**A DISSERTATION ON**

# Antimicrobial & Antioxidant activity of extracts of Pineapple (*Ananas comosus*) peel waste

**SUBMITTED TO THE**

**DEPARTMENT OF BIOENGINEERING**

**FACULTY OF ENGINEERING**

**INTEGRAL UNIVERSITY, LUCKNOW**

**IN PARTIAL FULFILMENT**

**FOR THE**

**DEGREE OF MASTER OF TECHNOLOGY**

**IN**

**FOOD TECHNOLOGY**

**BY**

**Faraz Ahmad Khan**

**M.Tech. Food Technology (IV Semester)**

**Roll No: 2101207005**

**UNDER THE SUPERVISION OF**

**Dr. Alvina Farooqui**

**Professor & Head**

**Department Of Bioengineering**

**Integral University, Lucknow**

**INTEGRAL UNIVERSITY, DASAULI, KURSI ROAD**

**LUCKNOW- 226026**

## DECLARATION FORM

I, **Faraz Ahmad Khan**, a student of **M.Tech. Food Technology (2nd year/ IV semester)**, Integral University have completed my six months dissertation work entitled **"Antimicrobial & Antioxidant activity of Pineapple (*Ananas comosus*) peel waste"** successfully from **Integral University**, Lucknow under the able guidance of **Dr. Alvina Farooqui**.

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

**Name and Signature of Student with Date**

**Name and Signature of Course Coordinator with Date**

INTEGRAL UNIVERSITY
LUCKNOW - INDIA
Phone No.: +91(0522) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809
Kursi Road, Lucknow-226026 Uttar Pradesh (INDIA)

## CERTIFICATE

Certificate that **Faraz Ahmad Khan** (Enrollment Number 1700100910) has carried out the research work presented in this thesis entitled **"Antimicrobial & Antioxidant activity of Pineapple (*Ananas comosus*) peel waste"** for the award of **M.Tech Food Technology** from Integral University, Lucknow under my supervision. The thesis embodies results of original work and studies carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of her **M.Tech Food Technology** degree.

I wish him good luck and bright future.

**Dr. Alvina Farooqui**
Professor and Head
Department of Bioengineering
Faculty of Engineering & Information Technology

INTEGRAL UNIVERSITY
LUCKNOW - INDIA
Phone No.: +91(0522) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809
Kursi Road, Lucknow-226026 Uttar Pradesh (INDIA)

## CERTIFICATE BY INTERNAL ADVISOR

This is to certify that **Faraz Ahmad Khan**, a student of **M.Tech. Food Technology (2nd/ IV semester)**, Integral University has completed his six months dissertation work entitled **"Antimicrobial & Antioxidant activity of Pineapple (*Ananas comosus*) peel waste"** successfully. He has completed this work from **Integral University Lucknow**, under the guidance of **Dr. Alvina Farooqui**. The dissertation was a compulsory part of his **M.Tech. Food Technology** degree.

I wish him good luck and bright future.

**Dr. Alvina Farooqui**
Professor and Head
Department of Bioengineering
Faculty of Engineering & Information Technology

INTEGRAL UNIVERSITY
LUCKNOW - INDIA
Phone No.: +91(0522) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809
Kursi Road, Lucknow-226026 Uttar Pradesh (INDIA)

**TO WHOM IT MAY CONCERN**

This is to certify that **Faraz Ahmad Khan,** a student of **M.Tech. Food Technology (2nd Year/ IV semester)**, Integral University has completed his six months dissertation work entitled **"Antimicrobial & Antioxidant activity of Pineapple (*Ananas comosus*) peel waste"** successfully. He has completed this work from **Integral University, Lucknow** under the guidance of **Dr. Alvina Farooqui**. The dissertation was a compulsory part of his **M.Tech. Food Technology degree**.

I wish him good luck and bright future.

**Dr. Alvina Farooqui**
Professor and Head
Department of Bioengineering
Faculty of Engineering & Information Technology

**ACKNOWLWDGEMENT**

Before I present my work I would like to gratefully acknowledge the contribution of all those people who have helped in the work described in this Dissertation. I am going to try anyway, and if your name is not listed, it is rest assured that my gratitude is not less than for those listed below. First, I would like to express my gratitude to **God** for providing me the blessing to complete this work. It gives me immense pleasure in expressing my gratitude to my supervisor **Dr. Alvina Farooqui, Professor & Head, Department of Bioengineering.** She has always been there for me throughout the work and helped me to overcome all odds. She has always taken a keen interest in my welfare. I gratefully acknowledge honourable **Chancellor prof. S. W. Akhtar, V.C. Prof. Javed Musarrat** for there unconditional support and encouragement to pursue my interest in the field ofFood Technology.

Special thanks to **Dr. Azhar Kamal** (Immunology Lab, Head Instructor) and all the staff members for their relentless help and advice.I would like to also thank my teachers **Dr. Mohammad Haneef**, **Er.Suhail Ahmad, Dr. Ashish, Dr. Rahul Singh, Er.Gyanendra Tripathi, Ms. Gazala Anjum**. They generously devoted their valuable time for guidance and without their kind efforts my work would not be possible. Their deeply protective, caring and loving nature has at times left me moved. In a student's life, thebest source of inspiration is always a teacher. So, I thank all my teachers in past and present for encouraging me and guiding me towards a bright future.

Other to whom I am grateful and also like the put on the record are Faculty and PhD Scholar (Department of Bioengineering) for providing me with all the support and requirements not only during my project but also during my whole academic session.

I also thank my group mates I must write about my family members for their unconditional love, support and encouragement. It is equally important to thank my parents...but this acknowledgement will never be complete if their name is not there.

**Date:** **Faraz Ahmad Khan**

**CONTENTS**

## LIST OF FIGURES

## List of Tables

## Abbreviations

- CAM photosynthesis- Crassulacean acid metabolism photosynthesis.
- DV- Daily Value
- Kg-Killograms
- gm-Grams
- ml-Millilitres
- µL-Microlitres
- mm-Millimetres
- DMSO-Dimethyl sulfoxide
- E. coli- *Escherichia coli*

# INTRODUCTION

Pineapple (Ananas *comosus*) is a tropical edible fruit, native to South America and one of the most important plants belonging to the family of Bromeliaceae (Wali N., 2019). The fruit Ananas describes as the same fruit as Nana made in the form of a Pine apple, and the meaning of Tupi word "excellent fruit". Many European languages and led to the plant science of the pineapple Ananas *comosus*, in which the comosus, "tuQed", means the stem of the plant. India is a country of diverse climatic conditions from the point of varied agricultural productions consisting of fruits, vegetables, ornamental plants, spices, medicinal herbs, tubers, roots, aromatic plants and plantation crops. During farming, handing out, harvesting and consumption of agricultural products, a large amount of lignocellulosic bio wastes are produced every year. For different applications, this biomass can be utilized for the production of less expensive biosorbent, biochemical and biofuels, different enzymes and metabolites along with the production of functional foods for the eradication of these wastes from the environment and for evading solid- wasteb1. Nearly 90% of horticultural production in India accounts to fruits and vegetables from which 31.2% share is from fruits . *Ananas comosus* (Pineapple) is from *Bromeliaceae* family and one of the leading members of the most important edible fruits in the world. Major producer countries are Brazil, Thailand, Philippines, Costa Rica, China, and India with a total cultivation area of 909.84 thousands ha and 19412.91 thousand tons production per annum in the world (Sarkar T et. al. 2018) From the total production, 7% share is of India with a total production of 1,415.00 thousand tons which was cultivated in 89 thousand ha area and is most abundantly grown in Karnataka, West Bengal, Bihar, Kerala, Goa, Maharashtra and other states. Approximately 75-85% of the production is discarded as crown, peel, and core; while out of the total waste, peel alone contributes about 30-35% (Varzakas T, et al.2016). Pineapples grow like small herbaceous shrubs growing to 1.0 to 1.5 m (3.3 ft to 4.11 ft)in length or more. This plant is usually propagated from the beginning produced by the top of the fruit, or from the side, and ripens within a year. In appearance, the plant has a short stem, with strong dark leaves. It usually produces up to 200 flowers, in some of the larger varieties, during the period of bloom, the individual fruits of the flower come together to form more fruit. After the first fruit is produced, lateral shoots (called ‘sweets’ by commercial growers) are produced on the axils of the

