Phytochemical Screening, Anti-oxidant and Anti-Microbial Activity of Polyphenolic Flavonoids Isolated from fruit of *Ananas comosus* in various solvents

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ABSTRACT

Ananas comosus, called Pineapple in common, has several beneficial medicinal properties counting antioxidant activity as well. The objective of the present study was to phytochemically screen the various pulp extracts for the presence of various secondary metabolites including Flavonoids as well as Phenols and to evaluate the antibacterial and antioxidant proceedings of the same. The fruit of pineapple was extracted with Ethanol, Methanol, Ethyl acetate, Acetone, n-Hexane, Chloroform and Distilled water by Maceration method. Primary phytochemical screening for the presence of various secondary metabolites was done in order, especially to check the presence of flavonoid glycosides. All the extracts prepared were screened positive except extracts in n-Hexane and chloroform. For the determination of antimicrobial activity of the extracts, bacterial *strains Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus amyloliquefaciens* were used and methods implemented were Agar well diffusion and broth dilution method. As in Agar well diffusion method, maximum Inhibition lengths were given by Aqueous extract in case of all four bacterial strains being 22 mm, 20mm, 22 mm and 19 mm *against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus amyloliquefaciens* respectively. Percentage of Inhibition, Protein Leakage and Nucleic Acid Leakage were assayed from the Broth dilution method. Antioxidant activity was determined by Electron Transfer Assay, Enzymatic and Non-Enzymatic Biochemical Assays. Antioxidant capacity of the extracts was measured as equivalent to Quercetin. Total Flavonoid and Total Phenolic Count were estimated by Spectrophotometric analysis and both were found to be maximum in Ethanolic extract – 122.28 and 575.67 mg QE/g of extract respectively. Enzymatic activity of the extracts was determined by Catalase Activity (CAT), Superoxide Dismutase Activity (SOD), and Glutathione-S-Transferase activity (GST). All the three activities were found to be maximum in the acetone extract, CAT being the highest, followed by GST and then SOD - 71.71 µmole of H_2O_2 consumed/min/mg of proteins, 64.228 Units/min/mg of proteins and 47.341 µmoles of CDNB-GSH conjugate formed/min/mg of proteins respectively. As far as Non-enzymatic biochemical assays are kept in consideration, Glutathione content was found to be maximum in Ethanolic extract – 1169 µg/mg of proteins and MDA content found to be maximum in acetone extract being 8.857 µg/mg of proteins. The results indicated that the extent of antioxidant activity of the extract is in accordance with the amount of phenolics present in the extract and the pineapple fruit being rich in polyphenols may provide a good source of antioxidant along with antibacterial properties.

Keywords: *Ananas comosus*, Phytochemicals, Antibacterial Activity, Leakage assays, Phenols, Antioxidants.

1.INTRODUCTION

1.1 Medicinal Plants:-

The term medicinal plants includes various types of plants used in herbalism and some of these plants have medicinal activities. The "backbone" of traditional medicine are the medicinal plants, which means more than 3.3 billion people in the less developed countries utilize them plants on a regular basis. In recent times, focus on plant research has increased tremendously all over the world and a large body of evidences collected has shown the great potential of medicinal plants used in various traditional systems [1]. The use of plants and plant products can be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is found in "Rigveda", which is said to be have written in 4500-1600 BC and is supposed to be the oldest repository of human knowledge [2]. Plants have been an important source of medicine and has helped human in the maintenance of health for thousands of years [3]. Medicinal plants, medicinal herbs, or simply herbs have been identified and used from prehistoric times. According to the World Health Organization estimates, up to 80 percent of people still rely mainly on traditional remedies such as herbs for their health needs due to better cultural acceptability, fewer side effects and better compatibility with the human body [4]. Its civilization is very ancient and the country as a whole has been long known for its rich resources of medical plants [5].

1.2 Pineapple (*Ananas comosus)*:-

Pineapple (*Ananas comosus*, Bromeliaceae) native to central and south America, is grown in several tropical and sub-tropical countries including Hawaii, India, China, Kenya, South Africa, Malaysia, Philippines and Thailand (6,7). Pineapple has been used as a medicinal plant in several native cultures. It has the characteristics such as interference with growth of malignant cells, inhibition of platelet aggregation, fibrinolytic activity, anti-inflammatory action and skin debridement properties (8,9,10,11).

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 [12]. It is the third most important tropical fruit in the world after Banana and Citrus[13]. Pineapples are consumed or served fresh, cooked, juiced and can be preserved. This fruit is highly perishable and is seasonal. Mature fruit contains 14% of sugar.

1.3 Antioxidants:-

Antioxidants are defined as compounds that protect against oxidation by increasing the oxidative stability of a system Antioxidants can be obtained either synthetically or naturally. There is a growing interest in naturally occurring antioxidants such as in fruits and vegetables due to the possible hazardous effect of synthetic antioxidants [14].

Antioxidants are often added to foods to prevent the radical chain reactions of oxidation by inhibiting the initiation and propagation step leading to the termination of the reaction and a delay in the oxidation process [15].

2.OBJECTIVE

The principle of the present study was to isolate the polyphenolic flavonoids from the pulp of *Ananas comosus* and to study the antibacterial and antioxidant activity of the extracts of the fruit. Flavonoid 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.

3.MATERIAL AND METHODS:-

3.1 Sample collection :

Ripened pineapples were bought from Reliance Fresh, Atta Market, Sector – 30, Noida, Uttar Pradesh, India. Pineapples were peeled, cut into pieces and washed with deionised water. The pieces were allowed to dry in direct sunlight for about one week. Then, the dried pieces were grounded with ordinary grinder to powder.

3.2 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.

3.3 Preparation of extract:

10g grounded powder of pulp was dissolved in 100ml of each of the solvents- ethanol, methanol, n-hexane, ethyl acetate, chloroform, acetone and distilled water to form crude extracts by maceration method by boiling continuously for 30 minutes. The conical flasks with extracts were covered by cotton plugs to avoid evaporation. The extracts were placed in shaking incubator at 250rpm for 48hrs. After shaking they were filtered with muslin cloth and again filtered with filter paper twice. Prepared crude extracts were evaporated to dryness, extract amount were measured [16, 17] and a final concentration of 1000 µg/ml was prepared.

3.4 Antimicrobial activity:

3.4.1. ANTIMICROBIAL SENSITIVITY TEST: This was performed by agar well diffusion method. LB agar media was prepared and autoclaved at 121°C for 15minutes at 15 PSI. The media was poured in sterile Petri plates up to a uniform thickness and the agar was allowed to set at ambient temperature. This method was suitable for organism to grow rapidly overnight at 37°C. The inoculation was done on the LB agar plates with microorganism. 100µl of inoculums were spread over LB agar plates using sterile spreader, after a few minutes four wells were made in each Petri plate and loaded with 100µl of extracts and control [18]. Plates were incubated at 37°C for 24hrs. Antimicrobial activity was observed and zone of inhibition was observed and measured. The experiments were done in quadruplicates [19].

3.4.2. BROTH DILUTION METHOD: This was used to determine the dose dependency of extracts against bacterial cells. Luria broth was prepared and sterilized. After sterilization, three different groups were formed which included the blank, control and reaction mixture. Blank was without bacterial cells, control containing the 200µl of respective Gram positive and Gram negative bacterial cells. Reaction mixture was containing extract in an increasing concentration from 100 µl to 500 µl as well as with defined cells. Broths were incubated for 24hrs in shaker at 37°C. Three parameters were calculated from this method:

3.4.2.1 Measurement of cellular Leakage – absorbance was checked after incubation at 620 nm and percentage of inhibition was calculated.

3.4.2.2 Protein leakage assay -Method was used to carry out the protein leakage assay [20].Bacterial cells after Incubation for 24 hrs were 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. Then 200 µl The optical density was measured at 760 nanometer. using Hitachi-U 2900 spectrophotometer. Bovine serum albumin (BSA) was used to prepare the standard curve.

3.4.2.3. Nucleic acid leakage assay - The nucleic acid leakage study was carried out by using Diphenylamine (DPA) method. Bacterial cells after Incubation for 24 hrs were centrifuged at 3000 rpm for 03 min. For each sample, 2ml of DPA was added to 100µl supernatant and further incubated at 95^{o}C for 10 min. Optical density was measured at 595 nm. Lyophilized DNA in the concentration of 200µg/ml was used for the preparation of the standard curve.

3.5 Anti oxidant assay :

3.5.1 ELECTRON TRANSFER ASSAY:

Total Flavonoid content and Total Phenolic content has been assayed to determine the oxidants which were reduced by transfer of electron from an antioxidant (oxidized).

