Abstract - Ananas comosus, commonly called pineapple is a very promising medicinal plant, which is being conventionally consumed by the people because of its delicious taste as well as high nutritional content. The endeavour of the present work conducted was to phytochemically screen the extracts made from the peels of the fruit as well as to evaluate the antimicrobial and antioxidant activity of the extracts made in different solvents. Methods: Both antioxidant and antimicrobial activity of the plant extract was analyzed with the final concentration of 30mg/ml. Escherichia coli, Bacillus amyloliquifaciens, Pseudomonas aureginosa and Staphylococcus aureus were used to determine the antimicrobial activity of the extracts prepared. Electron Transfer Assay, Enzymatic and non enzymatic assays were evaluated by various antioxidant methods. Results: Study reveals that flavonoids were screened positive in extracts made in ethanol, methanol, n-hexane and acetone. Highest antimicrobial sensitivity was observed in Ethanolic extract with an inhibition zone diameter of 15mm, 14mm, 14mm and 21mm against Bacillus amyloliquifaciens, Staphylococcus aureus, Pseudomonas aureginosa and Escherichia coli respectively. As far as antimicrobial activity by broth dilution method is concerned, Escherichia coli was the one which showed maximum percentages of inhibition i.e.98%, highest amount or proteins leaked was found to be in Staphylococcus aureus i.e. 400µg/ml of extract and maximum absorbance for nucleic acid leakage was observed in Escherichia coli, that was 0.085 at 595nm. Electron Transfer assay was performed by Total Flavonoids count and Total Phenolic count in which utmost results were given by methanolic and ethanolic extracts being 860µg/ml and6930 µg/ml respectively. All the three enzymatic activities CAT, SOD and GST were found to highest in extract made in acetone being 399.46 µmole of H2O2 consumed/min/mg of proteins, 246.15 Units/min/mg of proteins and 145.69 µmoles of CDNBS-GSH conjugate formed/min/mg of proteins respectively. MDA content was found to be maximum in acetic anion extract i.e. 27µg/mg of proteins. Conclusion: Peel extract of Ananas comosus have a broad antioxidant and antimicrobial activity both and have a potential in treatment of cancer and bacterial diseases.

Keywords: Ananas comosus, phytochemical screening, antimicrobial activity and antioxidants.

INTRODUCTION

Medicinal plants

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. The “backbone” of traditional medicine is the medicinal plants, which means more than 3.3 billion people in the less developed countries utilize them plants on a regular basis [1]. According to WHO, species of the plant which are used as medicaments were found about 70000. Actually what makes a plant medicinal is its property to cure disease [2, 3]. These plants have the special chemical properties by the virtue of which they can be used as conventional drugs. It was observed that there were 150 compounds identified in plants by which they can be used as medicine. Ethno botany is the study of traditional uses of plants and the type of plants are called Ethno medicinal plants.

Medicinal plant parts are commonly rich in phenolic compounds, such as phenolic acids, flavonoids, tannins, stilbenes, lignans, coumarins, and lignins. Multiple biological effects including the Anti oxidant activity are there in these compounds [4]. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health [5].

Ananas comosus

Pineapple [Ananas comosus (L.) Merr. Family: Bromeliaceae] is one of the most important commercial fruit crops in the world. It is known as the queen of fruits due to its excellent flavour and taste [6]. Pineapple is the third most important tropical fruit in the world after Banana and Citrus [7]. Pineapples are consumed or served fresh, cooked, juiced and can be preserved. This fruit is highly perishable and seasonal. Mature fruit contains 14% of sugar; a protein digesting Enzyme, bromelain, and good amount of citric acid, malic acid, vitamin A and B [8]. Pineapple juice's composition varies depending on geography, season, process and time of harvest. Its balance of sugar and acid contributes to the fruit's refreshing flavour [9]. Thailand, Philippines, Brazil and China are the main pineapple producers in the world supplying nearly 50 % of the total output [10]. Other important producers include India, Nigeria, Kenya, Indonesia, Mexico, Costa Rica and these countries provide most of the remaining fruit [11]. Green pineapple is also used for making pickles. After extraction of its juice, the left over is used as livestock feed and also the tender leaves are used for the same purpose. Various food items like squash, syrup, jelly are produced from pineapple. Vinegar, alcohol, citric acid, calcium citrate etc. are also produced from...
Pineapple. Pineapple is also recommended as medical diet for certain diseased persons [12]. The U.S. National Library of Medicine lists bromelain as a proteolytic digestive enzyme. When taken with meals, bromelain aids in the digestion of proteins, working to break proteins down into amino acids [13].

