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Nano-tribology: an overview

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1. Introduction:

According to, the pineapples original continent is South America, most likely in Argentina, Uruguay and southern and south-eastern Brazil. Its fruit is consumed natural, or in form of ice cream, candy, lollies, soft drinks and homemade juices. The Brazil cultivates over 70,000 hectares of pineapple (IBGE, 2011) and thus considered one of the largest producers in the world. In 2008, the production area was estimated in 62,862 hectares spread over several states, and minas gerais being one of the major producers, with an area of 7,600 hectares and production estimative of 597,895 t.

The pineapple fruit is the marketable part of the plant, representing only 23% of total plant, the remainder, composed of stem, leaf, bark and crown, is considered agricultural waste and is usually disposed or used in composting, losing completely its economic potential to extract high valued products (*crestani et al.,2010).*

The quality of pineapple fruits is referred to their physical features external, such as peel colour, size and shape of the fruit, and internal conferred by broad range of constituents, especially by the high levels of sugars and proteolytic enzymes, particularly bromelain (*abilio et al., 2009*).

The large percentage of waste generated during industrial processing, about 60 of total weight of the fruit, contains appreciable amounts of bromelain, a protease that has numerous applications in the food and pharmaceutical industries, which have high aggregate value and does not disappear when the fruit ripens (*Coelho et al.,2013*). Bromelain is chemically known since 1875 and used as a phytotherapeutical agent (*Pavan et al., 2012*). It's included in the classification of hydrolase, more specifically from the group of cysteine proteinase, being able to break peptide bonds, separating proteins and amino acids.

The raw material most often used to obtain bromelain are the mature stalks of pineapple utilizing them after harvesting the fruit, however, also leaves, juice, peel and waste can be utilized *(crestani et al.,2010*). Bromelain has a wide range of therapeutic benefits, but the mode of its action is not well understood. It is proved that bromelain is well absorbed in body after oral administration and has not important side effects even after prolonged use (*Pavan et al., 2012).*

This enzyme is used in different sectors, all based on your activity proteolytic, among which stand out the property to facilitate and digestion of proteins and are therefore added in digestive drugs, and the ability to soften meat (*abilio et al., 2009).* Its economic importance is related to the production of pharmaceuticals, their effect on the digestive system, replacing pepsin and

trypsin in treatment of pancreatic insufficiency. It is also used in the treatment of heart disease, rheumatoid arthritis, surgical trauma, sinusitis, due mainly to its ability to facilitate blood clotting, reducing edema and also provide an anti-inflammatory effect *(ketnawa et al., 2012).*

And furthermore, has an extensive use in the food industry, such as in clarification of beers, in cheese making, the softening of meat, in preparation of infant and dietetic foods, among others, in the treatment of digestive disorders, wounds and inflammations, preparation of hydrolysed collagens, in textile industries, for softening the fibres and also in the production of detergents (*Ferreira et al., 2011b).*

The commercial production process obtains bromelain from cooled pineapple juice using ultrafiltration, centrifugation and lyophilisation yielding a yellowish powder with 30 to 40% protein content, and uncertain biological activity (*Coelho et al., 2013, larocca et al., 2010),* which price exceeds 1800 US$/kg (sigma, 2014). The continuing interest in bromelain, due to its numerous applications, both in food industry, and in the pharmaceutical industry, makes this enzyme one of best protease of vegetal origin. But the Brazilian companies interested in its use must resort to importing bromelain, once it is not produced locally. Thus, several studies have been performed either to optimize bromelain biological activity (Godoi, 2007) or purify it using cross-flow filtration (*lopes et al., 2009*), reversed micelles *(fileti et al., 2009*) and aqueous two-phase systems (ATPS) *(Ferreira et al., 2011c, Ferreira et al., 2011a*). This, it is essential to develop a feasible process to purify bromelain based on national technology where the big challenge is obtaining bromelain enzyme and maintain its stability during all steps involved and fractional precipitation has been used successfully to obtain biomolecules (*longo et al.,2010).*

Pineapple has been used as a traditional medicine by several cultures throughout time and bromelain has been established since 1876. Bromelain gets its properties mainly due to the presence of its sulfhydryl proteolytic enzymes. Bromelain is classified as stem bromelain or fruit bromelain depending on the origin of the protease. Bromelain is present throughout the pineapple plant however the concentration and composition may vary depending on the part of the fruit and its variety (*Gautam et al., 2010*). Extraction of bromelain makes it feasible as its bioavailability rises during maturity of fruit rather than during development (Maurer, 2001).

Extensive studies have been done with bromelain to explore its clinical properties. Thus, finding effective extraction methods is necessary. Bromelain is found in pineapple wastes such as core, peel and leaves in reduced quantities compared to the fruit (*sriwatanapongse et al., 2000*). Various purification strategies have been discussed and developed for extraction of

bromelain. It precipitates at 20% to 60% with ammonium sulphate showing highest recovery at 20%. Dialysis to be followed after ammonium sulphate precipitation to concentrate and to purify bromelain further by a cost-effective manner (*pardhi et al., 2016).*

Acne is a skin condition affecting the skins oil glands is a very prominent condition. It can a lot of psychological disturbances and stress on the individual that it affects. P. acne is an opportunistic pathogen that plays an important role in the growth and cause of acne. S. aureus is a part of the normal skin microbiota and is associated with skin conditions such as folliculitis and also reported to enhance the effect of other microbes in acne lesions (*Kumar et al., 2016).*

Hence this study aims to evaluate the potential of bromelain in alleviation of acne owing to its diverse antimicrobial properties. Active bromelain was isolated from waste parts of pineapple and its effects on bacterial pathogens like acne was studied. Bromelain will aid as a potent anti-inflammatory agent that will act as beneficial additive to the formulation. Using bromelain from the waste parts of pineapple will help in waste recycling as well as make the whole process cost-effective.

