis. Mediat Inflamm 2015:918089. https://doi.org/10.1155/2015/918089

Aichele K, Bubel M, Deubel G, Pohlemann T, Oberringer M (2013) Bromelain down-regulates myofibroblast differentiation in an in vitro wound healing assay. Naunyn Schmiedeberg's Arch Pharmacol 386(10):853–863

Aiyegbusi AI, Duru FI, Awelimobor D, Noronha CC, Okanlawon AO (2010) The role of aqueous extract of pineapple fruit parts on the healing of acute crush tendon injury. Nig Q J Hosp Med 20:223–227

Amini A, Ehteda A, Masoumi S, Moghaddam AJ, Pillai K, Morris DL (2013) Cytotoxic effects of bromelain in human gastrointestinal carcinoma cell lines (MKN45, KATO-III, HT29-5F12, and HT29-5M21). Onco Targets Ther 6:403–409. https://doi.org/10.2147/OTT.S43072

Araujo e Silva AC, de Oliveira Lemos F, Gomes MTR, Salas CE, Lopes MTP (2015) Role of gastric acid inhibition, prostaglandins and endogenous-free thiol groups on the gastroprotective effect of a proteolytic fraction from Vasconcellea cundinamarcensis latex. J Pharm Pharmacol 67:133–141. https://doi.org/10.1111/jphp.12318

Arruda MS, Silva FO, Egito AS, Silva TMS, Lima-Filho JL, Porto ALF, Moreira KA (2012) New peptides obtained by hydrolysis of caseins from bovine milk by protease extracted from the latex Jacaratia corumbensis. LWT - Food Sci Technol 49:73–79. https://doi.org/10.1016/J.LWT.2012.04.001

Atacan K, Özacar M, Özacar M (2018) Investigation of antibacterial properties of novel papain immobilized on tannic acid modified Ag/CuFe2O4magnetic nanoparticles. Int J Biol Macromol 109:720–731. https://doi.org/10.1016/j.ijbiomac.2017.12.066

Auwal S, Zarei M, Tan C, Basri M, Saari N (2017) Improved in vivo efficacy of anti-hypertensive biopeptides encapsulated in chitosan nanoparticles fabricated by ionotropic gelation on spontaneously hypertensive rats. Nanomaterials 7:421. https://doi.org/10.3390/nano7120421

Bagga P, Ansari TM, Siddiqui HH, Syed A, Bahkali AH, Rahman MA, Khan MS (2016) Bromelain capped gold nanoparticles as the novel drug delivery carriers to aggrandize effect of the antibiotic levofloxacin. EXCLI J 15:772–780. https://doi.org/10.17179/excli2016-710

Balakireva AV, Kuznetsova NV, Petushkova AI, Savvateeva L, Zamyatnin AA (2017) Trends and prospects of plant proteases in therapeutics. Curr Med Chem. https://doi.org/10.2174/0929867325666171123204403

Barrett AJ, Fred Woessner J (2013) Handbook of proteolytic enzymes. Academic, London, pp li–liv

Baur X, Fruhmann G (1979) Allergic reactions, including asthma, to the pineapple protease bromelain following occupational exposure. Clin Allergy 9:443–450

Bernela M, Ahuja M, Thakur R (2016) Enhancement of anti-inflammatory activity of bromelain by its encapsulation in katira gum nanoparticles. Carbohydr Polym 143:18–24. https://doi.org/10.1016/j.carbpol.2016.01.055

Beuth J (2008) Proteolytic enzyme therapy in evidence-based complementary oncology: fact or fiction? Integr Cancer Ther 7:311–316. https://doi.org/10.1177/1534735408327251

Bhatnagar P, Patnaik S, Srivastava AK, Mudiam MKR, Shukla Y, Panda AK, Pant AB, Kumar P, Gupta KC (2014) Anti-cancer activity of bromelain nanoparticles by oral administration. J Biomed Nanotechnol 10:3558–3575

Bhatnagar P, Pant AB, Shukla Y, Chaudhari B, Kumar P, Gupta KC (2015) Bromelain nanoparticles protect against 7,12-dimethylbenz[a]anthracene induced skin carcinogenesis in mouse model. Eur J Pharm Biopharm 91:35–46. https://doi.org/10.1016/j.ejpb.2015.01.015

Bhatnagar P, Pant AB, Shukla Y, Panda A, Gupta KC (2016) Hyaluronic acid grafted PLGA copolymer nanoparticles enhance the targeted delivery of bromelain in Ehrlich's ascites carcinoma. Eur J Pharm Biopharm 105:176–192. https://doi.org/10.1016/j.ejpb.2016.06.002