3.5.1.1 Total Flavonoid Content - Aluminium Chloride Spectrophotometric method was choosen 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 thoroughly. 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 calibration plot was used to calculate the concentrations of flavonoids in the extracts and the values were expressed in mg QE/ g of extracts [17,21,28].

3.5.1.2 Total Phenolic Content - Flavonoids are polyphenolic compounds thus, the Total Phenolic content has been determined by Folin Ciocalteau method in order to quantify the polyphenolic Flavonoids. 0.1ml of Folin Ciocalteau reagent (0.5N) was added to 0.1ml of extracts or quercetin standard (10-100 µg/ml) 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 a period of 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 and were further calculated from the calibration plot. The values obtained were expressed in mg QE of phenol / g of extracts [17,21,20].

3.5.2 ENZYMATIC BIOCHEMICAL ASSAY

3.5.2.1 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 (O_2) by catalyzing the decomposition of hydrogen peroxide (H_2O_2) 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. The mixture was incubated for 30 min afterwards [30]. Absorbance was measured at 560nm at the intervals of 15 seconds for a minute. Superoxide dismutase activity was expressed as one unit of enzyme activity and defined as the enzyme concentration required for inhibition of the absorbance at 560nm of chromogen production by 50% in 1 min under assay conditions and expressed as specific activity in the unit of SOD per min per mg of protein [23, 24].

3.5.2.2 Catalase activity: CAT was assayed colorimetrically at 620 nm and expressed as µmoles of H_2O_2consumed/min/mg protein [29]To analyze the catalase activity, catalase buffer was prepared by adding 10mM H_2O_2 in the 1M phosphate buffer of pH6.5. Sample was added in the aliquots of catalase buffer and absorbance was measured at 260nm after definitive interval 15 seconds for a minute's time. Catalase activity was expressed in enzyme activity as µmoles of H_2O_2 oxidized per min per mg protein [25].

3.5.2.3 Glutathione-S-Transferase 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 thoroughly. Absorbance was measured at 340nm at every 15 seconds 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 [26,31]

3.5.3 NON ENZYMATIC BIOCHEMICAL ASSAY

3.5.3.1 Lipid Peroxidation: Malondialdehyde (MDA) content has been determined by the standardized protocol. Lipid Peroxidation i.e. Thiobarbituric Acid (TBA) reactive substances were measured spectrophotometrically at 532nm in extracts based on the principle of formation of Malondialdehyde (MDA) by breaking down of polyunsaturated fatty acids where the levels of lipid peroxidation were expressed as µg of Malondialdehyde formed per mg of extract[27].

3.5.3.2 Glutathione content : Glutathione content was estimated using dithio bis nitrobenzoic acid and expressed in µg per mg of protein [26].

3.6 Statistical Evaluation:

To estimate the accuracy of the experimental data, each experiment was performed in triplicates, and the result was expressed as the mean ± standard deviation of three replications. $P < 0.05$ was considered as statistically significant.

3.7. Results and Discussion:

Dried pineapple pulp was used to make various extracts using different solvents – Ethanol, Methanol, Ethyl acetate, Acetone, n-Hexane, Chloroform and Distilled water. Initially all the seven extracts were screened phytochemically for the presence of various secondary metabolites.

Phytochemicals, also well known as phytonutrients, are naturally occurring secondary metabolites found in plant life. These biochemicals have been found to be advantageous to human wellbeing as well as possessing antioxidant activity. Phytochemicals can act as an antioxidant, anti-inflammatory and anti-microbial agent. They play a vital role in detoxification of damaging and deleterious chemicals from the body. The phytochemical tests were carried out using standard methods of analysis of carbohydrates, tannins, saponins, flavonoids, alkaloids, glycosides, terpenoids, steroids, phytosteroids, phlobtanins, tannins, napthoquione, inulin and phenols [32].

As Flavonoids and Phenols were our major phytochemicals of concern, they were screened positive in extracts made in Ethanol, Methanol, Ethyl acetate, Acetone and in Distilled water (Table 01). Tannins were screened positive in extracts in Ethanol, Methanol and Acetone and Carbohydrates were found to be present only in extracts in acetone and n-hexane. Carbohydrates and tannins, both were found to be positive in aqueous extract [33].As far as Terpenoids and Napthoquione are considered, they were found to be present in extracts prepared in Ethanol,

Methanol and Distilled water only, showing their absence in rest of the extracts as in comparison to Phlobtannins, which were found to be absent in all the seven extracts, whereas Terpenoids were screened positive in aqueous extract as previously, and Phlobtannins were found to be absent [34]. Also, Saponnins were not screened negative [35] as compared to now, when they were present in Aqueous extract, Ethanolic extract and Methanolic extract and absent in extracts made in Ethyl Acetate, Acetone, n-Hexane and Chloroform. Inulin was screened positive in extracts prepared in Acetone, n-Hexane and Chloroform, whereas Alkaloids being absent only in Ethanol and n-Hexane extracts. Amino acids gave positive results only in extracts made in Distilled water. As far as Steroids are kept into consideration they were found to be negative in Ethanolic extract, as they were found to be positive, previously [34]

Extracts screened positive 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 and a final extracts of concentration of 1000µg/ml were made and used further during the rest of the studies.

Escherichia coli was found to be inhibited by extracts made in Methanol and Distilled Water giving a maximum inhibition length of 13 mm in aqueous extract as compared to extract prepared in Methanol giving a inhibition of length of 06 mm (Table 03 and 06). *Bacillus amyloliquifaciens* was inhibited to grow by all the extracts made except the extract prepared in Ethyl acetate, giving an inhibition length of 05 mm, 04 mm, 08 mm and 10 mm by extracts in Ethanol, Methanol, Acetone and Distilled Water respectively (Table 02, 03, 05 and 06) as compared by *Staphylococcus aureus* which was only inhibited by extracts prepared in Ethanol, Methanol and Distilled Water giving an inhibition length of 09 mm, 06mm and 13 mm respectively (Table 02, 03 and 06). Zone of inhibition for *Pseudomonas aeruginosa* and *Staphylococcus aureus* was found to be 17.7 mm and 23.2 in case of Ethanolic extract and 19.3 mm and 21.3 mm in Methanolic extract respectively [36], as in comparison to the Inhibition length given by *Pseudomonas aureginosa* now, which was inhibited by all the five extracts made were in the order 02 mm, 07 mm, 06 mm, 05 mm and 11 mm in extracts made in Ethanol, Methanol, Ethyl acetate , Acetone and Distilled Water respectively (Table 02, 03, 04, 05 and 06). Pineapple extract was more effective against E.coli with a zone of inhibition of 26mm diameter (at concentration 1000 µg/ml.), with *Pseudomonas aeruginosa*, and *Staphylococcus aureus* it was found to be 20mm, and 23mm respectively [37].

Cellular leakage was checked in the broth by measuring the optical density of the culture at 620 nm and then by calculating the Percentages of inhibition of the respective bacterial strain (Figure 01, 02, 03, 04 and 05). It was observed that E.coli was inhibited the most by the extract made in Acetone (98.36%), followed by Ethyl acetate (89.83%), Distilled Water (73.11%), Ethanol (66.22%) and Methanol (34.42%). *Bacillus amyloliquefaciens* was inhibited more as compared to *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Extract in Methanol, Ethanol and Acetone inhibited *Bacillus amyloliquefaciens* up to 55.71 %, 54.98 % and 55.53 % respectively as compared to extract in Ethyl acetate and Distilled Water being 42.98 % and 30.44 % respectively. As far as *Staphylococcus aureus* in considered, initially it was not inhibited by Methanolic extract and gradually was inhibited up to 55.34% with an increase in volume of extract. It was inhibited the most by aqueous extract (85.98 %). *Pseudomonas aeruginosa* was inhibited the most by extract made in acetone whereas methanolic extract inhibited it the least (25.48 %) out of all the five extracts.