2. Aim and objective:

2.1. Aim:

The aim of the research is to isolate bromelain enzyme from pineapple peel by using simple extraction and purification methods and its application towards cosmetic.

2.2. Objective:

- To extract the bromelain enzyme from pineapple peel
- To purify bromelain by ammonium sulphate method
- To measure the protein content by using Lowry's method
- To measure the enzyme activity by using kunitz method
- To formulate cosmetic by using extracted bromelain enzyme

3. Review of literature:

Taxonomical classification of *Ananas comosus*

Domain	Eukarya

Kingdom	Plantae
Phylum	Anthophyte
Class	Lilopsida
Order	Bromeliales
Family	Bromeliaceae
Subfamily	Bromelioideae
Genus	Ananas
Species	Ananas comosus

Pineapple is the common name of *Ananas comosus* (*syns. A. sativus, Ananassa sativa, Bromelia ananas, B. comosa*). Pineapple is the leading edible member of the family Bromeliaceae, grown in several tropical and subtropical countries including Philippines, Thailand, Indonesia, Malaysia, Kenya, India, and China. It has been used as a medicinal plant in several native cultures and these medicinal qualities of pineapple are attributed to bromelain, which is a crude extract from pineapple that contains, among other compounds, various closely related proteinases, exhibiting various fibrinolytic, antiedematous, antithrombotic, and anti-inflammatory activities *in vitro* and *in vivo*. Bromelain has been chemically known since 1875 and is used as a Phyto medical compound. Bromelain concentration is high in pineapple stem, thus necessitating its extraction because, unlike the pineapple fruit which is normally used as food, the stem is a waste by-product and thus inexpensive. A wide range of therapeutic benefits have been claimed for bromelain, such as reversible inhibition of platelet aggregation, sinusitis, surgical traumas, thrombophlebitis, pyelonephriti angina pectoris, bronchitis, and enhanced

Absorption of drugs, particularly of antibiotics. Several studies have been carried out indicating that bromelain has useful Phytomedical application. However, these results are yet to be amalgamated and critically compared so as to make out whether bromelain will gain wide acceptance as a Phyto medical supplement. Bromelain acts on fibrinogen giving products that are similar, at least in effect, to those formed by plasmin. Experiment in mice showed that antacids such as sodium bicarbonate preserve the proteolytic activity of bromelain in the gastrointestinal tract. Bromelain is considered as a food supplement and is freely available to the general public in health food stores and pharmacies in the USA and Europe. Existing evidence indicates that bromelain can be a promising candidate for the development of future

oral enzyme therapies for oncology patients. Bromelain can be absorbed in human intestines without degradation and without losing its biological activity. *(Rajendra et al., 2012)*

Enzymes plays an important role in digestion of food, as they consist of protein would help to perform various functions in body. Certain enzymes produce in the body, while some are provided by foods. The enzymes present naturally in plants are metabolised after ingestion and are considered safe. They are important for the quality of fresh fruits i.e. growth and ripening of fruits and also maintaining the same during the transportation & storage. Most of the enzymes are important to maintain the quality and metabolism of fruit, but some can have an undesirable effect on colour, flavour, taste etc of the fruits. Enzyme lipoxygenase can alter the development of flavour & odour of certain fruits. The other enzymes which affects the flavour and odour are lipase and peroxidase. The discoloration occurs in fruits and vegetables along with negative effect on their taste and nutrition is mainly due to phenol oxidase. The fruits and vegetables contain various substances which affects their physical and chemical appearance. The presence of pectic substances affects texture; activity of pectinase present in fruits ensures the fruit softening; presence of ascorbic acid affects vitamin availability. Pineapple is considered to be the main source from which bromelain is extracted. Bromelain is extracted from fruit as well as stem. The one extracted from fruit is known as fruit bromelain, while the one extracted from stem is called as stem bromelain. It consists of 212 amino acids, with a molecular weight of 33 kDA. This enzyme helps in protein breakage & re-main stable in pH ranges from 3 to 7 and temperature between 400C & 600C. The bromelain showed a maximum activity at pH 7 and 50°C at the simple ex-traction and most proteolytic activity at pH 8 and 60°C. (*Bandana et al.,2018*)

Bromelain is one of the few plant proteases that can be extracted from a variety of plant components, including the fruit pulp, stem, peel, and leaves. The concentration of bromelain is higher in the stem than in the fruit and, therefore, the stem is one of the most available and abundant sources of bromelain. Other parts outside the stem have also been investigated for their presence of bromelain, including the peel, core, and crown. Given this vast array of potential enzyme sources from the pineapple plant, researchers have now focused their efforts on the search for alternative, more efficient methods to obtain purified bromelain in fewer and more economical steps. Micropropagation processes, reverse micellar systems, membrane filtration, and aqueous two-phase extraction, are the most studied and promising extraction methods for bromelain. Moreover, scientists continue to utilize the advance recombinant DNA technology to yield large-scale production and purification of recombinant bromelain. *(Carolina et al.,2021).*

