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Economical Production of Lycopene, An Antioxidant, from Tomato Skin (Solanum Lycopersicum), using Pectinase Enzyme.

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KEYWORDS

Lycopene, Solanum lycopersicum, Tomato skin, Pectinase, Enzymatic extraction, Antioxidants

ABSTRACT:

Introduction: Lycopene, a significant nutrient that promotes health, is the main source of the by-product that is recovered from the wastes of the tomato processing industry. Polyphenols, carotenoids, and vitamins are among the bioactive chemicals that possess several physiological properties, such as anti-inflammatory, anti-allergenic, anti-bacterial, vasodilatory, anti-thrombotic, cardio-protective, and antioxidant effects. Because of its distinct acyclic structure, which confers lipophilic properties and makes it soluble in organic solvents, lycopene is an antioxidant carotenoid. But preserving lycopene's stability during extraction presents difficulties, and using traditional techniques also raises environmental issues.

Objectives: In this study, pectinase, an enzyme that was produced from banana peel using Aspergillus niger, was used to extract lycopene from wastes of tomato skin peel.Then purification and characterization of lycopene using thin layer chromatography and high performance liquid chromatography was studied and finally the lycopene's antioxidant activity was assessed utilising the DPPH, ABTS, and H2O2 assays.

Methods: The tomatoes (Solanum lycopersicum) were purchased from the local stores, and after peeling, the skin was allowed to dry for a few hours. The banana skin peels were then dried for 24 hours at 45°C before being pulverised with a mortar and pestle. Then, using Aspergillus niger, which was cultured in a lab using Sabouraud Dextrose Agar medium at room temperature, the resulting powdered banana peel was used to produce the pectinase enzyme. And the extraction of lycopene from tomato skin was done using the pectinase enzyme from banana skin wastes, followed by purification, characterization & finally, measuring the of antioxidant activity of the obtained lycopene.

Results: Pectinase from banana peel was found to be 10 mg with the absorbance at 750 nm, when measured spectrophotometrically. TLC is an effective method for identifying and separating substances in a mixture according to how differently they migrate across a thin layer of absorbent material. HPLC analysis showed a clear peak that corresponded to lycopene, with a distinctive absorption maximum at 475 nm.

Conclusions: The antioxidant assay demonstrates lycopene's remarkable scavenging activity against DPPH, ABTS, and H2O2 radicals, showcasing its potential therapeutic and industrial applications. These findings underscore the multifaceted role of lycopene as a

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natural antioxidant with significant health benefits. Overall, this study contributes valuable insights into the biological and industrial significance of pectinase enzyme and lycopene, paving the way for further research and development in food, pharmaceutical, and cosmetic industries.

1. Introduction

When fruits and vegetables are industrially processed, a significant amount of by-products rich in bioactive compounds are produced; these by-products may find application in other areas. One of the most important vegetables in the Mediterranean diet, both in terms of quantity and quality, is the tomato (Solanum lycopersicum),Fig 1. One of the most widely available crops of days, tomatoes have a long history. Tomatoes have been grown for food since the 16th century, despite the fact that they were formerly only used for adornment and were even considered hazardous in some locations. Tomatoes are a staple meal that are typically consumed raw due to their superior nutritional value, but they are also becoming more and more common in many well-known tomato products. More than 80% of tomatoes cultivated are used to produce processed foods such puree, ketchup, concentrate, juice, soup, salsa, and dry-concentrate [1].

Processing of any kind is usually necessary for several reasons: to make products available during off-season (e.g., canned tomatoes); to produce goods intended for home consumption (e.g., tomato ketchup); to develop new food products (e.g., sauces and soups) with enhanced or varied flavours and textures; to improve nutritional value; and to add value for extra profit [2].

Fig 1. Tomatoes (Solanum lycopersicum)

The majority of the by-products generated during the processing of tomatoes come from tomato pomace. Wet pomace has 33% seed, 27% skin, and 40% pulp, but dried pomace has 44% seed and 56% pulp and skin. Handling tomato leftovers is one of the biggest sustainability-related difficulties facing processing companies in agriculture today, as a result of the potential harm that unwanted elements released into the environment could create.sustainability and financial health of commercial enterprises [3].

One way to get around this problem would be to reuse the tomato by-products that were purchased from the tomato industry in order to capitalise on the wealth of compounds that could be beneficial. Despite the potential for providing dietary fibre, proteins, carotenoids, tocopherols, polyphenols, and other nutrients, the majority of these physiologically advantageous substances wind up in the garbage when they are extracted from tomatoes. These bioactive chemicals include polyphenols, carotenoids, and vitamins. They possess various physiological properties, such as anti-inflammatory, anti-allergenic, antibacterial, vasodilatory, anti-thrombotic, cardio-protective, and antioxidant effects [4].

Carotenoids, particularly lycopene, are the primary fruit colouring agents in tomato pericarp and tomato products, giving ripe tomatoes their characteristic orange and deep red colours, respectively. Similarly, tomato by-products include similar pigmenting compounds. Furthermore, lycopene and other carotenoids content in tomatoes depends on several factors, including variety and fruit ripeness at harvest. Like most fruits and vegetables, tomatoes have an overabundance during the growing season and a deficit during the off-season [5].