Bhui K, Tyagi S, Prakash B, Shukla Y (2010) Pineapple bromelain induces autophagy, facilitating apoptotic response in mammary carcinoma cells. Biofactors 36:474–482. https://doi.org/10.1002/biof.121

Bilheiro RP, Braga AD, Filho ML, Carvalho-Tavares J, Agero U, Carvalho M Das G, Sanchez EF, Salas CE, Lopes MTP (2013) The thrombolytic action of a proteolytic fraction (P1G10) from Carica candamarcensis. Thromb Res 131:e175–e182. https://doi.org/10.1016/j.thromres.2013.01.028

Brien S, Lewith G, Walker AF, Middleton R, Prescott P, Bundy R (2006) Bromelain as an adjunctive treatment for moderate-to-severe osteoarthritis of the knee: a randomized placebo-controlled pilot study. QJM 99:841–850. https://doi.org/10.1093/qjmed/hcl118

Burney RO, Giudice LC (2012) Pathogenesis and pathophysiology of endometriosis. Fertil Steril 98:511–519. https://doi.org/10.1016/j.fertnstert.2012.06.029

Bussadori SK, Castro LC, Galvão AC (2005) Papain gel: a new chemo-mechanical caries removal agent. J Clin Pediatr Dent 30:115–119

Büttner L, Achilles N, Böhm M, Shah-Hosseini K, Mösges R (2013) Efficacy and tolerability of bromelain in patients with chronic rhinosinusitis - a pilot study. B-ENT 9:217–225

Carrilho DM, Duarte IC, Francisco R, Ricardo CPP, Duque-Magalhães MC (2009) Discovery of novel plant peptides as strong inhibitors of metalloproteinases. Protein Pept Lett 16:543–551

Chay SY, Tan WK, Saari N (2015) Preparation and characterisation of nanoliposomes containing winged bean seeds bioactive peptides. J Microencapsul 32:488–495. https://doi.org/10.3109/02652048.2015.1057250

Chen Y-Y, Lu Y-H, Ma C-H, Tao W-W, Zhu J-J, Zhang X (2017) A novel elastic liposome for skin delivery of papain and its application on hypertrophic scar. Biomed Pharmacother 87:82–91. https://doi.org/10.1016/j.biopha.2016.12.076

Choi J-H, Kim D-W, Park S-E, Choi B-S, Sapkota K, Kim S, Kim S-J (2014) Novel thrombolytic protease from edible and medicinal plant Aster yomena (Kitam.) Honda with anticoagulant

activity: purification and partial characterization. J Biosci Bioeng 118:372–377. https://doi.org/10.1016/j.jbiosc.2014.03.004

Cordts T, Horter J, Vogelpohl J, Kremer T, Kneser U, Hernekamp J-F (2016) Enzymatic debridement for the treatment of severely burned upper extremities – early single center experiences. BMC Dermatol 16:8. https://doi.org/10.1186/s12895-016-0045-2

de Menezes Y, Félix-Silva J, da Silva-Júnior A, Rebecchi I, de Oliveira A, Uchoa A, Fernandes-Pedrosa M (2014) Protein-Rich Fraction of Cnidoscolus urens (L.) Arthur Leaves: Enzymatic Characterization and Procoagulant and Fibrinogenolytic Activities. Molecules 19:3552–3569. https://doi.org/10.3390/molecules19033552

Dąbrowska A, Szołtysik M, Babij K, Pokora M, Zambrowicz A, Chrzanowska J (2013) Application of Asian pumpkin (Cucurbita ficifolia) serine proteinase for production of biologically active peptides from casein. Acta Biochim Pol 60:117–122

DeClerck MP, Bailey Y, Craig D, Lin M, Auerbach LJ, Linney O, Morrison DE, Patry W, Auerbach PS (2016) Efficacy of topical treatments for Chrysaora Chinensis species: a human model in comparison with an in vitro model. Wilderness Environ Med 27:25–38. https://doi.org/10.1016/j.wem.2015.10.008

Déry O, Corvera CU, Steinhoff M, Bunnett NW (1998) Proteinase-activated receptors: novel mechanisms of signaling by serine proteases. Am J Phys 274:C1429–C1452

Dhandayuthapani S, Perez HD, Paroulek A, Chinnakkannu P, Kandalam U, Jaffe M, Rathinavelu A (2012) Bromelain-induced apoptosis in GI-101A breast cancer cells. J Med Food 15:344–349. https://doi.org/10.1089/jmf.2011.0145

Dittz D, Figueiredo C, Lemos FO, Viana CTR, Andrade SP, Souza-Fagundes EM, Fujiwara RT, Salas CE, Lopes MTP (2015) Antiangiogenesis, loss of cell adhesion and apoptosis are involved in the antitumoral activity of proteases from v. Cundinamarcensis (C. candamarcensis) in murine melanoma B16F1. Int J Mol Sci 16:7027–7044. https://doi.org/10.3390/ijms16047027