Vicente Marcano, a Venezuelan scientist, discovered bromelain for the very first time in 1891, and its extraction and investigation commenced in 1894. Bromelain is abundant in both the fruit and the stem of pineapple trees, with Heinecke revealing in 1957 that the pineapple stem contained significantly more bromelain than the actual fruit, enabling the monetization of a waste by-product that is stem bromelain. Crude pineapple aqueous extract is used to purify its defensive protein bromelain. This protein of the pineapple plant shields it during its growth, maturation, and ripening periods. Bromelain is extracted as a glycosylated monomeric single protein from both the stem and the fruit. (*Paridhi et al.,2022*)

Several cysteine endopeptidases (pineapple fruit- fruit bromelain, pineapple stem - ananain, stem bromelain, comosain) and other elements, such as phosphatases, peroxidases, carbohydrates, ribonucleases, protease inhibitors, cellulases, glycoproteins, and organically bound calcium are present in crude bromelain. A sulfhydryl moiety makes up its functional element. Bromelain from the stem has a stable secondary structure. In between pH 7 and 10 it is active, but it loses its action irreversibly above pH 10. At a pH of 14, stem bromelain forms a typical heated gelatinous mass configuration. Finally, bromelain has been shown to remain stable for a long time when kept at temperatures below 20 ºC. (*Paridhi et al.,2022)*

Besides its clinical applications bromelain has been subjected to many other industries due to its enormous benefits. Researchers thereby are trying various conventional as well as latest purification techniques to achieve bromelain in highest purified form at reduced cost (Arshad et al. 2014). As compared to fruit, bromelain concentration is high in stem and is thus a cheaply available source of bromelain (*Tochi et al. 2008*). Other parts of pineapple are also investigated for the presence of bromelain (*Ketnawa et al. 2012***)** including peel, core and crown etc. Extraction of bromelain from these parts is attractive not only from environmental point of view but also economically (*Novaes et al. 2013*). Bromelain can be easily extracted from the juice of pineapple by ultrafiltration (*Larocca et al. 2010*) but still FBM is not commercially available due to being different from SBM (*Pavan et al. 2012*). The marketable bromelain is mostly extracted from the stem of pineapple through centrifugation, ultrafiltration, lyophilization (*Corzo et al. 2011***)** and two-step Fast Protein Liquid Chromatography (FPLC) (*Harrach et al. 1998*).

Once extracted, the crude mixture containing required enzyme is then exposed to numerous purification stages to eradicate impurities that may interfere with bromelain to hinder its application and reduce the specific activity of the enzyme **(***Illanes 2008*). Product purity is the key factor which may constitute a large proportion of the total enzyme production cost

(*Lightfoot 1990*). Several conventional isolation and purification techniques are now obsoleted because of their low purification potential (*Soares et al. 2012*). So, the extraction and purification strategy designed should be selective for the desired product, cheap, high yielding and speedy (*Gupta et al. 2004***).** Inflammation is pivotal in the development of cancer during cellular transformation, proliferation, angiogenesis, invasion and metastasis. It has been demonstrated that suppression of chronic inflammation may reduce the cancer incidence and also inhibit cancer progression. Cyclooxigenase-2 (COX-2) is an important component of cancer-associated inflammation that is involved in the synthesis of prostaglandin E2 (PGE-2). PGE-2 is a pro-inflammatory lipid that also acts as an immunosuppressant, as well as a promoter of tumor progression. COX-2 converts arachidonic acid into PGE-2 and promotes tumor angiogenesis and cancer progression. It has been shown that bromelain downregulates COX-2 and PGE-2 expression levels in murine microglial cells and human monocytic leukaemia cell lines. Bromelain activates the inflammatory mediators, including interleukin (IL)-1β, IL-6, interferon (INF)-γ and tumor necrosis factor (TNF)-α in mouse macrophage and human peripheral blood mononuclear cells (PBMC). These results indicated that bromelain potentially activates the healthy immune system in association with the rapid response to cellular stress. Conversely, bromelain reduces IL-1β, IL-6 and TNF-α secretion when immune cells are already stimulated in the condition of inflammation-induced over production of cytokines. Studies have shown that bromelain reduced the expression of INF-γ and TNF-α in inflammatory bowel disease. A study demonstrated that bromelain diminished the cell damaging effect of advanced glycation end products by proteolytic degradation of receptor of advanced glycation end products and controlled the inflammation.

The cell surface marker, cluster of differentiation (CD) 44 is expressed by cancer and immune cells directly involved in cancer growth and metastasis. Furthermore, CD44 regulates lymphocyte requirement at the site of inflammation. Bromelain was shown to reduce the level of CD44 expression on the surface of mouse and human tumor cells, and regulate lymphocyte homing and migration to the sites of inflammation. Furthermore, bromelain modulates the expression of transforming growth factor (TGF)-β, one of the major regulators of inflammation in patients affected by osteomyelofibrosis and rheumatoid arthritis. There are various studies that report the immunomodulatory effect of bromelain. Bromelain activates natural killer cells and augments the production of granulocyte- macrophage-colony stimulating factor, IL-2, IL-6 and decreases the activation of T-helper cells. Thus, bromelain decreases the majority of inflammatory mediators and has demonstrated a significant role as an anti-inflammatory agent in various conditions. (*Vidhya et al., 2016*)

Bromelain is a common enzyme to evaluate the antibacterial activity of fruit or plant extracts. For example, Dutta and Bhattacharyya evaluated the antimicrobial activity of an aqueous extract of pineapple leaves which was active against strains of E. coli, S. aureus and B. subtilis. The aqueous extract inhibited 70 to 95% of microbial growth and its MIC varied from 1.65 to 4.95 mg/mL. Besides that, in previous research, the result of Herliani study using the minimum inhibitory concentration test of the dilution method was found that the concentration of bromelain enzyme capable of inhibiting Streptococcus mutants was 30% (v / v). Considering that, this research was conducted pineapple peel extract to get bromelain enzyme that can be formulated on the mouthwash for against Streptococcus mutants. In this study used an alternative surfactant, which is tween 80 which can be used as a surfactant and cleaning agent. Tween 80 belongs to non-inonic surfactants that have low toxicity so widely used in the eating industry, cosmetics and oral drug formula. (*h rahmi et al.,2019).*

