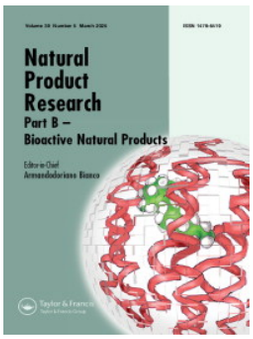

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Bromelain as a natural anti-inflammatory drug: a systematic review

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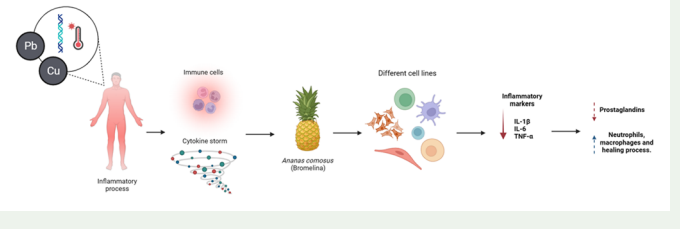
ABSTRACT

Inflammation is a complex and necessary mechanism of an organ's response to biological, chemical and/or physical stimuli. In recent years, investigations on natural compounds with therapeutic actions for the treatment of different diseases have increased. Among these compounds, bromelain is highlighted, as a cysteine protease isolated from the *Ananas comosus* (pineapple) stem. This review aimed to evaluate the anti-inflammatory activity of bromelain, as well as its pathways on inflammatory mediators, through a systematic review with *in vitro* studies on different cell lines. The search was performed in PubMed, Science Direct, Scopus, Cochrane Library and Web of Science databases. Bromelain reduced IL-1β, IL-6 and TNF-α secretion when immune cells were already stimulated in an overproduction condition by proinflammatory cytokines, generating a modulation in the inflammatory response through prostaglandins reduction and activation of a cascade reactions that trigger neutrophils and macrophages, in addition to accelerating the healing process.



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Abbreviations: A375: human melanoma cells; A431: human epidermoid carcinoma; AGS: human gastric adenocarcinoma cells; AKT: serine-threonine kinase; AMPK: adenosine monophosphate-activated protein kinase; BRO: bromelain; Caco-2: human colorectal adenocarcinoma cells; CCL4: carbon tetrachloride; CD's: classification of lymphocytes; COX-2: cyclooxygenase-2; CXCR: chemokine receptors; EECs: human endometriotic endothelial cells; ELISA: enzyme linked immunosorbent assay; ERK: extracellular signal-regulated kinases; GM-CSF: granulocyte-macrophage-colony stimulating factor; GRIESS: nitrite detection assay; HDMEC: human dermal microvascular endothelial cells; hDPCs: human dental pulp cells; IBD: inflammatory bowel disease; ICAM-1: intercellular adhesion molecule 1; IFN-γ: interferon-gamma; IL: interleukin; iNOS: inducible nitric oxide synthase; JNK: c-Jun N-terminal kinase; LA: alpha-lipoic acid; LPS: lipopolysaccharide; MAPK: mitogen-activated protein kinase; MCP-1: monocyte chemoattractant protein-1; MIP-1α: macrophage inflammatory protein-1 alpha; MMLC: mixed culture of modified lymphocytes; MMP: matrix metalloproteinases; MTT: 3[4-dimethylthiazol-2-71]-2-5-diphenyl tetrazolium bromide; Myt1: protein kinase, membrane associated tyrosine/threonine 1; MYT1: Fator de transcrição de mielina; NAC: N-acetyl cysteine; NF-κB: nuclear factor kappa B; NHDF: normal human dermal fibroblasts; NK: natural killers; NO: nitric oxide; p65: transcription factor; p-Stat3: phosphorylated transducer and activator of transcription-3; PBMC: peripheral blood mononuclear cells; PCR: polymerase chain reaction; PGE2: prostaglandin E2; Phospho-cdc25C: phospho-cdc25C (Ser216) Antibody; Plk1: polo- like kinase-1; PSGL-1: P-selectin glycoprotein ligand-1; RIPA: radioimmunoprecipitation assay; RNA: ribonucleic acid; RAW264.7: murine macrophages; SW1353: human chondrosarcoma cells; THP-1: monocytic leukaemia cells; TNF-α: tumour necrosis factor-alpha; TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labelling; U937: human monocytic cells; UtMECs: endothelial cells isolated from normal uterus; VCAM1: vascular cell adhesion molecule-1.