Divya G, Prasad MG, Vasa AAK, Vasanthi D, Ramanarayana B, Mynampati P (2015) Evaluation of the efficacy of caries removal using polymer bur, stainless steel bur, carisolv, papacarie - an in vitro comparative study. J Clin Diagn Res 9:ZC42–ZC46. https://doi.org/10.7860/JCDR/2015/12705.6202

Domsalla A, Melzig MF (2008) Occurrence and properties of proteases in plant latices. Planta Med 74:699–711. https://doi.org/10.1055/s-2008-1074530

Dutta S, Bhattacharyya D (2013) Enzymatic, antimicrobial and toxicity studies of the aqueous extract of Ananas comosus (pineapple) crown leaf. J Ethnopharmacol 150:451–457. https://doi.org/10.1016/j.jep.2013.08.024

Errasti M, Caffini N, Pelzer L, Rotelli A (2013) Anti-inflammatory activity of bromelia hieronymi: comparison with bromelain. Planta Med 79:207–213. https://doi.org/10.1055/s-0032-1328201

Errasti ME, Prospitti A, Viana CA, Gonzalez MM, Ramos MV, Rotelli AE, Caffini NO (2016) Effects on fibrinogen, fibrin, and blood coagulation of proteolytic extracts from fruits of Pseudananas macrodontes, Bromelia balansae, and B. hieronymi (Bromeliaceae) in comparison with bromelain. Blood Coagul Fibrinolysis 27:441–449. https://doi.org/10.1097/MBC.0000000000000531

Eskenazi B, Warner ML (1997) Epidemiology of endometriosis. Obstet Gynecol Clin N Am 24:235–258

Fitzhugh DJ, Shan S, Dewhirst MW, Hale LP (2008) Bromelain treatment decreases neutrophil migration to sites of inflammation. Clin Immunol 128:66–74. https://doi.org/10.1016/j.clim.2008.02.015

Fortelny N, Cox JH, Kappelhoff R, Starr AE, Lange PF, Pavlidis P, Overall CM (2014) Network analyses reveal pervasive functional regulation between proteases in the human protease web. PLoS Biol 12:e1001869. https://doi.org/10.1371/journal.pbio.1001869

Freitas KM, Barcelos LS, Caliari MV, Salas CE, Lopes MTP (2017) Healing activity of proteolytic fraction (P1G10) from Vasconcellea cundinamarcensis in a cutaneous wound excision model. Biomed Pharmacother 96:269–278. https://doi.org/10.1016/j.biopha.2017.09.109

Gajanan PG, Elavarasan K, Shamasundar BA (2016) Bioactive and functional properties of protein hydrolysates from fish frame processing waste using plant proteases. Environ Sci Pollut Res Int 23:24901–24911. https://doi.org/10.1007/s11356-016-7618-9

Golezar S (2016) Ananas comosus effect on perineal pain and wound healing after episiotomy: a randomized double-blind placebo-controlled clinical trial. Iran Red Crescent Med J 18:e21019. https://doi.org/10.5812/ircmj.21019

Gomes MTRTR, Mello VJ, Rodrigues KC, Bemquerer MP, Lopes MTP, Faça VM, Salas CE (2005) Isolation of two plant proteinases in latex from Carica candamarcensis acting as mitogens for mammalian cells. Planta Med 71:244–248. https://doi.org/10.1055/s-2005-837824

Gomes MTR, Turchetti AP, Lopes MTP, Salas CE (2009) Stimulation of fibroblast proliferation by the plant cysteine protease CMS2MS2 is independent of its proteolytic activity and requires ERK activation. Biol Chem 390:1285–1291. https://doi.org/10.1515/BC.2009.137

Gomes FSL, Spínola CDV, Ribeiro HA, Lopes MTP, Cassali GD, Salas CE (2010) Wound-healing activity of a proteolytic fraction from Carica candamarcensis on experimentally induced burn. Burns 36:277–283. https://doi.org/10.1016/j.burns.2009.04.007

Guimarães-Ferreira C, Rodrigues E, Mortara R, Cabral H, Serrano F, Ribeiro-dos-Santos R, Travassos L (2007) Antitumor effects in vitro and in vivo and mechanisms of protection against melanoma B16F10-Nex2 cells by fastuosain, a cysteine proteinase from Bromelia fastuosa. Neoplasia 9:723–733. https://doi.org/10.1593/neo.07427

Hafez MA, Elkateb M, El Shabrawy S, Mahmoud A, El Meligy O (2017) Microleakage evaluation of composite restorations following papain-based chemo-mechanical caries removal in primary teeth. J Clin Pediatr Dent 41:53–61. https://doi.org/10.17796/1053-4628-41.1.53