OBJECTIVE
The purpose of the present study was to isolate the flavonoid glycosides from the peel of Ananas comosus. Flavonoids glycosides were purified by Column chromatography. Pharmaceutical evaluation of isolated flavonoid glycosides was done by determining anti-microbial and anti-oxidant activity. To determine the anti-microbial activity both Agar Well Diffusion and Broth Dilution methods were implemented. For anti-oxidant activity Electron Transfer Assay, Enzymatic and Non- Enzymatic Biochemical Assays were quantified.

MATERIALS AND METHODS
Source of sample collection Fresh Pineapple was collected from the fruit vendor. It was washed and then peeled off. They were then washed twice, once with tap water and then with distilled water to remove dirt and soil particles. Peels were allowed to dry at room temperature at room temperature for a period of one week. The dried peels were grounded with ordinary grinder to form powder.

Bacterial cells: The bacterial cells of Escherichia coli (DH5α), Bacillus amyloliquefaciens, Staphylococcus aureus and Pseudomonas aeruginosa were obtained from Helix BioGenesis Pvt. Ltd., Noida, U.P. and were sub cultured freshly in Nutrient Broth and used further for research work.

Preparation of extract: 10g grounded powder of peels was dissolved in 100ml of ethanol, methanol, n-hexane, ethyl acetate, chloroform and acetone to form crude extracts by maceration method by boiling continuously for 30 minutes. The conical flasks of extract were covered by cotton plugs to avoid evaporation. The extracts were placed in shaking incubator at 250rpm for 48hrs. After shaking they were spread over LB agar plates using sterile spreader, after inoculation with microorganism. 200µl of respective Gram positive and Gram negative bacterial cells. Reaction mixture was containing extract as well as with defined cells. Broths were incubated for 24hrs in shaker at 37°C and their growth were observed at 620nm. Results were observed down in percentage of inhibition.

1. Measurement of cellular leakage
2. Protein leakage assay:
   The protein leakage assay was carried out using the method of Kim et al. (2011). The bacterial cells were treated with known concentration of extracts for about 24 hrs and then centrifuged at 3000 rpm for 03 min. For each sample, 100 µL of the supernatant was mixed with 900 µL of Lowry’s Reagent (A&B) and then incubated for 10 min. The optical density was measured at 700 Nanometre using Hitachi-U 2900 spectrophotometer. Bovine serum albumin (BSA) was used to prepare the standard curve.

3. Nucleic acid leakage assay
   The nucleic acid leakage study was done by Diphenylamine (DPA) method. Briefly, aliquots of bacterial cultures were exposed to extracts for 24 hours and then centrifuged at 5000 rpm for 5 minutes. For each sample, 2ml of DPA was added to 100µl supernatant and then was incubated at 95°C for 10 min. Optical density was measured at 595 nm. Lyophilised DNA in the concentration of 200µg/ml was used for the preparation for standard curve.

Antioxidant assay
1. Electron transfer assay: Total Flavonoids content and Total Phenolic content has been assayed to determine the oxidants which were reduced by transfer of electron from an antioxidant (oxidized).
   a. Total Flavonoid Content
   Aluminium Chloride Spectrophotometric method was used to determine the number of flavones present in the extracts. About 0.1ml of extract or Quercetin standard 10-100µg/ml, 1.5ml of methanol, 0.1ml Aluminium chloride (10%), 0.1ml Potassium acetate (1M) and 2.8ml of distilled water were added and mixed well. Sample blank was prepared by replacing sample with distilled water or solvent and absorbance was measured at 417nm. Standard calibration plot was made to determine the concentration of flavonoids in the extracts. The concentrations of flavonoids in the extracts were calculated from the calibration plot and were expressed in mg QE/ g of extracts [15,16].
   b. Total Phenolic Content
   Flavonoids are polyphenolic compounds so the total Phenolic content has been determined by Folin Ciocalteau method in order to quantify the polyphenolic Flavonoids. 0.1ml of extracts or quercetin standard (10-100 µg/ml) and 0.1ml of Folin Ciocalteau reagent (0.5N) was added and incubated at room temperature for 30min. About 2.5ml of 20% saturated sodium carbonate was added in to the
solution and further incubated for 30min. After incubation, the absorbance was measured at 760nm against blank reagent. The standard calibration plot was made to determine the concentration of polyphenolic component in the extracts were calculated from the calibration plot and were expressed in mg QE of phenol g⁻¹ of extracts [15,16,17].