1.1 Carotenoids

A family of molecules known as carotenoids, which includes more than 600 distinct chemicals, is produced by higher plants and algae. They are members of a class of naturally occurring pigments that are well known for the orange-red to yellow colour of many fruits, vegetables, and flowers, as well as for having provitamin A activity. Their feature is a conjugated double-bond

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linear polyisoprene structure, which is present in lycopene and xanthophylls (lutein and zeaxanthin) that are produced by cyclizing the two extremities either with or without oxidation (carotenes). Carotenoids, especially those without hydroxyl groups, are lipophilic substances [6].

Carotenoids are thought to be the cause of fruits and vegetables' capacity to shield individuals from illness. Over 90% of all carotenoids are present in the body and food, mostly as lutein, β-, α-, and lycopene carotene, as well as α-cryptoxanthine. Recent epidemiological and animal research have directly connected a high dietary intake of carotenoids to a lower risk of cardiovascular diseases, age-related macular degeneration, and numerous types of cancer. It has been suggested that the main way that carotenoids contribute to health is via their antioxidant properties. Indeed, of the following, lycopene is believed to be the most potent antioxidant: α-tocopherol, α-carotene, β-cryptoxanthin, and lutein are ranked lower than lycopene. However, nothing is understood on how certain carotenoids concentrations, structures, and ratios impact their absorption and action in the human body [7].

1.2 Lycopene

Lycopene, a red pigment, is found in many red fruits and vegetables, particularly in tomatoes and tomato-based products. Lycopene, acting as a natural pigment, imparts orange, red, and yellow hues and is essential to the photosynthetic mechanisms in plants. Lycopene is found in pink grapefruit,watermelons, tomato, and tomato-based food products. In particular lycopene accounts for nearly 80 % of total carotenoids in fully ripe tomatoes [8]. Though it hasn't received much attention as a therapeutic element, lycopene has long been utilised as a natural food pigment. Due to its many health benefits and popularity as a food additive, it is one of the most extensively used hues in the food industry. The use of lycopene as a red colouring and antioxidant is growing in popularity. According to reports, lycopene consumption has tripled from 5,000 metric tons in 1995 to 15,000 metric tons in 2004 [9].

The chemical structure of lycopene was first proposed by Willstatter and Escher. Lycopene is an isoprenoid polyene ($C_{40}H_{56}$) with a core skeleton consisting of 22 carbon atoms and two ends with nine additional carbon atoms each and it has been shown to possess a molecular mass of 536.89 g mol^{-1} . It has a distinctive unsaturated acyclic carotenoid of 13 double bonds, with 11 linear conjugated and an 8-isoprene-based tetraterpene. However, due to steric hindrance, only a limited number of double-bond groups undergo geometric isomerisation [10].

As with other carotenoids, lycopene's double bonds can undergo isomerization from trans to mono or poly-cis isomers by light, heat energy, and chemical processes, even though the all-trans form of lycopene is present naturally in food products and is the most thermodynamically stable isomer. Thus, 94% to 96% of red tomatoes contain all-trans lycopene, which is the most thermodynamically stable form of lycopene. Lycopene is a lipid-soluble antioxidant that is a member of the carotenoid family of phytochemicals. While many plants and bacteria produce it to protect themselves from photosensitization and to absorb light during photosynthesis, animals and the human body do not [11].

One of the main components of the carotenoids that give these molecules their eye-catching hues is their extended conjugated double bond structure, which forms the light-absorbing chromophore. Lycopene is lost more when heat treatments like blanching, sterilising, and drying are used. However, human plasma and tissues contain at least 50% cis-isomers; the most common isomeric forms of lycopene are 5-cis, 9-cis, 13-cis, and 15-cis. The all-trans isomer of lycopene, which is the most stable form, is best found in fresh tomatoes. Pigment is degraded during processing, though, as it is liberated from the tissue matrix and undergoes spontaneous isomerization and oxidation [12].

Lycopene was found to have a higher level of antioxidant efficacy than βcarotene, α-carotene, and α-tocopherol. An increasing number of in vivo and in vitro clinical experiments have shown that this molecule is also effective as a nutraceutical due to its great antioxidant activity, which lowers the risk of atherosclerosis and coronary heart disease. Moreover, epidemiological research has connected lycopene consumption to a lower prevalence of some cancer types [13]

1.3 Lycopene Extraction

The process of extracting lycopene from various sources is critical due to the presence of numerous physicochemical hindrances. In recent decades,

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extensive endeavours have been undertaken to enhance lycopene extraction methods. The recovery from various plant sources continues to be suboptimal, as numerous physical and chemical obstacles impede the mass transfer of lycopene during extraction. Eighty to ninety percent of the pigment content comes from ripe tomatoes, which are the primary source of lycopene extraction [14]. 15,000 metric tonnes of lycopene were produced globally in 2004 at a cost of $54.5 million. Since lycopene is soluble in organic solvents such as benzene, chloroform, and methylene chloride, chemical extraction is usually employed to obtain it. Chemical synthesis is another commercial method for producing it.