Hale LP, Greer PK, Trinh CT, Gottfried MR (2005) Treatment with oral bromelain decreases colonic inflammation in the IL-10-deficient murine model of inflammatory bowel disease. Clin Immunol 116:135–142. https://doi.org/10.1016/j.clim.2005.04.011

Hale LP, Chichlowski M, Trinh CT, Greer PK (2010) Dietary supplementation with fresh pineapple juice decreases inflammation and colonic neoplasia in IL-10-deficient mice with colitis. Inflamm Bowel Dis 16:2012–2021. https://doi.org/10.1002/ibd.21320

Harada T, Yoshioka H, Yoshida S, Iwabe T, Onohara Y, Tanikawa M, Terakawa N (1997) Increased interleukin-6 levels in peritoneal fluid of infertile patients with active endometriosis. Am J Obstet Gynecol 176:593–597. https://doi.org/10.1016/S0002-9378(97)70553-2

Heinicke RM, Gortner WA (1957) Stem bromelain---a new protease preparation from pineapple plants. Econ Bot 11:225–234. https://doi.org/10.1007/BF02860437

Ho D, Jagdeo J, Waldorf HA (2016) Is there a Role for Arnica and Bromelain in prevention of post-procedure ecchymosis or Edema? A systematic review of the literature. Dermatol Surg 42(4):445–463

Huffaker RC (1990) Proteolytic activity during senescence of plants. New Phytol 116:199–231. https://doi.org/10.1111/j.1469-8137.1990.tb04710.x

Iida H, Takai T, Hirasawa Y, Kamijo S, Shimura S, Ochi H, Nishioka I, Maruyama N, Ogawa H, Okumura K, Ikeda S (2014) Epicutaneous administration of papain induces IgE and IgG responses in a cysteine protease activity-dependent manner. Allergol Int 63:219–226. https://doi.org/10.2332/allergolint.13-OA-0621

Iram S, Zahera M, Khan S, Khan I, Syed A, Ansary AA, Ameen F, Shair OHM, Khan MS (2017) Gold nanoconjugates reinforce the potency of conjugated cisplatin and doxorubicin. Colloids Surf B Biointerfaces 160:254–264. https://doi.org/10.1016/j.colsurfb.2017.09.017

Kim D-W, Choi J-H, Park S-E, Kim SS-J, Sapkota K, Kim SS-J (2015) Purification and characterization of a fibrinolytic enzyme from Petasites japonicus. Int J Biol Macromol 72:1159–1167. https://doi.org/10.1016/j.ijbiomac.2014.09.046

Kong X, Guo M, Hua Y, Cao D, Zhang C (2008) Enzymatic preparation of immunomodulating hydrolysates from soy proteins. Bioresour Technol 99:8873–8879

Kush A, Thakur R, Patil SDS, Paul ST, Kakanur M (2015) Evaluation of antimicrobial action of Carie Care™ and Papacarie Duo™ on Aggregatibacter actinomycetemcomitans a major periodontal pathogen using polymerase chain reaction. Contemp Clin Dent 6:534–538. https://doi.org/10.4103/0976-237X.169860

Lacroix IME, Li-Chan ECY (2012) Dipeptidyl peptidase-IV inhibitory activity of dairy protein hydrolysates. Int Dairy J 25:97–102. https://doi.org/10.1016/J.IDAIRYJ.2012.01.003

Lemos FO, Ferreira LAM, Cardoso VN, Cassali GD, Salas CE, Lopes MTP (2011) Skin-healing activity and toxicological evaluation of a proteinase fraction from Carica candamarcensis. Eur J Dermatol 21:722–730. https://doi.org/10.1684/ejd.2011.1466

Levecke B, Buttle DJ, Behnke JM, Duce IR, Vercruysse J (2014) Cysteine proteinases from papaya (Carica papaya) in the treatment of experimental Trichuris suis infection in pigs: two randomized controlled trials. Parasit Vectors 7:255. https://doi.org/10.1186/1756-3305-7-255

Lewinsohn TM (1991) The geographical distribution of plant latex. Chemoecology 2:64–68. https://doi.org/10.1007/BF01240668

Li-Chan ECY, Hunag S-L, Jao C-L, Ho K-P, Hsu K-C (2012) Peptides derived from Atlantic salmon skin gelatin as dipeptidyl-peptidase IV inhibitors. J Agric Food Chem 60:973–978. https://doi.org/10.1021/jf204720q

Luoga W, Mansur F, Lowe A, Duce IR, Buttle DJ, Behnke JM (2015) Factors affecting the anthelmintic efficacy of papaya latex in vivo: host sex and intensity of infection. Parasitol Res 114:2535–2541. https://doi.org/10.1007/s00436-015-4456-5

