Published by :
http://www.ijert.org

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 09, September - 2017

Anti-Microbial, Anti-Oxidant Activity and Phytochemical Screening of Polyphenolic Flavonoids Isolated from Peels of Ananas Comosus

Namrata[1], Yash Sharma[1] and Tripti Sharma[2]
[1.] Research Assistant, Helix Bio Genesis Pvt Ltd, Noida, Uttar Pradesh, India.
[2.] Associate Professor, Department of Bio-Sciences and Biotechnology, Banasthali University, Rajasthan, India.

***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 anti microbial 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 H_2O_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. MDA content was found to be maximum in acetonic 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

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 09, September - 2017

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 weak. 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 filtered with muslin clothe and again filtered with filter paper twice. Prepared crude extracts were evaporated to dryness and extract amount were measured [14, 15].

Antimicrobial activity

Antimicrobial Sensitive Test: Agar well diffusion method- LB agar media was prepared and autoclaved at 121°C for 15minutes at 15 PSI and poured in sterile Petri plates up to a uniform thickness and the agar was allowed to set at ambient temperature. This method is suitable for organism to grow rapidly overnight at 37°C. The wells were made in medium after inoculation with microorganism. 200µl of inoculums were spread over LB agar plates using sterile spreader, after few minutes four wells were made in each Petri plate and loaded with 100µl of extracts and control (Kirby Bauer method). Plates were incubated at 37°C for 24hrs. Antimicrobial activity was observed and zone of inhibition was measured. The experiments were done in quadruplicates.

Broth micro dilution method 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 such as 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 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 95ºC 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.

Anti oxidant 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

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 09, September - 2017

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 aa 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. 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 was 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 [18, 19].

b. Catalase activity:To analyze the catalase activity, catalase buffer has been 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 taken at 260nm after definite interval of time. Catalase activity was expressed in enzyme activity as µmoles of H_2O_2 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. Saponnins and Napthoquinone 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 Terpernoids 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 aureginosa* 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. 6mm 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

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 09, September - 2017

acetone. *Escherichia coli* was inhibited up to 38% and 20% in extracts made in n-hexane and acetone respectively. *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_2O_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 acetonic 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.Pneumoniea* 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_2O_2 consumed/min/mg proteins respectively as compared to ours that was found to be 245 units/mg proteins and 400 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 [35].

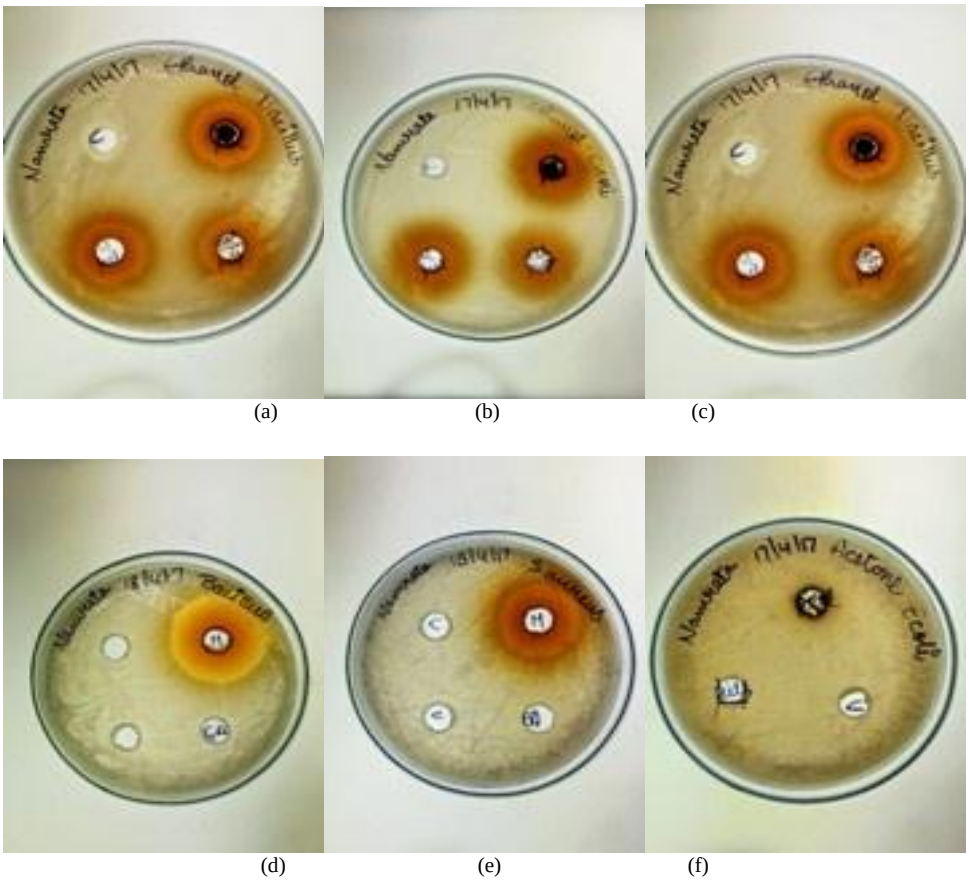
International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 09, September - 2017

Table 01: Primary phytochemical screening of extracts.