1. Introduction

Inflammation is a complex and necessary mechanism of an organ's response to either biological, chemical or physical stimuli (Ishaq et al. 2022; El-Shawi et al. 2023). Often, it can be described as acute and chronic responses to extraneous agents, even if there is an overlap of these processes. The mechanism of inflammation represents a dynamic and coordinated response chain that includes vascular and cellular events with specific humoral secretions (Huether and McCance 1994; Germolec et al. 2018). These immune pathways recruit white blood cells, plasma and fluids at inflamed site while mediators and others signalling molecules - histamine, prostaglandins, leukotrienes, serotonin, oxygen and nitrogen-derived free radicals - are released to contribute with these mechanisms (Anwikar and Bhitre 2010).

During inflammation, pathogenic microorganisms and death cells resulting from injury are eliminated by strong oxidants produced by neutrophil and macrophages may cause adjacent tissue damage. The inflammation mechanism represents an organised and dynamic chain of responses that includes cellular and vascular events with specific humoral secretions. These pathways involve migration of white blood cells (monocytes, basophils, eosinophils and neutrophils), plasma and fluids at the inflamed site (Huether and McCance 1994).

There is a large variety of natural molecules with antioxidant properties that constitutes an exogenous defense system that helps to maintain homeostasis (Jamaddar et al. 2023). These molecules are known as bioactive compounds that are resulted from plants secondary metabolism against ultraviolet radiation, insects attack or pathogens (Manach et al. 2004). In complex organisms, these molecules act on different physiological targets through distinct mechanisms of action (Bappi et al. 2024; Kamli et al. 2024). The antioxidant activity is the oxidation- reduction ability of these compounds, which causes a stabilisation of free radicals (Abdelazim et al. 2024; El-Nashar et al. 2024). In addition, they compete for active sites and receptors in several cellular structures or modulate gene expression that encode proteins involved in the defense against oxidative and degenerative processes of these structures (Ferreira et al. 2009).

In recent years, investigations on natural compounds have increased (Rabie et al. 2023; El-Nashar et al. 2023b). These substances with therapeutic effects, low or no toxicity have been applied in the prevention and treatment of different diseases (Ashmawy et al. 2023; El-Nashar et al. 2023a). Among them, bromelain is highlighted, which is a cysteine protease isolated from the pineapple (*Ananas comosus*) stem (Ramli et al. 2017).

Traditionally, bromelain has anti-inflammatory and healing effects on arthritis and soft tissue injuries. Experimental studies show that bromelain has unique immunomodulatory activity, such as proinflammatory prostaglandin E2 (PGE-2) downregulation by inhibiting nuclear factor kappa B (NF-kB) and cyclooxygenase 2 (COX-2) (Rathnavelu et al. 2016), as well as anti-inflammatory PGE-1 upregulation, activation of inflammatory mediators interleukin-1β (IL-1β) and IL-6, tumour necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) as an acute response to cellular stress, but also the inhibition of inflammatory mediators in situations of evident cytokine production (Bhui et al. 2009; Pavan et al. 2012). Modulation of T cells responses *in vitro* and *in vivo* and enhancement of T11-dependent antigen-specific B cell antibody responses have been evidenced (Kritis et al. 2020).

Considering the need to deepen the use of bromelain as an anti-inflammatory compound with low or no toxicity and no evidence of a compilation containing these findings in a systematic review, this work sought subsidies to evaluate its anti-inflammatory activity, as well as its pathways on inflammatory mediators *in vitro* studies using different cell lines.

Furthermore, the findings of this review may provide support for the development of more targeted *in vivo* studies and, in the future, clinical trials, since there is a constant need benefits without effects on human health.

