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An overview of the Properties, medicinal uses, extraction and purification of Bromelain

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Abstract :

Bromelain is a significant proteolytic enzyme that is present in pineapple *Ananas comosus* plants and has several medical applications. Bromelain is in high demand on the market, particularly in the health sector where it is utilized as a medicine or nutraceutical. Bromelain contains a number of characteristics, including anti-inflammatory, anti-oedema, antithrombotic and fibrinolytic, mucolytic, anticancer, even if the entire molecular mechanism behind these benefits is yet unknown.Scientists have an unending interest in it because of its excellent availability, high efficiency, limited toxicity, and relative easy of purchase. The scientific literature on the biochemical characterization and structure of bromelain, extraction and purification, potential use in medicine as anti-inflammatory activity, antioxidant , antibiotic therapy, Anticancer,effects on cardiovascular disease and circulation ,and toxicity of bromelain is summarized in this review.

Keywords: bromelain, anti-inflammatory activity, antioxidant , antibiotic, anti-cancer , cardiovascular disease.

نظرة عامة على خصائص البروميلين واستخداماته الطبية واستخلاصه وتنقيته

حنين إبراهيم علي ، وبيداء حسين مطلك
جامعة بغداد / كلية التربية للعلوم الصرفة \ابن الهيثم - قسم علوم الحياة

مستخلص

البروميلين هو انزيم محلل للبروتينات مهم يوجد في نبات الاناناس *Ananas comosus* و يمتلك العديد من التطبيقات الطبية . هناك طلب كبير على البروميلين في السوق، خاصة في القطاع الصحي اذ يتم استخدامه كدواء أو كمواد مغذية. يحتوي البروميلين على عدد من الخصائص، بما في ذلك خصائص مضادة للالتهابات، ومضادة للوذمة، ومضادة للتخثر وحال للفيبرين، وحال للبلغم، ومضادة للسرطان، حتى لو كانت الآلية الجزيئية الكاملة وراء هذه الفوائد غير معروفة بعد لدى العلماء اهتمام لا ينتهي به بسبب توفره الممتاز، وكفاءته العالية، وسميته المحدودة، والسهولة في الشراء. تم تلخيص الأدبيات العلمية حول التوصيف الكيميائي الحيوي وبنية البروميلين، واستخلاصه وتنقيته، والاستخدام المحتمل في الطب كنشاط مضاد للالتهابات، ومضادات الأكسدة، والعلاج بالمضادات الحيوية، ومضاد للسرطان، والتأثيرات على أمراض القلب والأوعية الدموية والدورة الدموية، وسمية البروميلين تم تلخيصها في هذه المراجعة.

الكلمات المفتاحية: بروميلين، نشاط مضاد للالتهابات، مضاد للأكسدة، مضاد حيوي، مضاد للسرطان، أمراض القلب والأوعية الدموية.

1. Introduction

Bromelain is a well-known mixture of proteolytic enzymes that is mostly isolated from pineapples (*Ananas comosus*) and employed in many industries (de Lencastre Novaes *et al*.,2016).The pineapple is the Bromeliaceae-family member , first detected in pineapple juice by Marcano in 1891, Heinecke found in 1957 that the stem contained more bromelain than the fruit (Pavan *et al*.,2012) ,While the name "bromelain" originally referred to a combination of enzymes found in fruit, it is now used to refer to any protease that has been isolated from any plant in the Bromeliaceae family . The most popular plant from which bromelain is derived is the pineapple. The hydrolysis of the peptide bonds in non-terminal amino acids is catalyzed by cysteine endopeptidases, which are the proteases that make up bromelain (Ramli *et al*.,2016). Bromelain include enzymes that break peptide bonds, mainly those of the alanyl, glycyl, and leucyl types; these include glycosidases, phosphatases, ribonucleases, cellulases, peroxidases, and glycoproteases. These enzymes are found in varying proportions depending on the plant's location, the kind of land and cultivation technique, the extraction process, and the portion of the plant that is utilized (Arshad *et al*.,2014)