Majid OW, Al-Mashhadani BA (2014) Perioperative Bromelain reduces pain and swelling and improves quality of life measures after mandibular third molar surgery: a randomized, double-blind, placebo-controlled clinical trial. J Oral Maxillofac Surg 72(6):1043–1048

Manosroi A, Chankhampan C, Manosroi W, Manosroi J (2012) Toxicity reduction and MMP-2 stimulation of papain and Bromelain loaded in elastic Niosomes. J Biomed Nanotechnol 8(5):720–729

Mansur F, Luoga W, Buttle DJ, Duce IR, Lowe A, Behnke JM (2014) The anthelmintic efficacy of natural plant cysteine proteinases against two rodent cestodes Hymenolepis diminuta and Hymenolepis microstoma in vitro. Vet Parasitol 201:48–58. https://doi.org/10.1016/j.vetpar.2013.12.018

Mazorra-Manzano MA, Ramírez-Suarez JC, Yada RY (2017) Plant proteases for bioactive peptides release: a review. Crit Rev Food Sci Nutr 10:1–17. https://doi.org/10.1080/10408398.2017.1308312

Medeiros AF, Costa IS, Carvalho FMC, Kiyota S, Souza BBP, Sifuentes DN, Serquiz RP, Maciel BLL, Uchôa AF, Santos EA, Morais AHA (2018) Biochemical characterisation of a Kunitz-type inhibitor from Tamarindus indica L. seeds and its efficacy in reducing plasma leptin in an experimental model of obesity. J Enzyme Inhib Med Chem 33:334–348. https://doi.org/10.1080/14756366.2017.1419220

Mello VJ, Gomes MTR, Lemos FO, Delfino JL, Andrade SP, Lopes MTP, Salas CE (2008) The gastric ulcer protective and healing role of cysteine proteinases from Carica candamarcensis. Phytomedicine 15:237–244. https://doi.org/10.1016/j.phymed.2007.06.004

Mendonça RJ, Maurício VB, Teixeira LB, Lachat JJ, Coutinho-Netto J (2010) Increased vascular permeability, angiogenesis and wound healing induced by the serum of natural latex of the rubber tree Hevea brasiliensis. Phytother Res 24:764–768. https://doi.org/10.1002/ptr.3043

Menzel C, Bernkop-Schnürch A (2018) Enzyme decorated drug carriers: targeted swords to cleave and overcome the mucus barrier. Adv Drug Deliv Rev 124:164–174. https://doi.org/10.1016/j.addr.2017.10.004

Miranda ÍKSPB, Miranda AFS, Souza FVD, Vannier-Santos MA, Pirovani CP, Pepe IM, Rodowanski IJ, Ferreira KTSE, Vaz LMS, de Assis SA (2017) The biochemical characterization, stabilization studies and the antiproliferative effect of bromelain against B16F10 murine melanoma cells. Int J Food Sci Nutr 68:442–454. https://doi.org/10.1080/09637486.2016.1254599

Morimatsu F, Ito M, Budijanto S, Watanabe I, Furukawa Y, Kimura S (1996) Plasma cholesterol-suppressing effect of papain-hydrolyzed pork meat in rats fed hypercholesterolemic diet. J Nutr Sci Vitaminol 42:145–153

Motta LJ, Bussadori SK, Campanelli AP, Silva AL, Alfaya TA, Godoy CHL, Navarro MFL (2014) Randomized controlled clinical trial of long-term chemo-mechanical caries removal using Papacarie™ gel. J Appl Oral Sci 22:307–313. https://doi.org/10.1590/1678-775720130488

Mugita N, Nambu T, Takahashi K, Wang P-L, Komasa Y (2017) Proteases, actinidin, papain and trypsin reduce oral biofilm on the tongue in elderly subjects and in vitro. Arch Oral Biol 82:233–240. https://doi.org/10.1016/j.archoralbio.2017.04.035

Muhammad ZA, Ahmad T (2017) Therapeutic uses of pineapple-extracted bromelain in surgical care - a review. J Pak Med Assoc 67:121–125

Müller A, Barat S, Chen X, Bui KC, Bozko P, Malek NP, Plentz RR (2016) Comparative study of antitumor effects of bromelain and papain in human cholangiocarcinoma cell lines. Int J Oncol 48:2025–2034. https://doi.org/10.3892/ijo.2016.3411

Nongonierma AB, FitzGerald RJ (2014) Susceptibility of milk protein-derived peptides to dipeptidyl peptidase IV (DPP-IV) hydrolysis. Food Chem 145:845–852. https://doi.org/10.1016/j.foodchem.2013.08.097