Secondary metabolite	Ethanol	Methanol	Acetone	n-hexane	Chloroform	Ethyl acetate
Saponin	-ve	-ve	-ve	-ve	-ve	-ve
Tannin	+ve	-ve	-ve	-ve	-ve	-ve
Flavonoid	+ve	+ve	+ve	+ve	-ve	-ve
Terpenoid	+ve	+ve	-ve	-ve	-ve	-ve
Napthoquinone	-ve	-ve	-ve	-ve	-ve	-ve
Inulin	+ve	-ve	-ve	-ve	+ve	+ve
Glycoside	+ve	-ve	+ve	-ve	-ve	-ve
Alkaloid	+ve	+ve	+ve	+ve	+ve	+ve
Phenolic compound	+ve	+ve	+ve	+ve	+ve	+ve

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

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



(a) (b) (c)

(d) (e) (f)

Fig 01: Antimicrobial Sensitivity Test Results of

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 09, September - 2017

(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

Bacterial Cells	well diameter(mm)	Zone of inhibition (mm)	Inhibition length(mm)
Bacillus amyloliquefaciens	9	15	6
Staphylococcus aureus	9	14	5
Pseudomonas aeruginosa	9	14	5
Escherichia coli	9	21	10

Table 03: Antimicrobial Sensitivity Test of Extract prepared in Methanol

Bacterial Strain	well diameter(mm)	Zone of inhibition (mm)	Inhibition length(mm)
Bacillus amyloliquefaciens	9	15	6
Staphylococcus aureus	9	15	6
Pseudomonas aeruginosa	9	0	0
Escherichia coli	9	20	11

Table 04: Antimicrobial Sensitivity Test of Extract prepared in n Hexane

Bacterial Strain	Well diameter(mm)	Zone of inhibition (mm)	Inhibition length(mm)
Bacillus amyloliquefaciens	9	18	9
Staphylococcus aureus	9	0	0
Pseudomonas aeruginosa	9	0	0
Escherichia coli	9	13	4

Table 05: Antimicrobial Sensitivity Test of Extracts prepared from Acetone

Bacterial Strain	well diameter(mm)	Zone of inhibition (mm)	Inhibition length(mm)
Bacillus amyloliquefaciens	9	0	0
Staphylococcus aureus	9	0	0
Pseudomonas aeruginosa	9	0	0
Escherichia coli	9	0	0

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 09, September - 2017

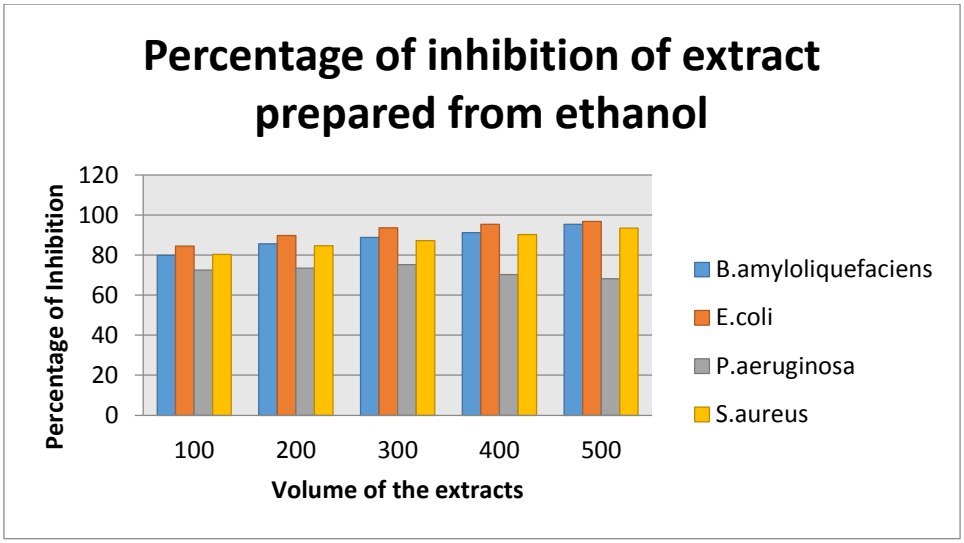


Fig. 02: Percentage of Inhibition of Ethanolic Extract against *B.amyloliquefaciens, E.coli, P.aeruginosa, S.aureus*

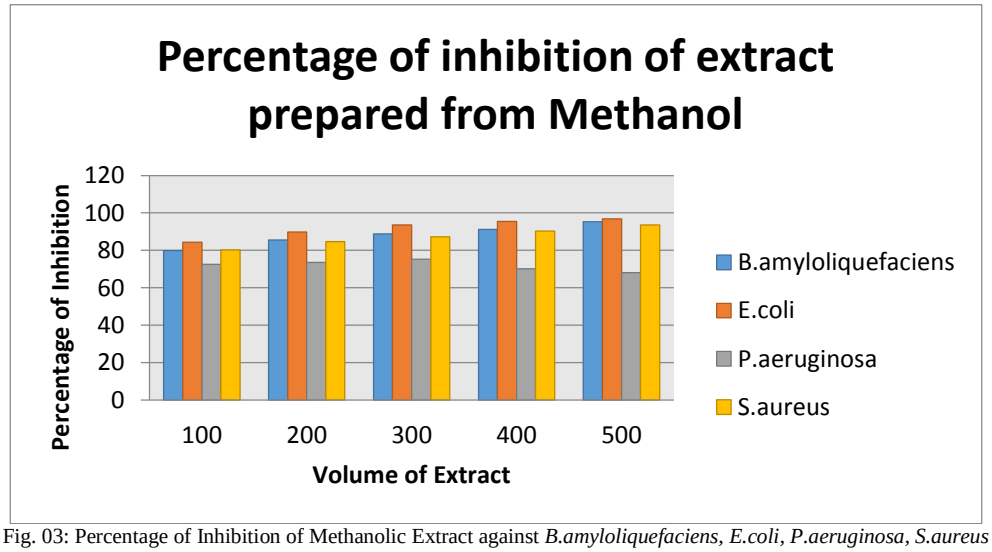


Fig. 03: Percentage of Inhibition of Methanolic Extract against *B.amyloliquefaciens, E.coli, P.aeruginosa, S.aureus*