Ananas comosus is mostly grown in equatorial areas of the world and is recognized to have wide-ranging positive benefits.An extract from pineapple stems which is an agro-waste that is incredibly abundance of complex enzymes that have been found by the enzyme bromelain and are crucial for several therapeutic applications, including the control of tumor development, wound healing, anti-inflammatory effects, antidiarrheal effects, and digestive system support (Bharat,2019). There are several industrial applications for bromelain, including those in the food sector, pharmaceuticals (including cosmetics), dietary supplements, and the manufacturing of protein hydrolysates (Ketnawa *et al*.,2011).

Bromelain may be used orally for an extended period of time without harm, and the human digestive tract can readily absorb it without breakdown or loss of function (Chobotova *et al*.,2010; Pavan *et al*.,2012) . An alternate method is to use pineapple residues as a bioactive resource substances, particularly proteolytic enzymes.

2. Extraction and Purification of bromelain

There are two different variety of enzymes, Fruit bromelain (FBM) has an EC number of EC 3.4.22.33, while stem bromelain (SBM) has, EC 3.4.22.32 (Table 1), based on the fruit particular region where the enzyme was extracted. There is a possibility that their slightly unfortunate names indicate that there are two different enzyme types. However, studies have found that the physicochemical characteristics, biological activity, and structure of SBM and FBM varied (Pang *et al*.2020). In the pharmaceutical, medicinal, and food industries, SBM is essentially the only material used. Because the pineapple stems have a higher enzyme concentration than the fruit, getting bromelain from them is significantly more profitable. Additionally, the difficult extraction and purification of SBM is less expensive (Ramli *et al*.2016 and Arshad *et al*.2014) . SBM has a molecular weight range of 26–37 kDa, while FBM has a range of 24.5–32 kDa (Zhou *et al*.2021).

Bromelain concentration is high in the stem of the pineapple, which necessitates its extraction, as it isn't the same as pineapple Fruit. The stem is typically used as food, is cheap because it is a waste byproduct (Brien *et al*.2004). Preparation in the pure form of the proteolytic enzyme has always been shown to be difficult, bromelain seems to be no exception (Ota *et al*.1964). Crude commercial bromelain used in pharmaceutical manufacturing is non-chemical homogeneous. The main ingredient in raw bromelain is a proteolytic enzyme called glycoprotein, besides materials such as insoluble substances, for example color pigments ,minerals, organic acids, protease inhibitors, organic solvents and excipients used for enzyme recovery have been reported (Murachi *et al*.1964).

Investigation of partial purification and isolation of the enzyme have been began since 1849 (Mohan *et al*.,2016) , and was initially recognized by Marcano in 1891 (Nor *et al*. 2015), Bromelain can be found throughout the entire plant, which refers by the scientific name (EC3.4.22.33), also is a monomer of glycosylated enzyme that has significant proleolytic activity against the peptide bonds in foods like milk and meat (Nor *et al*.2016) . The pineapple stem is used to extract the marketable bromelain primarily using two-step

Fast Protein Liquid Chromatography (FPLC) (Harrach *et al*., 1998), centrifugation, ultrafiltration, and lyophilization (Corzo *et al*., 2011). After being extracted, the crude mixture containing the necessary enzyme is subjected to a number of purification steps in order to remove any contaminants that might interfere with the application of bromelain and lower the enzyme specific activity (Illanes 2008). According to Soares *et al*. (2012), a number of traditional methods of isolation and purification have become outdated due to their limited capacity for purification. According to Gupta *et al*. (2004), the extraction and purification technique (Figure 1) should be inexpensive, quick, high yielding, and selective for the intended product.