2. Methodology

2.1. Study design and problem identification

This review was conducted according to PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Rethlefsen et al. 2023), PRISMA-Search (Rethlefsen et al. 2013). Table 1 presents the variables applied on the guiding question 'What evidence is available regarding the anti-inflammatory activity of bromelain and its signaling pathways?' based on PICOS criteria [P: population; I: intervention; C: Comparator; O: Outcome; S: study] (Galvão and Pereira 2022).

Table 1. Variables used to define the research question.

	Variables
P	Cell lines associated with inflammation process
I	Supplementation with isolated or associated bromelain
C	Control and/or placebo group
O	Anti-inflammatory activity
S	*In vitro* studies

2.2. Search strategy

Searches in PubMed, Science Direct, Scopus, Cochrane Library and Web of Science databases were performed by search terms 'Bromelain' and 'Inflammation' registered in MeSH, combined by the Boolean operators 'AND' in January 20th, 2023, by four independent reviewers (T.A.N., A.A.S., A.M.C.P.S., I.C.P.).

2.3. Eligibility criteria

Eligibility criteria included studies developed with the application of isolated or bromelain in association with any substance, in cell lines associated with the inflammatory process, with no limitation for publication period. Studies that did not report the concentration of bromelain, the time of exposure or the cell line were excluded. Inclusion and exclusion criteria are detailed in Table 2.

2.4. Study selection

The analysis and selection of the publications were carried out by two independent researchers (T.A.N., A.A.S., A.M.C.P.S., I.C.P.) and, in case of divergence, a consensus was reached. The selection followed the order: 1) identification of the number of studies found in each database; 2) titles and abstracts reading and 3) full article reading, focusing on the inclusion criteria. The articles were exported to the EndNote Web software for reference management (Thomson Research Software, Carlsbad, CA, USA).

2.5. Data extraction

Data extraction was made independently (T.A.N., A.A.S., A.M.C.P.S., I.C.P.) and included the authors, year of publication, outcomes, method applied, concentration tested, cell lines and main results. Discrepancies were resolved by consensus.

Table 2. Eligibility criteria.

	Inclusion criteria	Exclusion criteria
P	Tumour or non-tumour cell lines	Animals or humans with inflammatory injuries
I	Bromelain isolated or in association with natural or synthetic substances	No mention of applied concentration and/or exposure time
C	Control group	Absence of control group
O	Outcomes related to inflammatory parameters	Absence of inflammatory parameters
S	Studies in Portuguese, Spanish or English	Other languages
	In vitro	Cross-sectional, case series, case-control, reviews, randomised or non-randomised clinical trials, preclinical studies
	Any publication period	

3. Findings

Figure 1 highlights the study selection process. 1434 publications were identified which 47 were duplicated and removed. A total of 1387 articles were eligible for title and abstract reading, which 56 were selected for full-text review and 21 of them met the inclusion criteria.

The cell lines analysed were: human blood cells (endometriotic endothelial cells and isolated from normal uterus) (Agostinis et al. 2015), peripheral blood mononuclear cells (PBMC) (Kleef et al. 1996; Barth et al. 2005), primary microglial cells (Abbasi Habashi et al. 2016), human dermal microvascular endothelial cells (HDMEC) (Aichele et al. 2013), colonic mucosa (Onken et al. 2008), human dental pulp cells (hDPCs) (Hong et al. 2021), osteoarthritic synovial cells (Brochard et al. 2021), mixed culture of modified lymphocytes (MMLC) (Rose et al. 2006) and melanoma-A375 (Bhui et al. 2009), cholangiocarcinoma (Müller et al. 2016), human colorectal adenocarcinoma cells (Caco-2) (Mynott et al. 2002), stomach, intestinal and chondrocytes cells models (AGS, Caco-2 and SW1353) (Bottega et al. 2021), human monocytes (U937) (Kasemsuk et al. 2018), and immune cells, such as normal human dermal fibroblasts (NHDF) (Aichele et al. 2013), leukocytes (Foroncewicz et al. 2012), neutrophils (Banks et al. 2013), T cells (Mynott et al. 2002), murine macrophages (RAW264.7) (Insuan et al. 2021) and inflammatory exudate (Gaspani et al. 2002) (Table 3).