As far as Protein leakage (by Lowry's method) assay is considered, for all the four bacterial strains, maximum amount of proteins were found to be leaked in case of *Staphylococcus aureus* - 496 µg/ml of Acetonic extract followed by Ethanolic extract (490 µg/ml of extract), Aqueous extract (468 µg/ml of extract), Ethyl acetate extract (440.5 µg/ml of extract) and Methanolic extract (545 µg/ml of extract) (Figure 08). As in order, protein leakage by *Staphylococcus aureus* was followed by *Bacillus amyloliquefaciens*. Highest amount of protein was found to be leaked in Methanolic extract - 430 µg/ml of extract, least was found to be in extract of Acetone 359.5 µg/ml of extract (Figure 07). As among *Pseudomonas aeruginosa* and *Escherichia coli*, more amount of proteins were leaked in case of *Escherichia coli* –Methanolic extract showing the maximum leakage - 377.5 µg/ml of extract and minimum leakage was showed by extract in Ethyl acetate - 70 µg/ml of extract (Figure 06), as compared to

Pseudomonas, in which Methanolic extract gave the maximum amount of leakage -295.5 µg/ml of extract and minimum amount was given by Acetonic extract - 105 µg/ml of extract (Figure 09).

The next parameter for broth dilution was Nucleic acid Leakage assay, which was performed by standard Diphenylamine (DPA) estimation. Following protein leakage assay, maximum amount of Nucleic acid was also found to be leaked in *Staphylococcus aureus*, highest being in Ethyl acetate (23.25 µg/ml of extract), followed by Methanol (17 µg/ml of extract), Distilled water (15.5 µg/ml of extract), Ethanol (12.5 µg/ml of extract) and Acetone (11.5µg/ml of extract) (Figure 12) . Amount of nucleic acid leaked in *Pseudomonas* was in order - 30 µg/ml of extract (Methanol) > 17.75 µg/ml of extract (Ethanol) > 15.5 µg/ml of extract (Aqueous) > 13.25 µg/ml of extract (Acetone) > 07.5 µg/ml of extract (Ethyl acetate) (Figure 13). As compared to *Bacillus amyloliquefaciens*, more amount of Nucleic acid was found to be leaked in *Escherichia coli*, highest being in case of extract prepared in Methanol (10.75µg/ml of extract) and lowest in Ethyl acetate (3.5 µg/ml of extract) (Figure 10). As in *Bacillus amyloliquefaciens*, maximum amount of leakage was found to be in Ethanolic extract (9.75 µg/ml of extract) and minimum in Acetonic extract (2.5 µg/ml of extract) (Figure 11).

Electron transfer assay was performed by Total Flavonoids Content (TFC) and Total Phenolic Content (TPC), by Aluminium chloride and Sodium bi carbonate spectrophotometric analysis. The decreasing order of Flavonoids in the extracts was found to be in Ethanol, Aqueous, Methanol, Ethyl acetate and acetone that were 122.28 mg QE/ g of extract,71.14mg QE/ g of extract, 43.43mg QE/ g of extract, 7.35mg QE/ g of extract and 6.85mg QE/ g of extract respectively (Figure 14), whereas previously the total flavonoid contents varied from 39.4 to 55.2 mg quercetin/g weight. The variation may be due environmental conditions, which can modify the constituents of the plant [38].

The phenolic compounds are the central antioxidants that display the scavenging efficiency on free radicals and reactive oxygen species. They are numerous and widely distributed in the plant kingdom [39]. Since Quercetin contributes to the TPC reading, it has the ability to reduce the Folin reagent, thus resulting in the Prussian blue chromophore [40].

TPC in the present study was found to be 575.67 mg QE/ g of extract, 108.67 mg QE/ g of extract, 106.67 mg QE/ g of extract, 88.67 mg QE/ g of extract and 26.17 mg QE/ g of extract, in extracts made in ethanol, distilled water, methanol, acetone and ethyl acetate respectively (Figure 15). The TPC of the fibre concentrates, expressed as gallic acid equivalent, ranged ($p< 0.05$) from 129 mg/100 g for pineapple to 546 mg/100 g for mango, in the methanol:acetone extractions as compared to the present phenolics which were reported to be 108.67 mgQE / g of extract [41].

Several reports have conclusively shown close relationship between total phenolic contents and antioxidative activity of the fruits and vegetables [42]. 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 [43].

Enzymatic activity of the extracts made, were determined by Catalase Activity (CAT) , Superoxide Dismutase Activity (SOD), 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 GST and then SOD being 71.71 µmole of H_2O_2 consumed/min/mg of proteins, 64.228 Units/min/mg of proteins and 47.341 µmoles of CDNB-GSH conjugate formed/min/mg of proteins respectively as followed by the extracts made in ethanol, methanol, ethyl acetate and Distilled water. CAT was found to be 6.56, 7.041, 6.42, 6.069 µmole of H_2O_2 consumed/min/mg of protein, SOD 1.064, 3.991, 2.104, 1.294 Units/min/mg of proteins and GST being 6.8, 7.285, 25.412, and 5.759 µmoles of CDNB-GSH conjugate formed/min/mg of proteins in extracts prepared in Ethanol, Methanol, Ethyl acetate and distilled

water respectively (Figure 16). as in comparison with the peel extracts, in which all the three activities were found to be maximum in the extract made in acetone from pineapple peel, CAT being the highest, followed by SOD and then GST being 399.46 µmole of H2O2 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 [19]. 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_2O_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 µmoles of CDNB-GSH conjugate formed/min/mg protein [44].

The basis of the GSH determination method is the reaction of Ellman's reagent 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) with the thiol group of GSH at pH 8.0 to give yellow color of 5-thiol-2-nitrobenzoate anion [45]. Glutathione content was found to be maximum in Ethanolic extract, followed by extracts made in Distilled water, Methanol, Acetone and least being in Ethyl acetate – 1169, 1107, 1085, 377.5 and 36 µg/mg of proteins respectively. Malondialdehyde is the most abundant individual aldehyde resulting from Lipid Peroxidation breakdown in biological systems and used as an indirect index of Lipid Peroxidation [46]. As far as MDA content was kept in to consideration it was found to be maximum in acetone extract being 8.857 µg/mg of proteins, followed by methanol – 1.184 µg/mg of proteins, ethyl acetate 1.153 µg/mf proteins, distilled water – 0.355 µg/mg of proteins and least being in ethanolic extract- 0.195 µg/mg of proteins (Figure 17)

Table 01: Primary Phytochemical screening for the Presence of Secondary Metabolites in the Prepared extracts.

Secondary Metabolite	Ethanol	Methanol	Acetone	n-hexane	Chloroform	Ethyl acetate	Aqueous
Saponins	+ve	+ve	-ve	-ve	-ve	-ve	+ve
Tannins	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Steroids	-ve	+ve	-ve	-ve	-ve	-ve	+ve
Flavonoids	+ve	+ve	+ve	-ve	-ve	+ve	+ve
Terpenoids	+ve	+ve	-ve	-ve	-ve	-ve	+ve
Napthoquinone	+ve	+ve	-ve	-ve	-ve	-ve	+ve

Inulin	-ve	-ve	+ve	+ve	+ve	-ve	-ve
Alkaloids	-ve	+ve	+ve	-ve	+ve	+ve	+ve
Phenols	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Amino acids	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Carbohydrates	-ve	-ve	+ve	+ve	-ve	-ve	-ve
Phlobtannins	-ve	-ve	-ve	-ve	-ve	-ve	-ve

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

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

Table 02: Antibacterial Activity of extract made in Ethanol against the Bacterial Strains

Bacterial strain	Zone of Inhibition (mm)	Well diameter (mm)	Inhibition length (mm)
Escherichia coli	00	09	00
Pseudomonas aeruginosa	11	09	02
Staphylococcus aureus	18	09	09
Bacillus amyloliquefaciens	14	09	05

Table 03: Antibacterial Activity of extract made in Methanol against the Bacterial Strains

Bacterial strain	Zone of Inhibition (mm)	Well diameter (mm)	Inhibition length (mm)
Escherichia coli	15	09	06
Pseudomonas aeruginosa	16	09	07
Staphylococcus aureus	15	09	06
Bacillus amyloliquefaciens	13	09	04

Table 04: Antibacterial Activity of extract made in Ethyl Acetate against the Bacterial Strains

Bacterial strain	Zone of Inhibition (mm)	Well diameter (mm)	Inhibition length (mm)
Escherichia coli	00	09	00
Pseudomonas aeruginosa	15	09	06
Staphylococcus aureus	00	09	00
Bacillus amyloliquefaciens	00	09	00

Table 05: Antibacterial Activity of extract made in Acetone against the Bacterial Strains

Bacterial strain	Zone of Inhibition (mm)	Well diameter (mm)	Inhibition length (mm)
Escherichia coli	00	09	00
Pseudomonas aeruginosa	14	09	05
Staphylococcus aureus	00	09	00
Bacillus amyloliquefaciens	17	09	08

Table 06: Antibacterial Activity of extract made in Distilled water against the Bacterial Strains