Ochsner A, Storck A (1936) The prevention of peritoneal adhesions by papain: a clinical study. Ann Surg 104:736–747

Oliveira de Lima VC, de Araújo Machado RJ, Vieira Monteiro NK, de Lyra IL, da Silva Camillo C, Coelho Serquiz A, Silva de Oliveira A, da Silva Rufino FP, Leal Lima Maciel B, Ferreira Uchôa A, Antunes Dos Santos E, de Araújo Morais AH (2017) Gastroprotective and antielastase effects of protein inhibitors from Erythrina velutina seeds in an experimental ulcer model. Biochem Cell Biol 95:243–250. https://doi.org/10.1139/bcb-2016-0034

Oliveira CP, Prado WA, Lavayen V, Büttenbender SL, Beckenkamp A, Martins BS, Lüdtke DS, Campo LF, Rodembusch FS, Buffon A, Pessoa A, Guterres SS, Pohlmann AR (2017) Bromelain-functionalized multiple-wall lipid-core nanocapsules: formulation, chemical structure and antiproliferative effect against human breast cancer cells (MCF-7). Pharm Res 34:438–452. https://doi.org/10.1007/s11095-016-2074-2

Onken JE, Greer PK, Calingaert B, Hale LP (2008) Bromelain treatment decreases secretion of pro-inflammatory cytokines and chemokines by colon biopsies in vitro. Clin Immunol 126:345–352. https://doi.org/10.1016/j.clim.2007.11.002

Ordesi P, Pisoni L, Nannei P, Macchi M, Borloni R, Siervo S (2014) Therapeutic efficacy of bromelain in impacted third molar surgery: a randomized controlled clinical study. Quintessence Int 45(8):679–684. https://doi.org/10.3290/j.qi.a32237

Parodi A, Haddix SG, Taghipour N, Scaria S, Taraballi F, Cevenini A, Yazdi IK, Corbo C, Palomba R, Khaled SZ, Martinez JO, Brown BS, Isenhart L, Tasciotti E (2014) Bromelain surface modification increases the diffusion of silica nanoparticles in the tumor extracellular matrix. ACS Nano 8:9874–9883. https://doi.org/10.1021/nn502807n

Patel DV, Sawant MG, Kaur G (2015) Evaluation of anti-osteoarthritic activity of Vigna mungo in papain induced osteoarthritis model. Indian J Pharm 47:59–64. https://doi.org/10.4103/0253-7613.150340

Patil PA, Ankola AV, Hebbal MI, Patil AC (2015) Comparison of effectiveness of abrasive and enzymatic action of whitening toothpastes in removal of extrinsic stains - a clinical trial. Int J Dent Hyg 13(1):25–29

Pepe A, Frey ME, Muñoz F, Fernández MB, Pedraza A, Galbán G, García DN, Daleo GR, Guevara MG (2016) Fibrin(ogen)olytic and antiplatelet activities of a subtilisin-like protease from Solanum tuberosum (StSBTc-3). Biochimie 125:163–170. https://doi.org/10.1016/j.biochi.2016.03.015

Phares K, Kubik J (1996) The growth factor from plerocercoids of Spirometra mansonoides is both a growth hormone agonist and a cysteine proteinase. J Parasitol 82:210–215

Pillai K, Akhter J, Chua TC, Morris DL (2013) Anticancer property of bromelain with therapeutic potential in malignant peritoneal mesothelioma. Cancer Investig 31:241–250

Pu C, Tang W (2017) The antibacterial and antibiofilm efficacies of a liposomal peptide originating from rice bran protein against *Listeria monocytogenes*. Food Funct 8:4159–4169. https://doi.org/10.1039/C7FO00994A

Rakashanda S, Qazi AK, Majeed R, Andrabi SM, Hamid A, Sharma PR, Amin S (2015) Plant-derived protease inhibitors LC-pi (Lavatera cashmeriana) inhibit human lung cancer cell proliferation in vitro. Nutr Cancer 67:156–166. https://doi.org/10.1080/01635581.2015.967876

Rakhimov MR (2001) Anti-inflammatory activity of domestic papain. Eksp Klin Farmakol 64:48–49

Romano B, Fasolino I, Pagano E, Capasso R, Pace S, De Rosa G, Milic N, Orlando P, Izzo AA, Borrelli F (2014) The chemopreventive action of bromelain, from pineapple stem (Ananas comosus L.), on colon carcinogenesis is related to antiproliferative and proapoptotic effects. Mol Nutr Food Res 58:457–465. https://doi.org/10.1002/mnfr.201300345