leaves of the main stem. It has 30 or more leaves, which are small, with rhizome-shaped leaves with sharpened edges 30 to 100 cm (1 to 3 ft) in length, around the thick stem. In the first year of growth, the axis is enlarged and thickened, carrying many leaves at close quarters. After 12 to 20 months, the stem grows into a spike-shaped stem up to 15 cm (6 inches) long with more than 100 wind-shaped flowers, each of which is uniformly distributed. The ovaries grow into berries, blending into a large, compact fruit. Pineapple fruits are usually arranged in two interlocking helices. There are usually 8 on one side and 13 on the other, each with a Fibonacci number. Due to its outstanding flavour and taste, it ranks third in World's most important tropical fruits (Chaudhary V et al. 2019). . A fresh pineapple contains about 80-85% of moisture content and around 60% of edible portion. It contains 0.6% acid, 0.5% ash, 12-15% sugars, 0.4% protein, 0.1% fat, the remaining part consists of fibre, vitamin A, C and antioxidants, mainly flavonoids. It also contains a proteolytic enzyme named bromelain (Tochi B N et al. 2008) which is beneficial for the breakdown of protein molecules into amino acids along with helping in digestion Bresolin (I R A P, et al. 2013) A comosus plant is classified under the kingdom Plantae; phylum Tracheophyta; division Spermatophyte; sub-division Angiospermae; class Lilopsida, sub-class Magnoliales; order Bromeliales; family Bromeliaceae; genus Ananas and; species A. comosus (Debnath B, et al. 2021) Pineapple makes CAM photosynthesis, prepares carbon dioxide at night and stores it as an acid malate, and releases it during the day to aid photosynthesis. The six varieties of *A. comosus* include the former species are as Ananas *comosus* var. *ananassoides,* Ananas *comosus* var. *bracteatus,* Ananas *comosus* var. *comosus,* Ananas *comosus* var. *erectifolius,* Ananas *comosus* var. *parguazensis.* In the wild, pineapples are pollinated primarily by hummingbirds.(Chaudhary V, et al. 2019) Certain wild pineapples are foraged and pollinated at night by bats (Aziz SA et al. 2016). Under cultivation, because seed development diminishes fruit quality, pollination is performed by hand, and seeds are retained only for breeding. In Hawaii, where pineapples were cultivated and canned industrially throughout the 20th century, Bresolin (I R A P et al. 2013) importation of hummingbirds was prohibited. (Debnath B, et al. 2021) Pineapple waste is an agricultural waste which is normally described as waste produced from farming activities; it can be from natural resources (organic) or unnatural sources (inorganic). Major industrial activitie s such as food and agricultural-based account for approximately 30% of total commercial waste generated including liquid, residues, and

refuse (Ashworth andAzevedo 2009). Techniques employed in managing waste include the following methods: landfilling, incineration, pyrolysis and gasification, composting, and anaerobic digestion. Pineapple waste can be sustainably managed using the thermochemical or the organic conversion technique. This consists of pyrolysis and gasification in addition to fermentation. In developing nations like Nigeria, pyrolysis is used in generating charcoal for domestic cooking and as a waste management technique in pineapple management to produce char, gases, and bio-oil, which can be applied in the production of other high-value-added merchandise. through gasification, pineapple waste can also be managed in a sustainable manner, to produce syngas and combustion fuels, electricity, and other renewable technologies. Anaerobic digestion can be used in waste management of pineapple under controlled conditions. Bromelain supplementation protects animals from diarrheal diseases caused by bacterial enterotoxins from *Escherichia coli* and *Vibrio cholerae*. Bromelain acts as an anti-adhesion agent by modifying receptor attachment sites and has an effect on intestinal secretory signaling pathways. In addition to its ability to fight off the effects of certain intestinal infections and its synergism with antibiotics, these two methods demonstrate the benefits of bromelain against certain diseases. Bromelain has been reported to increase the blood and urine levels of certain antibiotics in humans. Pineapple peel extract when undergone through Soxhlet extraction method with Methanol and Hexane as the solvents shows antibacterial activities in different bacterial cultures with different sizes of zone of inhibitions. Where, the compounds extracted in Methanol solvent shown more antibacterial activities than the compounds extracted in Hexane solvent.

**Aims and Objectives-**

- Extraction of bioactive compound from pine apple peels using Soxhlet apparatus.
- To evaluate the antimicrobial potential of extracted compounds from pine apple peels against gram-negative bacteria.
- To validate the antioxidant property of extracted compound from pine apple peels.

## REVIEW OF LITERATURE

### Pineapple (*Ananas comosus*)

Pineapple is one of the most important fruits because it shows strong antibacterial activity and should be considered antimicrobials and contains a large number of bioactive compounds, dietary fibre, minerals and nutrients. In addition, pineapple has been shown to have a variety of health benefits, including anti-inflammatory activity, antioxidant activity, monitoring nervous system function, and healing bowel movements. The antimicrobial compounds that are active in *Ananas comosus* L.Merr include saponin and bromelain. Both saponin and bromelain act as antimicrobials through membranolitic structures. Bromelain acts primarily as a proteolytic enzyme when bound to a bacterial cell membrane, causing injury and reducing mortality of cells. *Ananas comosus* L. Merr has been reported to be highly potent in both Gram-negative bacteria.

The pineapple plant has many specific behavioral characteristics, body composition and physiological factors that determine important aspects of the management of pineapple plants, such as flower induction, water use and vegetative propagation methods. The use of pineapple reproduction is limited to breeding objectives developed by research institutes looking for new varieties with improved agronomic properties. Seeds are produced only in the case of pollination between varieties. Commercial pineapple must be propagated by vegetable materials, asexual reproduction, without the addition of new genetic compounds. Some types of propagules are naturally produced by plants and are commonly called plant material. Its availability and quality depend on many factors, especially the combination with nature. Management measures for this have been developed and will be addressed. In addition to conventional planting materials, in many cases not enough to ensure expansion or at least care of the cultivated area, many other methods of dispersal of pineapple vegetables have been studied and made available over the past decades.

**Characteristic of pineapple fruit:**

This plant and its main features-Pineapple (Ananas comosus var. comosus) (COPPENS D'EECKENBRUGGE and LEAL, 2003; COPPENS D'EECKENBRUGGE and GOVAERTS, 2015) is a tropical plant in the Bromeliaceae family with 56 genes 2794 species. (LUTHER and SIEFF, 1998). In addition to the nutritional value of its fruit, many species are cultivated for other purposes, such as the production of fibre for the manufacture of baggage material and used as ornaments (COLLINS, 1960; CUNHA et al., 1999). Reports by Sabahel khier et al. (2010) pineapple recommended for the treatment and recovery of certain diseases. The purpose of this study is to compare the antibacterial properties of pineapple peels and to investigate the anti-microbial properties of these products and byproducts. In addition, peels are often discarded as a waste product, which is why research may highlight the potential use of this waste product, which may be significant.

The degree of ripeness, type of cultivator, climate and post-harvest handling are several factors that contribute to the chemical and biochemical properties of pineapple (Ancos, S´anchez-Moreno, & Gonz´alez-Aguilar, 2016; Chaumpluk, Chaiprasart, & Vilaivan, 2012). Pineapple planted with good agricultural practice will produce good fruit with a pleasant aroma while fruits infected with pests and diseases will produce tasteless fruit (Sipes & Wang, 2016). In recent years, pineapple has received much attention since the its composition has contributed to the potential uses of nutritious foods has influenced the use of various pineapple-based products. Previous studies by Lasekan and Hussein (2018) have shown that pineapple is rich in ester compounds including methyl-2methylbutanoate, methyl hexanoate, methyl-3- (methylthiol) -propanoate, methyl octanoate, and 2-methoxy-4-vinyl phenol associated with the taste quality of a wide variety of pineapple species. In this sense, the different ripening levels of pineapple bring about changes in the chemical composition and different aroma profiles of the fruit, especially during storage.