The industrial synthesis of lycopene has drawn attention towards the development of environmentally friendly technologies, since the current processes involve extremely hazardous chemical solvents. Supercritical fluid extraction (SFE) has emerged as a competitive alternative to traditional, questionable, and insufficiently selective separation techniques employing organic solvents since carbon dioxide (CO2) is inert and has a comparatively low critical temperature (Tc = 304.1 K). Thus, optimising lycopene yields through changes in temperature, pressure, flow rate, addition of a modifier or co-solvent, and enhancement of SC-CO2 fluid extraction conditions is the primary objective of research on lycopene extraction by SFE [15].

According to these scientists, trans-lycopene extraction from tomato industrial wastes (skins and seeds) using SFE increased with increasing pressure, solvent flow rate, and particle size decrease, and was influenced by the compound's quantity in the starting material. Furthermore, at 60 ºC, 300 pressure, 0.59 g/min of solvent flow, 0.36 mm of particle size, and 4.6% of feed moisture content, the maximum trans-lycopene recovery of 93% was reached. The yield of lycopene extracted from tomato skin varied between 0.639 and 1.98 mg/100 g. This wide range of extraction was influenced by the solvent/meal ratio, number of extractions, temperature, particle size, and length of the extraction process. The extraction of tomato skin in four extractions at 50 ºC, 0.15 mm particle size, and 8 minutes of extraction time, using a 30:1 v/w solvent/meal ratio, produced the maximum amount of lycopene [16].

Extraction of lycopene from tomato skin yield has increased as a function of both temperature and pressure, however temperature had a stronger effect. Lycopene yields with pure CO2 ranged from 11.0 to 25.5 µg/g, depending on temperature and pressure. The largest concentration of lycopene was recovered at the highest temperature (75^{oC}) and pressure (35MPa). The quantity of useful compounds that were extracted by SFE from industrial tomato waste, including carotenoids (lycopene, β-carotene). The product obtained at 460 pressure and 80oC using supercritical CO2 extraction exhibited the highest concentration of carotenoids, with 90.1% lycopene. a cutting-edge method that extracts lycopene from tomato juice without the need to dry the raw material and instead uses supercritical CO2 as a solvent [17].

The tomato juice was extracted by centrifuging it multiple times and rinsing it with 100% ethanol to remove the water present in the solid part of the juice. With an extraction efficiency ranging from 7.7% to 76.7%, the only factor that statistically significantly affected the process was the extraction temperature. Two further non-SFE lycopene extraction methods are ultrasonic/microwave assisted extraction (UMAE) and ultrasonic assisted extraction (UAE). So, using UMAE and UAE, lycopene was extracted from tomato paste. The results showed that the optimal parameters for UMAE were 40 KHz ultrasonic processing, 98 W microwave power, 10.6:1 (V/W) solvent to tomato paste ratio, and 367 s extraction duration. The optimal extraction temperature for the United Arab Emirates was 86.4 ºC, the appropriate solvent to tomato paste ratio was 8.0:1 (V/W), and the optimal extraction time was 29.1 minutes. Under these conditions, the lycopene yields of UAE and UMAE were 89.4% and 97.4%, respectively [18].

1.4 Enzyme aided extraction of Lycopene

Enzymes have been used extensively to improve the yield and quality of a number of naturally occurring plant products, including flavourings, colourants, and bioactives. Using mechanical or organic solvent-based methods, cell wall hydrolase pretreatments have been demonstrated to increase the rate and calibre of oily product extraction from a variety of seeds and fruits [19].

Most investigations in this field improve the release of intracellular contents by hydro-distillation or conventional solvent extraction (CSE) by using cocktails of cellulase, xyloglucanase, and pectinase to hydrolyze

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and breakdown the polysaccharide network surrounding the cell. This is due to the fact that cellulose, hemicelluloses, and pectin make up the majority of the primary cell wall in dicotyledonous plants. Enzymatic mixtures have been studied using this technique to enhance the extraction of lutein from marigold flowers, lycopene from tomato tissues, lutein and capsaicinoids from chilli peppers, α- and β-carotene from orange peel, sweet potatoes, and carrots, and flavonoids from the leaves of ginkgo biloba or pigeon pea.Nevertheless, as far as we are aware, not much work has been done on the combination of employing supercritical fluids and cell-wall hydrolytic enzymes to extract plant components [20].

Recently, a similar technique was assessed to enhance grape seed oil supercritical fluid extraction, resulting in a significant increase in oil output (+44%) as compared to untreated seeds. The current study describes a novel enzyme-assisted technique for lycopene extraction-friendly matrix creation by dissolving the main cell wall of tomato parenchyma cells. Pectic enzymes, which include polygalacturonase, pectin lyase, and pectin methyl esterase, are referred to as pectinase when combined. These break down pectin, a polysaccharide substrate found in plant cell walls [21]. Extracellular enzymes produced by the fungi Aspergillus, Rhizopus, and Penicillium are easily recovered and regarded as safe. The increased need for energy has brought attention to the issue of renewable industrial and agricultural wastes because of the environmental challenges associated with their disposal. A range of fruit and vegetable industry byproducts have been used to make pectinases [22].