Bacterial strain	Zone of Inhibition (mm)	Well diameter (mm)	Inhibition length (mm)
Escherichia coli	22	09	13
Pseudomonas aeruginosa	20	09	11
Staphylococcus aureus	22	09	13
Bacillus amyloliquefaciens	19	09	10

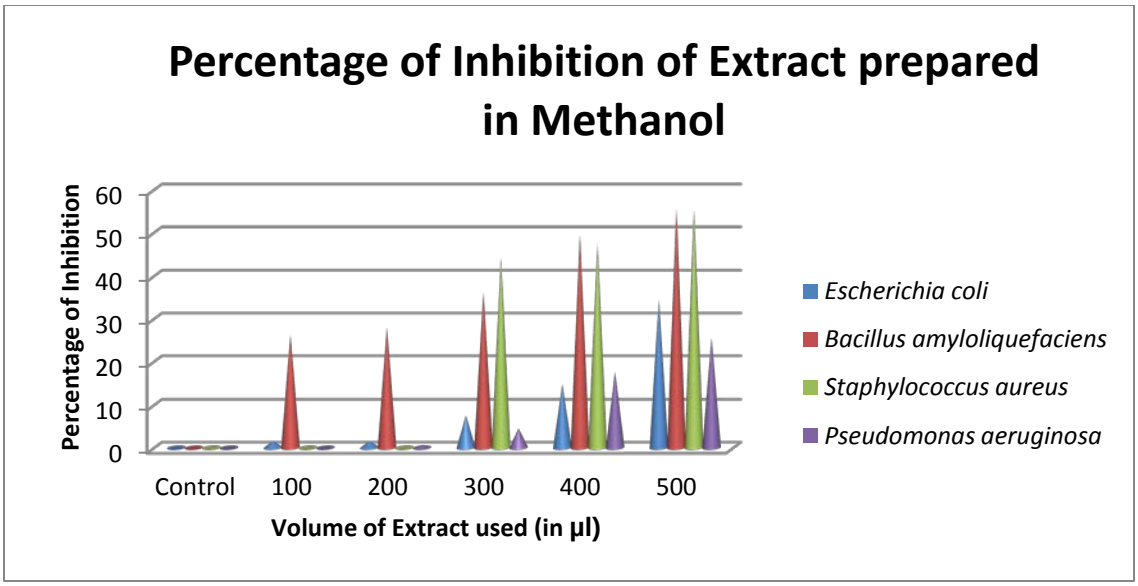

Figure 01: Percentage of Inhibition of Extracts prepared in Methanol against all the four Bacterial Strains used for Broth Dilution.

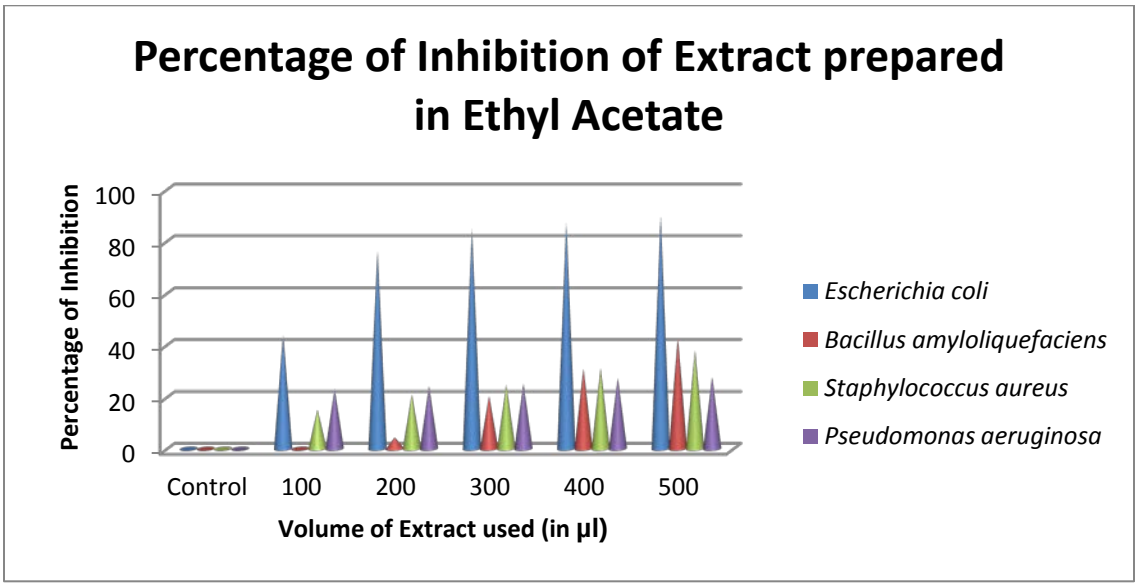

Figure 02: Percentage of Inhibition of Extracts prepared in Ethyl Acetate against all the four Bacterial Strains used for Broth Dilution.

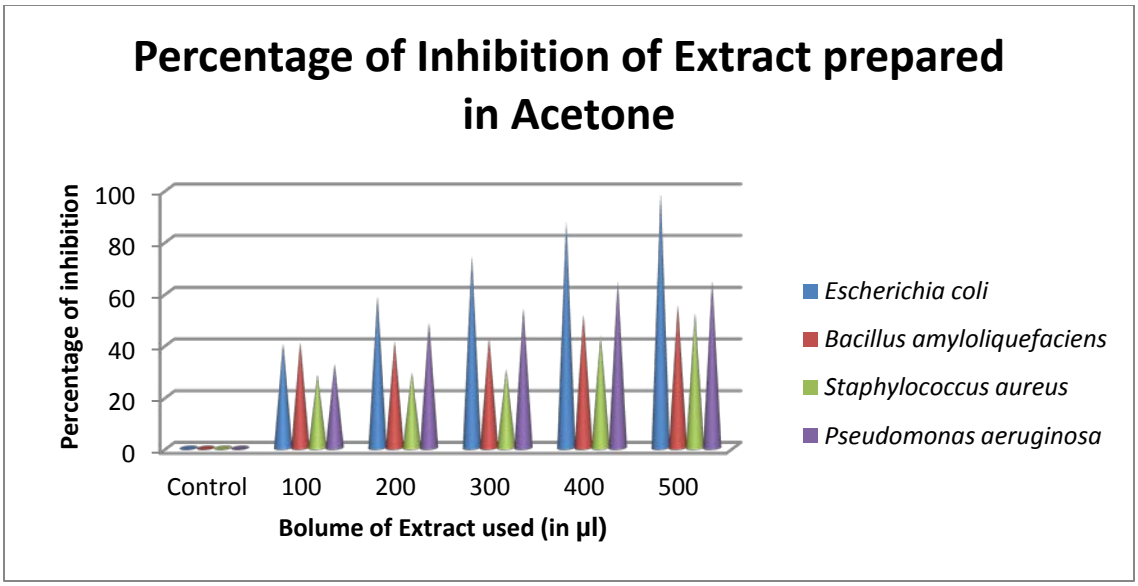

Figure 03: Percentage of Inhibition of Extracts prepared in Acetone against all the four Bacterial Strains used for Broth Dilution.

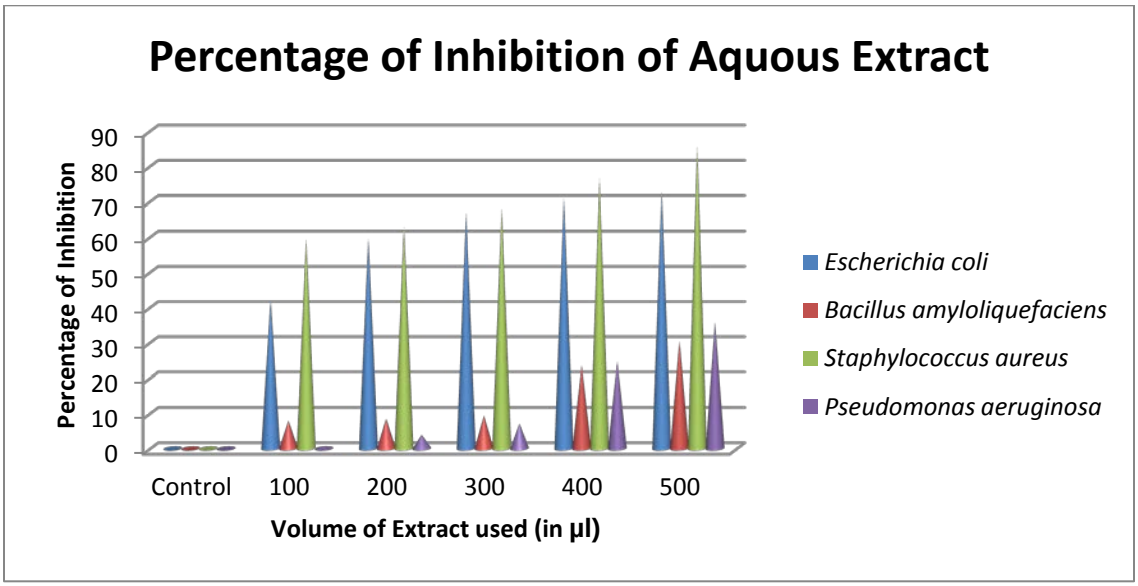

Figure 04: Percentage of Inhibition of Extracts prepared in Distilled water against all the four Bacterial Strains used for Broth Dilution.