George et al. (2016) examined ascorbic acid content and antioxidant activity of Sarawak pineapple at five different stages of ripening. Significant reductions were observed in the content of ascorbic acid, while antioxidant activity increased at all levels of maturity. In a previous research project, Sharma et al. (2016) developed a functional process parameter for total phenolic content, total flavonoid, ascorbic acid, and antioxidant activity of pineapple using response surface methodology. Depending on the ideal conditions at 68

◦C and screw speed of 70.3 rpm, the estimated test values were the complete phenolic content (46.91 mg Gallic Acid Equivalents / 100 g), the perfect flavonoid (48.75 mg quercetin / g ), ascorbic acid (51.97 mg / 100 g), and antioxidant activity (95.95%), respectively. Chaudhary et al. (2019) investigated the presence of calcium, potassium carbohydrate, water content, vitamin C, and fiber essential for maintaining a healthy diet and a healthy digestive system. As pineapple is rich in bromelain, the possible use of chemical content has been studied to reduce inflammation in inflammatory conditions including sour throat, gout, and arthritis (Khalid et al., 2016). The chemical structure of bromelain in pineapple is shown as follows:

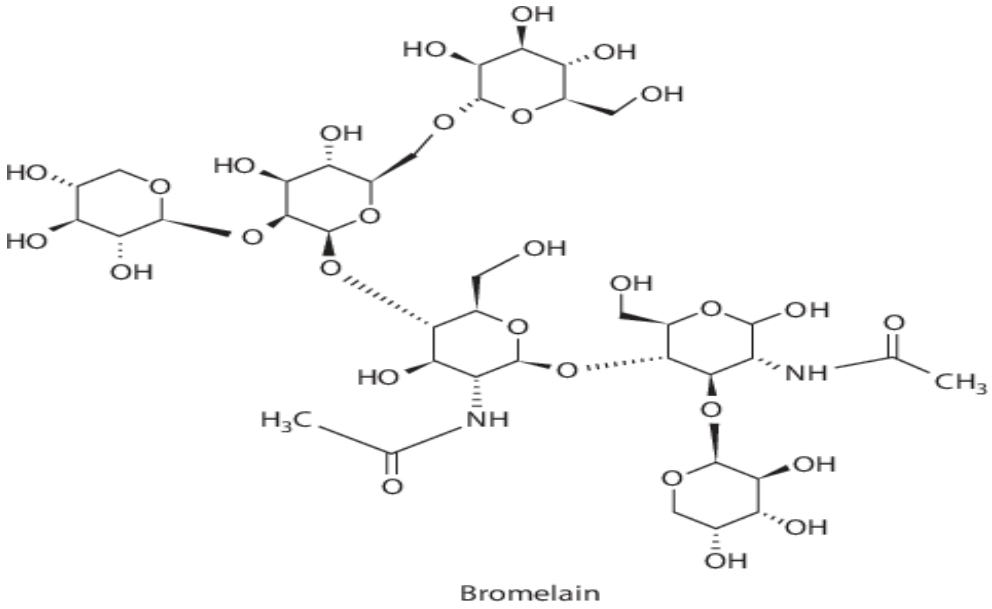



**Figure 1**: Chemical structure of Bromelain

**Anti-inflammatory activities of bromelain-**

There are various studies reporting the immunomodulatory effect of bromelain. Bromelain activates natural killer cells and increases the production of granulocyte-macrophage-colony stimulating factor, IL-2, IL-6 and reduces the production of T-helper cells. Therefore, bromelain reduces the number of inflammatory agents and plays an important role as an anti-inflammatory agent in various conditions.

**Anti-Cancer activities of Bromelain-**

It has been shown that bromelain inhibits nuclear factor-kB (NF-kB) translocation through G2 / M arrest to apoptosis in human epidermoid carcinoma and melanoma cells. The process of apoptosis is important in the developmental and homeostatic care of complex biological systems. Failure of normal apoptotic processes contributes to cell turnover and contributes to the growth of cancer cells. Bromelain reduces the activity of cell survival regulators, such as Akt and extracellular signal-regulated kinases, thereby promoting apoptotic cell death in tumors. Bromelain treatment of established mouse tumor cell lines resulted in inhibition of cell growth and matrigel invasion capacities.

**Clinical applications-**

Bromelain is currently being offered in many clinical settings because of its therapeutic effects in the treatment of inflammation and soft tissue damage. Clinical studies have shown that bromelain given to boxers completely removes all facial scratches and haematomas of the larynx, lips, ears, chest and arms in four days. It has been shown that oral bromelain is absorbed by the intestines without losing its biological properties and significantly reduces edema in the edema of the posterior leg which has been painfully treated in mice.

**Various Applications of Pineapple Waste**

**Energy Generation and as Carbon Source**

Oranusi et al. (2015) stated the technology of biogas (71% $CH_4$, 18% $CO_2$, 7.0% $N_2$, 1.5% $H_2$, 1.5% $H_2S$, 1% $O_2$) using codigestion of pineapple peels with food waste (1:1) where livestock rumen was used as the inoculums.

**Antioxidant Activity**

Pineapple waste has been discovered to have a excessive content of phytochemical activity and antioxidant ability that may be harnessed and utilized in various methods. Oliveira et al. (2009) stated that the usage of

methanolic extract from pineapple waste containing overall phenolic contents of 10 mg/g GAE confirmed tremendous dietary, therapeutical functionality and antioxidant activities (DPPH free radical scavenging and superoxide anionscavenging homes).

**Pharmaceutical and Food Industry**

Bromelain is a proteolytic enzyme present inside the stem of pineapple, called stem bromelain, and also in fruit. it is extensively used in pharmaceutical and food industries mainly as a tenderizer and a dietary complement (Hebbar et al. 2008).

**Citric Acid and Lactic Acid Production**

Pineapple waste has been used in food industries to enhance flavor (citrus). Kumar et al. (2003) stated using pineapple waste as a substrate to produce citric acid by A. niger under solid state fermentation where variation in the methanol concentration led to yield growth from 37.8% to 54.2%.

**Production of Ethanol**

Pineapple waste can be further metabolized to produce ethanol. Saccharomyces cerevisiae and Zymomonas mobilis are a few examples of the microorganisms used at commercial scale for this motive. however, due to the low availability of fermentable sugars, pretreatment step the use of enzymes (cellulase, hemicellulase) is necessary. both organisms were capable of producing approximately 8% ethanol from pineapple waste in 48 h after pretreatment with enzymes (Ban-Koffi and Han 1990).

**Heavy Metal Removal from Waste/Wastewater**

Pineapple fruit residues also act as effective biosorbent to dispose of toxic metals like Hg, Pb, Cd, Cu, Zn, and Ni where the addition of phosphate groups improved the adsorbent capacities at decrease pH (Senthilkumaar et al. 2000).

**Production of Fibers**

Larrauri et al. (1997) have stated that dietary fiber powder prepared from pineapple waste consists of 70.6% overall dietary fiber (TDF) that is similar tocommercial dietary fibers from apple and citrus fruits. however, pineapple has a better sensory property such as neutral coloration and taste.

**Vinegar Production**

Conversions of pineapple peel to vinegar were reported by Praveena and Estherlydia (2014) via simultaneous fermentation by Saccharomyces boulardii and Acetobacter where the resulting vinegar were further proven to have phytochemical property and antioxidant capacity.

**The Nutritional content of raw pineapples**

Raw pineapple contains 86% water, 13% carbohydrates, 0.5% protein, with a very low fat content. In about 100-gram, raw pineapple provides 209 kilojoules (50 kilocalories) of food energy, and is a rich source of manganese (44% Daily Value, DV) and vitamin C(58% DV), but otherwise you do not have micronutrients at significant values.

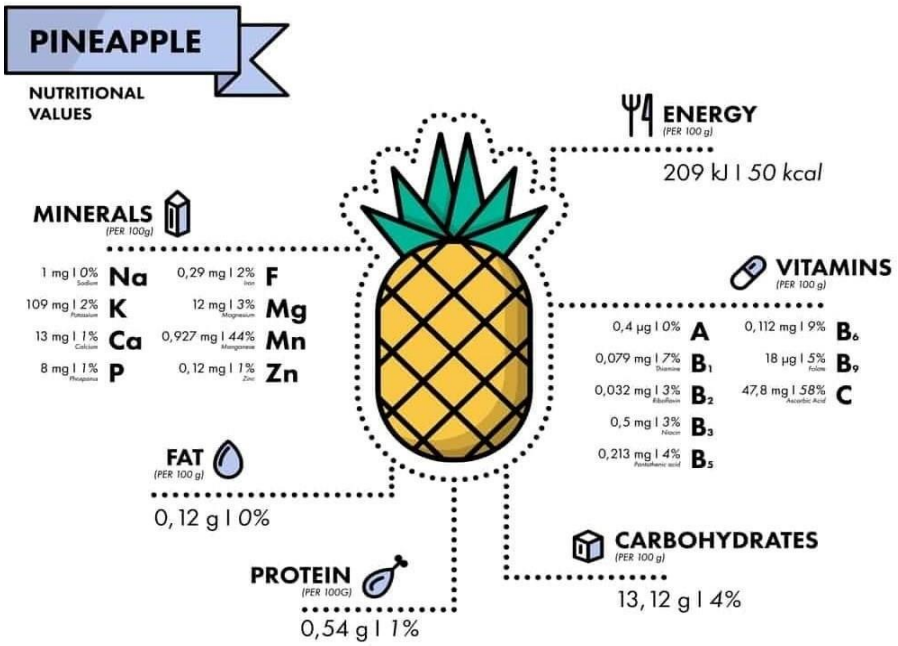



**Figure 2**: Nutritional content of raw pineapples.

**Table 1**: Top 10 countries of Pineapple production around the World

(All quantities are in million tons)

| Rank | Country | 2017 | 2016 |
|---|---|---|---|
| 1 | Costa Rica | 3.01 | 2.93 |
| 2 | Philippines | 2.67 | 2.61 |
| 3 | Brazil | 2.25 | 2.69 |
| 4 | Thailand | 2.12 | 1.77 |
| **5** | **India** | **1.86** | **1.92** |
| 6 | Indonesia | 1.81 | 1.39 |
| 7 | Nigeria | 1.64 | 1.57 |
| 8 | China | 1.57 | 1.55 |
| 9 | Colombia | 1.09 | 0.98 |
| 10 | Mexico | 0.94 | 0.87 |