2. ENZYMATIC BIOCHEMICAL ASSAY

a. Superoxide dismutase activity: This is an enzymatic assay that catalyses the dismutation of free radicals at a rate 10 times higher than that for spontaneous dismutation at pH 7.4. SOD mainly act by quenching of superoxide (O₂⁻) by catalyzing the decomposition of hydrogen peroxide (H₂O₂) to water and oxygen. To analyze the SOD activity, 2ml of phosphate buffer (0.17 M, pH 8.3), 0.1ml of Nitroblue tetrazolium (1.2mM), 0.1ml of Phenazine methosulphate (28mM) and 0.1ml of NADH was added with 0.1ml – 0.5ml of extract. Mixture was allowed to incubate for 30 min afterwards. Absorbance was taken at 560nm at different intervals of time i.e. after every 15 seconds. Superoxide dismutase activity was expressed as one unit of enzyme activity as µmoles of H₂O₂ oxidized per min per mg protein [20].

b. Catalase activity: To analyze the catalase activity, catalase buffer has been prepared by adding 10mM H₂O₂ in the 1M phosphate buffer of pH6.5. Sample was added in the aliquots of catalase buffer and absorbance was taken at 260nm after definite interval of time. Catalase activity was expressed in enzyme activity as µmoles of H₂O₂ oxidized per min per mg protein [20].

c. Glutathione S Tranferase activity: To determine the detoxification of extracts, 2ml of 1M of phosphate buffer pH6.5, 0.1ml of CDNB, 0.1ml of GSH and 0.1ml of extract/buffer was added and mixed well. Absorbance was taken at 340nm at every interval of time. The GST assay was based on glutathione conjugation to 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate and measured spectrophotometrically at 340 nm. The specific activity of the enzyme was expressed as µmole of CDNB-GSH conjugate formed per min per mg protein [21].

3. NON ENZYMATIC BIOCHEMICAL ASSAY

a) Lipid peroxidation: Malonaldehyde (MDA) content has been determined by standardized protocol. Lipid peroxidation i.e. thiobarbituric acid (TBA) reactive substances were measured spectrophotometrically at 532nm in extracts on the principle of formation of Malondialdehyde (MDA) by breaking down of polyunsaturated fatty acids where the levels of lipid peroxidation were expressed as nano mole of Malondialdehyde formed per mg of extract[22].

Statistical Evaluation: To assure the accuracy of the experimental data, each experiment was performed in triplicate and the result was expressed as mean ± standard deviation of three replications. p value < 0.05 was regarded as significant.

RESULTS and DISCUSSION

Extracts were prepared from ethanol, methanol, acetone ethyl acetate, chloroform and n-hexane. First of all primary phytochemical screening was done to check the presence of various secondary metabolites present (Table 1). Flavonoids screened positive in extracts made from ethanol, methanol, acetone and n-hexane being absent in the extracts made in chloroform and ethyl acetate. Saponins and Naphthoquinone were absent whereas total Phenolic count and alkaloids showed positive results in all the six extracts. Tannins were screened positive only in ethanolic extract whereas Terpenoids showed positive results in both ethanolic and methanolic extracts. Inulin was positive in extracts prepared in ethanol, chloroform and ethyl acetate as compared to Glycosides which were only present in extracts prepared in ethanol and acetone. Extracts showing positive results for the presence of flavonoids were further purified by column chromatography using 60-120 mesh silica, which can retain the other constituents present other than flavonoids in the column itself. Final extracts of concentration 30mg/ml were made and used further during the rest of the studies.

Escherichia coli was found to be inhibited by extracts made in ethanol, methanol and n-hexane giving a maximum inhibition length of 11 mm in methanolic extract as compared to extracts prepared in ethanol and n-hexane giving a inhibition of length of 10mm and 4mm respectively (Table 02, 03, 04). Bacillus amyloliquifaciens was inhibited to grow by extracts made in ethanol, methanol and n hexane a compared by Staphylococcus aureus and Pseudomonas aereginosa which were inhibited by only ethanolic and methanolic extracts. Inhibition length for Bacillus amyloliquefaciens was maximum in n hexane extract i.e. 9mm as compared to extracts in ethanol and methanol that were found to be 6 mm and 6mm respectively. Staphylococcus aureus gave more inhibition length in methanolic extract as compared to ethanolic extract i.e. 5mm and 5mm respectively. Pseudomonas aeruginosa gave a inhibition length of 5mm in ethanolic extract only whereas extract made in acetone gave no zone of inhibition in any of the bacterial strains (Figure 01 and Table 05).