Methods is of great interest, aiming to obtain more economically feasible processes (*Leite et al., 2012)* The commercial production of bromelain from pineapple consists of several steps such as extraction, purification, drying and packing in the powder form (*Nor et al, 2015*). The isolation and purification stages require the most expensive materials and operations or are exceedingly laborious. In fact, considering both economic and technical aspects, the purification step corresponds to 70-90% of the total production cost (*Soares et al., 2012*).

It is desirable that the purification is cost-effective, rapid, high-yielding and robust. Moreover, it should allow continuous product recovery, with a high capacity and selectivity for the desired products (*Bala et al., 2012*). Many approaches have been used to increase the purity and activity of bromelain enzyme preparations (*Amid et al., 2011*) and these strategies include ion exchange chromatography, ammonium sulphate fractionation, aqueous two-phase systems, as well as membrane filtration processes *(Nor et al., 2015).*

In downstream processing, it is difficult and expensive to selectively recover a targeted enzyme from a crude extract due to the low protein concentration among various contaminants and the similarity of their physical properties (*Soares et al., 2012*). In bromelain separation, for instance, there are other compounds such as phosphatases, glucosidases, peroxidases, cellulases, glycoproteins and carbohydrates (*Silvestre et al., 2012*) that may decrease the yield of purification. Considering the increase of biotechnology, novel purification technologies are demanded to improve the overall enzyme yields and reduce the number of steps involved in the production of a specific one (*Wu et al., 2017*), as well as produce highly active biocatalysts. Thus, the study of both purification and alternative extraction). (*Danielly et al., 2019*).

Ammonium sulphate precipitation, the crude extract was subjected to ammonium sulphate precipitation at 40% saturation (226 g/l). The extraction was carried out in an ice box over a magnetic stirrer. Ammonium sulphate was gradually added pinch by pinch in a period of 30 min. It was allowed to settle for 24 h at 4 °C. The solution was then centrifuged at 6000 rpm for 10 min at 4 °C. The pellet was later reconstituted in minimum volume of 10 mM Tris, (pH–7.0) and then subjected to dialysis. The supernatant obtained was further precipitation at 60% saturation (120g/l). The solution was allowed to stand for 24 h at 4 °C and then centrifuged at 6000 rpm for 10 min at 4 °C. The pellet obtained was reconstituted in minimum volume of 10 mM Tris, (pH – 7.0).

Dialysis, 7 cm of dialysis membrane was added into boiling distilled water with 2% sodium carbonate for 45 min. The membrane was boiled again for 45 min in distilled water, the membrane was left overnight in Acetate buffer (pH-7.0). The fractions from ammonium precipitation were loaded into the activated dialysis membranes and tagged. They were equilibrated into a beaker with acetate buffer. The process was carried out for 24 h in an ice box with the replacement of buffer every 6 h. The samples from membranes were then unloaded and labelled as purified bromelain samples (*Sukaina et al., 2021)*

Bromelain in various applications with up-to-date literature on the purification of bromelain from pineapple fruit and waste such as peel, core, crown, and leaves. Bromelain, a cysteine protease, has been exploited commercially in many applications in the food, beverage, tenderization, cosmetic, pharmaceutical, and textile industries. Researchers worldwide have been directing their interest to purification strategies by applying conventional and modern approaches, such as manipulating the pH, affinity, hydrophobicity, and temperature conditions in accord with the unique properties of bromelain. The amount of downstream processing will depend on its intended application in industries. (*zatul et al.,2014*)

Pineapple *(Ananas comosus)* has been used as a traditional medicine by several cultures throughout time and bromelain has been established since 1876. Bromelain gets its properties mainly due to the presence of its sulfhydryl proteolytic enzymes. Bromelain is classified as stem bromelain or fruit bromelain depending on the origin of the protease. Bromelain is present throughout the pineapple plant however the concentration and composition may vary depending on the part of the fruit. Extraction of bromelain makes it feasible as its bioavailability rises during maturity of fruit rather than during fruit development (*Maurer, 2001).*

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finding effective extraction methods is necessary. Bromelain is found in pineapple wastes such as core, peel and leaves in reduced quantities compared to the fruit (*Sriwatanapongse et al., 2000*). Various purification strategies have been discussed and developed for extraction of bromelain. It precipitates at 20% to 60% with ammonium sulphate showing highest recovery at 20%. Dialysis to be followed after ammonium precipitation to concentrate and to purify bromelain further by a cost-effective manner (*Pardhi et al., 2016*).

Skin problems like wrinkles, acne and dry skin seemed to be solved using proteases such as papain and bromelain (*Ozlen and Chatsworth, 1995*). *Loon et al., (2018)* observed sensitivity of *S. aureus* against pineapple extract. Partially purified bromelain from the pineapple core also inhibited the growth of *S. epidermidis* and *P. acne* (*Hidayat et al., 2018*). Bromelain has also been extensively used as a line of treatment for periodontal gingivitis and found to be effective against periodontal pathogens namely, *S. mutants, A. actinomycetemcomitans, P. gingivalis* (*Praveen et al., 2014*). Edema caused by post-surgical trauma also has been effectively treated by bromelain (*Mackay and Miller, 2003*).