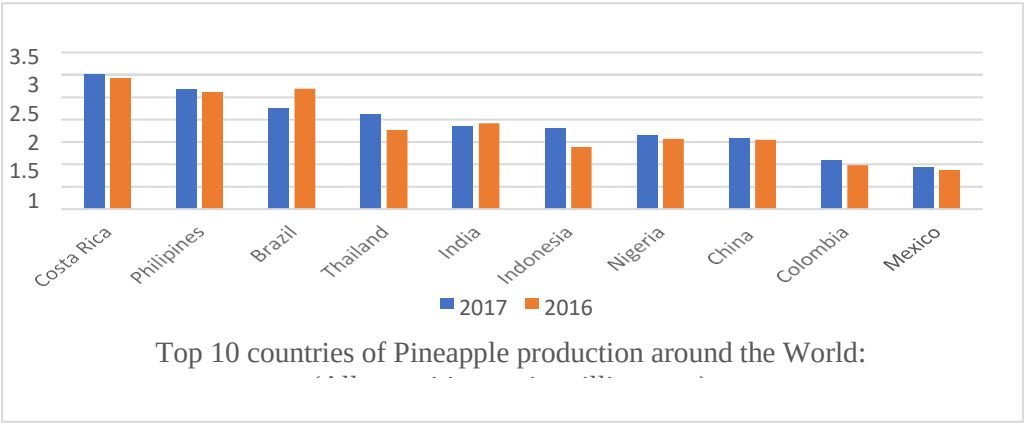



**Figure 3** -Top 10 countries of Pineapple production around the World:(All quantities are in million tons)

It is well-known that a balanced diet is essential to human health. Pineapple is known to have important bioactive properties for medicinal purposes. The fruit acts as a contraceptive, diuretic and for the removal of intestinal worms (Hossain, 2016). In addition, pineapple is often used to increase appetite for food nourishment and boost the excretion of fat for topical debridement. As a source of bromelain, pineapple is used to supplement proteolytic enzyme as an anti-inflammatory of soft tissue (Siow & Lee, 2012). (Kargutkar and Brijesh, 2018) successfully investigated the ability of anti- inflammatory activity from the pineapple leaf sac to identify phytochemical structures that were responsible for inflammatory diseases. In that study, the components identified in the extract included proteins, flavonoids, tannins, carbohydrates, glycosides and phenols. Pineapple is rich in vitamins and micronutrients that are recommended for daily consumption. In addition to this, pineapple also has low calories and is often included in the diet of the weight-watcher. The potential health benefits of pineapple are illustrated as follows:

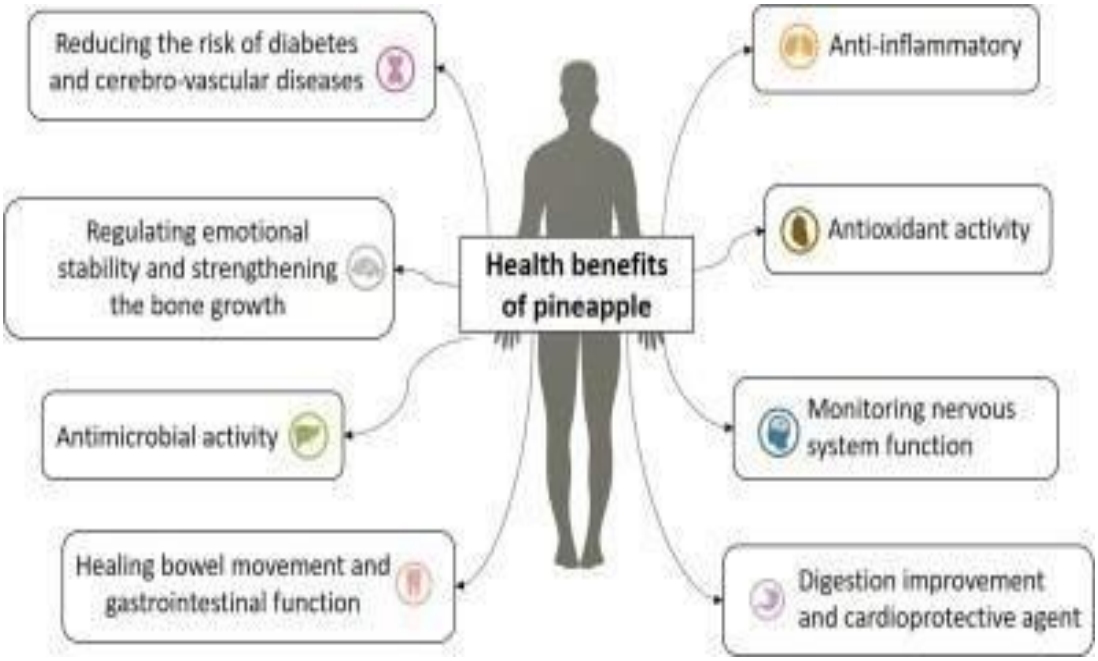



**Figure 4:** Health benefits of pineapple.

**Table 2:** List of Pineapple verities of species *Ananas comosus*, their names, place oforigin and front view of fruits-

| Name of Varity | Origin/Grown | Fruit Images |
|---|---|---|
| Abacaxi Pineapple | Bahamas, Florida, and Brazil. |  |
| Queen Pineapple | Philippines, Queensland, and South Africa. |  |
| Red Spanish Pineapple | West Indies, Mexico, and Venezuela. |  |
| Smooth Cayenne Pineapple | Whole world. |  |
| Brecheche Pineapple | Southern Venezuela. |  |

| | | |
|---|---|---|
| Cayena Lisa Pineapple | India, Sri Lanka, Malaysia and Thailand |  |
| Panare Pineapple | Venezuela. |  |
| Mauritius Pineapple | India, Ceylon, and Malaya. |  |
| Sugarloaf Pineapple | Puerto Rico, Philippines, South America, and Cuba. |  |
| Maipure Pineapple | Whole world. |  |
| Pernambuco Pineapple | Ecuador, Colombia, Brazil, Peru, and Venezuela. |  |

| | | |
|---|---|---|
| Roja<br>Espanola Pineapple | Whole world. |  |
| RipleyPineapple | Queensland |  |
| James Queen Pineapple | South Africa. |  |
| Cabezona Pineapple | Whole world. |  |
| Champaka Pineapple | Whole world. |  |
| Monte Lirio Pineapple | Whole world. |  |

| | | |
|---|---|---|
| PR-1-67 Pineapple | Whole world. |  |
| Montufar Pineapple | Whole world. |  |
| Spanish Jewel Pineapple | Spain. |  |

## MATERIAL AND METHODS

**Place of work-**

The present study was carried out in the Department of Bioengineering, Integral University, Lucknow.

**Collection of sample-**

Fresh Pineapple peel (weight = 1.7 Kgs) was collected from a juice corner near Integral University, Kursi Road, Lucknow.

**Washing-**

- Wash the sample with clean water.
- Remove leaves, chunks and contaminants from the pine apples's peel. (Pure weight of pine apples's peel = 1.5 Kgs)

**Air Drying-**

• Spread the peels sample for air-drying for 10 to 12 hours.

**Figure 5**: Fresh pineapple peel

**Grinding-**

- Crush the sample using grinder.

**Figure 6**: Crushed pine apple peel

**Extraction**

- The extraction of the pine apples's peel was obtained using muslin cloth and collected 780 ml liquid extract in a beaker from 1.5 kg pine apple's peel.