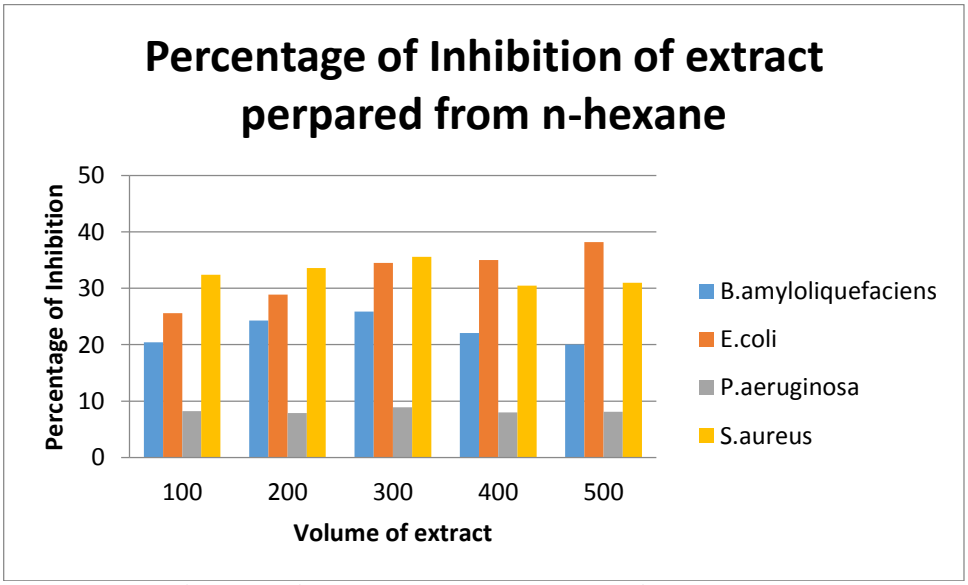


Fig. 04: Percentage of Inhibition of n-hexane Extract against *B.amyloliquefaciens, E.coli, P.aeruginosa, S.aureus*

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 09, September - 2017

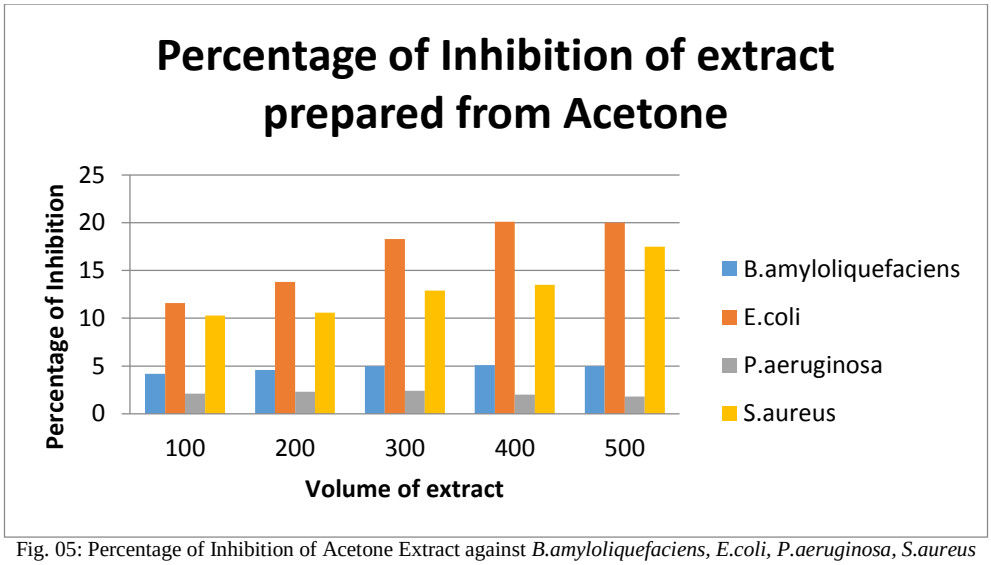


Fig. 05: Percentage of Inhibition of Acetone Extract against *B.amyloliquefaciens, E.coli, P.aeruginosa, S.aureus*