Acne is a skin condition affecting the skin's oil glands is a very prominent condition. It can create a lot of psychological disturbances and stress on the individual that it affects. *P. acne* is an opportunistic pathogen that plays an important role in the growth and cause of acne. *S. aureus* is a part of the normal skin microbiota and is associated with skin conditions such as folliculitis and also reported to enhance the effect of other microbes in acne lesions (*Kumar et al., 2016*).

Hence this study aims to evaluate the potential of bromelain in alleviation of acne owing to its diverse antimicrobial properties. Active bromelain was isolated from waste parts of pineapple and its effects on bacterial pathogens like acne was studied. Bromelain will aid as a potent anti-inflammatory agent that will act as beneficial additive to the formulation. Using bromelain from the waste parts of pineapple will help in waste recycling as well as make the whole process cost-effective. *(sukaina et al.,2021*)

Proteases (also known as peptidases or proteinases), their substrates and inhibitors are of great relevance to biology, medicine, and biotechnology. Proteases are referred to as a group of enzymes that hydrolyse the protein bonds of amino acids (proteolysis). Proteases have evolved multiple times, and different classes of protease can perform the same reaction by completely different catalytic mechanisms (*Gupta and Khare, 2007; Kalpana Devi et al., 2008*). Proteases constitute the largest group of enzymes in bioindustry with an array of applications. They play an important role in industrial biotechnology, especiallyin detergents, foods, pharmaceuticals,

and in PCPs. Proteolytic enzyme is essential for several physiological processes like digestion of food proteins, protein turnover, cell division, blood clotting cascade, signal transduction, processing of polypeptide hormones, etc. (*Li et al., 2013*).

The vast variety of proteases, with specificity of their action and application, have attracted worldwide attention to exploit their physiological as well as biotechnological applications (*Poldermans, 1990*). They are considered eco-friendly because the appropriate producers of these enzymes for commercial exploitation are nontoxic and nonpathogenic and are designated as safe (*Gupta et al., 2002*). Proteases are used extensively in the pharmaceutical industry for preparation of medicines, such as ointments for debridement of wounds. They are also used in denture cleaners and as contact lens enzyme cleaners (*Ogunbiyi et al., 1986*). Proteases that are used in the food and detergent industries are prepared in bulk quantities and are used as crude preparations; whereas those used in medicine are produced in small amounts but require extensive purification before application (*Bholay and Patil, 2012*). The thermostability and their activity at high pH and the alleviation of pollution characteristics have made proteolytic enzymes an ideal candidate for laundry applications. Alkaline proteases are supplemented in different brands of detergents for use in home and commercial establishments. Enzymes have been added to laundry detergents for the last 50 years to facilitate the release of proteinaceous material in stains, such as those of milk and blood. The proteinaceous dirt coagulates on the fabric in the absence of proteinases as a result of washing conditions. (*kiran et al.,2016).*

4. Materials and methods:

4.1. Sample collection:

Fresh pineapple peel was collected from nearby fruit shop in Coimbatore.

4.2. Extraction:

4.2.1. Preparation of solutions:

4.2.2. Preparation of monobasic sodium phosphate solution:

Monobasic sodium phosphate (2.75 g) was dissolved in distilled water and the volume was make up to (100 ml) in the volumetric flask to give (0.2 M) solution. It was labelled as solution A.

4.2.3. Preparation of dibasic sodium phosphate solution:

Dibasic sodium phosphate (7.16 g) was dissolved in distilled water and the volume was make up to (100 ml) in the volumetric flask to give (0.2 M) solution. It was labelled as solution B.

4.2.4. Preparation of pH 7.2 sodium phosphate buffer solution:

Solution A (29.40 ml) was mixed with (70.60 ml) of solution B. then the volume was made up to (200 ml) with distilled water. The pH of this solution (0.1 M) was measured by a pH meter and it was adjusted to pH 7.2. it is necessary by using (0.1 M) sodium hydroxide or (0.1 M) hydrochloric acid solution.

5. Procedure:

Pineapple peel was cut into small pieces and then grind using a blender, then it was filtered and the juice will be extracted. After that 200 ml of phosphate buffer was added into the extracted pineapple juice, again this mixture was filtered through muslin cloth. The filtrate was collected and left overnight at 4^{o} Celsius. After that, it was filtered and the filtrate was use for extraction of enzyme.

6. Purification:

6.1. Ammonium sulphate precipitation:

The crude extract containing the enzyme was used for the isolation of bromelain. The filtrate was brought to 20% saturation (22.68 g) by slow addition of solid ammonium sulphate. The filtrate was collected by centrifugation at 3000 rpm for 20 minutes. The ammonium sulphate (49.40 g) was then added into the supernatant to achieve 60% saturation. The partially purified bromelain was then collected by centrifugation at 6000 rpm for 30 minutes.

7. Dialysis:

7.1. Activation of dialysis membrane:

7 cm of dialysis membrane was added into boiling distilled water with 2% sodium carbonate for 45 minutes in distilled water, the membrane was left overnight in acetate buffer (pH-7.0).

7.2. Dialysis:

The fractions from ammonium sulphate precipitation were loaded into the activated dialysis membranes and tagged. They were equilibrated into a beaker with acetate buffer. The process

was carried out for 24 h in an ice box with the replacement of buffer every 6 h. the samples from membranes were then unloaded and labelled as purified bromelain sample.