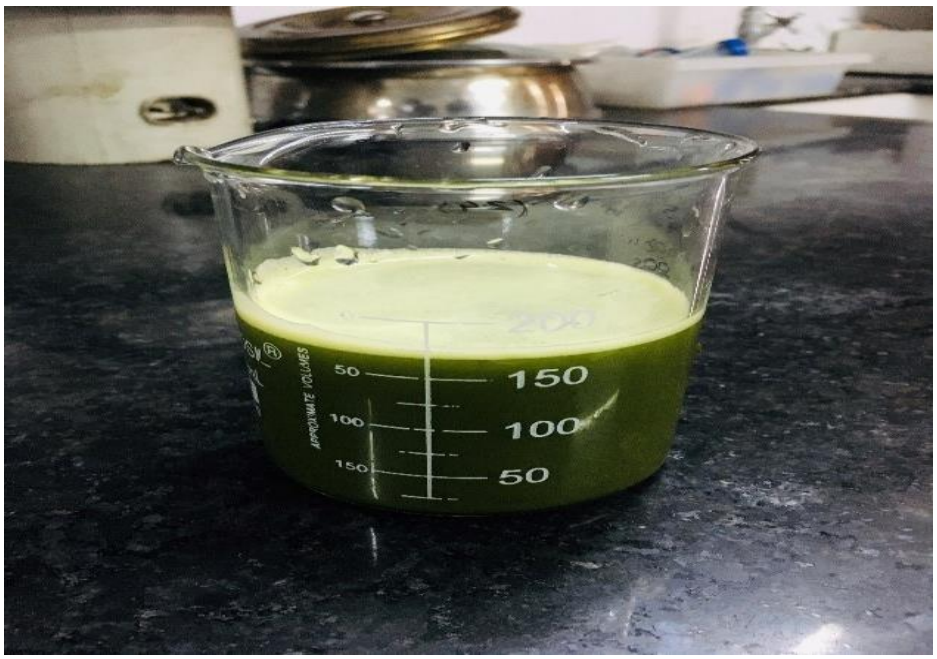



**Figure 7:** Extract of pine apple's peel

**DRYING-**

- Sample was dried under the 80°C temperature for evaporation of moisture.

**SCRAPING-**

- Scrape the dried extract from petri plates and collect it in falcon tubes. (33 grams of powdered extract was obtained from 780ml of liquid extract).

## SOXHLET EXTRACTION

The extraction of Soxhlet from solid and powder samples has been used for extracting polyphenolic compounds. This is a very simple process that involves limited preparation. In accordance with new standards it has been updated. In the case of extraction of hot-water bath, the water bathtub is the means of maintaining the necessary temperature for extraction of the phytochemical in the surrounding solvent. The performance of solvent-based extraction methods is determined by many variables, including existence and form of solvent, extraction period and retained temperature during extraction. In other instances, though, the time taken may be greater than or less than 6 hours.

**Figure 8**: Soxhlet apparatus

**Table 3:** Solvent used in soxhlet extraction

| Contents | METHANOL | HEXANE |
|---|---|---|
| Solvent | 200ml | 150ml |
| Powdered extract | 10gm | 6gm |
| Extract obtained | 1.77gm | 0.4gm |

Extraction was undergone at controlled temperature until all compounds were extracted.

**Figure 9:** Hexane crude extract after Soxhlet extraction

**DMSO-**

Collection of dried  hexane crude extract, DMSO solution was prepared by dissolving 20% w/w i.e. 0.4gm extract in 2 ml DMSO.

**Figure 10:** Hexane DMSO solution

## ANTI-MICROBIAL ACTIVITY

Testing both DMSO solutions (Methanol & Hexane) on different bacteria cultures by spreading the bacteria on Nutrient Agar media in petri dishes and cutting 4 wells in each petri dish to pour the DMSO solution into them with different concentrations to see the antimicrobial action of pineapple peel extracted compounds.

**Table 4**: Names of bacterial cultures used in the testing.

| S.No. | Bacteria culture |
|---|---|
| 1 | *Pseudomonas aeruginosa* |
| 2 | *E. coli* |
| 3 | *Bascillus Subtilis* |
| 4 | *Streptococcus aureus* |

## ANTIOXIDANT ACTIVITY

### DPPH Assay

2,2-diphenyl 2- picrylhydrazyl is a stable nitrogen-centered radical that is widely used to test the free radical scavenging ability of various samples; the higher the DPPH-scavenging activity, the higher the antioxidant activity of the sample. Scavenging activity also slightly high in extract of *pineapple peel*, development of suitable antioxidant. The reducing capacity of the extract may serve as a significant indicator its potential antioxidant activity. This activity may be due to phenolic compounds and flavonoids present in the extract as also indicated by Velioglu et al., 1998(Velioglu et al. 1998).

### FRAP Assay

The FRAP assay provides a direct estimation of the antioxidants or reductants present in a sample based on its ability to reduce the ferric (Fe3+)/(Fe2+) ferrous couple (Tanvir et al. 2015). The FRAP assay is also an important indicator of the antioxidant potential of a sample.

## RESULTS AND DISCUSSIONS

Petri dishes were kept in an incubator for 24 hours at 37°**C** to see the growth of bacteria and inhibition (if any) by the extracts.

The results are as follows:

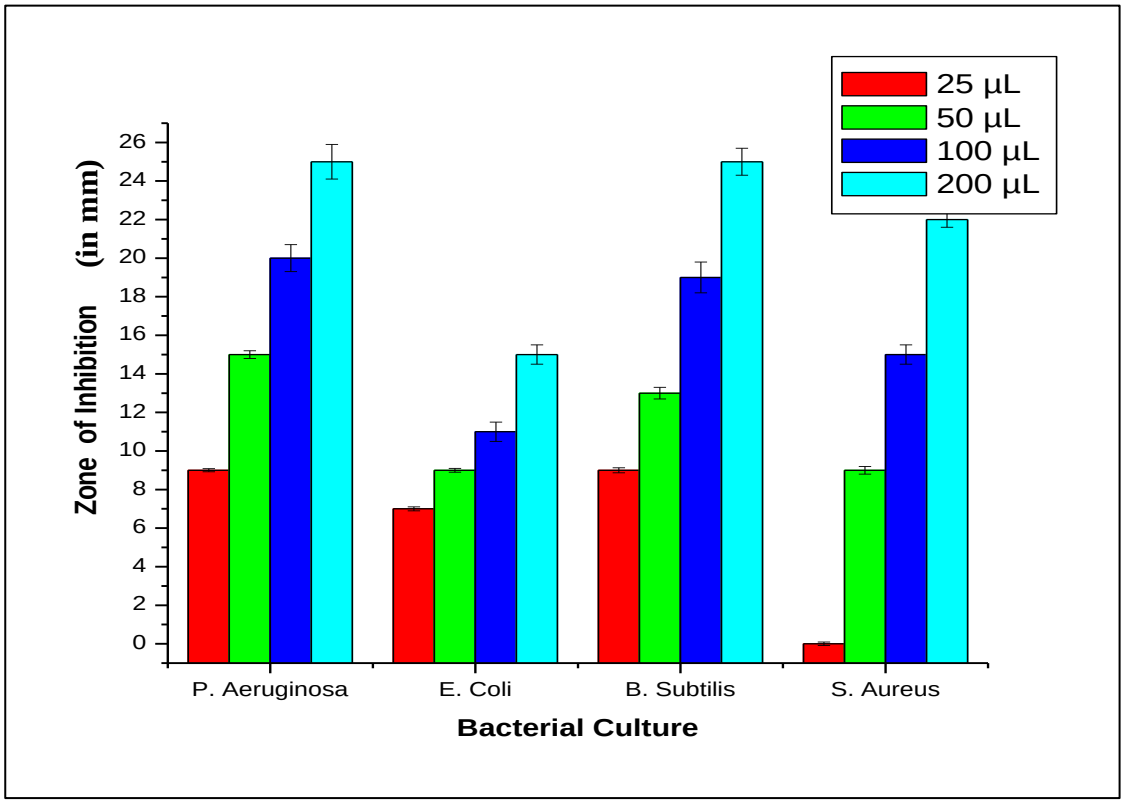



**Figure 11**: Antimicrobial activity of pineapple peel extract (Methanol) on different bacterial cultures

**Table 5:** Zones of inhibition at different concentrations of Methanol-DMSO solution on the plates of gram negative bacteria

.

| **Bacteria** | **25µL** | **50µL** | **100µL** | **200µL** |
|---|---|---|---|---|
| **Pseudomonas *Aeruginosa*** | 9mm | 15mm | 20mm | 25mm |
| **E.*coli*** | 7mm | 9mm | 11mm | 15mm |
| **Bascillus *Subtilis*** | 9mm | 13mm | 19mm | 25mm |
| **Streptoco *Aureus*** | — | 9mm | 15mm | 22mm |

***Pseudomonas aerginosa***

***E.Coli***

***Bascillus Subtilis***

***Streptococcus***

**Figure 12:** Zones of inhibition at different concentrations of Methanol-DMSO solution on the plates of gram negative bacteria