Percentage of inhibition was calculated by measuring the optical density of the cultures after incubation at 620 nm. High percentages of inhibition was shown by the methanolic extract as compared to extract made in ethanol which showed comparatively low percentage of inhibition in all the four bacterial strains. Escherichia coli was inhibited the most in both the extracts made up to 99% in methanolic extract and up to 98% in ethanolic extract (Figure 02 and 03) whereas Pseudomonas aeruginosa was inhibited the least in all the four strains; up to 70% and 65% in methanolic and ethanolic extract respectively. Extracts in n-hexane and acetone (Figure 04 and 05) showed less percentages of inhibition being least in extract made in...
Electron transfer assay was performed by Total Flavonoids (TFC) and Total Phenolic Count (TPC) by spectrophotometric analysis. The decreasing order of Count (TFC) and Total Phenolic Count (TPC) by methanolic, n-hexane and acetone respectively. 300µg/ml and 170µg/ml respectively (Table 06), whereas GST being 399.46 µmole of H$_2$O$_2$ consumed/min/mg of proteins, CAT being the highest, followed by SOD and then GST activities were found to be maximum in the extract made in ethanol, methanol, n-hexane and acetone respectively (Figure 07). Enzymatic activity of SOD and CAT of medicinal plant, *Pseudomonas aeruginosa* was inhibited the least in actinic extract i.e., 2% only as compared to extract made in n-hexane that was 9%.

As far as protein leakage assay is concerned maximum protein was found to be leaked in case of *Staphylococcus aureus* (Figure 07) as compared to *Bacillus amyloliquefaciens* (Figure 08) that was approximately 410 µg/ml of proteins by ethanolic extract and 250 µg/ml of proteins by ethanolic extract respectively. As was as *Escherichia coli* (Figure 06) and *Pseudomonas aeruginosa* (Figure 09) are concerned, proteins from *Pseudomonas aeruginosa* were found to be leaked more as compare to *Escherichia coli*, that was approximately 70 µg/ml of proteins by ethanolic extract and 50 µg/ml or proteins by ethanolic extract respectively, whereas optical density at 595nm for nucleic acid was found to be leaked in *E.coli* the most by extract made in ethanol, followed by methanol, n-hexane and acetone that was 0.085, 0.08, 0.07 and 0.04 respectively. Optical density for nucleic acid leakage in case of *S.aureus* was observed to be 0.04 for both ethanolic and methanolic extracts and 0.03 for extract made in n-hexane and0.025 for extract made in acetone. Nucleic acid in case of *B.amyloliquefaciens* was found to be leaked the least as compared to all the four bacterial strains that was 0.036, 0.031, 0.3 and 0.25 for the extracts made in ethanol, methanol, n-hexane and acetone respectively as compared to *P.aeruginosa* that was observed 0.0357,0.356,0.33 and 0.3 in extracts in ethanol, methanol, n-hexane and acetone respectively.

Electron transfer assay was performed by Total Flavonoids Count (TFC) and Total Phenolic Count (TPC) by spectrophotometric analysis. The decreasing order of flavonoids in the extracts was found to be in methanol, n-hexane, ethanol and acetone that were 860µg/ml, 350µg/ml, 300µg/ml and 170µg/ml respectively (Table 06), whereas TPC was found 6930µg/ml, 6830 µg/ml, 1570µg/ml and 230µg/ml in extracts made in ethanol, methanol, acetone and n-hexane respectively (Table 07). Enzymatic activity of the extracts made, were determined by Superoxide Dismutase Activity (SOD), Catalase Activity (CAT) and Glutathione S Transferase activity (GST). All the three activities were found to be maximum in the extract made in acetone, CAT being the highest, followed by SOD and then GST being 399.46 µmole of H$_2$O$_2$ consumed/min/mg of proteins, 246.15 Units/min/mg of proteins and 145.69 µmoles of CDNB-GSH conjugate formed/min/mg of proteins respectively as compared to the extracts made in ethanol, methanol and acetone, whereas n-hexane showed the least activity (Figure 15). MDA content was found to be maximum in aceticonic extract, followed by methanolic, ethanolic and extract prepared in n-hexane, being approximately 27µg/mg of proteins, 3 µg/mg of proteins, 2.5µg/mg of proteins and 2 µg/mg of proteins respectively (Figure 16).