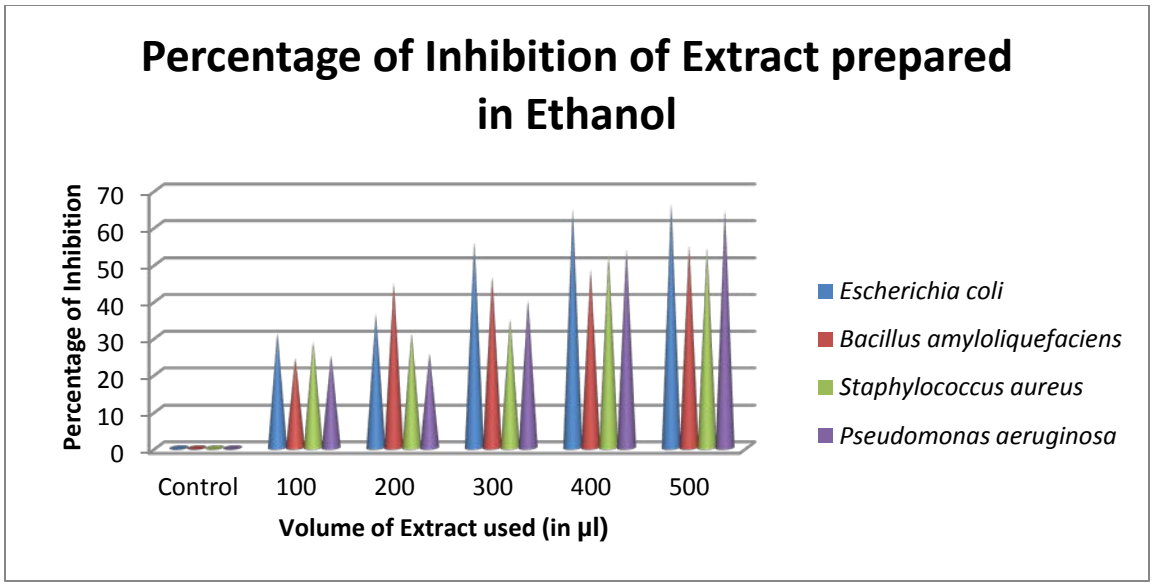

Figure 05: Percentage of Inhibition of Extracts prepared in Ethanol against all the four Bacterial Strains used for Broth Dilution.

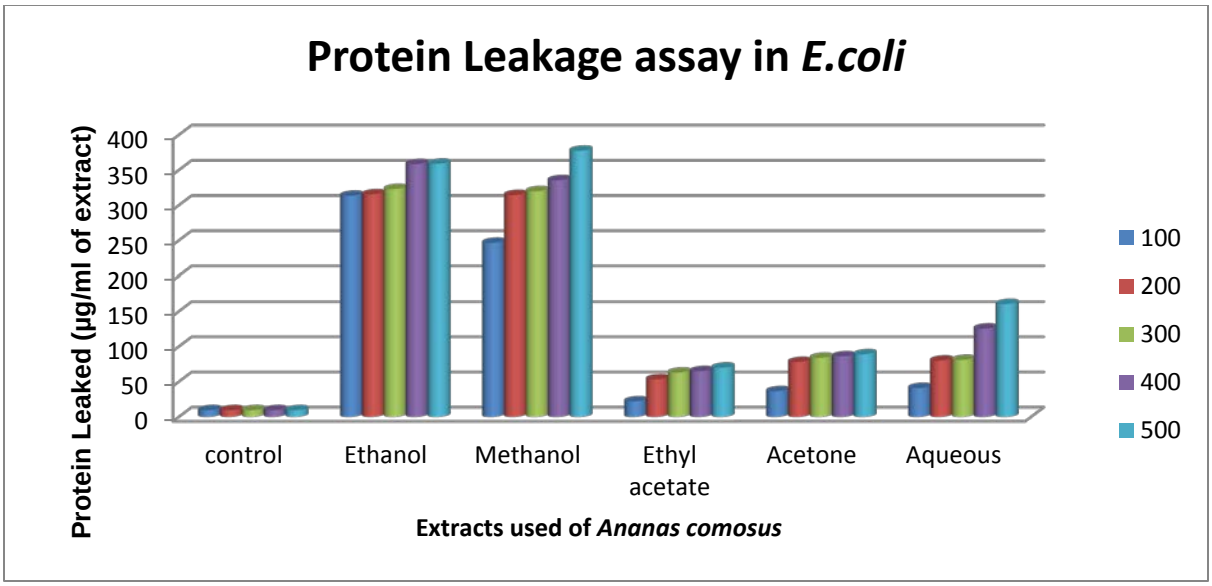

Figure 06: Protein Leakage Assay for all the Five Extracts used against *E.coli*

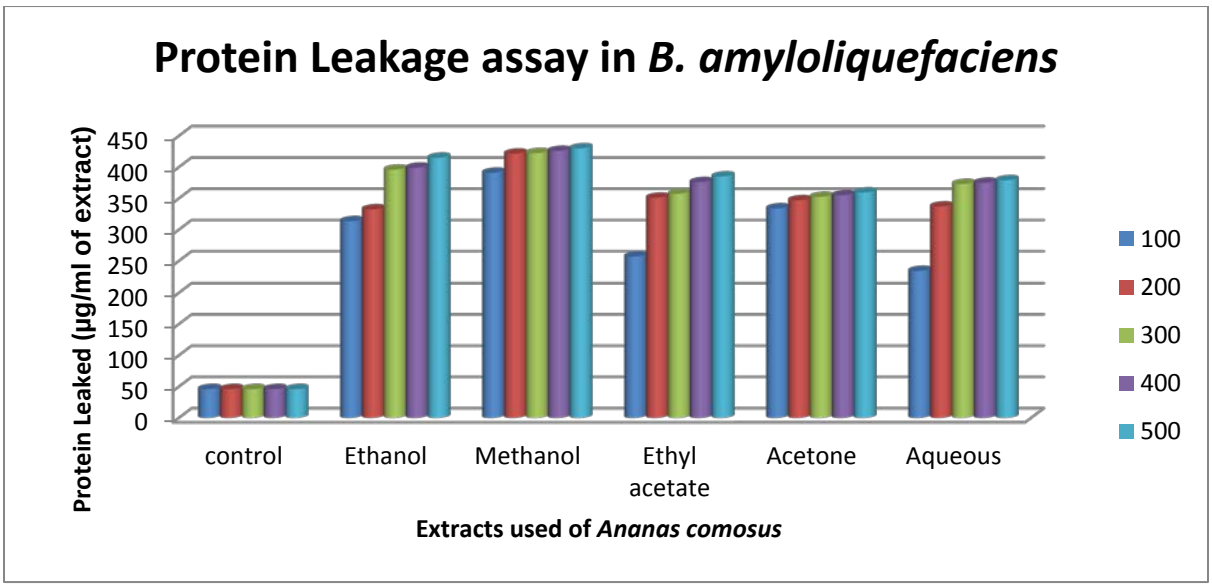

Figure 07: Protein Leakage Assay for all the Five Extracts used against *B.amyloliquefaciens*