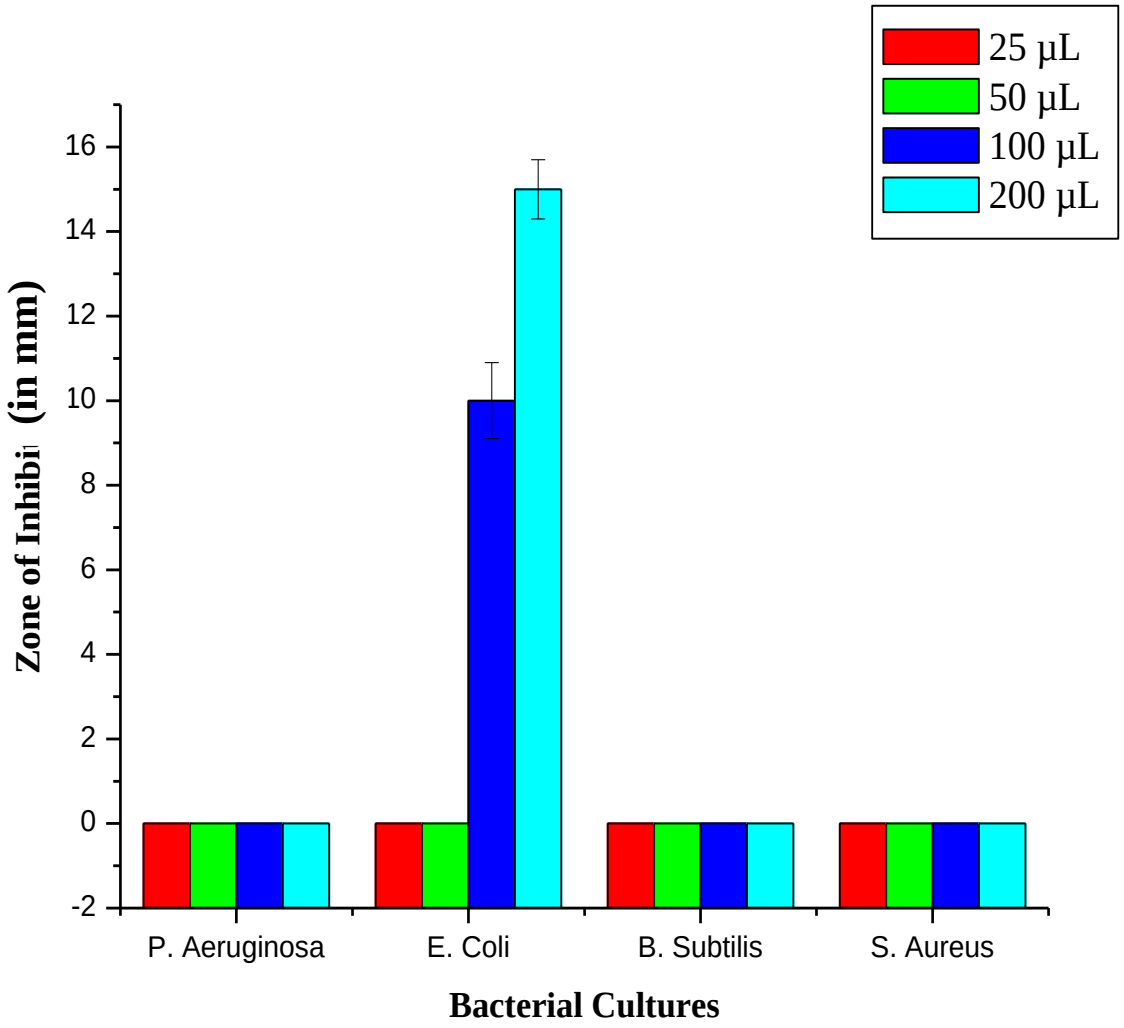
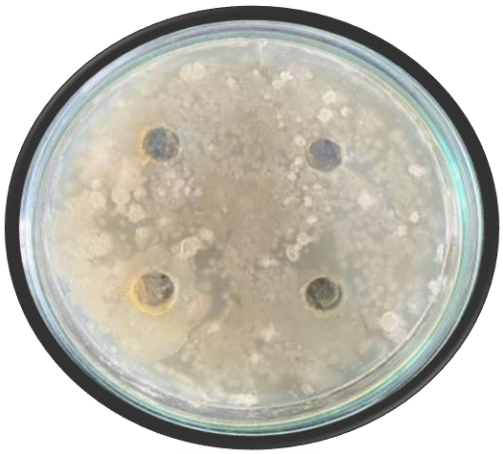



**Figure 13:** Antimicrobial activity of pineapple peel extract (Hexane) on different bacteria culture

**Table 6:** Zones of inhibition at different concentrations of Hexane-DMSOsolution on the plates of gram negative bacteria

| Bacteria | 25µL | 50µL | 100µL | 200µL |
|---|---|---|---|---|
| **Pseudomonasa *Aeruginosa*** | — | — | — | — |
| **E.*coli*** | — | — | 10mm | 15mm |
| **Bascillus *subtilis*** | — | — | — | — |
| **Streptococcus *aureus*** | — | — | — | — |



***Pseudomonas aerginosa***

***E.Coli***

***Bascillus subtilis***

***Streptococcus***

**Figure 14**: Zones of inhibition at different concentrations of Hexane-DMSO solution on the plates of gram negative bacteria

**Analysis of Antioxidant activity**

Freshly cut fruits and vegetables lose some of their overall antioxidant activity due to - oxidative stress brought on by cutting, which also damages membranes and affects the content and concentration of bioactive chemicals, primarily phenolics and vitamin C.

The DPPH (2,2-diphenyl-1-picrylhydrazy!) assay, a popular technique for evaluating a substance's capacity to scavenge free radicals and counteract oxidative stress, can be used to measure antioxidant activity. The DPPH assay is based on the idea that when a free radical combines with an antioxidant, it loses some of its stability and turns a deep purple color. A change from purple to yellow as a result of this reduction can be seen using spectrophotometry. The antioxidant activity of coated and uncoated sample was analyzed, coated sample exhibit better antioxidant activity than uncoated sample due to the presence of CEO and AEO in the form of nano emulsion. The antioxidant activity ranges from 70-90% in samples. Sample shows good antioxidant activity due to the presence of antioxidant compounds which have good antioxidant property, while standard shows not good in comparison of sample. Primarily phenolics and vitamin C, changing the level of the overall antioxidant. in this study on freshly cut pineapple fruit, antioxidant activity increased during the first few days of storage but gradually decreases over time. The higher stress level in the tissue caused by pine apple fruit cutting is shown by the bigger increase in antioxidant activity. In order to counteract the effects of minimal processing, effective antioxidants may be lost as storage duration increases as a result of their interactions with free radicals (Sharma & rao, 2018).

The antioxidant content of one samples were determined by DPPH scavenging method. The DPPH is frequently used to refer is an organic substance with formula 2,2-diphenyl-1- picrylhydrazyl. It is in the form of a powder in crystalline form with a black color and made of free radical particles that are stable. It is employed to assess the capacity of pine apple peel components to scavenge and destroy free radicals. This method is completely based on spectrophotometer analysis of the change and alteration in DPPH concentration that are brought due to the Interaction with an antioxidant. 1 mg/ml methanolic extract is prepared further DPPH and methanol are added to extract, the sample is kept in dark for 30 mins and absorbance is checked 540nm.