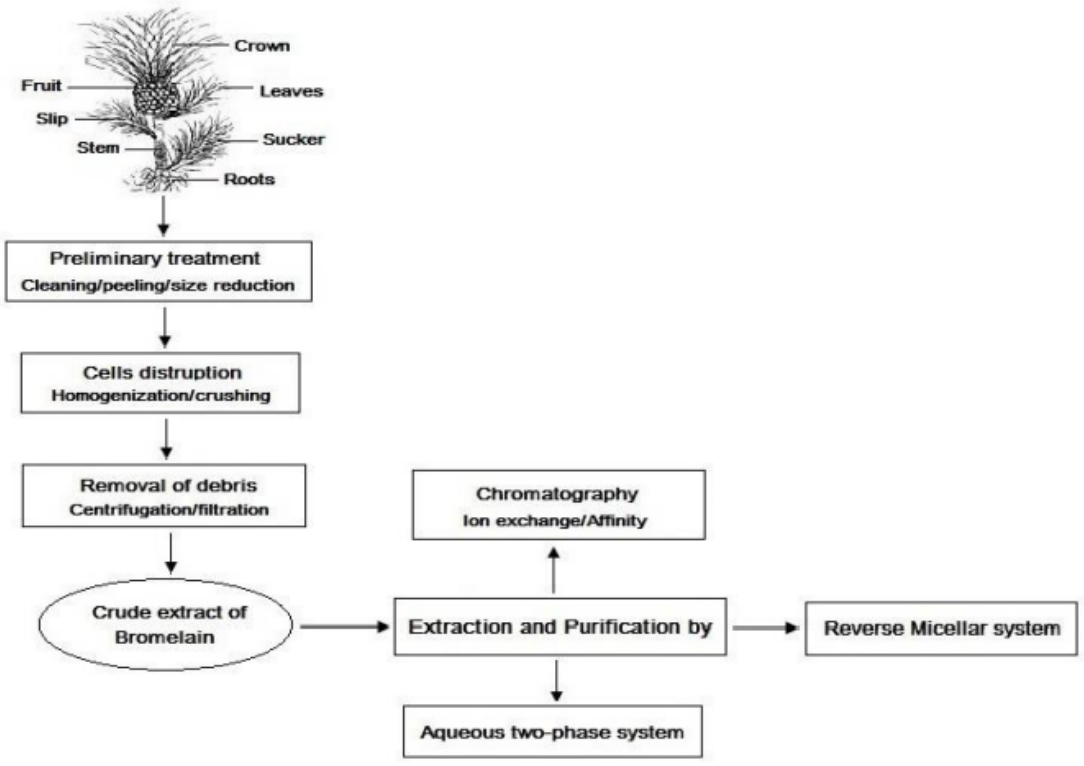

Figure 1 . Overview of extraction and purification of Bromelain.

As a result of scientists growing interest in bromelain, a number of new purification methods have been developed for its extraction and purification. Among them are:

1- Aqueous two phase systems (Babu *et al*. 2008; Ferreira *et al*. 2011; Coelho *et al*. 2013; Spir *et al*. 2015).
2- Membrane processes
3- Precipitation (Soares *et al*. 2012).

4- Reversed micellar systems (Hemavathi *et al*. 2007; Navapara *et al*. 2011; Kumar *et al*. 2011).
5- Different chromatographic techniques (Doko *et al*.,2005)