Tannins were positive only in ethanolic extract and Saponin were negative in all the solvents whereas previously Tannins and Saponin both were positive in only ethanol solvents of pineapple and negative in methanol solvent[26]. But these results are not matching with similar work [27], because presence or absence of secondary metabolites may be depends on type of solvents, nature of extraction or climatic condition of the zone from where plant samples were collected.

The methanolic extract of *A.comosus* peel was inactive to *B.subtilis*, *E.coli* at a concentration of 50mg/ml (50,000ug/ml)[28], as compared to the present study where inhibition length against *E.coli* was 11mm. The methanolic extract of *A.comosus* peel showed activity against *S. aureus* with inhibition zone of 12mm, *K.Pneumonia* with inhibition zone of 9mm [29]. The hexane extract of *A.comosus* peel showed activity against *S. subflava*, *S.typhimurium*, but no activity against *S. aureus* [29], as now also there was no anti microbial activity was observed against *S.aureus*. It also reported that the ethanolic extract of *A.comosus* peel has activity on *S.typhi* with inhibition zone of 15mm [30].Also ethanolic extract of *A.comosus* peel showed antibacterial activity against *A.hydrophi* and *Salmonella species* [31].

Main sources of antioxidant vitamins (vitamin E, vitamin C, precursor of vitamin A i.e., β -carotene) are fruits and vegetables, which act as free radical scavengers, making these foods essential to human health[32]. However, more than 80% of the total antioxidant capacity in fruits and vegetables comes from the ingredients other than antioxidant vitamins, indicating the presence of other potentially important antioxidants in these foods [33]. Critical review [34] reported high amount of polyphenolic compounds in pineapple peels.

Activity of SOD and CAT of medicinal plant, *Evolvulus alsinoides* were found to be 49.8 units/mg protein and 180.3 µmole of H$_2$O$_2$ consumed/min/mg proteins respectively as compared to ours that was found to be 245 units/mg proteins and 400 µmole of H$_2$O$_2$ consumed/min/mg proteins respectively. As far as GST was considered in *Evolvulus alsinoides* it was found to be 353.1 µmole of CDNB –GSH conjugate formed/min/mg protein as compared to *Ananas comosus* which was 145 µmole of CDNB-GSH conjugate formed/min/mg protein [35].
<table>
<thead>
<tr>
<th>Secondary metabolite</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Acetone</th>
<th>n-hexane</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
</tr>
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<tbody>
<tr>
<td>Saponin</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Tannin</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
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<td>-ve</td>
</tr>
<tr>
<td>Napthoquinone</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Inulin</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
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<tr>
<td>Glycoside</td>
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<tr>
<td>Alkaloid</td>
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<td>+ve</td>
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<tr>
<td>Phenolic compound</td>
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<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

+ve: Represents the presence of the mentioned phytochemical constituent.

-ve: Represents the absence of the mentioned phytochemical constituent.

Fig 01: Antimicrobial Sensitivity Test Results of
(a) Zone of inhibition of extract prepared in ethanol on Bacillus amyloliquefaciens.
(b) Zone of inhibition of extract prepared in ethanol on Escherichia coli.
(c) Zone of inhibition of extract prepared in methanol on Bacillus amyloliquefaciens.
(d) Zone of inhibition of extract prepared in n Hexane on Bacillus amyloliquefaciens.
(e) Zone of inhibition of extract prepared in methanol on Staphylococcus aureus.
(f) Zone of inhibition of extract prepared in acetone on Escherichia coli.

Table 02: Antimicrobial Sensitivity Test of extract prepared in Ethanol

<table>
<thead>
<tr>
<th>Bacterial Cells</th>
<th>diameter (mm)</th>
<th>Zone of inhibition (mm)</th>
<th>Inhibition length (mm)</th>
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<tbody>
<tr>
<td>Bacillus amyloliquefaciens</td>
<td>9</td>
<td>15</td>
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<tr>
<td>Staphylococcus aureus</td>
<td>9</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>9</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Escherichia coli</td>
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<td>21</td>
<td>10</td>
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</table>