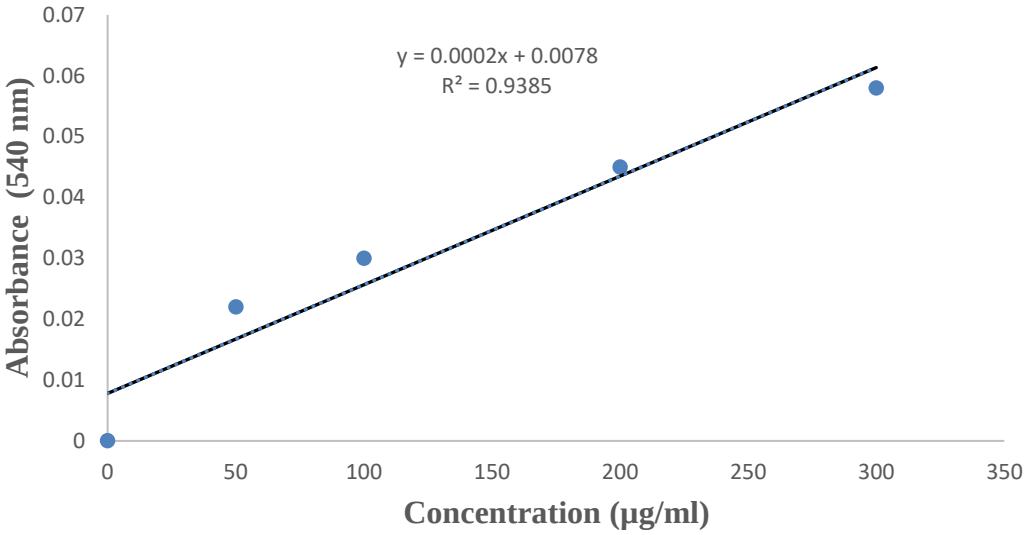



**Figure 15**: Scavenging of DPPH in different concentration of Ascorbic acid (Standard)

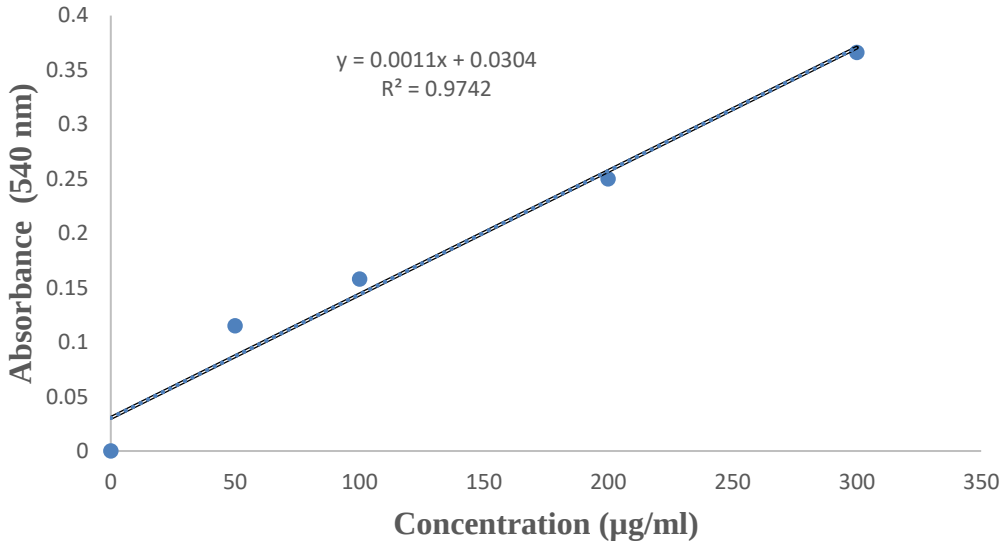



**Figure 16:** Scavenging effect of methanolic crude extract of pine apple peel on DPPH in different concentration

**Ferric reducing antioxidant activity (FRAP)**

The method described in Gue et al. (2003) was used with slight modifications. Briefly, FRAP reagent was prepared with sodium acetate buffer solution (300 mmol L−1, pH 3.6), a tripyridyltriazine (TPTZ) solution (20 mmol L−1 in 36 mmol L−1 HCl) and FeCl3 solution (10 mmol L−1) in the proportion of 10:1:1 (v:v:v). 1 ml of pineapple peel extracts prepared in distilled water was added reagent. The antioxidant capacity was determined using the calibration curve and represented as mmol FeSO4 equivalents per 1 g-1 of sample in dry weight (µmol FeSO4 . g-1DW).

The results of FRAP test is presented in Graph--- It was observed that the highest reducing power pine apple peel extract in comparison of standard. Therefore, the FRAP assay is a precise and common method for measurement of antioxidant capacity in many natural sources including fruits and vegetables as well as their wastes.

The FRAP value was also evaluated and found to be significantly higher in methanolic extract, (378.50±6.98) µmol Fe(II)/g

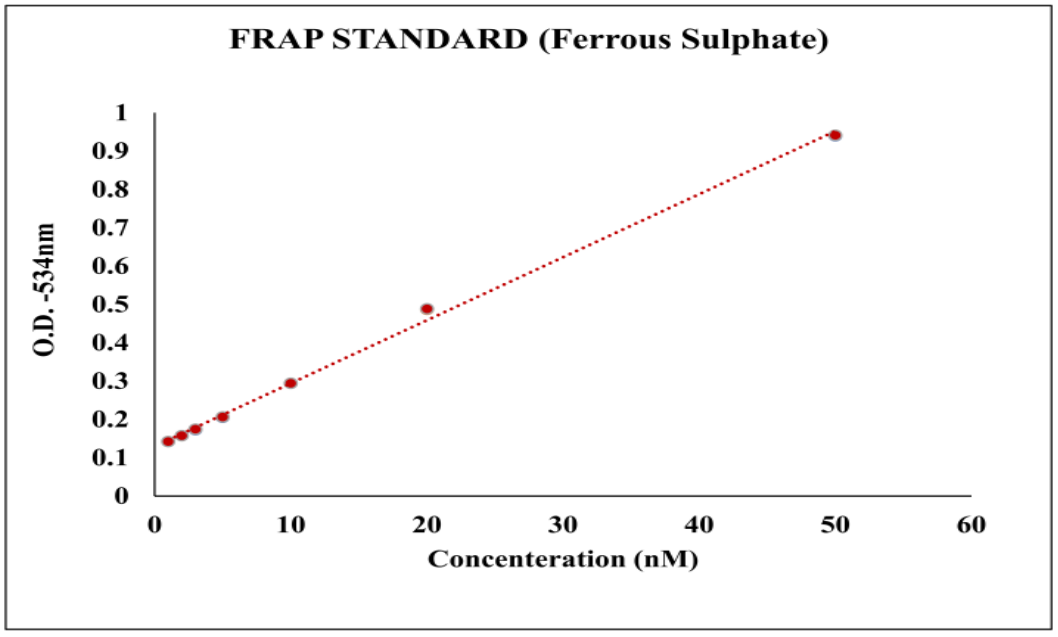



**Figure 17-** FRAP assay of methanolic extract of pineapple peel.

## CONCLUSION

Preparation of extracts from pineapple peel is eco-friendly and cost-effective process as it involves the use of food waste materials. The antimicrobial potential exhibited by hexane and methanol extract of pineapple peel was significant against gram negative bacterial strains which encourages for the search of newer active compounds from pineapple peel responsible for its antimicrobial potential.

The presence of phenolics and flavonoids in the methanolic pineapple peel extract emphasizes its importance & warrants further research as an antioxidant agent.

Thus this study paves way for the use of fruit waste to derive lead compounds that can be used as antioxidant and antimicrobial agents.

## REFERENCES


- Pathak P D, Mandavgane S A and Kulkarni B D, Fruit peel waste: Characterization and its potential uses, Curr Sci, 2017, 113(3), 444-454.

- International Diabetes Federation (IDF) Atlas, Available from www.diabetesatlas.org 2019.

- Sarkar T, Nayak P and Chakraborty R, Pineapple [Ananas comosus (L.)] product processing techniques and packaging: A review, IIOAB J, 2018, 9(4), 6-12.

- Horticultural Statistics at a Glance, 2018, www.agricoop.nic.in

- Varzakas T, George Z and Francis V, Plant food residues as a source of nutraceuticals and functional foods, Foods, 2016,

- Upadhyay A, Jeewan P L and Shinkichi T, Utilization of pineapple waste: A review, J Food Sci Technol Nepal, 2010

- Chaudhary V, Kumar V, Vaishali S, Singh K, Kumar R, et al., Pineapple (Ananas comosus) product processing: A review, J Pharmacogn Phytochem, 2019, 8(3), 4642-4652.

- Tochi B N, Wang Z, Xu S Y and Zhang W, Therapeutic application of pineapple protease (Bromelain): A review, Pak J Nutr, 2008, 7(4), 513-520.

- Bresolin I R A P, Bresolin I T L, Silveira E, Tambourgi E B and Mazzola P G, Isolation and purification of Bromelain from waste peel of pineapple for therapeutic application, Braz Arch Biol Technol, 2013, 56(6), 971-979.

- Debnath B, Singh W S and Manna K, A phytopharmacologica review on Ananas comosus, Adv Tradit Med, 2021, 1-8.

- Ancos. B., S´anchez-Moreno, C., & Gonz´alez-Aguilar, G. A. (2016). Archived from the original on 4 March 2016.

- Aziz SA, Olival KJ, Bumrungsri S, Richards GC, Racey PA (2016). The Conflict Between Pteropodid Bats and Fruit Growers: Species, Legislation and Mitigation. In Voigt C, Kingston T (eds.). Bats in the Anthropocene: Conservation of Bats in a Changing World. Springer.