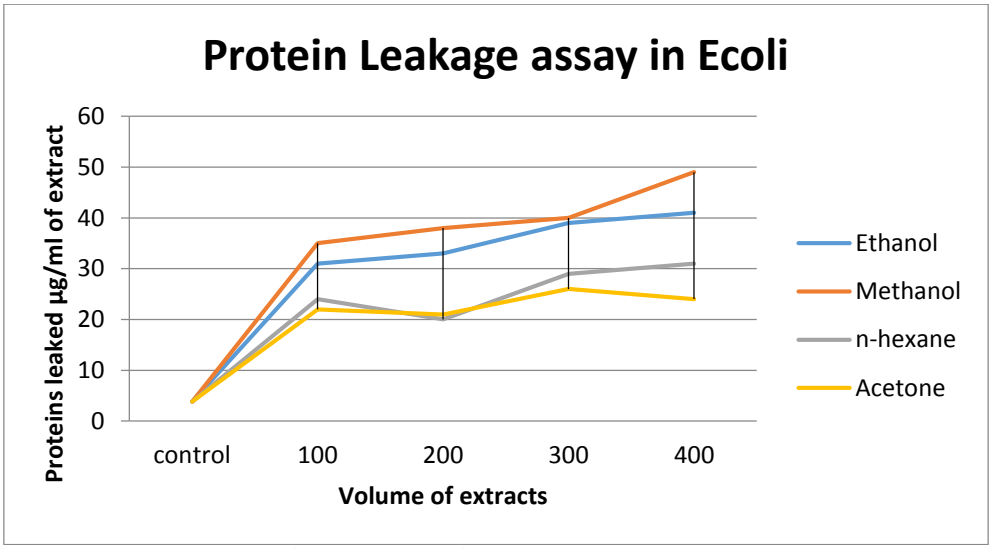


Fig. 06: Protein Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *E.coli*

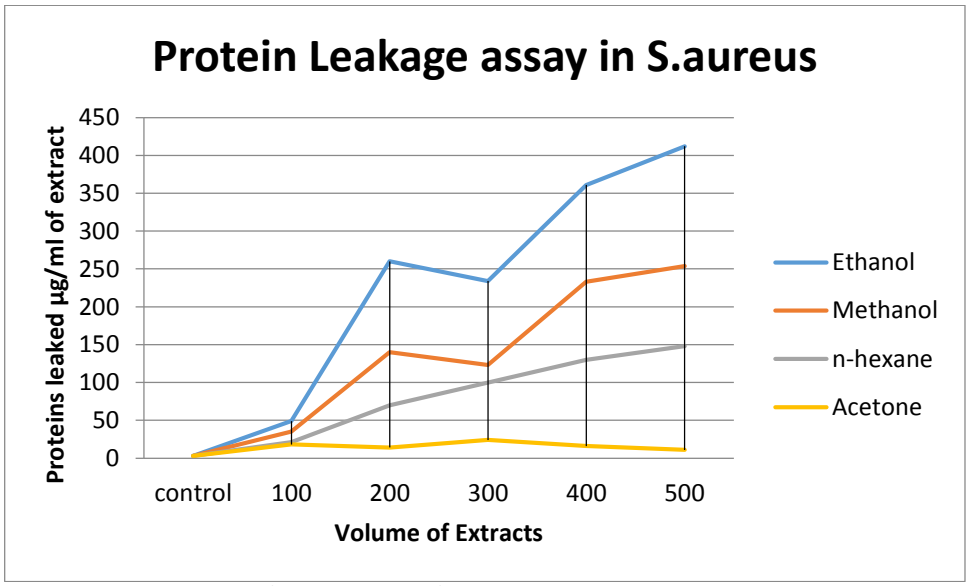


Fig. 07: Protein Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *S.aureus*

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 09, September - 2017

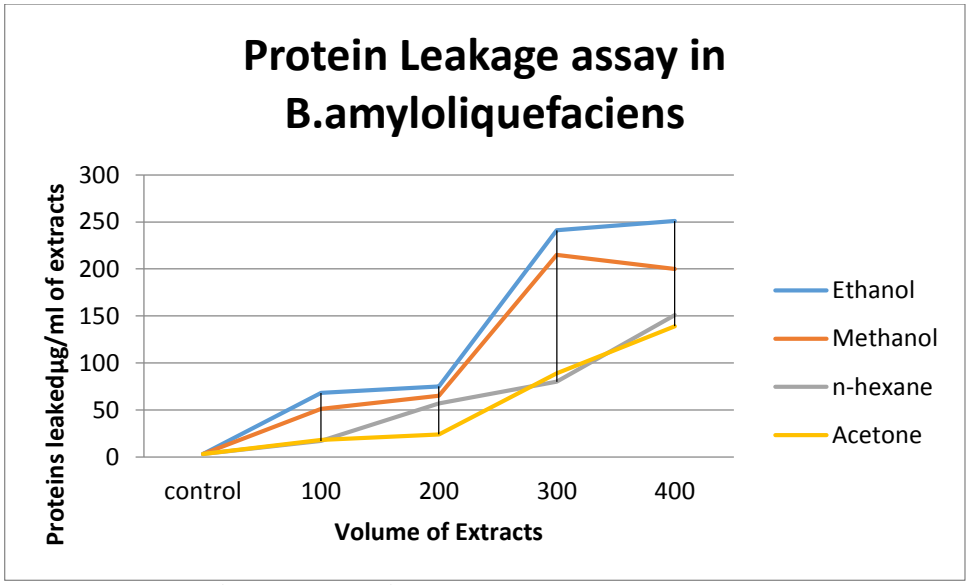


Fig. 08: Protein Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *B.amyloliquefaciens*

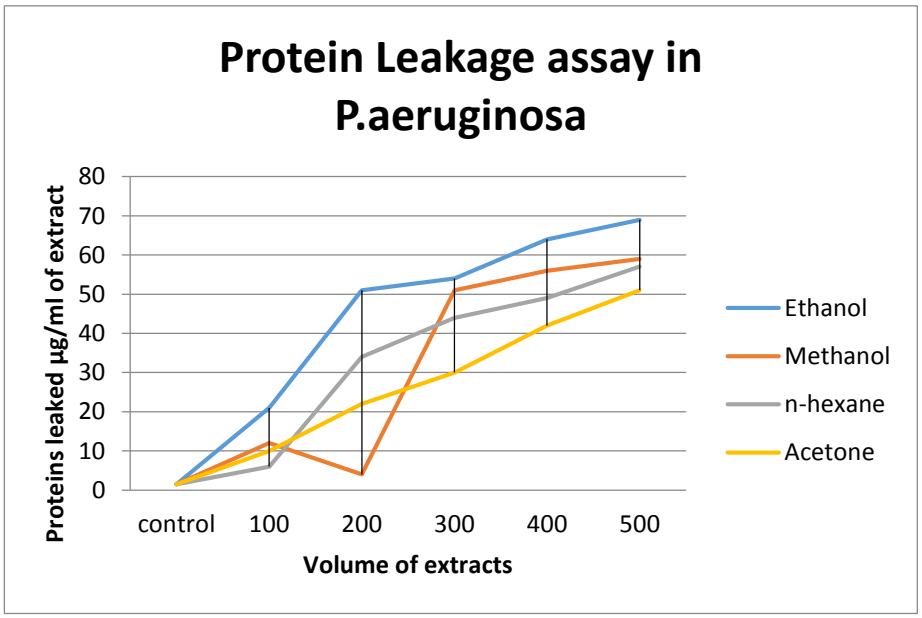


Fig. 09: Protein Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *P.aeruginosa*