3. Biochemical characteristic of bromelain

The pineapple plant *Ananas comosus*, is the primary source of the bromelain enzyme, which is one of the important proteolytic enzymes of plant origin and is classified as an endopeptidases. The bromelain enzyme belongs to the class of proteases, also known as proteins or peptidases, which is a group of enzymes that catalyze proteolytic reactions, as the breakdown of proteins into smaller peptides occurs,Or a small, single amino acid, and more specifically, it is classified as a cysteine protein (CP,EC 3.4.22) also known as thiol protein because of the cysteine thiols present in its active site, meaning it works to break down and digest proteins that contain cysteine residues in their composition, such as blood clotting proteins (Gautam *et al*., 2010). The bromelain enzyme are also known as Proteolytic enzymes, often known as proteinases or peptidases.They are an interesting collection among the enzymes that have been discovered in a significant range of entities, including animals, plants, and microbes. They act a variety of crucial activities for different processes, such as digesting, regulating protein synthesis, controlling cell growth and death, and controlling proliferation. They also have a significant impact on the growth and transmission of bacteria, parasites, and viruses. Proteolytic enzymes are a subset of hydrolases (EC 3.4.). In the midst of the chain of amino acids (endopeptidases) or at its ends, they hydrolyze the peptide bond (exopeptidases) (Mohammad,2023). Crude bromelain may be used to produce at least four evolutionarily and structurally similar cysteine endopeptidases: stem bromelain (EC 3.4.22.32), fruit bromelain (EC 3.4.22.33), ananain (EC 3.4.22.31), and comosain (Table 1) (Mamo and Assefa ,2019; Ramli *et al*. ,2018).

The substrate and the hydrolyzed bond position are responsible for the initial division. the second is about the catalytic mechanism . Proteases are the grouped into the third category based on the pH level at which they are best active. Over 500 of these chemicals have been discovered in the human body to date, and about 2% of all genes

encode them (Suarez-Puente ,2003). Plant proteases have gained significantly more interest in biotechnology and modern medicine because of their useful features. Bromelain from pineapple is among the most widely known phytoproteases that have excellent marketing values (Ramli *et al*.,2018).

In terms of biochemistry, bromelain is not toxic substance having therapeutic advantages that is categorized as a protease, a digestive enzyme for proteins. Actually, it is important to note that the extract of bromelain also contains glycoproteins, phosphatases, glucosidase, cellulases, and peroxidases, polysaccharides, numerous inhibitors of protease, and organically bound Ca2+ in addition to different thiol endopeptidases (Chakraborty *et al*. 2021). According to assumptions, the bromelain extract's percentage composition is made up of 5% ananain, 10% fruit bromelain, 80% stem bromelain, and additional components. (Wan *et al*.2016).

Table 1: The physiochemical characteristics of pineapple plant-derived cysteine endopeptidases (Mamo and Assefa ,2019; Ramli *et al*. ,2018).

	Stem Bromelain	Fruit Bromelain	Ananain	Comosain
Source	Pineapple stem	Pineapple fruit	Pineapple stem	Pineapple stem
Molecular weight [kDa]	23.8–37.0	23.0–32.5	23.4–25.0	24.4–24.5
Isoelectric point	≥9.5	4.6	>10	>10
Amino acid sequence	212, 291, 285	326, 351	216	186
Optimum T [°C]	40–60	37–70	/	/
Optimum pH	4–8	3–8	/	/
Presence of Glycoproteins	Yes	Yes/No	No	Yes

Harrach *et al*. (1995) provide significant information on the biochemical examination of the SBM extract. Nine fundamental components of the proteolytically active SBM extract were able to be isolated using two-step cation-exchange chromatography. Two major constituents , 24.4 kDa for F4 and F5, are thought to be 50% glycosylated. Disulfide bridges and many hydrogen bonds stabilize them (Soares *et al*.2019). The portion designated F9 (ananain; 23.5 kDa) in the SBM extract, which accounts for around 2% of all proteins, appears to be the most active protease. F9 is not glycosyl-

ated, according to studies (Harrach *et al.*1995). The ideal pH and ambient temperature, two variables that determine the action of pineapple extracts, are not in balance, which is another indication of differences in how SBM and FBM function (De Lencastre Novaes *et al.*2016).