The concentrations tested ranged from 0.001 to 1000 mg/mL. The inflammatory biomarkers evaluated were TNF-α (Barth et al. 2005; Huang et al. 2008; Onken et al. 2008; Banks et al. 2013; Insuan et al. 2021), NF-Kb (Kleef et al. 1996), IFN-γ(Banks et al. 2013) (Mynott et al. 1999; Barth et al. 2005; Onken et al. 2008), IL-1β (Huang et al. 2008; Onken et al. 2008; Kasemsuk et al. 2018), IL-2 (Mynott et al. 1999; Onken et al. 2008), IL-4 (Barth et al. 2005), IL-5(Onken et al. 2008), IL-6 (Barth et al. 2005), IL-7, IL-8, IL-10 (Onken et al. 2008), IL-12 (p70), IL-17, IL-13 (Onken et al. 2008), different protein kinases (ERKs) ERK-1, ERK-2, PGRK, AKT, PLK1 and AMPK.

Other proteins were also evaluated, as CD7, CD8, CD14, CD16, CD21, CD41, CD42a, CD44, CD45RA, CD48, CD57, CD62L, CD128a and CD128b14, CD44, CD62-L, CD16, CD56, CD49 and CD11a-c16, CD128b, CXCR1 and CXCR227, MYT-19, PSGL128, immunoglobulins ICAM-120 and VCAM-112,20, prostaglandins PGE221, PGE15 and COX-2, and, finally, the antibodies cyclin-B1, phospho-cdc2, phospho-cdc25C and Myt.9.

Furthermore, the growth factors GM-CSF (Onken et al. 2008), matrix metalloproteinases (MMP) (Brochard et al. 2021), proinflammatory proteins MIP-1α (Kasemsuk et al. 2018), MIP-1β (Onken et al. 2008), MCP-1 (Onken et al. 2008; Kasemsuk et al. 2018) and others, such as substance P (Gaspani et al. 2002), nitric oxide (NO) (Abbasi Habashi et al. 2016; Bottega et al. 2021; Insuan et al. 2021) and thromboxanes (Huang et al. 2008).

The developed tests were ELISA (enzyme linked immunosorbent assay) (Aichele et al. 2013) (Rose et al. 2006; Bottega et al. 2021), flow cytometry (Banks et al. 2013) (Hale 2004; Barth et al. 2005), immunofluorescenc (Barth et al. 2005), immunocytochemistry (Bhui et al. 2009), immunoassay (Gaspani et al. 2002; Foroncewicz et al. 2012), PCR (polymerase chain reaction) (Kasemsuk et al. 2018), apoptosis assay (Müller et al. 2016), immunoreactivity assay (Mynott et al. 2002), cell viability assay by MTT (3[4-dimethylthiazol-2-71]-2-5- diphenyl tetrazolium bromide) method (Abbasi Habashi et al. 2016) and Western-blotting (Hong et al. 2021; Insuan et al. 2021).