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 09, September - 2017

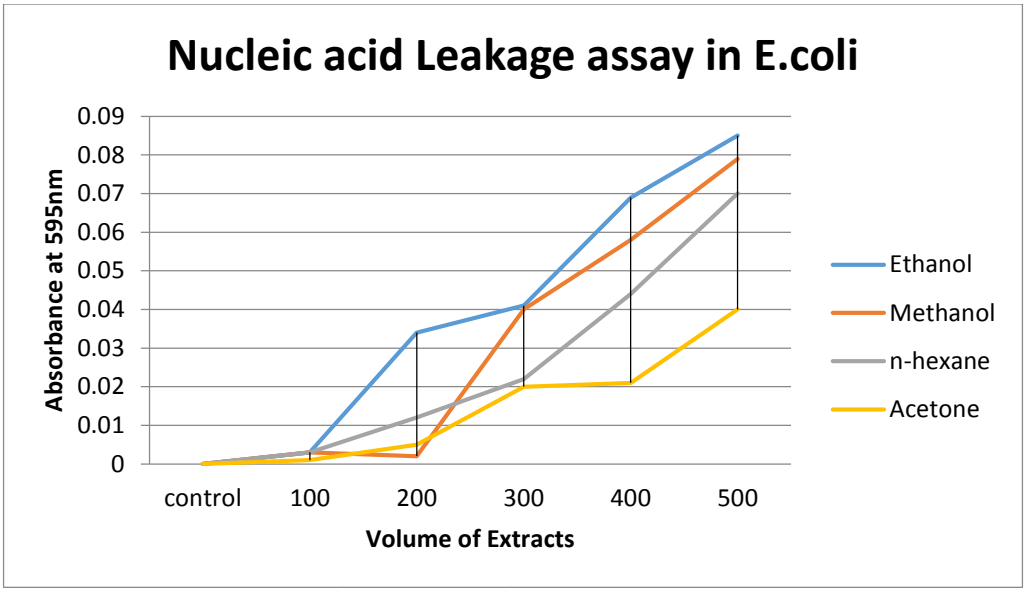


Fig. 10: Nucleic Acid Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *E.coli*

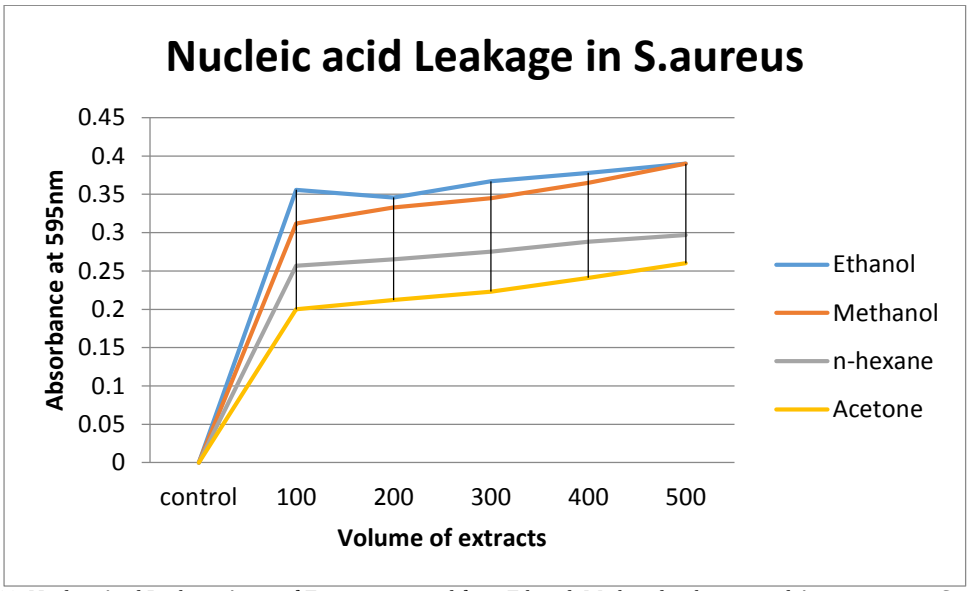


Fig. 11: Nucleic Acid Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *S.aureus*

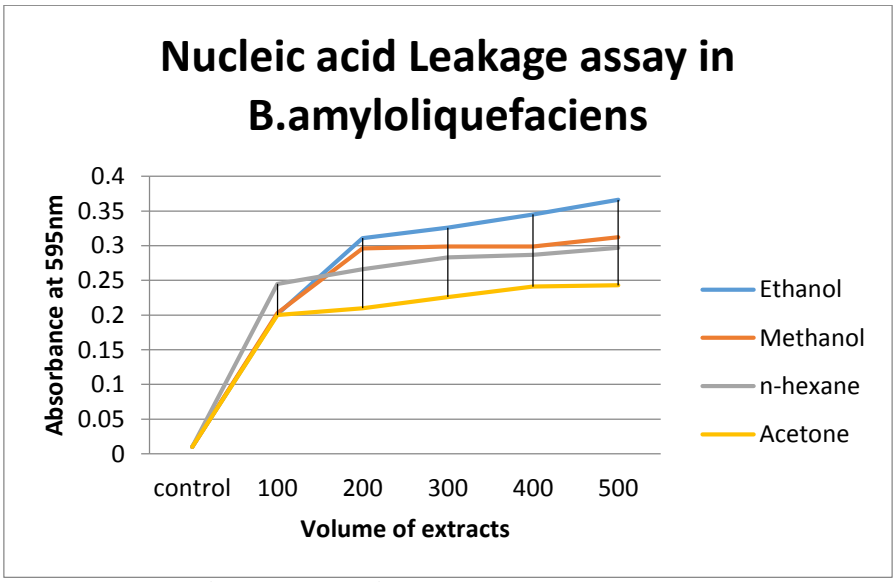


Fig. 12: Nucleic Acid Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *B.amyloliquefaciens*