After citrus fruits, bananas are the second most widely produced fruit worldwide. Bananas are mostly eaten raw, but they can also be processed to create different products. They are cultivated by the majority of small-scale farmers and are essential to the socioeconomic growth of many developing tropical and subtropical countries. Banana peels are an inexpensive and abundant agricultural waste byproduct. It is easily obtainable in large quantities. When the fruit is raw, it accounts for over 40% of its weight and is rich in protein, carbohydrates, and a number of vitamins and minerals. However, because there is currently no large commercial use for banana peels, they are usually thrown away in open locations, which could have negative environmental repercussions. It is imperative to look at its industrial reuse. The pectinase enzyme is extracted from banana peel using Aspergillus niger in the current work, and lycopene is then recovered using the pectinase enzyme [23,24].

1.5 Bioavailability

Bioavailability is the proportion of consumed carotenoids that may be used by the body for normal physiological functions or stored there. One of the most important factors to consider when assessing the contribution of dietary components to human health is their bioavailability. Interest in the bioavailability of vitamins and other phytochemicals has significantly increased due to the existence of undernourished populations, populations at risk of micronutrient deficiencies (e.g., the elderly), and epidemiological evidence supporting protective effects against noncommunicable diseases (e.g., cancer, cardiovascular disease, age-related eye diseases) [25,26].

Our knowledge of the bioavailability of lycopene in people today has been hindered by the difficulty to distinguish recently administered lycopene from the body's stores of the same. The sort of carotenoid, molecular connections, carotenoid amount, matrix, effectors, nutritional condition, genetics, host-related factors, and interactions between these variables all appear to have an impact on the bioavailability of carotenoids. Within the thylakoid membranes of the chromoplasts in intact tomato cells, lycopene is discovered to be contained within cellular compartments [27,28]. As a result, not much lycopene is absorbed after being released from raw tomatoes. Nonetheless, the bioavailability of lycopene can be significantly increased by thermal and mechanical processing that disrupts the tomato matrix. These treatments may help spread lycopene and other liposoluble tomato components throughout the food matrix in addition to potentially increasing lycopene accessibility. Concentrated tomato extracts include largely crystallised poorly soluble lycopene, and it has been found that this crystalline state is one of the primary factors reducing the bioavailability of carotenoids [29].

Similar to lipids, lycopene is absorbed by a system that includes emulsification, liberation from the physical matrix, solubilization in mixed micelles, diffusion, and penetration through the enterocyte membrane. The cis

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isomers of lycopene make up more than half of the compound found in human serum and other tissues. This is in contrast to the food sources they originate from, as 79% to 91% of the total lycopene found in tomatoes and tomato-based products is made up of all-trans lycopene. The cis isomers of lycopene are more accessible than trans-lycopene because they are more soluble in bile acid micelles and may be absorbed into chylomicrons preferentially [30]. Lycopene transport in the intestinal lymph and triglyceride transport in the lymph were highly associated. Oral bioavailability of lycopene may be enhanced by formulation methods, such as lipid-based formulations including lecithin or long-chain fatty acids (LCFAs), that facilitate intestinal lymphatic absorption. Other factors that influence the absorption of carotenoids include the physical form of the carotenoid, an individual's health, and the availability of dietary fibre [31,32].

Rats can easily withstand lycopene at a dose of 10 mg/kg, or two servings of tomatoes or tomato derivatives per day and moreover bioavailable, lycopene builds up in tissues [33]. Participants in a human dietary intervention study reported noticeably greater serum lycopene levels after drinking tomato juice, spaghetti sauce, and lycopene pills for one week After 4 weeks of consuming oleoresin or lycopene beadlets, respectively, lycopene in buccal mucosa cells increased dramatically (<2-fold) to 4.95 and 3.75 mg/g protein. Nevertheless, there was no change in the antioxidant and absorption qualities of consuming 5 to 20 mg of lycopene [34].

1.6 LYCOPENE AS AN ANTIOXIDANT

Studies have shown that lycopene is the most potent carotenoids at quenching singlet oxygen; this impact is mostly determined by the quantity of conjugated double bonds and, to a lesser degree, by the presence of cyclic or acyclic end groups. This suggests that lycopene supplementation and a high-lycopene diet may provide protection against ROS products. Experimental research suggests that lycopene may also have the ability to scavenge free nitrogen dioxide (NO·2), thiol (RS·), sulfonyl (RSO·2), singlet oxygen and peroxyl radical (LOO•). Lycopene degradation may lead to pigment loss when exposed to free radicals or oxidizing agents [35].

The primary cause of lycopene's antioxidant qualities is its eleven conjugated double bonds, which facilitate chemical interactions with substances that have unpaired electrons. This can happen when the radical joins the polyene chain, transfers electrons from lycopene to the radical, or lycopene loses an atom of hydrogen [36]. β-carotene and α-tocopherol are thought to neutralise ROS ten times and twice as well, respectively, as lycopene. It has strong effects on the nitrogen dioxide (NO2•), thiyl radical (RS•), and hydroxyl radical (OH•) [37]. The following three possible mechanisms [38] could account for lycopene's effects on reactive species: i) adduct formation: Lycopene + R• → R-Lycopene•; ii)electron transfer to the radical: Lycopene + R• → Lycopene•+ + R−; iii) allylic hydrogen abstraction: Lycopene + R• → Lycopene• + RH. Lycopene's bioactivity can be expanded to include immunomodulatory, anticancer, and protective effects against neurological, ocular, and cardiovascular diseases due to its potent antioxidation capacity [39].