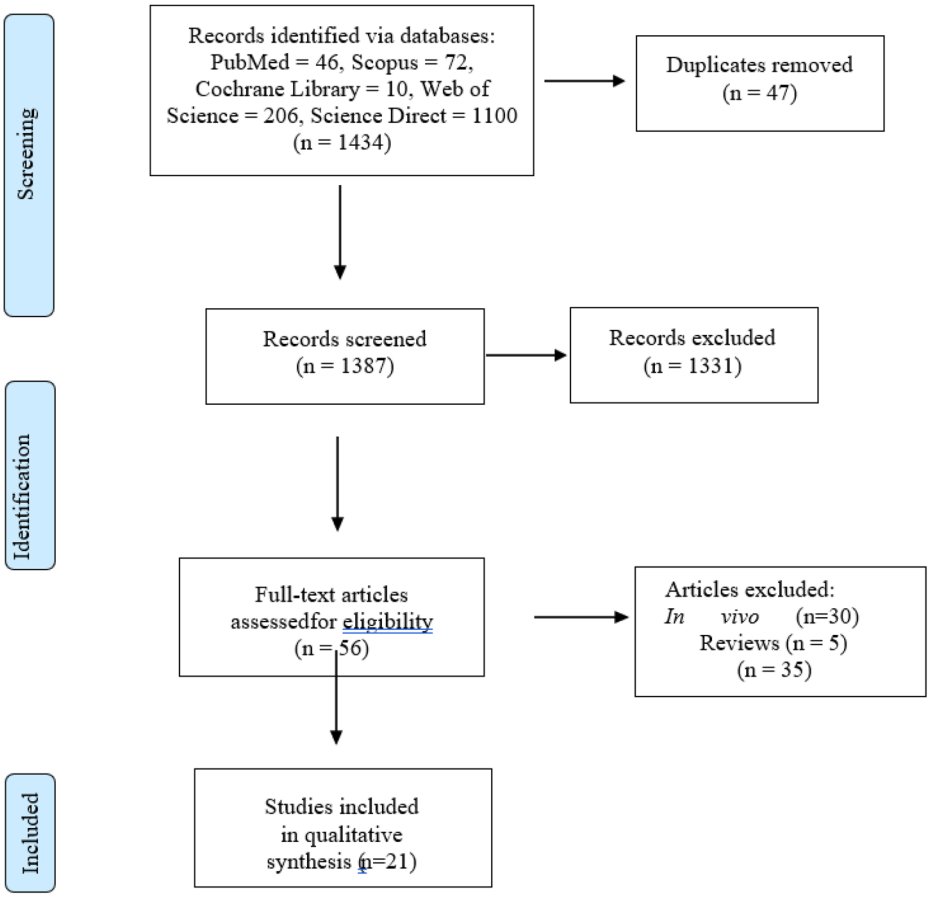

Figure 1. Screening flow diagram based on PRISMA.

Among the selected studies, 82.61% evaluated the effect of isolated bromelain, while 17.39% associated bromelain with N-acetyl cysteine (NAC) and alpha-lipoic acid (LA) (Agostinis et al. 2015), trypsin (Barth et al. 2005) and papain (Rose et al. 2006).

When evaluating the bromelain effect on proinflammatory parameters, it was observed the ability to suppress cytokines, chemokines, prostaglandins and the cyclo-oxygenase pathways, such as COX2 (Huang et al. 2008; Bottega et al. 2021), MIP-1α, MCP-1 (Kasemsuk et al. 2018), IL-8 (Kasemsuk et al. 2018; Bottega et al. 2021), IL-1β, IL-6 (Aichele et al. 2013) (Barth et al. 2005; Brochard et al. 2021) and TNF-α (Barth et al. 2005), PGE2, CCL2, CCL3, CCL4 and CXCL8 (Brochard et al. 2021) (Gaspani et al. 2002), ICAM-1 e VCAM (Agostinis et al. 2015; Hong et al. 2021). A decrease in neutrophil migration to sites of acute inflammation and removal of the CD128 chemokine receptor were verified (Foroncewicz et al. 2012).

Another modification observed by bromelain treatment was a decrease in iNOS (inducible nitric oxide synthase) production at the mRNA level (Abbasi Habashi et al. 2016). Bromelain acted on adhesion proteins, interfering with rolling, adhesion and migration, modulating ICAM- 1, VCAM-1 (upregulation), PECAM-1 and P-selectin (reduction of mediated neutrophils), E-selectin and L-selectin (Hong et al. 2021).

Table 3. Bromelain effects on inflammatory process *in vitro*.