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 09, September - 2017

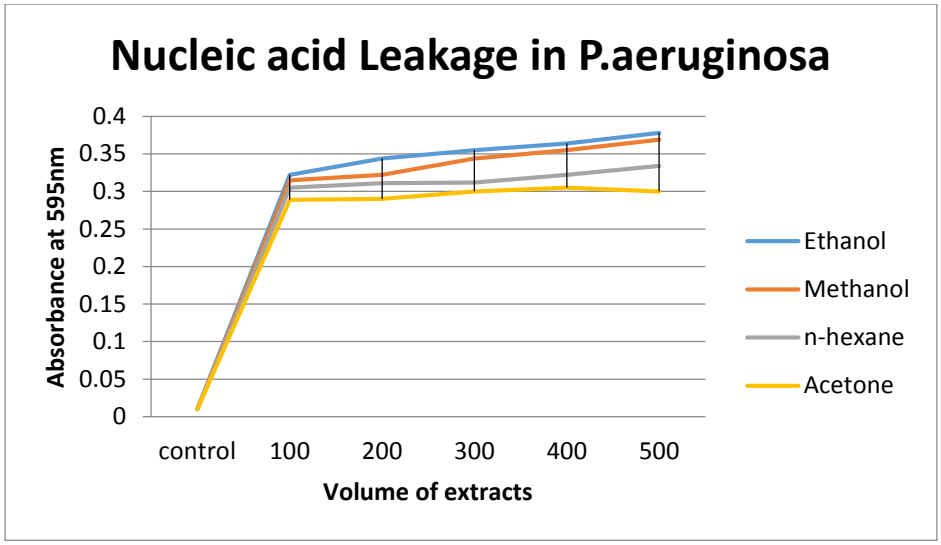
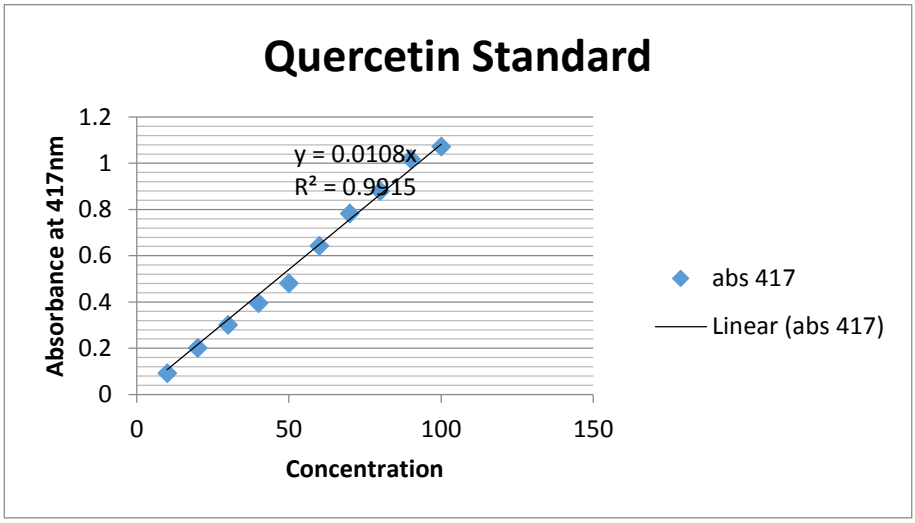


Fig. 13: Nucleic Acid Leakage Assay of Extracts prepared from Ethanol, Methanol, n-hexane and Acetone against *P.aeruginosa*

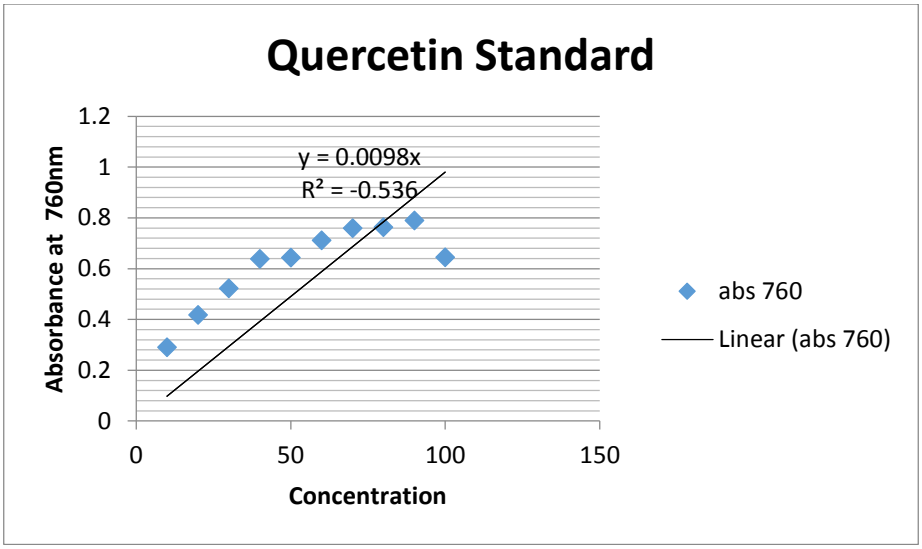


Graph 1: Quercetin Standard Curve for TFC

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

Extract	Mg QE/g of extract	µg/ml
Ethanol	3	300
Methanol	8.6	860
Acetone	1.7	170
n-hexane	3.5	350

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 09, September - 2017



Graph 2: Quercetin Standard Curve for TPC

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

Extract	Mg QE/g of extract	µg/ml
Ethanol	69.3	6930
Methanol	63.8	6380
Acetone	15.7	1570
n-hexane	2.3	230