Additionally, the complex conjugated polyene system in its chain structure influences its biological properties, including its susceptibility to oxidative degradation. Studies have been conducted to demonstrate the in vivo, ex vivo, and in vitro protective effects of lycopene against oxidative stress. Oxidative stress and oxidation of proteins, lipids, and DNA are closely related in this context. Together, lycopene and S-allylcysteine enhanced protection against oxidative stress. This was connected to altered lipid peroxidation, reduced glutathione levels, and the glutathione-dependent enzymes glutathione reductase, glutathione S-transferase, and glutathione peroxidase [40].

At final concentrations of 10, 25, and 50 µM, lycopene (97%) reduced the frequency of micro nucleated cells induced by the three mutagens. However, this chemotherapy preventative effect depended on the amounts and regimens used. examined the possible protective benefits of 10 mg/kg of lycopene given to male rats in a 2007 study against spermatozoal and testicular damage associated with oxidative stress from cyclosporine A. Lycopene's ability to interact with metabolites of free oxygen may be responsible for its protective effects against the increase in lipid peroxidation caused by cyclosporine A [41]. The antioxidant and pro-oxidant qualities of lycopene have a tendency to be dose-dependent. It was found that the radioprotective impact of lycopene on γ-radiation-induced toxicity. Lycopene pretreatment (1, 5 and 10 µg/mL) significantly decreased the frequency of

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micronuclei, dicentric aberration, and translocation in comparison to the γ-radiation control [42].

Hydroperoxide and thiobarbituric acid reactive compound levels decreased relative to the γ-radiation control, whereas glutathione peroxidase, catalase, and superoxide dismutase activities significantly increased in tandem with GSH levels. Thus, lycopene's ability to reduce oxidative stress was also demonstrated when UV radiation was administered to human skin. Lycopene was found to be preferentially removed as compared to β-carotene, suggesting that it might have a more active or protective role [43].

Industrial processing of fruits and vegetables results in a large quantity of byproducts that are rich in bioactive chemicals; these byproducts may find use in other contexts. Treatment costs may be lowered by recycling or reusing byproducts produced during processing that are generally available in large quantities. Tomato pomace is the primary source of by-products produced during the processing of tomatoes. Reusing the tomato by-products that were bought from the tomato business would be one approach to get over this issue and take advantage of the abundance of potentially helpful substances.

Even though they have the ability to supply nutrients such as dietary fibre, proteins, carotenoids, tocopherols, polyphenols, and other beneficial components, most of these physiologically beneficial substances are wasted during the extraction process from tomatoes. These bioactive substances consist of vitamins, carotenoids, and polyphenols. These include anti-inflammatory, anti-allergenic, antibacterial, vasodilatory, anti-thrombotic, cardio-protective, and antioxidant actions, among other physiological attributes. Bananas are the second most widely cultivated fruit in the world, after citrus fruits. Although most people eat bananas fresh, they can also be processed to make a variety of goods. Banana peels, however, are currently discarded in public areas due to their lack of significant commercial usage, which may have detrimental effects on the environment. It is imperative to look at its industrial reuse.

2. Objectives

The current study's scope comprises of

- i)Enzymatic extraction of lycopene from tomato skin using pectinase enzyme derived from banana peel using Aspergillus niger.
- ii)Purification and characterization of lycopene using thin layer chromatography and high performance liquid chromatography .
- iii)Determining the antioxidant activity of Lycopene using DPPH assay , ABTS aasay , H2O2 assay .

2.1 MATERIALS AND METHODS

2.1.1 Collection of plant material

The tomatoes (Solanum lycopersicum) were picked up from local stores, and after peeling them, they were allowed to dry for a few hours. After being washed and given time to air dry, the peels were placed in the freezer to await further analysis.

2.2. Extraction of lycopene using pectinase enzyme produced from banana peel

2.2.1 Preparation of banana peel powder

Bananas, or musa balbisiana, were bought from the local market. The banana skins were washed under tap water and then carefully peeled. The peels were then dried for 24 hours at 45°C before being pulverised with a mortar and pestle. Then, using Aspergillus niger, which was cultured in a lab using Sabouraud Dextrose Agar medium at room temperature, the resulting powdered banana peel was used to produce the pectinase enzyme.

2.2.2 Pectinase production

The potassium dihydrogen phosphate, magnesium sulphate, ferrous sulphate, and manganese sulphate were combined with 100 mL of distilled water in 250 mL Erlenmeyer flasks for the experiments. Two grams of powdered banana peel were added to this. The medium was cleaned, allowed to cool at room temperature, and then the pH was raised to 5.8 using citrate buffer. To this are added 2x108 Aspergillus niger spores, which are shaken at a speed of 150 rpm in the incubator. The mixture was then allowed to cool in a refrigerator set at 4°C. The contents were moved into a centrifuge tube, and the centrifuge was run for five minutes at 10,000 rpm. After the supernatant was collected, the protein content

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of the partially purified and crude enzymes was measured using, Lowry's technique.