According to studies bromelain is used so widely in the food, pharmaceutical, biotechnology, and medical sectors, so it should be highly biologically active, have less systemic toxicity, and be well absorbed by the body. Based on research conducted on animals, bromelain has been found to have extremely low toxicity, with a lethal dosage (LD) of greater than 10 g/kg body weight. The cytotoxic or carcinogenic properties of the extract have not been demonstrated in animals given it, such as rats and dogs (Taussig *et al*.1975). Additionally, bromelain was found to have no negative side effects in human clinical trials. It is important to remember that prolonged bromelain treatment may cause tachycardia in persons with hypertension (Gutfreund *et al*.1978). According to research, taking bromelain may cause allergic reactions in certain people with pineapple allergies are particularly at risk (Knox *et al*.2019). Bromelain can result in IgE-mediated respiratory allergies in these persons, which can lead to breathing problems, sinus congestion, angioedema, wheezing, and coughing (McWilliam *et al*.2017). The digestive system is the primary route by which bromelain is absorbed. According to Castell *et al*. (1997) and White *et al*. (1988), the highest concentration of functionally intact bromelain is observed up to an hour after oral administration of the extract, with an estimated 40% of the rat population potentially carrying this protein in their blood. According to Castell *et al.* (1997) bromelain has a 6–9-hour half-life. Additionally, bromelain in the plasma maintained its proteolytic characteristics and was linked to alpha 1-antichymotrypsin and alpha 2-macroglobulin, two blood proteins that stabilize bromelain even at low quantities. After 4 hours, bromelain was still present in synthetic blood at a 2.44 mg/mL concentration. Its concentration in artificial stomach juice was 3.66 mg/mL (Setiasih *et al*.2018). The fundamental structure of bromelain is a single polypeptide chain including two structural domains formed by folded amino acids: the α-helix The antiparallel β-sheet domain (domain peptidase

C1) and the domain (domain cathepsin propeptide inhibitor—I29) are shown in Figure 2. Bromelain has become a staple in multiple industries, including food, pharmaceuticals, cosmetics, and biotechnology, as a result of its superior biological capabilities, absence of systemic cytotoxicity,and frequently great advantages that it provides. Bromelain extracts have intriguing anticancer characteristics, which promote the action of existing chemotherapeutic drugs, according to growing body of scientific evidence. The advancement of efficient techniques for pineapple stem removal of the enzyme as a result of bromelain commercial use has led to preparations that are ultra-pure.

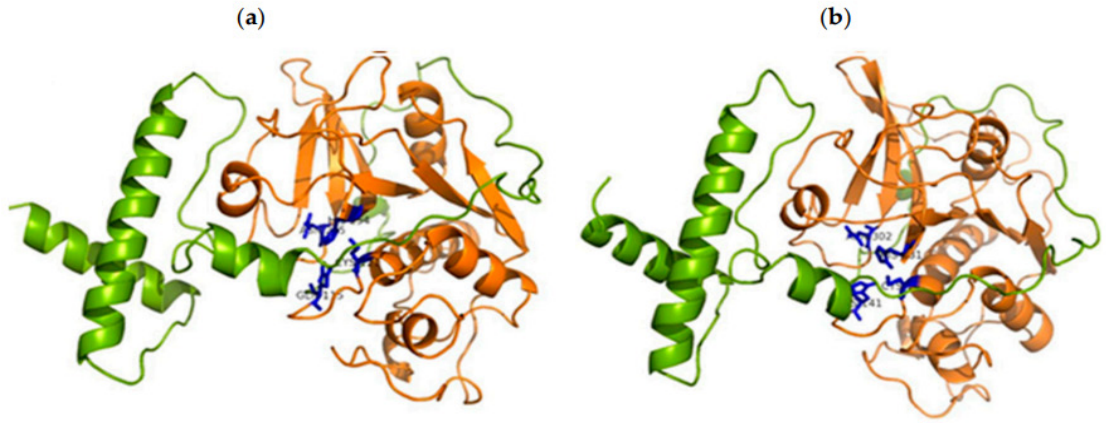

Figure 2.Model domain organization for (a) fruit bromelain (sequences with the NCBI Genbank database accession number of BAA21848 and 352 amino acids) and (b) stem bromelain (sequences with the NCBI Genbank database accession number of CAA08861 and 357 amino acids). The β-sheet domain (domain peptidase C1 at the C-terminal region) is colored orange, while the α-helix domain (domain I29 at the N-terminal region) is colored green. In both models, the catalytic amino acids are shown as sticks. (Reproduced with permission from Ramli *et al.*2018, Elsevier, Amsterdam, The Netherlands, 2018).