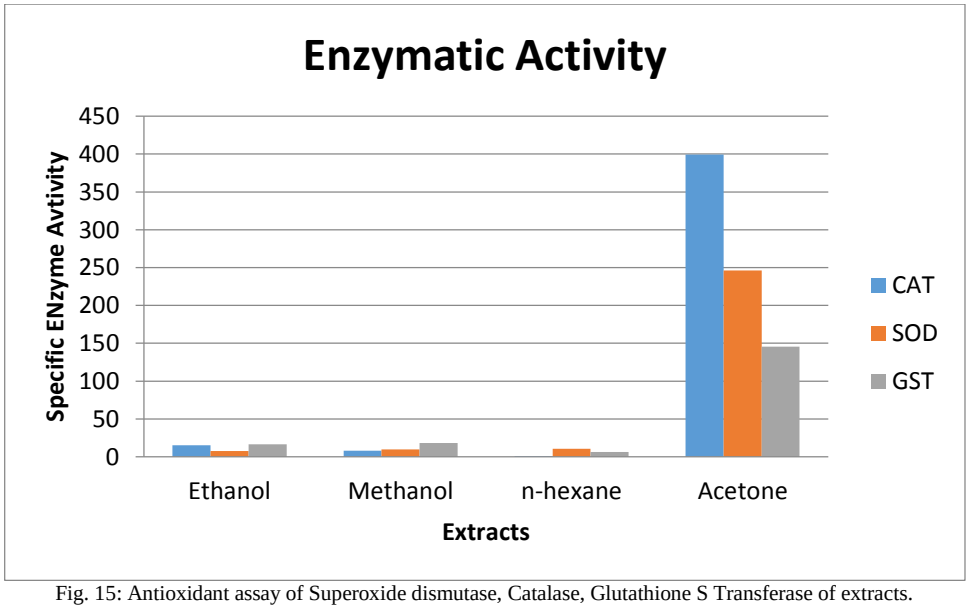


Fig. 15: Antioxidant assay of Superoxide dismutase, Catalase, Glutathione S Transferase of extracts.

Units for CAT: µmole of H_2O_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.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 09, September - 2017

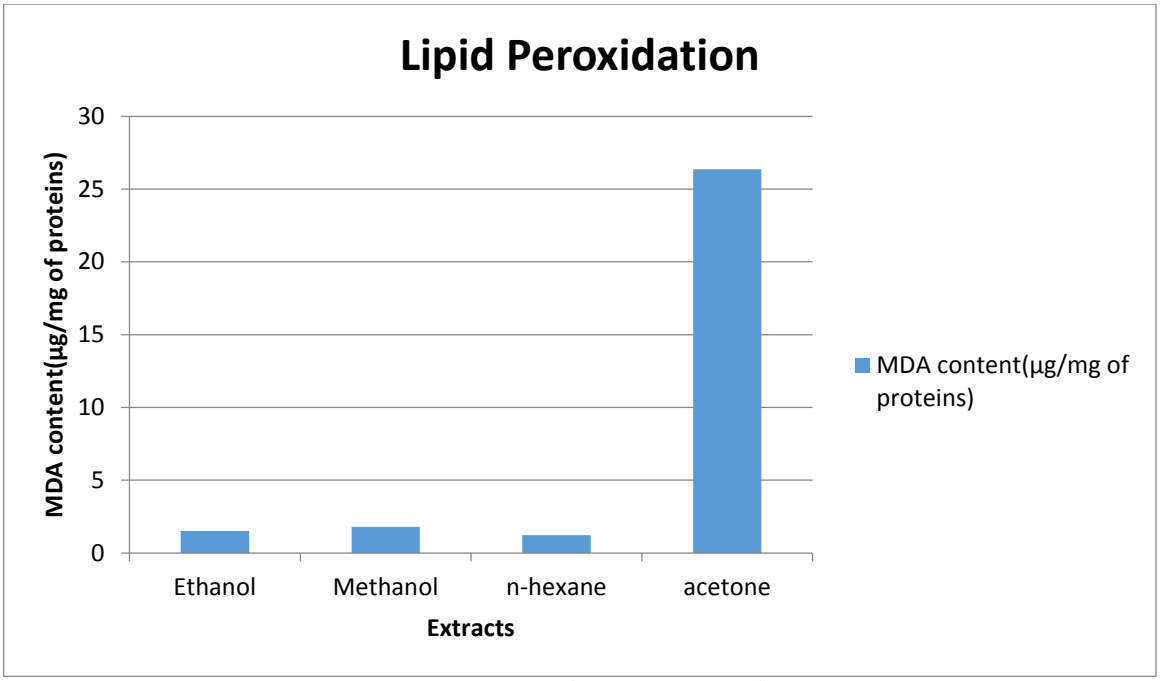


Fig. 16: Antioxidant assay of lipid per oxidation of extracts

CONCLUSION

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.

ACKNOWLEDGMENT

We, the authors extend heartfelt thanks to Mr. Ravi Kumar, Research Director, Helix Biogenesis Pvt Ltd, India for his continuous support and guidance.