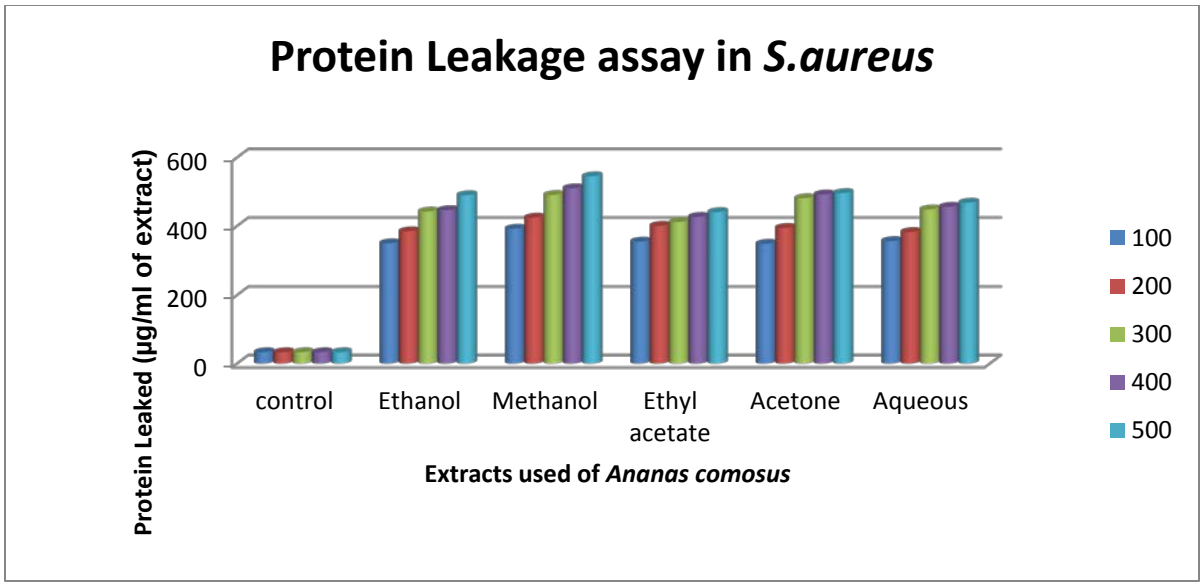

Figure 08: Protein Leakage Assay for all the Five Extracts used against *S.aureus*

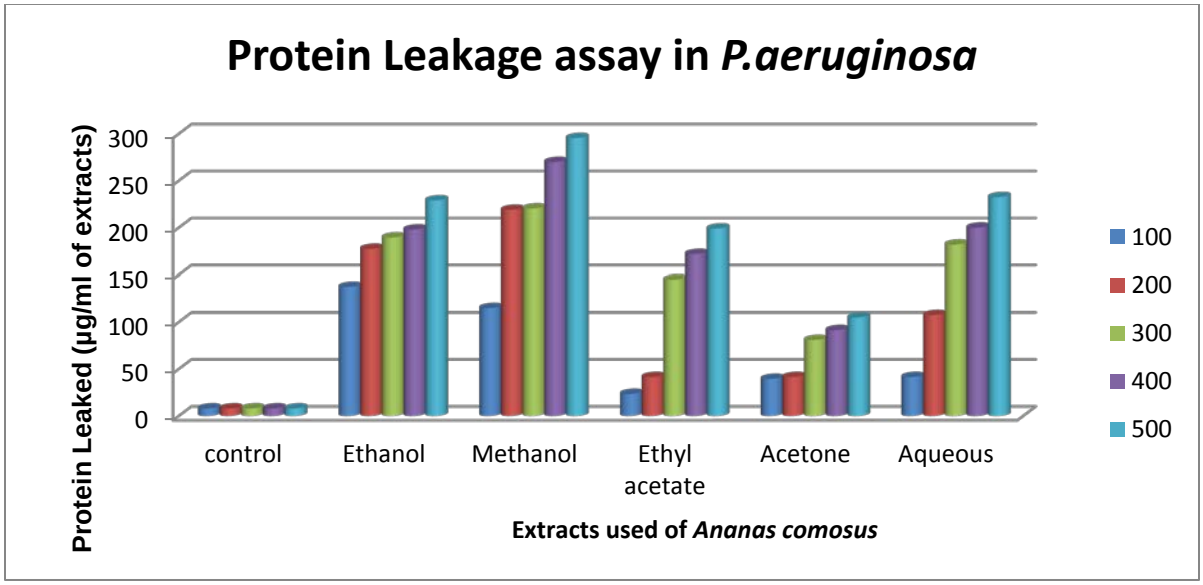

Figure 09: Protein Leakage Assay for all the Five Extracts used against *P.aeruginosa*

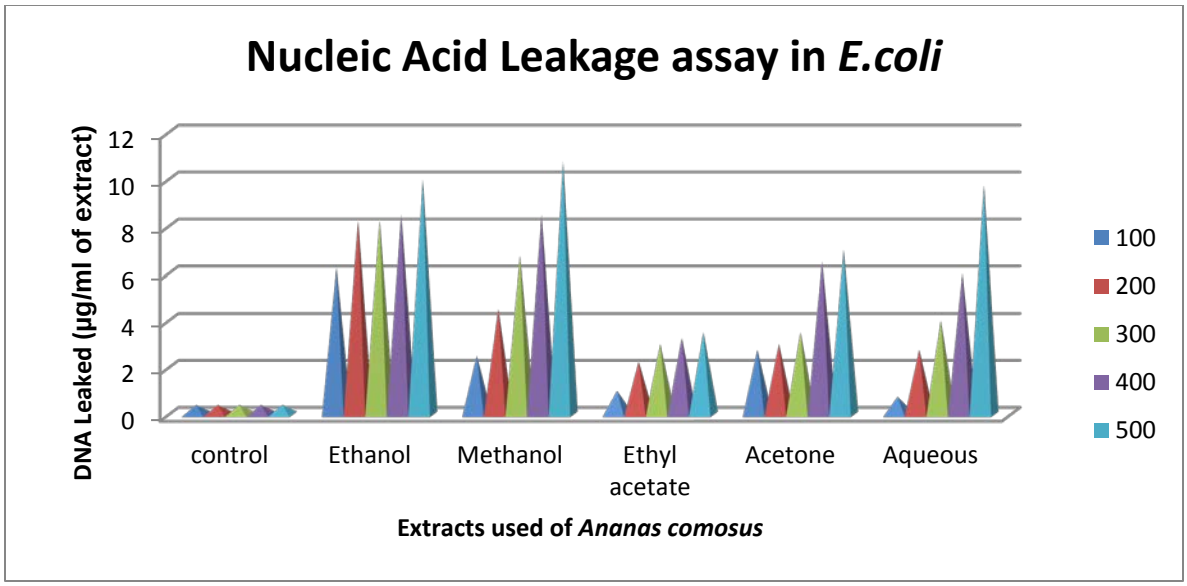

Figure 10: Nucleic Acid Leakage Assay for all the Five Extracts used against *E.coli*

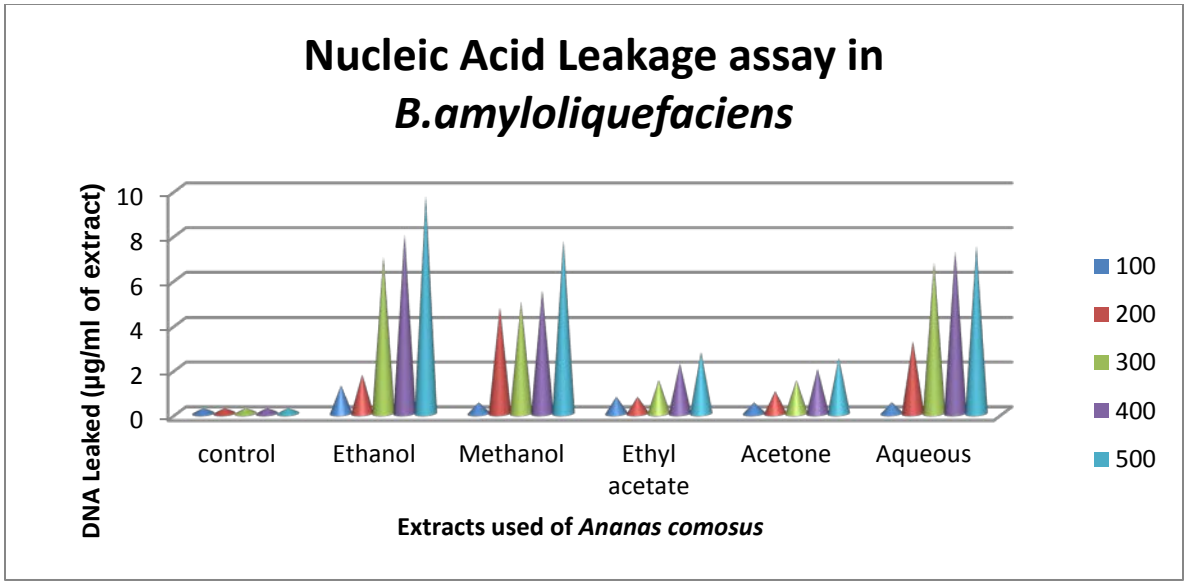

Figure 11: Nucleic Acid Leakage Assay for all the Five Extracts used against *B.amyloliquefaciens*