Reference	Cell lines	Inflammatory biomarkers Investigated	Effect assessment method	Compounds concentration	Main results
Agostinis et al. (2015)	EECs, UtMECs	VCAM1	Immunofluorescen ce	BRO (0.05 mg/mL) + NAC (1 mg/mL) + LA (0.5 mg/mL)	BRO + NAC + LA association promoted VCAM1 upregulation.
Aichele et al. (2013)	NHDF, HDMEC	IL-6	Immunocytochemi stry and ELISA	BRO (1; 3; 6; 9; 18; 36 and 72×10^3 UI/mL)	In NDHF under conditions of hypoxia and normoxia an increase of IL-6 in bromelain supplementation after 18h was observed. In HDMEC under normoxia there was an increase in IL-6, but in hypoxia there was a decrease.
Banks et al. (2013)	Human neutrophils	PSGL-1	Flow cytometry and Trypan blue	BRO (50 mg/mL)	BRO acted on PSGL-1 selective cleavage to reduce P- selectin-mediated neutrophil recruitment.
Barth et al. (2005)	PBMC	IL-6, GM-CSF, TNF-α, IFN-γ, IL-4 and IL-5	ELISA and flow cytometry	BRO + trypsin (10 to 1000 mg/mL)	BRO induced IL-6, GM-CSF, TNF-α and IFN-γ increase. In contrast, type-2 cytokines (IL- 4, IL-5) were not induced. A significant secretion of macrophage/ monocyte- associated cytokines IL-6, GM-CSF and TNF-α, as well as the production of type-1 cytokine related to IFN-γ by PBMC in healthy controls was also observed. When analysing its association with trypsin, no significant differences were observed.
Bottega et al. (2021)	AGS, SW1353 Caco-2,	COX-2, iNOS and IL-8	ELISA	BRO (3 mg/mL)	Digested bromelain showed a strong anti-inflammatory activity on Caco-2, reaching approximately 100% IL-8 inhibition. On SW1353, BRO exerted an anti-inflammatory effect under different experimental conditions.
Brochard et al. (2021)	Human osteoarthritis synovial cells	PGE2, MMP and IL-6	Protein RNA (RIPA), and PCR extraction	BRO (147 mg/mL)	Significant reduction in gene expression and/or release of proteins involved in inflammation (IL-6, PGE2).
Foroncewicz et al. (2012)	Human leukocytes	CD128a / CXCR1 e CD128b / CXCR2, IL-8	Immunoassay	BRO (100 mg/mL)	Decrease in neutrophil migration to sites of acute inflammation and specific removal of CD128 as a potential mechanism of action.

(*Continued*)

Table 3. Continued.

Reference	Cell lines	Inflammatory biomarkers Investigated	Effect assessment method	Compounds concentration	Main results
Gaspani et al. (2002)	Inflammatory exsudate	PGE2, neuromodulator substance P	PGE2 immunoenzymatic assay	BRO (25; 50 and 100 mg/mL)	Significant decrease in PGE2 concentrations and substance P in the exsudate.
Abbasi Habashi et al. (2016)	Primary microglial cells	iNOS and NF-kB	MTT assay, nitrite assay, RNA extraction, PCR, Western Blotting	BRO (5; 10; 20 and 30 mg/mL)	Pre-treatment with LPS reduced iNOS production. Significant decrease in iNOS expression at mRNA level and at NF-kB protein level.
Hale et al. (2002)	PBMC	CD7, CD8, CD14, CD16, CD21, CD41, CD42a, CD44, CD45RA, CD48, CD57, CD62L, CD128a and CD128b	Immunofluorescen ce and flow cytometry	BRO (0.001 mg/mL to 1 mg/mL)	BRO treatment on PBMC altered proteolytically 14 of 59 leukocyte markers.
Huang et al. (2008)	PBMC, THP-1	TNF-α, IL-1β, IL-6, PGE2, thromboxane B2 and COX-2	ELISA	BRO (1 to 100 mg/mL)	BRO significantly reduced TNF-α, IL-β and IL-6 of PBMC induced by LPS and THP-1.
Insuan et al. (2021)	RAW264.7	NO, IL-6 and TNF-α	MTT assay, ELISA, GRIESS and Western blotting	Crude bromelain (CB) (0.01; 0.02 and 0.04 mg/mL) or purified bromelain (PB) (0.02; 0.04 and 0.08 mg/mL)	The inhibitory potency of PB was stronger than CB. NO release was inhibited by PB in a dose-dependent manner. The level of IL-6 produced was drastically reduced by CB, however TNF-α level did not changed.
Hong et al. (2021)	hDPCs	IL-1β, IL-6, IL-8, ICAM- 1, VCAM-1, ERK, p65 e	Western blotting, ELISA and immunofluorescen	BRO (0.0025; 0.005; 0.01; 0.02 and 0.04	BRO significantly decreased IL-1β, IL-6, IL-8, ICAM-1 and VCAM-1 levels, p65
Huang et al. (2008)	PBMC, THP-1	TNF-α, IL-1β, IL-6, PGE2, thromboxane B2 and COX-2	ELISA	BRO (1 to 100 mg/mL)	BRO significantly reduced TNF-α, IL-β and IL-6 of PBMC induced by LPS and THP-1.
Insuan et al. (2021)	RAW264.7	NO, IL-6 and TNF-α	MTT assay, ELISA, GRIESS and Western blotting	Crude bromelain (CB) (0.01; 0.02 and 0.04 mg/mL) or purified bromelain (PB) (0.02; 0.04 and 0.08 mg/mL)	The inhibitory potency of PB was stronger than CB. NO release was inhibited by PB in a dose-dependent manner. The level of IL-6 produced was drastically reduced by CB, however TNF-α level did not changed.