4. Medicinal Uses

4-1 Antioxidant properties of bromelain

Bromelain has antioxidant properties that include the ability to prevent the oxidation of lipids and scavenge free radicals (Manosroi *et al.*,2014). Various protease inhibitors, cellulases, glycoproteins, phosphatases, glucosidase, peroxidases, and thiol endopepti-

dases are all present in bromelain (Al-Jubori *et al.,*2016,Hussein *et al*.,2020).

Determining antioxidative activity is crucial thus it may be used to create a substance that prevents degenerative diseases (Young and Woodside,2001). When evaluated against the DPPH radical 1,1-diphenyl-2-picrylhydrazyl bromelain antioxidant activity provided a quick and simple technique to assess its antiradical properties (Ahmad and Albakri,2022 ; Muhsen *et al*.,2020; Abdalrazaq *et al.*2022).

4-2 Impact of Bromelain on Circulation and Cardiovascular Disease

Despite inconsistent clinical studies that call for more research, bromelain is useful as an adjuvant in the treatment of cardiovascular disorders, particularly given its proven antithrombotic and anticoagulant properties (Giles *et al*.,2003). Bromelain has been shown to improve Akt phosphorylation, which inhibits cell death, and diminish platelet aggregation as triggered by adenosine phosphate, blood viscosity, and the risk of thrombus formation (Juhasz *et al*.,2008). Transient ischemic attack (TIA) and angina pectoris are prevented or treated with bromelain to reduce their severity. It helps in thrombophlebitis treatment and prevention. It has a strong fibrinolytic effect and may help dissolve cholesterol plaques. Bromelain and other nutrients work together to protect skeletal muscle against ischemia/reperfusion damage (WHO,2011). Heart and blood vessel problems, Cardiovascular illnesses (CVDs) include coronary heart disease (heart attacks), cerebrovascular disease (stroke), hypertension (high blood pressure), peripheral artery disease, rheumatic heart disease, heart failure, and congenital heart disease (Neumayer *et al*.2006) .

King *et al*.(2009) reported that between 1988–1994 and 2001–2006, there was a 121% rise in the use of medications to treat the symptoms of diabetes, hypertension, and hypercholesterolemia (P 0.05), and this increase was higher among patients who had less of a healthy lifestyle. Any risk factor that contributes to the development of cardiovascular disease may be decreased by taking supplements that include bromelain.

4-3 Bromelain in Antibiotic Therapy

Bromelain has a wide range of applications in the treatment with antibiotics as a chemical that enhances their activity because of its good proteolytic characteristics and very low systemic

cytotoxicity. It has been shown that the combination of bromelain and antibiotic therapy is more effective in treating a variety of disease states, including sinusitis, bronchitis, pneumonia, pyelonephritis, thrombophlebitis, perirectal and rectal obsessions, and cutaneous infections caused by Staphylococcus. (in comparison to the antibiotic used alone) (Ibrahim,2017 and Varilla *et al*.,2021) . The concentrations of gentamicin and penicillin have been found to rise in rat tests (Moss *et al*.1963).

Correspond Bromelain treatment with antimicrobial therapy produced results in people.Higher levels of antibiotics were detected in the blood and urine as a result of amoxicillin and tetracycline levels in the blood and tissues (Al-Jebouri ,2010;Saleh and Najim,2020). As demonstrated by Shahid *et al.* (2002) when utilize bromelain as adjuvant therapy to treat pediatric sepsis, bromelain, rutin, and trypsin were found to boost the efficacy of antibiotics, The outcomes show that utilizing trypsin, rutin, and bromelain in combination with antibiotics is a successful an adjunct therapy for promoting earliest recovery in sepsis-afflicted children and adolescents.