REFERENCES

[1] Davidson-Hunt I.2000: Ecological ethno botany: stumbling toward new practices and paradigms. MASA J. ,16:1–13,2000
[2] Tapsell LC, Hemphill I,Cobiac L et al. (August 2006).Health benefits of herbs and spices: the past, the present, the future.Med. J. Aust.185 (4 Suppl): S4-24.
[3] Lai PK, Roy J (June 2004). Antimicrobial and chemopreventive properties of herbs and spices.Curr. Med.Chem.11(11): 1451–60.
[4] Sharma SK, Singh Lalit, Singh Suruchi, A review on medicinal plants having antioxidant potential, Indian J of Research in Pharmacy and Biotechnology,Vol1(3)May-Jun, 2013,PP404-409.
[5] Dabelstein, Werner; Reglitzky, Arno; Schütze, Andrea; Reders, Klaus (2007). "Automotive Fuels". Ullmann's Encyclopedia of Industrial Chemistry. doi:10.1002/14356007.a16_719.pub2. ISBN 3527306730.
[6] Debnath, P.; Dey. P.; Chanda, A. and a Bhakta, T. 2012. A Survey on ineapple and its medicinal value. Scholars Academic J. Pharm.1 (1).
[7] Baruwa, O.I. 2013. Profitability and constraints of pineapple production in Osun State, Negeria.2013. Journal of Horticultural Research. 21(2):59-64. [DOI: .2478/johr-2013-0022].
[8] Kakkar P, Das B. and Viswanathan PN: A modified spectrophotometric assay of superoxide dismutase. Indian Journal of Biochemistry & Biophysics 1984; 21:130-132.
[9] Joy, P.P. 2010. Benefits and uses of pineapple. Pineapple Research Station, Kerala Agricultural University, Kerala, India. [http://www.kau.edu/prsvkm/Html/BenefitsofPA.htm]
[10] FAO. 2005. Food and Agriculture Organization of United Nations.
[11] Fransworth NR, Akerele O, Bingel AS, Soejarto DD and Guo: Medicinal plants in therapy. Bulletin of the World Health Organization 1985; 63:965–981.
[12] Nascimento GG, Locatelli J, Freitas PC and Silva GL, (2000), Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria, Braz. J. Microbiol, Vol -31, pg 247 – 256.
[13] Duangsri P, Juntarapun K, and Satirapipathkul C: The tobacco leaf extract and antibacterial activity in textile. International Conference: Textiles & Fashion 2012; 3-4.
[14] Siddhuraju, P.; Vijayakumar, K. and Janardhanan, K 1996.Food. Chem. 57(3) 385 – 391.
[15] Yash Sharma, Anshita Nagar, Nupur Sinha Srivastava and Kumud Bala, 2017. antioxidant Activity of Polyphenolic Flavonoid of Stem of Nicotiana tabacum. American Journal of Drug Discovery and Development, 7: 25-32.
[16] Maurya, U.; Srivastava, S. Traditional Indian Herbal Medicine Used As Antipyretic, Antiulcer, Anti-Diabetic And Anticancer: A Review. International Journal Of Research In Pharmacy And Chemistry. 1(4): 1152-1159; 2011.
[17] Sinha N, Dua D, Wadhwa D. Antioxidant activity, free radical scavanging capacity and phytochemical screening of some selected Indian plants of medicinal importance. International journal of Innovative Pharmaceutical Sciences and Research 2015; 3: 151-163.
[18] Kiranmai M, Kumar CBM and Ibrahim M: Comparison of total flavonoid content of Azadirachta indica root bark extracts prepared by different methods of extraction. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2011; 2(3):254-261.
[19] Vinoth B, Manivasagaperumal R and Rajaravindran M, Phytochemical analysis and antibacterial activity of Azadirachta indica A Juss. International Journal of Research in Plant science, 2(3): 50 – 55, (2012).
[20] Hemalatha, R. and Anbuselvi, S. 2013. Physicochemical constituents of pineapple pulp and waste. J. Chem. Pharm. Res. 5(2):240-242.
[21] Owens CWI and Belcher RV: A colorimetric micro-method for the determination of glutathione. Biochemical Journal Mar 1965; 94(3):705–711.
[22] Srivastava NS and Dua D: Neuroprotective effect of Potent Antioxidant Lycopene in stress induced rat PC-12 cells. International Journal of Pharma and Bio Sciences 2015; 6(2):740-751.
[23] Ngele K. K., Olugbue V. U., Okorie U. V. (2014). Phytochemical constituents and Antimicrobial effect of unripe epicarp of orange friuts (Citrus sinensis) against Escherichia coli and Staphylococcus aureus.Int. J. of Sci. and Nature , 5(3): 418-422.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 09, September - 2017

[24] Dabesor A. P., Asowata-Ayodele A. M., Umoiette P. Phytochemical Compositions and Antimicrobial Activities of Ananas comosus Peel (M.) and Cocos nucifera Kernel (L.) on Selected Food Borne Pathogens. American Journal of Plant Biology. Vol. 2, No. 2, 2017, pp. 73-76. doi: 10.11648/j.ajpb.20170202.15

[25] Atul U, Jeewan P, Shinkichi T.2010. Utilization of pineapple waste: a review.Journal of Food Scienceand Technology 6, 10-18.

[26] Biradar Balasaheb Gunwantrao, Sonawane Kaveri Bhausaheb, Barge Sagar Ramrao* and Kharade Sachin Subhash (2016). Antimicrobial activity and phytochemical analysis of orange (Citrus aurantium L.) and pineapple (Ananas comosus (L.) Merr.) peel extract. Annals of Phytomedicine 5(2): 156-160, 2016

[27] Huda, K.; Dilnawaz, S.S.; Baqir, S. and Naqvi Ishaq, H. (2016). Antidepressant activity on methanolic extract of Ananas comosus Linn. Peel (MeACP) by using forced swim and tail suspension apparatus in mice, Sci. Int. (Lahore), 28(3):2525-2531.

[28] Ishii, R., Yoshikawa, K., Minakata, H. andKada, T. (1984): Specificities of Bio-Antimutagens in plant Kingdom. J. Agr. Biol. Chem. 4810: 2587-2591.

[29] Chanda, S., Baravalia, Y., Kaneria, M. and Rakholiya, K. (2010): Current Research Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. A. Mendez-Vilas (Ed). Pp 444 - 450.

[30] Taura, D.W., Okoli, A.C. and Bichi, A.H. (2005): In-vitro Antibacterial Activities of Ethanolic Extracts of AnnonaComosus L. Allium Sativum L. And Aloe Barbadensis L. in Comparison With Ciprofloxacin. BEST Journal 1(1): 36-41.

[31] Lawal, D., Mukhtar, M.D., Taura, D.W. and Bukar, A. (2011).Phytochemical screening and invitro anti-bacterial studies of the ethanolic extract of A. comosus (Linn) Merr. Peels on Aeromonashydrophila and S a l m o n e ll a s p e c i e s .Bayero J o u r n a l o f P u r e a n d Applied Sciences .4(1): 168-172.

[32] Elliot, J. G. (1999). Application of antioxidant vitamins in foods and beverages.
FoodTechnology, 53 , 46 −48

[33] Miller, N. J., & Rice-Evans, C. A. (1997). The relative contributions of ascorbic acid andphenolic antioxidants to the total antioxidant activity of orange and apple. FoodChemistry , 60, 331−337

[34] Atul U, Jeewan P, Shinkichi T. 2010. Utilization of pineapple waste: a review. J. Food Sci. Technol. Nepal, Vol. 6 (10-18), 2010

[35] D.Gomati, M.Kalaiselvi, G.Ravikumar and C.Uma. Evaluation of Enzymatic and Non-Enzymatic Antioxidant potential of *Evolvulus alsinoides* (L.). Asian Journal of Pharmaceutical and Clinical Research Vol 5, Suppl 2, 2012