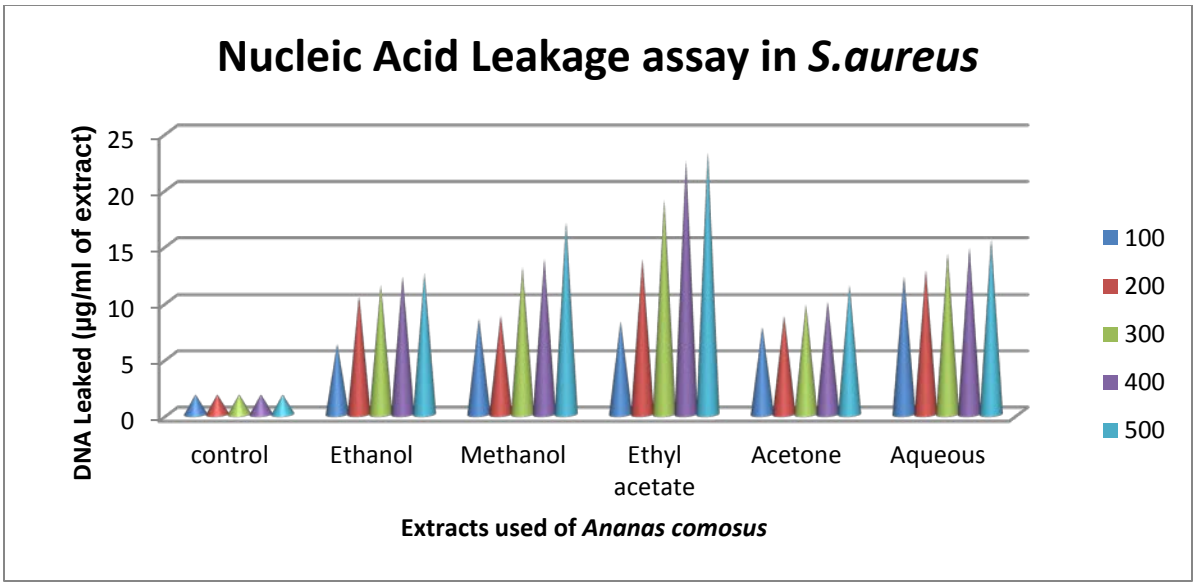

Figure 12: Nucleic Acid Leakage Assay for all the Five Extracts used against *S.aureus*

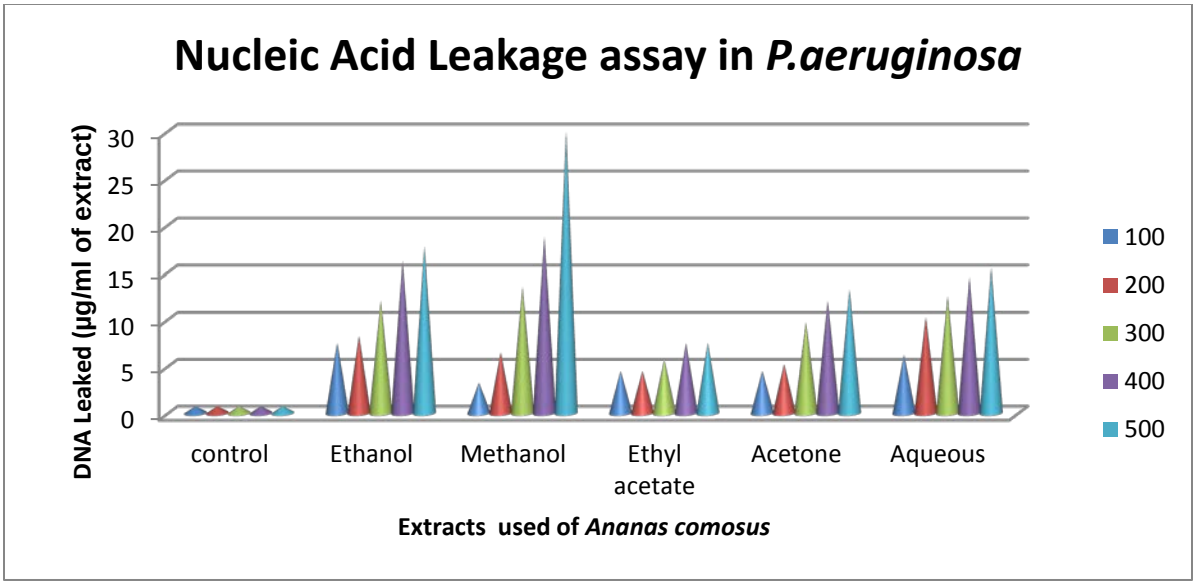

Figure 13: Nucleic Acid Leakage Assay for all the Five Extracts used against *P.aeruginosa*

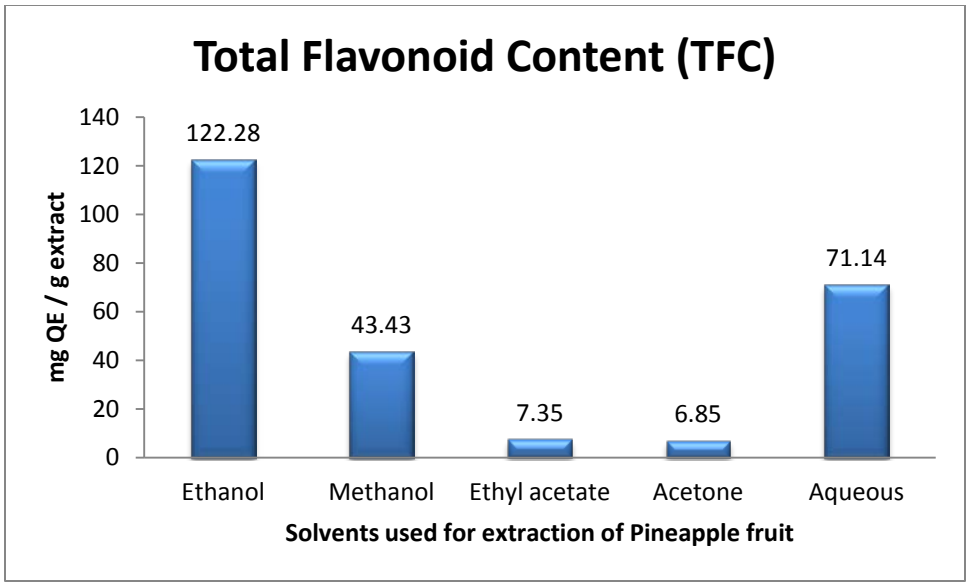

Figure 14: Total Flavonoid Content in Extracts prepared in various Solvents.

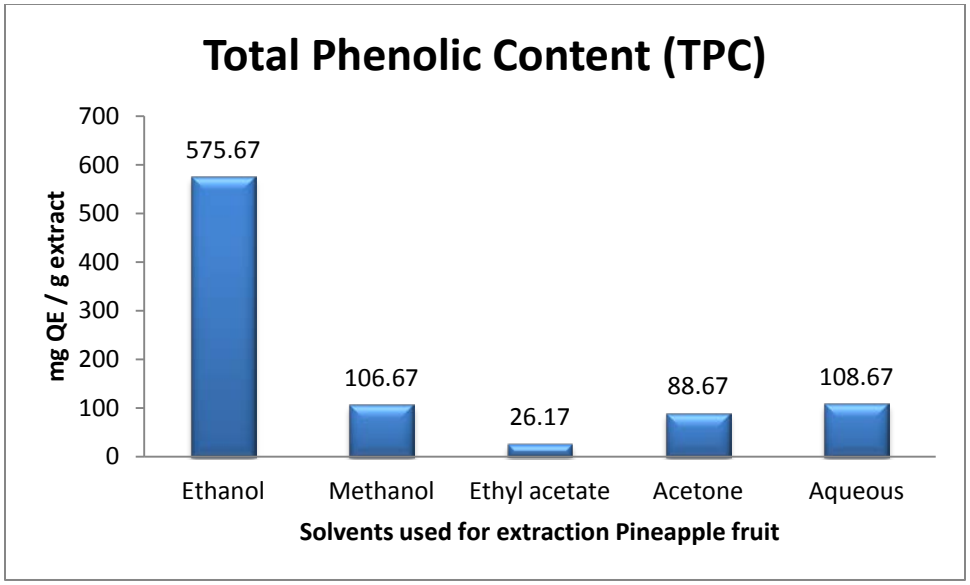

Figure 15: Total Phenolic Content in Extracts prepared in various Solvents.