7.3. Estimation of protein content by folin-lowry assay:

The protease mixture was estimated for the protein content by using Folin-Lowry assay. Bovine serum albumin (BSA) was used as a standard at 1mg/ml. freshly prepared alkaline copper sulphate solution, Folin-Ciocalteu phenol reagent (FC) 1N was used. 1 ml of protein was added to 5.5 ml alkaline copper sulphate solution and incubated for 10 minutes. 0.5 ml of FC reagent was added to the mixture and incubated in the dark for 30 minutes and absorbance was recorded in 660 nm.

8. Measurement of enzyme activity:

The fractions of enzyme were determined by proteolytic activity according to the kunitz method using casein as a substrate. The fraction was added with Tris--HCL buffer pH 8.0 and 1% casein substrate solution. The mixture was incubated for 30 minutes at 37^{o} Celsius. The reaction was stopped by the addition of 10% TCA solution. The control solution was prepared by mixing the fraction to be tested with Tris-HCL buffer pH 8.0 and then added the 10% TCA solution. The mixture was incubated for 30 minutes at 37 ^{o} Celsius. Then 1% solution of casein substrate was added. The mixture was incubated again for 30 minutes at 4^{o} Celsius. The sample and control solutions were measured using a UV-Vis spectrophotometer at 280 nm. The specific activity of the enzyme was obtained by comparing the amount of activity unit (U/mL) with the enzyme protein content (mg/ml)

8.1. Drying:

The purified bromelain was powdered using evaporation method. The purified bromelain extract will pour into glass plates and then it was placed on the water bath for evaporation in particular temperature. After evaporation the dried extract will scrape and collect in a glass container for further use.

8.2. Antibacterial activity test (well diffusion method)-extract:

Mueller Hinton agar plates were prepared and a well was drilled at the center of the media. The sample taken from acne were swabbed on separate petri plates and the bromelain crude extract were transferred using a micropipette into the hole. Then the plates were incubated for 24 hours at 37 degree Celsius and the results were recorded.

8.3. Product formulation (face cream):

Materials	Functions
Bromelain (1 gram)	Antimicrobial
Sheabutter (20 grams)	Soothing
Almond oil (20 ml)	Hydration
Beeswax (10 grams)	Emulsifier
Vitamin E oil (2 ml)	Antioxidant
Jojoba oil (0.15 ml)	Preservative
Coconut oil (10 ml)	Moisturizer
Rose extract	Pigment
Rose flavour	Fragrant

Place all ingredients in a glass jar. Bring a pot filled about 3-4 inches with water to a simmer. Put the jar, without its lid, in the pot and let it wait until the ingredients have melted. Stir occasionally. Once the mixture has melted and all is evenly combined, pour into a glass jar and let it place at room temperature until the cream hardens, then store it for use.

8.4. Antibacterial activity test (well diffusion method)-product:

Mueller Hinton agar plates were prepared and a well was drilled at the center of the media. The sample taken from acne were swabbed on separate petri plates and the formulated product were transferred using a micropipette into the hole. Then the plates were incubated for 24 hours at 37 degree Celsius and the results were recorded.

9. Result:

9.1. Collection of sample:

The pineapple peel sample was collected from nearby fruit shop in coimbatore.

Sample-Pineapple peel

9. 2. Extraction:

The pineapple peel was cut into small pieces and then grind, then it was filtered and the juice will be extracted. After that phosphate buffer was added into the extracted pineapple juice, again this mixture was filtered through muslin cloth. The filtrate was collected and left overnight at 4^{o} Celsius. After that, it was filtered and the filtrate was use for extraction of enzyme.

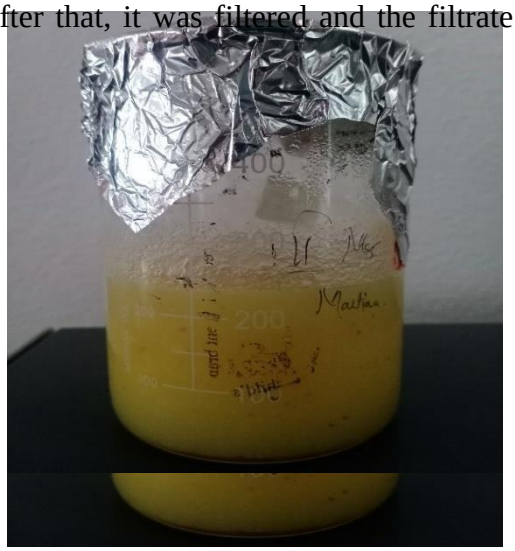


Figure. 1: extract

10. Purification:

10.1. Ammonium sulphate precipitation:

Purification is done by ammonium sulphate precipitation and dialysis. The crude extract containing the enzyme was used for the isolation of bromelain. The filtrate was brought to 20%

saturation (22.68 g) by slow addition of solid ammonium sulphate. The filtrate was collected by centrifugation at 3000 rpm for 20 minutes. The ammonium sulphate (49.40 g) was then added into the supernatant to achieve 60% saturation. The partially purified bromelain was then collected by centrifugation at 6000 rpm for 30 minutes.

10.2. Dialysis:

The fractions from ammonium sulphate precipitation were loaded into the activated dialysis membranes and tagged. They were equilibrated into a beaker with acetate buffer. The process was carried out for 24 h in an ice box with the replacement of buffer every 6 h. the samples from membranes were then unloaded and labelled as purified bromelain sample.