Table 03: Antimicrobial Sensitivity Test of Extract prepared in Methanol

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<th>Bacterial Strain</th>
<th>diameter (mm)</th>
<th>Zone of inhibition (mm)</th>
<th>Inhibition length (mm)</th>
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<tbody>
<tr>
<td>Bacillus amyloliquefaciens</td>
<td>9</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>9</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>9</td>
<td>20</td>
<td>11</td>
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Table 04: Antimicrobial Sensitivity Test of Extract prepared in n Hexane

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<th>Bacterial Strain</th>
<th>diameter (mm)</th>
<th>Zone of inhibition (mm)</th>
<th>Inhibition length (mm)</th>
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</thead>
<tbody>
<tr>
<td>Bacillus amyloliquefaciens</td>
<td>9</td>
<td>18</td>
<td>9</td>
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<tr>
<td>Staphylococcus aureus</td>
<td>9</td>
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<td>0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>9</td>
<td>13</td>
<td>4</td>
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</table>

Table 05: Antimicrobial Sensitivity Test of Extracts prepared from Acetone

<table>
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<tr>
<th>Bacterial Strain</th>
<th>diameter (mm)</th>
<th>Zone of inhibition (mm)</th>
<th>Inhibition length (mm)</th>
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</thead>
<tbody>
<tr>
<td>Bacillus amyloliquefaciens</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Fig. 02: Percentage of Inhibition of Ethanolic Extract against *B. amyloliquefaciens*, *E. coli*, *P. aeruginosa*, *S. aureus*

Fig. 03: Percentage of Inhibition of Methanolic Extract against *B. amyloliquefaciens*, *E. coli*, *P. aeruginosa*, *S. aureus*

Fig. 04: Percentage of Inhibition of n-hexane Extract against *B. amyloliquefaciens*, *E. coli*, *P. aeruginosa*, *S. aureus*
Fig. 05: Percentage of Inhibition of Acetone Extract against *B. amyloliquefaciens, E. coli, P. aeruginosa, S. aureus*

Fig. 06: Protein Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *E. coli*

Fig. 07: Protein Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *S. aureus*
Fig. 08: Protein Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *B.* amyloliquefaciens

Fig. 09: Protein Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *P.* aeruginosa
Fig. 10: Nucleic Acid Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *E.coli*

Fig. 11: Nucleic Acid Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *S.aureus*

Fig. 12: Nucleic Acid Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *B.amyloliquefaciens*
**Fig. 13:** Nucleic Acid Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *P. aeruginosa*

**Graph 1:** Quercetin Standard Curve for TFC

**Table 06:** Total Flavonoid Content in Extracts prepared from Ethanol, Methanol, n-hexane and Acetone

<table>
<thead>
<tr>
<th>Extract</th>
<th>Mg QE/g of extract</th>
<th>µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>3</td>
<td>300</td>
</tr>
<tr>
<td>Methanol</td>
<td>8.6</td>
<td>860</td>
</tr>
<tr>
<td>Acetone</td>
<td>1.7</td>
<td>170</td>
</tr>
<tr>
<td>n-hexane</td>
<td>3.5</td>
<td>350</td>
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</table>
Graph 2: Quercetin Standard Curve for TPC

Table 07: Total Phenolic Content in Extracts prepared from Ethanol, Methanol, $n$-hexane and Acetone.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Mg QE/g of extract</th>
<th>µg/ml</th>
</tr>
</thead>
<tbody>
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<td>Ethanol</td>
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</tr>
<tr>
<td>Methanol</td>
<td>63.8</td>
<td>6380</td>
</tr>
<tr>
<td>Acetone</td>
<td>15.7</td>
<td>1570</td>
</tr>
<tr>
<td>$n$-hexane</td>
<td>2.3</td>
<td>230</td>
</tr>
</tbody>
</table>

Fig. 15: Antioxidant assay of Superoxide dismutase, Catalase, Glutathione S Transferase of extracts.

Units for CAT: µmole of H$_2$O$_2$ consumed/min/mg of proteins.

Units for SOD: Units/min/mg of proteins.

Units for GST: µmoles of CDNB-GSH conjugate formed/min/mg of proteins.
The present work proves that the extracts from peels of Ananas comosus possessed antimicrobial activity that can be utilized as an active antimicrobial agent against the many bacterial diseases. Preliminary phytochemical screening as well as the Anti-oxidant activity of the extracts suggests that it can be used as a natural and very effective anti-oxidant.

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Fig. 16: Antioxidant assay of lipid per oxidation of extracts


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