2.2.3 Estimation of pectinase protein by Lowry's Method

A popular approach for determining the amount of protein in a sample is the Lowry method, which involves a few steps that must be completed in order. To create a calibration curve, standard protein solutions with known concentrations are first made. The sample is then appropriately diluted to make sure it is within the linear range of the curve. Following dilution, Lowry reagents—copper ions, alkaline copper tartrate, and Folin-Ciocalteu reagent—are added to the sample and standard solutions. This causes a colour development reaction that yields a complex with a blue colour. The amount of protein present in the sample is directly correlated with the colour's intensity, Fig.2.

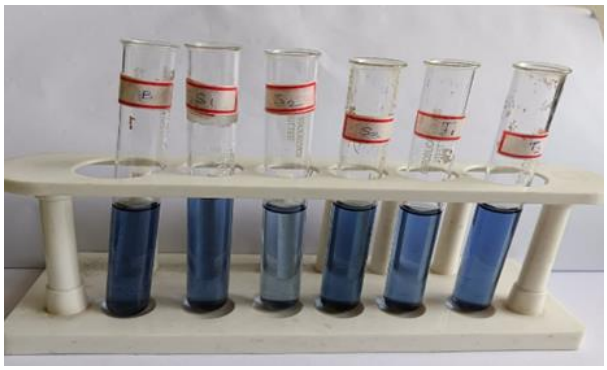

Fig.2 Protein Estimation by Lowry's Method

2.2.4 Enzymatic extraction of the Lycopene extract using produced pectinase

To 2.5 grams of tomato peel powder, 20 milliliters of acetone is added and kept for an hour at 250 degrees without light, with continuous magnetic stirring. Following a cellulose membrane filter, the solutions were placed into centrifuge tubes together with 20 ml of petroleum ether and 10 ml of deionized water. The tubes were centrifuged for 10 minutes at 1620 rpm. The mixtures were poured into a 50 ml volumetric flask, and made up to the entire capacity with petroleum ether. Finally, a rotatory evaporator was used to get rid of the solvents. The extract was then kept for later use out of direct sunlight. Regarding the lycopene's enzymatic extraction 90ml of hexane, 10mL of an enzyme solution, and 4g of tomato peel are put to a conical flask. The flasks were incubated at 250 C for one hour after being magnetically agitated. After passing the mixture through a muslin cloth filter, 2 milliliters of the hexane layer were taken and its lycopene concentration was measured.The schematic representation of lycopene extraction using pectinase enzyme is shown in Fig.3.

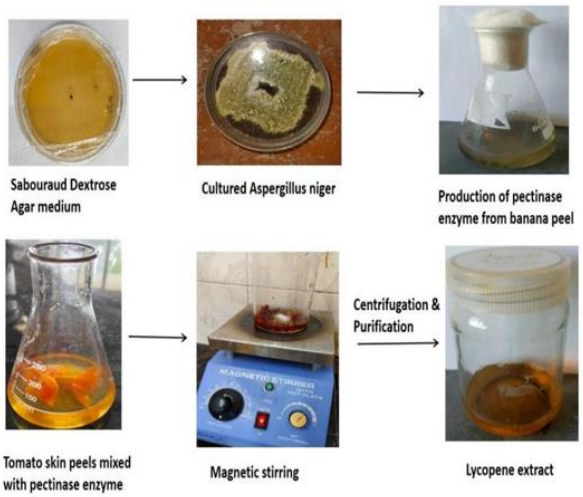

Fig.3 Schematic representation of lycopene extraction using pectinase enzyme

2.3 Purification and characterization of Lycopene

2.3.1 Thin layer chromatography

Hexane : toluene solvent was used as a mobile phase to identify the component in the sample. The stationary phase was a TLC plate covered in silica slurry.The sample was positioned over the line drawn at the bottom of the TLC plate using a capillary tube. The TLC plate was placed in a beaker containing the mobile phase and left undisturbed to allow the solvent to reach the top of the plate. The TLC plate was removed and allowed to air dry. Ten minutes are spent with a little quantity of iodine crystals in a different beaker. The TLC plate is then put in the beaker and left for 20 minutes so that the band can form.

2.3.2 High Performance Liquid Chromatography

The lycopene was extracted using hexane and acetone. The amount of lycopene was then measured using reverse phase HPLC with a C18 column absorbance

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detector set to 475 nm and methanol, acetonitrile, methylene chloride, and water (700:700:200:16, v/v) as the mobile phase. During measurement, lycopene's trans and cis isomers were discovered in a single peak. β-carotene served as an external reference for the identification and measurement of lycopene peaks.

2.4 Determination of Antioxidant activity Lycopene

2.4.1 DPPH Free Radical Scavenging Assay

The ability of the ethanolic lycopene extract extract to scavenge free radicals was determined using the DPPH [44]. Using 95% methanol, a 200µM DPPH solution was created. One milliliter (mL) was produced by adding distilled water to five test tubes containing lycopene extract solutions (100, 200, 300, 400, and 500µg/ml) from the stock. After adding 0.5 milliliters of freshly prepared DPPH solution to the extract, the tubes were incubated for approximately half an hour. The absorbance at 517 nm was then measured using a spectrophotometer. Standard ascorbic acid was used as a reference.