- Báez R, Lopes MT, Salas CE and Hernández (2016). In vivo antitumoral activity of stem pineapple (*Ananas comosus*) bromelain. Planta Med 73: 1377-1383,2007.
- Bartholomew DP; Hawkins RA; Lopez JA (2012). "Hawaii Pineapple: The Rise and Fall of an Industry". *HortScience.* 47 (10):
- Bartholomew, D. P.; Paull, Robert E.; Rohrbach, K. G. (2002). The Pineapple: Botany, Production, and Uses. *CABI. p*. 23. ISBN 9780851999791.
- Blonstein JL: Control of swelling in boxing injuries. Practitioner 185
- Chandler DS and Mynott TL (1998). Bromelain protects piglets from diarrhoea caused by oral challenge with K88 positive enterotoxigenic *Escherichia coli*. Gut43: 196-202
- Chaumpluk, P., Chaiprasart, P., & Vilaivan, T. (2012). Postharvest non- destructive determination of fruits: A model on fruit maturity assay via biosensor based on colorimetric change of gold nanoparticles. *Acta Horticulturae,* 945, 205–212. 10.17660/ *ActaHortic*.2012.945.27.
- COLLINS, J.L(1960). The pineapple, botany, utilization, cultivation. *London: Leonard Hill*. 294 p.
- COPPENS D'EECKENBRUGGE, G.; GOVAERTS, R (2015). Synonymies in Ananas (Bromeliaceae*). Phytotaxa, Auckland,* n.239, p.273-279.
- COPPENS D'EECKENBRUGGE, G.; LEAL, F (2003). Morphology, anatomy and taxonomy. In: BARTHOLOMEW, D.P.; PAULL, R.E.; ROHRBACH, K.
- (Ed.). The pineapple: botany, production and uses. *New York: CABI Publishing*.p.13-32.
- CUNHA, G.A.P. da; CABRAL, J.R.S.; SOUZA, L.F. da S (1999). (organizadores). O abacaxizeiro – cultivo, agroindústria e economia. Brasília, DF*: Embrapa Comunicação para Transferência de Tecnologia*. 480p.
- Dalimartha S (2000). Atlas tumbuhan obat Indonesia; vol 2. *Jakarta: Trubus Agriwidya. p*. 1405
- Davidson A. (2008) The Penguin Companion to Food. Penguin Books.
- Eshamah, H., Han, I., Naas, H., Rieck, J., Dawson, P. Bactericidal
- George, D. S., Razali, Z., & Somasundram, C. (2016).
- Gibson, Arthur C(2017). "Pineapple – The Plant That Ate Hawai'i". UCLA.

- Grant, Jason R.; Zijlstra, Gea (1998). "An Annotated Catalogue of the Generic Names of the Bromeliaceae". *Selbyana.* 19 (1): 91– 121. ISSN 0361-185X.JSTOR 41759978.
- Hossain, M. F. (2016). World pineapple production: An overview. *African Journal of Food, Agriculture, Nutrition and Development*, 16(4), 11443–11456. 10.18697/ ajfand.76.15620.
- https://doi.org/10.1590/0100-29452018302
- https://www.crazymasalafood.com/top-20-different-types-ofpineapple-you- should-know/
- https://www.homestratosphere.com/types-of-pineapple/
- Inoue K, Motonaga A, Dainaka J, Nishimura T, Hashii H, Yamate K, Ueda F and Kimura K: Effect of etodolac on prostaglandin E2 biosynthesis, active oxygen generation and bradykinin formation.
- Jones, J.; Wilson, W (2006). "Chapter 11: Science". An Incomplete Education. *Ballantine*. p. 544. ISBN 978-0-7394-7582-9.
- Khalid, N., Suleria, H. A. R., & Ahmed, I. (2016). Pineapple Juice. In F. Shahidi & C. Alasalvar (Eds), *Handbook of Functional Beverages and Human Health (1st Editio, pp. 489–500).* CRC Press. 10.1201/ b19490-43.
- Lasekan, O., & Hussein, F. K. (2018). Classification of different pineapple varieties grown in Malaysia based on volatile fingerprinting and sensory analysis. *Chemistry Central Journal*, *12*(1), 1–12. https://doi.org/10.1186/s13065-018-0505-3
- List of prohibited animals"(PDF). Government of Hawaii (2013), Department of Agriculture. Listeria monocytogenes. *Journal of Food Research*. Vol. 2, No. 1
- Luerti M and Vignali ML (2006). Influence of bromelain on penetration of antibiotics in uterus, salpinx and ovary. *Drugs Exp Clin Res* 4: 45-48, 1978.
- LUTHER, H.E.; SIEFF, (1998). An alphabetical list of bromeliad bionomials. 6th ed. *Newberg: The Biomeliad Society,* 138 p. Morphology, Anatomy, and Taxonomy". In Bartholomew, DP; Paull,
- Morton, Julia F (1987). "Pineapple, Ananas comosus". Retrieved 22 April 2011.
- Mynott TL, Guandalini S, Raimondi F and Fasano A: Bromelain prevents secretion caused by *Vibrio cholerae* and *Escherichia coli* enterotoxins in rabbit ileum in vitro.

Gastroenterology 113: 175-184, 1997.

- Mynott TL, Luke RK and Chandler DS (1996). Oral administration of protease inhibits enterotoxigenic *Escherichia coli* receptor activity in piglet small intestine. Gut 38: 28-32.
- Nutrient data for pineapple (2012), raw, all varieties, per 100 g serving". *Nutritiondata.com, USDA SR-21*.

- Oh-ishi S, Uchida Y, Ueno A and Katori M: Bromelian, a thiolprotease from pineapple stem, depletes high molecular weight kininogen by activation of Hageman factor (Factor XIII). Thromb Res 14: 665-672, 1979.
- Oxford English Dictionary. Physiochemical Changes during Growth and Development of Pineapple (Ananas comosus L Merr. cv. Sarawak). *Journal of Agricultural Science and Technology, 18*(2), 491–503.

- Pineapple composition and nutrition. *Handbook of Pineapple Technology: Postharvest Science, Processing and Nutrition, 221– 239*. https://doi.org/10.1002/9781118967355.ch12.
- Pineapple Definition | Definition of Pineapple at Dictionary.com"
- Pineapple Growing"(2012). Tropical Permaculture.com (Birgit Bradtke). Archived from the original . Pineapple Working Group-International Horticultural Society.Retrieved 15 August 2010.[permanent dead link] pp. 377–426. doi:10.1007/978-3-319-25220-9_13. ISBN 9783319252209.

- Pratiwi, Dewi (1994). Aktivitas Antibakteri Ekstrak Etanol Kelopak Rosela (Hibiscus sabdariffa Linn) Terhadap Pseudomonas aeruginosa Multiresisten dan Shigella dysenteriae. Seminar Nasional. 2011 PERHIPBA *Prostaglandins Leukot Essent Fatty Acids* 51: 457-462.

RE; Rohrbach, KG (1994). The Pineapple: Botany, Production, and Uses. Wallingford, UK*: CABI Publishing*. p. 21. ISBN 978-0-85199-503-8.

- Sabahelkhier, K.M., Hussain, A.S., Ishag, K.E.A. (2010). Effect of maturity stage on protein fractionation, in vitro protein digestibility and anti-nutrition factors in pineapple (Ananas comosus) fruit grown in Southern Sudan. *African Journal of Food*

*Science, 4(8), 550-552.*

- Shahid SK, Turakhia NH, Kundra M, Shanbag P, Daftary GV and Schiess W: Efficacy and safety of phlogenzym - a protease formulation, in sepsis in children. J Assoc Physicians India 50
- Sharma, P., Ramchiary, M., Samyor, D., & Das, A. B. (2016). Study on the phytochemical properties of pineapple fruit leather processed by extrusion cooking. *LWT – Food Science and Technology, 72,* 534– 543. https://doi.org/10.1016/j.lwt.2016.05.001.
- Sipes, B., & Wang, K. H. (2016). Pests, diseases and weeds. *In Handbook of Pineapple Technology: Postharvest Science, Processing and Nutrition* (pp. 62– 88). Wiley Blackwell.
- Stahl, JM; Nepi, M; Galetto, L; Guimarães, E; Machado, SR (2012). "Functional aspects of floral nectar secretion of Ananas ananassoides, an ornithophilousbromeliad from the Brazilian savanna". *Annals of Botany*. 109 (7): 1243–1252. doi:10.1093/aob/mcs053. PMC 3359915. PMID 22455992.
- Tinozzi S and Venegoni A (1978). Effect of bromelain on serum and tissue levels of amoxicillin. Drug1s Exp Clin Res 4: 39-44.
- Uhlig G and Seifert J (1981). The effect of proteolytic enzymes (traumanase) on posttraumatic edema. Fortschr Med 99: 554-556.
- Vellini M, Desideri D, Milanese A, Omini C, Daffonchio L, Hernandez A and Brunelli G (2017). Possible involvement of eicosanoids in the pharmacological action of bromelain. *Arzneimittelforschung 36: World list/Production Quantity (pick lists)". UN Food and Agriculture Organization, Corporate Statistical Database (FAOSTAT).*