Rosenberg L, Krieger Y, Silberstein E, Arnon O, Sinelnikov IA, Bogdanov-Berezovsky A, Singer AJ (2012) Selectivity of a bromelain based enzymatic debridement agent: a porcine study. Burns 38:1035–1040. https://doi.org/10.1016/j.burns.2012.02.011

Rosenberg L, Krieger Y, Bogdanov-Berezovski A, Silberstein E, Shoham Y, Singer AJ (2014) A novel rapid and selective enzymatic debridement agent for burn wound management: a multi-center RCT. Burns 40:466–474. https://doi.org/10.1016/j.burns.2013.08.013

Sahana S, Vasa AAK, Geddam D, Reddy VK, Nalluri S, Velagapudi N (2016) Effectiveness of chemo-mechanical caries removal agents Papacarie(®) and Carie-Care™ in primary molars: an in vitro study. J Int Soc Prev Community Dent 6:S17–S22. https://doi.org/10.4103/2231-0762.181162

Sahbaz A, Aynioglu O, Isik H, Ozmen U, Cengil O, Gun BD, Gungorduk K (2015) Bromelain: a natural proteolytic for intra-abdominal adhesion prevention. Int J Surg 14:7–11. https://doi.org/10.1016/j.ijsu.2014.12.024

Sahu K, Kaurav M, Pandey RS (2017) Protease loaded permeation enhancer liposomes for treatment of skin fibrosis arisen from second degree burn. Biomed Pharmacother 94:747–757. https://doi.org/10.1016/j.biopha.2017.07.141

Salampessy J, Phillips M, Seneweera S, Kailasapathy K (2010) Release of antimicrobial peptides through bromelain hydrolysis of leatherjacket (Meuchenia sp.) insoluble proteins. Food Chem 120:556–560. https://doi.org/10.1016/J.FOODCHEM.2009.10.054

Salas CE, Gomes MTR, Hernandez M, Lopes MTP (2008) Plant cysteine proteinases: evaluation of the pharmacological activity. Phytochemistry 69:2263–2269. https://doi.org/10.1016/j.phytochem.2008.05.016

Salu BR, Pando SC, Brito MV, Medina AF, Odei-Addo F, Frost C, Naude R, Sampaio MU, Emsley J, Maffei FHA, Oliva MLV (2018) Improving the understanding of plasma kallikrein contribution to arterial thrombus formation using two plant protease inhibitors. Platelets 14:1–9. https://doi.org/10.1080/09537104.2018.1428738

Schulz A, Shoham Y, Rosenberg L, Rothermund I, Perbix W, Christian Fuchs P, Lipensky A, Schiefer JL (2017) Enzymatic versus traditional surgical debridement of severely burned hands: a comparison of selectivity, efficacy, healing time, and three-month scar quality. J Burn Care Res 38:e745–e755. https://doi.org/10.1097/BCR.0000000000000478

Seaf M, Ben-Zimra M, Mankuta D, Dayan N, Levi-Schaffer F (2016) Papain activates human mast cells to release proinflammatory mediators via its enzymatic activity. J Invest Dermatol 136:1523–1525. https://doi.org/10.1016/j.jid.2016.03.030

Secor ER, Shah SJ, Guernsey LA, Schramm CM, Thrall RS (2012) Bromelain limits airway inflammation in an ovalbumin-induced murine model of established asthma. Altern Ther Health Med 18:9–17

Shimizu M, Sawashita N, Morimatsu F, Ichikawa J, Taguchi Y, Ijiri Y, Yamamoto J (2009) Antithrombotic papain-hydrolyzed peptides isolated from pork meat. Thromb Res 123:753–757. https://doi.org/10.1016/j.thromres.2008.07.005

Shing CM, Chong S, Driller MW, Fell JW (2015) Acute protease supplementation effects on muscle damage and recovery across consecutive days of cycle racing. European Journal of Sport Science 16(2):206–212

Shivalingu BR, Vivek HK, Priya BS, Soujanya KN, Swamy SN (2016) Purification and characterization of novel fibrin(ogen)olytic protease from Curcuma aromatica Salisb.: role in hemostasis. Phytomedicine 23:1691–1698. https://doi.org/10.1016/j.phymed.2016.09.007

Shivaprasad HV, Rajesh R, Nanda BL, Dharmappa KK, Vishwanath BS (2009) Thrombin like activity of Asclepias curassavica L. latex: action of cysteine proteases. J Ethnopharmacol 123:106–109. https://doi.org/10.1016/j.jep.2009.02.016

Shoba E, Lakra R, Syamala Kiran M, Korrapati PS (2017) Fabrication of core-shell nanofibers for controlled delivery of bromelain and salvianolic acid B for skin regeneration in wound therapeutics. Biomed Mater 12:35005. https://doi.org/10.1088/1748-605X/aa6684