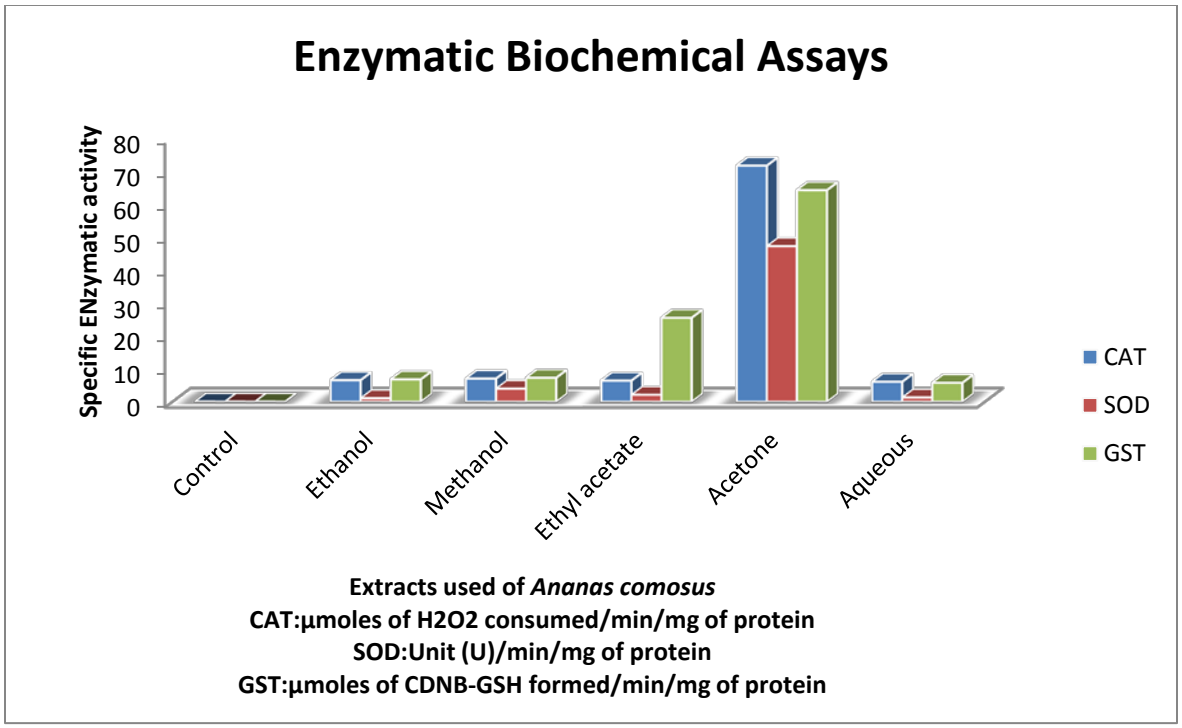

Figure 16: Enzymatic Biochemical Assays for all the Five Extracts used

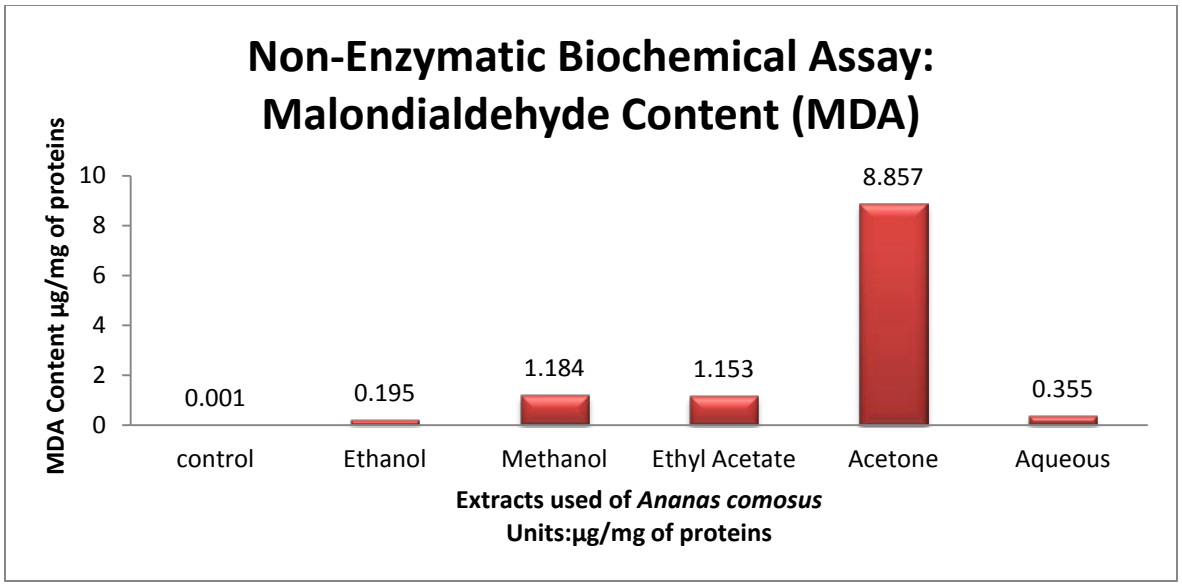

Figure 17: Non- Enzymatic Biochemical Assay – MDA content for all the Five Extracts used

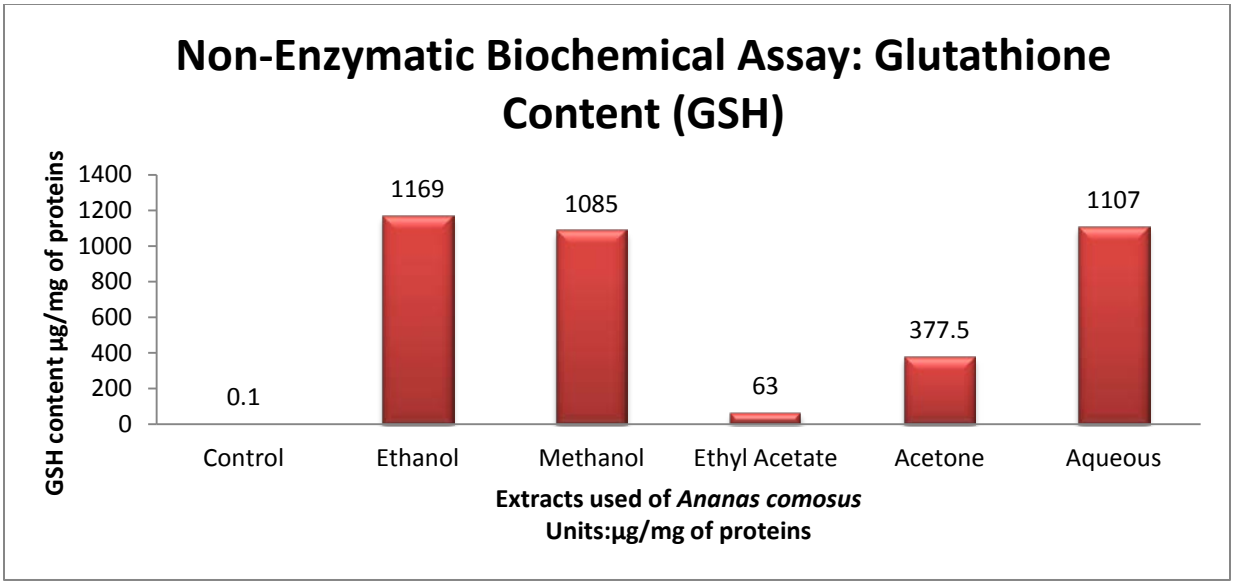

Figure 18: Non- Enzymatic Biochemical Assay – GSH content for all the Five Extracts used

3.8 Conclusion:

Authors reached to a conclusion that the fruit extracts of *Ananas comosus* possess various beneficial properties. Extracts have the ability to inhibit the bacterial strains used during the research work. However, the percentage of inhibition varied from extract to extract as well as from bacterial strains to bacterial strains.

Along with the antibacterial activity, fruit also contains Glycoflavonoids, which contributes to the Antioxidant activity.

Therefore, it may be utilized in the bacterial infections and as naturally effective antioxidant. Furthermore, these potentials may support to discover new chemical structures biochemically, that may contribute towards the alleviation or cure for some illness.

3.9 Acknowledgment:

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