Hong et al. (2021)	hDPCs	IL-1β, IL-6, IL-8, ICAM- 1, VCAM-1, ERK, p65 e MAPK	Western blotting, ELISA and immunofluorescen ce	BRO (0.0025; 0.005; 0.01; 0.02 and 0.04 mg/mL	BRO significantly decreased IL-1β, IL-6, IL-8, ICAM-1 and VCAM-1 levels, p65 phosphorylation in the cytoplasm and nucleus. It also reduced ERK and mitogen- activated protein kinases levels.
Kasemsuk et al. (2018)	U937	COX2, MIP-1α, MCP-1, IL-8 and IL-1β	MTT assay and PCR	BRO 100 mg/mL	BRO suppressed proinflammatory cytokines, chemokines and COX pathway.
Kleef et al. (1996)	PBMC	CD44, CD62-L, CD16, CD56, CD49 and beta(2)-integrins CD11a- c	Immunocytometry	BRO (10 mg/mL)	Lymphocytes treated with BRO had their CD44 and CD62-L molecules reduced. Moderate increase in beta(2)- integrins CD11a-c expression. A selective modulation of cell adhesion molecules (CAM) has been observed in T cells and NK cells.
Mynott et al. (2002)	Caco-2	ERK-1, ERK-2, JNK and MAPK	Immunoreactivity assay and measurement of IL- 8 secretion	BRO (0.001; 0.025 and 0.005 mg/mL)	BRO blocked ERK-1, ERK-2 and JNK activation induced by *sorovar Typhimurium* in Caco- 2. It also inhibited carbachol and anisomycin-induced MAPK signalling.
Mynott et al. (1999)	T cells	ERK-2, IL-2, IL-4 and IFN-γ	Evaluation of extracellular proteases in blocking signal transduction	BRO (50 or 100 mg/mL)	BRO inhibited T cell signal transduction.
Muller et al. (2016)	Human cholangiocarcinoma cells	NFκB, AMPK, p-AKT, p-ERK, p-Stat3	Analysis of proliferation, invasion and apoptosis assay	BRO (150 and 200 μM)	BRO inhibited NF-κB/AMPK signalling as well as its downstream signalling proteins such as p-AKT, p-ERK, p- Stat3.
Onken et al. (2008)	Colonic mucosa	G-CSF, GM-CSF, IFN-γ, IL1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12 (p70), IL-13, IL-17, MCP-1, MIP-1β and TNF-α	Immunoassay	BRO (1 mg/mL)	BRO decreased G-CSF, GM- CSF, IFN-γ, CCL4/ macrophage inhibitory protein (MIP)-1β, and TNF-α secretion in inflamed tissue by IBD.
Rose et al. (2006)	MMLC	IL-6 and IL-10	ELISA	BRO and papain (0.003; 0.01 and 0.03 mg/mL (both substances together)	Both were able to increase IL- 6 expression in a dose- dependent manner. IL-10 levels were not modulated.

4. Discussion

By highlighting the biomarkers evaluated in the studies, it was observed that bromelain presented an anti-inflammatory effect, decreasing the activity of cytokines, interleukins and prostaglandins (Huang et al. 2008; Kasemsuk et al. 2018) in addition to contribute to the healing process by reducing the massive presence of fibroblasts for example, but also acting against tumour cells leading to apoptosis (Müller et al. 2016; Brochard et al. 2021).