Recent *in-vivo* research by Cai *et al*.(2016) investigated the effects of levofloxacin combined with the extract of *Serenoa repens*, selenium, lycopene, bromelain, and methylsulfonylmethane in patients with chronic bacterial prostatitis (CBP). Levofloxacin clinical effectiveness in CBP patients was enhanced by the combination of extracts. The anti-inflammatory characteristics of bromelain may also be responsible for the observed improvement in life quality. Notably, there were no negative effects while receiving therapy.

4-4 Anticancer Properties of Bromelain

Unfortunately, little is known about bromelain anticancer activity from the clinical studies it has already undergone for the treatment of cancer. Bromelain, however, is the subject of ongoing both *in-vivo and in-vitro* research as an possible chemical therapy drug. It is important to note considering that the precise methods underlying its antitumor molecule activity remains a mystery. According to some theories, bromelain capacity to prevent tumor cell growth and spread as well as to trigger cancer cell death might be a result of its immunomodulatory and proteolytic characteristics(Pillai *et al.*2013). One of bromelain key properties that

effectively inhibits cancer growth and proliferation is unquestionably its capacity to cause apoptosis. Numerous pieces of evidence point to bromelain as a prospective anticancer drug that alters The appearance of several genes associated in metastasis, proliferation, or programmed cell death. According to (Figure 3), numerous biochemical pathways are involved in the molecular mechanisms underlying bromelain anticancer action. In a variety of ovarian cancer *in-vivo and in-vitro* models, lung, melanoma, digestive system cancer (Park *et al.*2018 ;Chang *et al.*2019) , and leukemia (Al-Jumaily *et al.*,2013; Debnath *et al.*2019) , Molecular research has been done on the therapeutic potential of bromelain, as well as its efficacy in inducing PCD processes and modifying the expression of proteins linked to apoptosis.