Figure. 2: Ammonium sulphate precipitation

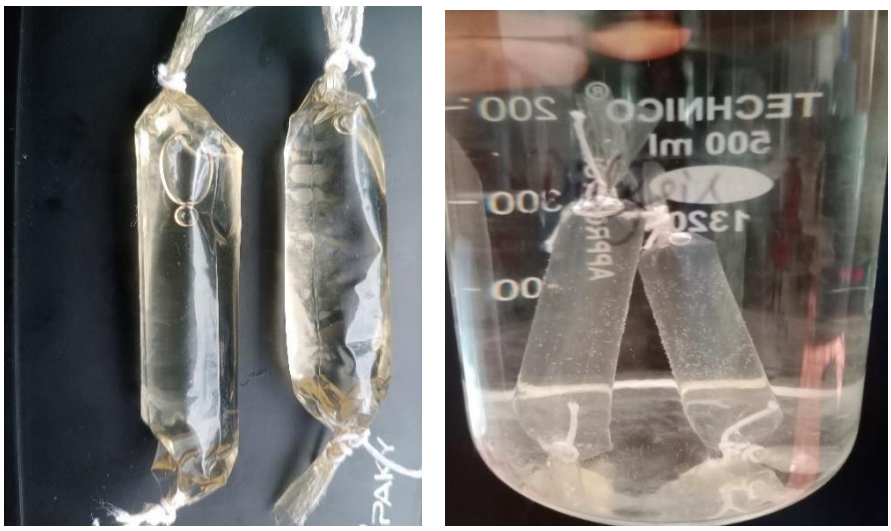
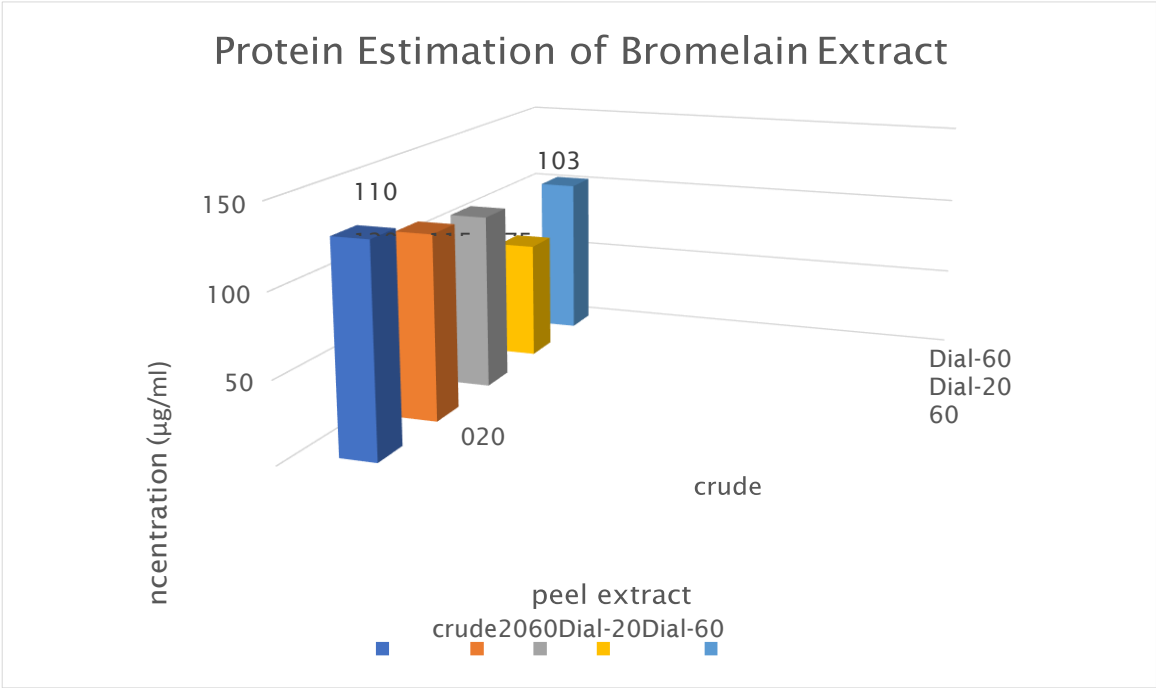


Figure. 3: Dialysis

10.3. Estimation of protein content by folin-lowry method:

The protein content was estimated by folin-lowry method. The values obtained in the crude extract from peel is (128 µg /ml). The crude extract was purified by ammonium sulphate precipitation and dialysis. The highest protein content after purification was observed in peel (103 µg/ml).



10.4. Measurement of enzyme activity:

Addition of ammonium sulphate, enzyme fraction was produced with the specific activity and

highest purity. However, other fractions also exhibit fluctuating specific activities. At this stage, fraction 2 (20-50%) as his purest fraction from the other fractions has purity 1.72 times greater than its crude enzyme. However, after further purification using the dialysis, fraction 2 has an increase specific activity, it was 120.83 units per mg with a purity of 2.32 times.

Table. 1 and 2 represents the activity of enzyme.

Fractionation with ammonium sulphate

Fraction	***Volume(ml)***	***Protein content (mg)***	***Proteolytic activity (U)***	***Specific activity (U/mg)***	***Purity***
Crude extract	155	2.88	149.83	52.03	
F1(0-20%)	7.2	0.26	6.60	52.03	0.49
F2(20-50%)	11	0.73	65.27	89.32	1.72
F3(50-80%)	242	0.04	1.07	24.89	0.48
F4(Residual filtrate)	224	1.84	44.71	6.09	0.12

Before and after Dialysis

Fraction	Volume(ml)	Protein content(mg)	Proteolytic activity (U)	Specific activity (U/mg)	Purity
F2(20-80%) Before dialysis	7	0.47	41.51	88.32	1.7
F2(20-80%) After dialysis	10	0.37	44.71	120.83	2.32

10.5. Drying:

Drying and powdering process is done by evaporation method. The purified sample was poured into glass plates then it was placed on the water bath for few minutes in particular temperature. After drying the sample was scraped and stored in powder form.