2.4.2 ABTS Radical Scavenging Assay

To generate ABTS radical action cation (ABTS +), 2.45mM potassium persulfate was mixed with 7mM ABTS stock solution in water. Before using, the combination was left for a full day at room temperature in the dark. The ABTS+ solution was then supplemented with ethanol until the absorbance at 734 nm reached 0.7. One milliliter of diluted ABTS+ solution was mixed with several quantities (100, 200, 300, 400, and 500µg/ml) of lycopene extraction ethanol. A minute later, a spectrophotometer was used to detect the absorbance at 734 nm [45].

2.4.3 Hydrogen peroxide Scavenging Activity

The efficiency of the lycopene extract and vitamin C in scavenging free radicals was investigated using the hydrogen peroxide (H_2O_2) scavenging method [46]. Different amounts of the extract (100, 200, 300, 400, and 500 µg/mL) and vitamin C (100, 200, 300, 400, and 500 µg/mL) were prepared and added to the H_2O_2 solution (1mL, 43 mM). The test tube designated as the blank included phosphate buffer but no hydrogen peroxide. The absorbance of hydrogen peroxide was measured at 230 nm following a 10 minute incubation period against a blank solution [47].

3. Results & Discussion

3.1 Pectinase content from banana peel

The sample's protein concentration of pectinase from banana peel was found to be 10 mg with the absorbance at 750 nm, when measured spectrophotometrically. The obtained absorbance values were then compared to the calibration curve, Fig.4.

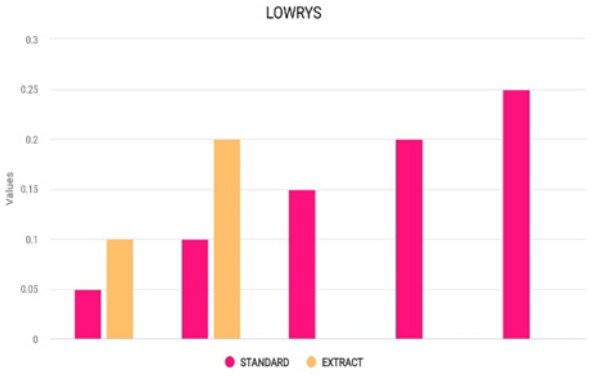

Fig.4 Pectinase protein content in Banana peel

3.2 Lycopene Characterization using Thin layer chromatography (TLC)

TLC is an effective method for identifying and separating substances in a mixture according to how differently they migrate across a thin layer of absorbent material. The organic mixture was put on a silica gel-coated plate to create the TLC plate, which was then submerged in a developing chamber that was filled with a toluene and hexane solvent mixture. After development, the plate was taken out of the chamber, dried, and then its visualization was assessed. Different bands could be seen on the TLC plate upon visualization, which showed that the mixture's components were separated. Each band's Rf (retention factor) value was computed, revealing details about the compounds' relative polarity and affinity for the stationary phase. The retention factor (Rf) of the compound was calculated using the formula, Fig.5.

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

Sample ⟶ Rf = 5.7 / 8 =0.71

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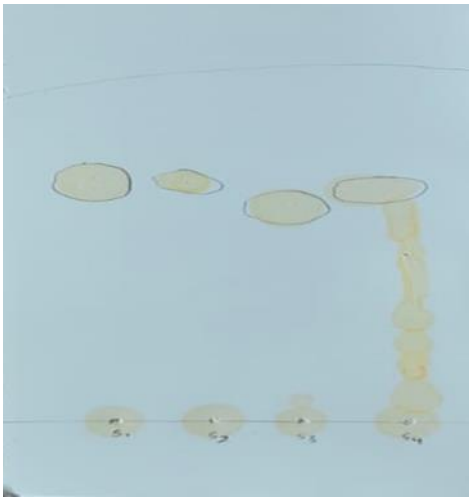

Fig.5 Thin Layer Chromatogram for lycopene

3.3 Lycopene Characterization using HPLC

After 14.20 minutes of retention, the sample's HPLC analysis showed a clear peak that corresponded to lycopene, with a distinctive absorption maximum at 475 nm. By comparing the height of the peak with the area under the curve, which was created using standard lycopene solutions, one may ascertain the concentration of lycopene in the sample. This finding establishes the presence of lycopene in the sample and quantifies its quantity, offering important information on its makeup and possible uses in a range of sectors, such as food, medicine, and cosmetics, Fig.6.