Singer AJ, Taira BR, Anderson R, McClain SA, Rosenberg L (2010) The Effects of rapid enzymatic debridement of deep partial-thickness burns with Debrase® on wound reepithelialization in swine. J Burn Care Res 31:795–802. https://doi.org/10.1097/BCR.0b013e3181eed48e

Siritapetawee J, Sojikul P, Klaynongsruang S (2015) Biochemical characterization of a new glycosylated protease from Euphorbia cf. lactea latex. Plant Physiol Biochem 92:30–38. https://doi.org/10.1016/j.plaphy.2015.04.012

Soplin SP, Millones EA, Campos JLA, Deza LG, Ríos EJ, Sánchez IB, Benítez MR, Pando LG, Panizo RS, Merino C del C, Rivera JC (1995) Perú: Informe nacional para la conferencia técnica internacional de la FAO sobre los recursos fitogenéticos (Leipzig, 1996). Peru, p 253

Stevens LE (1968) A reassessment of papain in preventing peritoneal adhesions. Am J Surg 115:535–539

Tadikonda A, Pentapati K-C, Urala A-S, Acharya S (2017) Anti-plaque and anti-gingivitis effect of Papain, Bromelain, Miswak and Neem containing dentifrice: a randomized controlled trial. J Clin Exp Dent 9:e649–e653. https://doi.org/10.4317/jced.53593

Tavares T, Contreras MDM, Amorim M, Pintado M, Recio I, Malcata FX (2011) Novel whey-derived peptides with inhibitory effect against angiotensin-converting enzyme: in vitro effect and stability to gastrointestinal enzymes. Peptides 32:1013–1019. https://doi.org/10.1016/j.peptides.2011.02.005

Tharakan A, Dobzanski A, London NR, Khalil SM, Surya N, Lane AP, Ramanathan M (2018) Characterization of a novel, papain-inducible murine model of eosinophilic rhinosinusitis. Int Forum Allergy Rhinol 8(4):513–521. https://doi.org/10.1002/alr.22072

Uday P, Maheshwari M, Sharanappa P, Nafeesa Z, Kameshwar VH, Priya BS, Nanjunda Swamy S (2017) Exploring hemostatic and thrombolytic potential of heynein - a cysteine protease from Ervatamia heyneana latex. J Ethnopharmacol 199:316–322. https://doi.org/10.1016/j.jep.2016.12.047

van der Hoorn RAL (2008) Plant proteases: from phenotypes to molecular mechanisms. Annu Rev Plant Biol 59:191–223. https://doi.org/10.1146/annurev.arplant.59.032607.092835

van Wijk KJ (2015) Protein maturation and proteolysis in plant plastids, mitochondria, and peroxisomes. Annu Rev Plant Biol 66:75–111. https://doi.org/10.1146/annurev-arplant-043014-115547

Wang B, Li Z-R, Chi C-F, Zhang Q-H, Luo H-Y (2012) Preparation and evaluation of antioxidant peptides from ethanol-soluble proteins hydrolysate of Sphyrna lewini muscle. Peptides 36:240–250. https://doi.org/10.1016/j.peptides.2012.05.013

Wei B, He L, Wang X, Yan GQ, Wang J, Tang R (2017) Bromelain-decorated hybrid nanoparticles based on lactobionic acid-conjugated chitosan for in vitro anti-tumor study. J Biomater Appl 32:206–218. https://doi.org/10.1177/0885328217715537

Wu S-Y, Hu W, Zhang B, Liu S, Wang J-M, Wang A-M (2012) Bromelain ameliorates the wound microenvironment and improves the healing of firearm wounds. J Surg Res 176:503–509. https://doi.org/10.1016/j.jss.2011.11.1027

Yariswamy M, Shivaprasad HV, Joshi V, Nanjaraj URSAN, Nataraju A, Vishwanath BS (2013) Topical application of serine proteases from Wrightia tinctoria R. Br. (Apocyanaceae) latex augments healing of experimentally induced excision wound in mice. J Ethnopharmacol 149:377–383. https://doi.org/10.1016/j.jep.2013.06.056

Zetter BR, Chen LB, Buchanan JM (1976) Effects of protease treatment on growth, morphology, adhesion, and cell surface proteins of secondary chick embryo fibroblasts. Cell 7:407–412. https://doi.org/10.1016/0092-8674(76)90170-7

Zhou Z, Wang L, Feng P, Yin L, Wang C, Zhi S, Dong J, Wang J, Lin Y, Chen D, Xiong Y, Peng J (2017) Inhibition of epithelial TNF-α receptors by purified fruit bromelain ameliorates intestinal inflammation and barrier dysfunction in colitis. Front Immunol 8:1468. https://doi.org/10.3389/fimmu.2017.01468