Studies suggest that the anti-inflammatory effects of bromelain may be attributed, in part, to its ability to process in a proteolytical way the PSGL-1 and thereby reduce the number of cells that interact with P-selectin presented in the inflamed endothelial during the initial phases of the inflammatory response (Banks et al. 2013). Bromelain presented a similar effect when applied in an inflammatory exudate, reducing significantly PGE2 and substance P concentrations (Gaspani et al. 2002).

Bromelain treatment decreased cytokine production, affecting mediating inflammatory processes and ERK-2-mediated signal transduction by leukocytes and colonic epithelial cell lines *in vitro* (Mynott et al. 2002). A later concomitant study reported that bromelain decreased intestinal inflammation in humans with ulcerative colitis (Kane and Goldberg 2000).

When evaluating the anti-inflammatory activity of bromelain on A431 cells, a modulation in the expression of COX-2 levels was evidenced, which is a key mediator in the inflammatory response, suggesting that COX-2 is one of the molecular targets of bromelain (Bhui et al. 2009).

Bromelain exerted an inhibitory effect on the expression of several chemokines, including MIP-1α, MIP-1β, MCP-1 and IL-8 in human monocytes activated by LPS. In addition, it downregulated the expression of the cytokines IL-1β, IL-6 and the COX-2 enzyme, attenuating the inflammatory response (Kasemsuk et al. 2018).

Cytokines such as TNF-α and IL-1β act as key initiators to stimulate inflammatory events that lead to the tissue destruction, a common condition in chronic inflammatory diseases (Andreakos et al. 2004). Bromelain reduced the serum production of some cytokines in the evaluated studies. Thus, the inhibition of pro-inflammatory and proximal cytokines, such as IL-1β, could suppress the production of several inflammatory mediators and the immune response amplification in various diseases (Hou et al. 2006; Bottega et al. 2021).

The healing process is a product of inflammation. In this context, based on the results observed in different studies included in this review, the potential of bromelain in this process was evidenced, since it interferes in chemical mediators related to the chain of underlying reactions. It was observed that bromelain accelerated wound healing in different experimental studies, which one of the evaluated pathways was the migration decrease of normal human dermal fibroblasts under hypoxic conditions (Singer et al. 2011; Rosenberg et al. 2012). *In vitro* bromelain supplementation can be notably useful in preventing a massive presence of fibroblasts in wounds, which are accompanied by hypoxic conditions, burns and radiation wounds, for example (Yarnold and Brotons 2010).

The studies presented in this systematic review promote discussion and provide subsidies to continue research on the therapeutic potential of bromelain and its

possible applications in the pharmaceutical industry. *In vitro* studies demonstrated positive effects, however, more studies are needed, to establish parameters such as dosage, frequency and possible effects of its supplementation.

Furthermore, it is important to highlight that the effects differed according to each cell line employed, concentration, exposure time, and whether the application of bromelain was isolated or in combination with other bioactive or chemotherapeutic compounds. *In vitro* studies present limitations since they need to be corroborated in *in vivo* studies, to establish the best protocol for clinical trials, including the dosage, route, and combination or not with other compounds. All these variables limit the results found in the present review.

5. Conclusion

From the results found in this review, it was possible to verify that bromelain presents a satisfactory activity on inflammatory processes *in vitro*, since it has mechanisms capable to modulate them by reducing inflammation mediators and can be used isolated or in combination with other substances, in different concentrations and cell lines.

Authors' contributions

T.A.N. and A.A.S. conducted the literature review, database search, data extraction and edited drafts of the manuscript as well as the final version. I.C.P. and H.A.S.E. conducted the text review, database search, data extraction, graphic formatting and contributed to the final revision. A.M.C.P.S., M.T.I. conducted database search and data extraction. L.O.L., M.E., N.D., M.T.I. provided content input into draft versions, conducted the translation and edited drafts of the manuscript. J.M.C.S. and F.L.T.L.S. guided the research, contributed to the data extraction, provided intellectual input into draft versions and reviewed the drafts and final version.

Disclosure statement

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