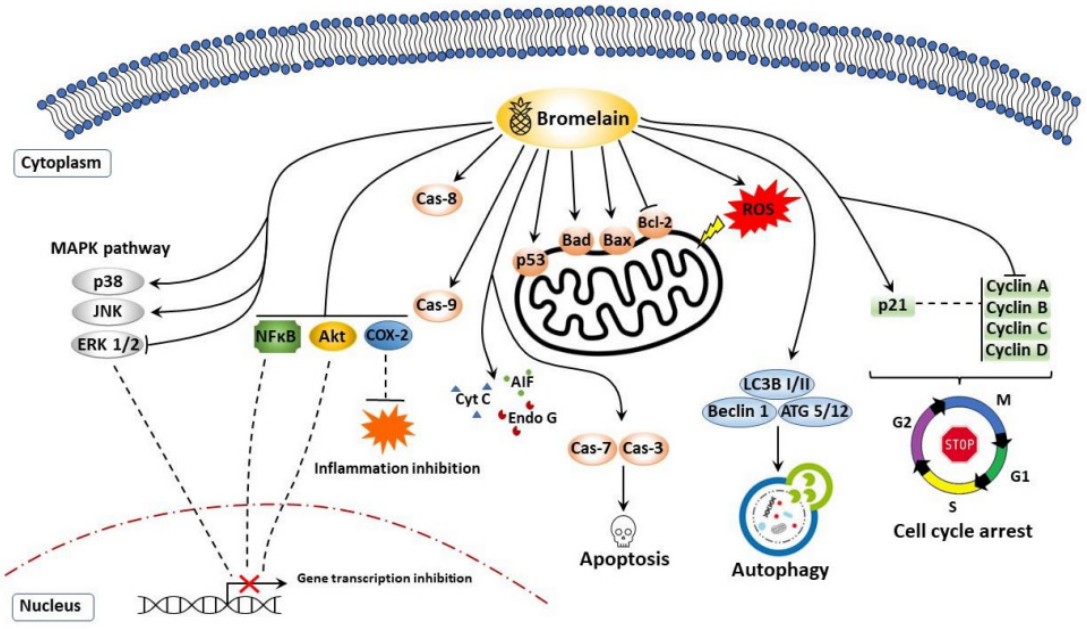

Figure 3. shows the three stages of cellular metabolism that may be involved in the molecular processes that underlie bromelain anticancer effects. Bromelain primarily inhibits the growth of cancer cells by I) changing the expression of genes necessary for cell differentiation and proliferation (MAPK signaling pathway, Akt, Cox-2, NF-B), II) inducing cell death through apoptosis/autophagy, and III) stopping the cell cycle by inhibiting cyclins that are required for this process, according to studies done in the lab and on animals (Hikisz and Bernasinska-Slomczewska ,2021).

4-5 Anti-inflammatory activity

Angiogenesis, invasion, metastasis, cellular transformation, and proliferation are all critical stages in the development of cancer where inflammation plays a key role. Research indicates that the management of persistent inflammation can both lower the risk of developing cancer and impede its advancement (Huang *et al*.,2008). Prostaglandin E2 (PGE-2) production is aided by cyclooxigenase-2 (COX-2), a crucial element of inflammation linked to cancer. Pro-inflammatory lipid PGE-2 also functions as an immunosuppressant and accelerates the growth of tumors (Bhui *et al*.,2009). COX-2 promotes tumor angiogenesis and the advancement of cancer by converting arachidonic acid into PGE-2 (Gaspani *et al*.,2002). Bromelain has been demonstrated to downregulate the expression levels of PGE-2 and COX-2 in human monocytic leukemia cell lines and murine microglial cells (Desser *et al*.,1994). Interleukin (IL)-1β, IL-6, interferon (INF)-γ, and tumor necrosis factor (TNF)-α are among the inflammatory mediators that bromelain activates in human peripheral blood mononuclear cells (PBMC) and mice macrophages (Engwerda *et al*.,2001; Engwerda *et al.*,2001 and Barth *et al*.,2005). These findings suggested that bromelain may stimulate the immune system in a healthy person in connection with a quick reaction to cellular stress. On the other hand, when immune cells are already excited due to inflammation-induced overproduction of cytokines, bromelain decreases the release of IL-1β, IL-6, and TNF-α (Hale *et al*.,2005 and Onken *et al*.,2008).

In addition to increasing the synthesis of IL-2, IL-6, and granulocyte-macrophage-colony stimulating factor, bromelain also stimulates natural killer cells and reduces the activation of T-helper cells (Giacca ,1995). As a result, bromelain significantly functions as an anti-inflammatory agent in a variety of diseases and reduces most inflammatory mediators (De-Giuli and Pirotta,1978).

4-6 Toxicity of Bromelain

According to Taussig *et al.* (1975), bromelain has very low toxicity, with an LD50 (lethal dosage) in mice, rats, and rabbits of more than 10 g/kg. Tests for toxicity on dogs using daily doses of bromelain up to 750 mg/kg revealed no harmful effects after six months. Rats given daily dosages of 1500 mg/

kg exhibited no teratogenic or carcinogenic effects, nor did they cause any changes in food consumption, growth, spleen, kidney, or hematological parameters (Luerti and Vignali,1978). Zavadova *et al*.,1995 observed no appreciable changes in blood coagulation parameters following a ten-day administration of bromelain (3000 FIP units/ day) to humans.

Conclusion

Despite the many therapeutic benefits that bromelain provides, its mechanism of action is still not widely understood. Oral administration of bromelain has been shown to effectively absorb the substance and to have no discernible side effects, even after prolonged use. The evidence reviewed in this paper supports the potential of bromelain as anti- cancer, anti-cardiovascular disease, antioxidant, and antibacterial supplement.

Bromelain is a possible candidate for the development of oral enzyme therapies for cancer patients. This work clearly shows that bromelain is a multifunctional enzyme, but further research is required to pinpoint the enzyme's exact mode of action so that its multifunctional qualities may be fully used.

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