Figure. 4: powdered sample

10.6. Aantibacterial activity test (well diffusion method) - extract:

Mueller Hinton agar plates were prepared and a well was drilled at the center of the media. The sample taken from acne were swabbed on separate petriplates and the extract were transferred using a micropipette into the hole. Then the plates were incubated for 24 hours at plastic economy: Rethinking the future of plastics. Ellen MacArthur Foundation. 37 degree Celsius and the results were recorded. Zone of inhibition were observed for extract.

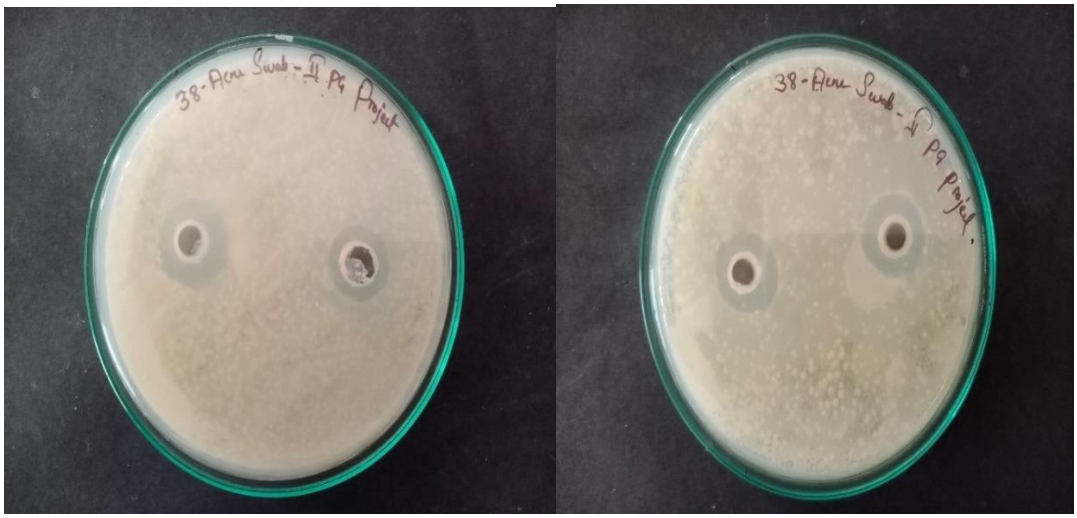


Figure. 5: Zone of inhibition (extract)

11. Production formulation:

Bromelain enzyme is act against acne so the face cream was formulated using isolated bromelain. It's also acts as anti- aging agent.

Figure. 5: Product (face cream)

11.1. Aantibacterial activity test (well diffusion method) – product:

Mueller Hinton agar plates were prepared and a well was drilled at the center of the media. The sample taken from acne were swabbed on prepared petri plate and the formulated product were transferred using a micropipette into the hole. Then the plates were incubated for 24 hours at 37 degree Celsius and the results were recorded. Zone of inhibition were observed.

Figure. 6: zone oh inhibition (product)

12. Discussion:

Pineapple is an age-old fruit, consumed and used traditionally for many of its medicinal properties. It has been used for debridement of skin (Houck et al., 1983). Bromelain is also used as a replacement during deficiency of pepsin and trypsin due to its activity and stability over a wide pH range (Balakrishnan et al., 1981). Generally, only the fruit is considered as the only edible member and the rest is treated as waste like the peel, core, crown, and the stem. Pineapple wastes are usually discarded and then allowed to biodegrade which leads to environmental degradation mainly due to carbohydrate rich contents. According to our study, active bromelain was obtained from the waste part of the pineapple. Peel demonstrated highest proteolytic activity. Ammonium sulphate precipitation and dialysis were applied for purification for the ease of usage, feasibility and the easy recovery of the enzyme with highest activity.

Measurement of enzyme activity is done by kunitz method, Protein estimation is done by Lowry's method. Acne is a skin condition affecting the skins oil glands is a very prominent condition. It can create a lot of psychological disturbances and stress on the individual that it affects. Purified extract and product exhibited highest inhibitory effect towards acne swab. A face cream was prepared using peel bromelain extract. This was a herbal face cream acclaimed to be used for anti-acne and anti-aging.

13. Summary and conclusion:

- Bromelain is a proteolytic enzyme found in pineapple fruit and also other parts and it has many beneficial effects and its also used as a dietary supplement.
- The sample (fresh pineapple peel) was collected from nearby fruit shop in Coimbatore.
- Peel was cut into small pieces and grinded then filtered using phosphate buffer and then the filtered juice was purified using ammonium sulphate precipitation and dialysis.
- Then the purified bromelain crude extract was tested for protein activity and enzyme activity by Lowry's method and kunitz method.
- The purified bromelain was dried and powdered using evaporation method and then the face cream was formulated using purified bromelain powder.

- The purified bromelain and formulated face cream were tested for antibacterial Activity against acne swab, zone of inhibition was observed

This work is exhibited that the purification of bromelain by Ammonium sulphate precipitation and Dialysis from the peel of the pineapple. Recovered extract maintain the better proteolytic activity, and the product were formulated using extracted bromelain. Moreover, a lot of attempts are required to be made to develop a simple and economical, effective technique to produce bromelain.

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