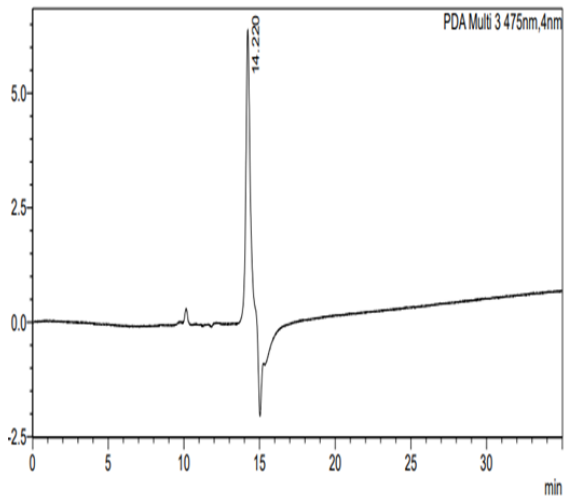

Fig.6 Chromatogram obtained by HPLC analysis

3.4 Antioxidant activity of Lycopene

A variety of methods have been developed for determining the antioxidant activity of lycopene from tomato. DPPH, ABTS, and H2O2 scavenging assays, which are based on the ability of an antioxidant to decrease a stable radical, are commonly used to measure the antioxidant activity in fruits. Thus, these assays were used to determine their potential free radical scavengers that function by reducing hydrogen- or electrondonating agents. The ethanolic extract of lycopene was tested in triplicate for its ability to scavenge DPPH radicals. The results were represented as a percentage of the standard deviation and computed as a percentage of radical scavenging activity. For highest activity of the lycopene extract, was observed as 69.0 µmol, Fig.7a.

Free radicals or highly reactive oxygen species are produced by the human body either through endogenous metabolic processes or exogenous substances. Antioxidants are substances that thwart the onslaught of free radicals, reducing the incidence of certain illnesses. Enzymatic antioxidants like glutathione, catalase, and superoxide dismutase (SOD) as well as non-enzymatic antioxidants like ascorbic acid and tocopherol are vital for cellular defense because of their capacity to remove free radicals, as demonstrated by this study. Due to their ability to reduce oxides and to adsorb or neutralize free radicals, phenolic compounds are key components of antioxidant activity[48-49].

The reaction involving ABTS radicals involves a single electron transfer event, and they are more reactive than DPPH radicals. The pre-formed radical monocation of ABTS radical, or ABTS radical assay, is produced when ABTS radicals are oxidized with potassium persulfate and is reduced in the presence of antioxidants that donate hydrogen. The quantity and location of hydroxyl and other functional groups, such as carboxyl and the phenolic ring system, affect the antioxidant activity of various lead compounds[50]. The results were represented as a percentage of the standard deviation and computed as a percentage of radical scavenging activity. The ABTS activity of lycopene extract, was found to be 76.058 µmol, Fig.7b.

When different doses of lycopene are used in the H2O2 experiment, a dose-dependent decrease in H2O2 absorbance is seen, which suggests that lycopene has scavenging action for hydrogen peroxide. With an

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increase in lycopene concentration, the absorbance values dropped, indicating a greater capacity for antioxidant activity. This drop in absorbance shows that lycopene can counteract hydrogen peroxide, which lowers oxidative stress. The results were represented as a percentage of the standard deviation and computed as a percentage of radical scavenging activity. The H2O2 scavenging activity of lycopene extract was found to be 84.0 µmol, Fig.7c.

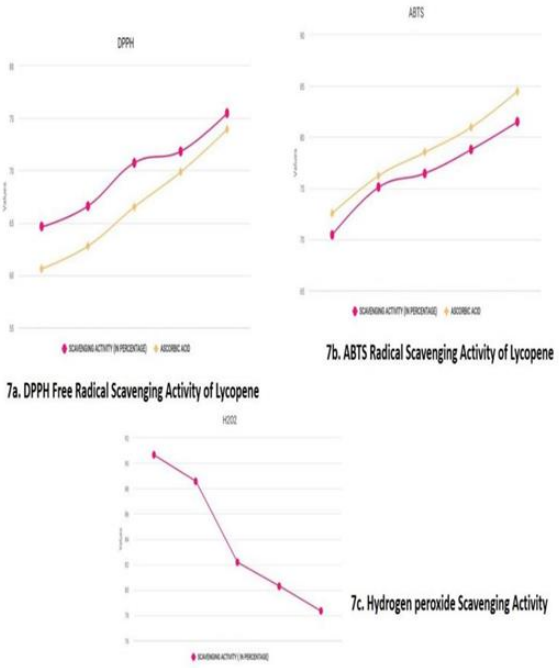

Fig.7 Antioxidant activity of Lycopene extracted from tomato skin

4. Conclusion

In conclusion , the analysis of pectinase enzyme's protein content, coupled with TLC, HPLC, and antioxidant activity assays, provided comprehensive insights into its composition and functional properties. Spectrophotometric determination of protein concentration allowed for precise quantification, aiding in quality assessment and standardization of enzyme preparations. TLC characterization revealed distinct separation of organic mixture components, elucidating their relative polarity and affinity for the stationary phase. HPLC analysis identified lycopene as a prominent constituent, with implications for various industries due to its distinct absorption properties. Furthermore, antioxidant assays demonstrated lycopene's remarkable scavenging activity against DPPH, ABTS, and H2O2 radicals, showcasing its potential therapeutic and industrial applications. These findings underscore the multifaceted role of lycopene as a natural antioxidant with significant health benefits. Additionally, the correlation between antioxidant activity and lycopene concentration suggests potential avenues for enhancing its efficacy through extraction and formulation techniques. Overall, this study contributes valuable insights into the biological and industrial significance of pectinase enzyme and lycopene, paving the way for further research and development in food, pharmaceutical, and cosmetic industries.

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