International Journal of Advanced Biotechnology and Research
ISSN 0976-2612, Online ISSN 2278–599X,
Vol 3, Issue 3, 2012, pp 670-679
http://www.bipublication.com

ANTIMUTAGENIC AND ANTIBACTERIAL ACTIVITY OF *Aegle marmelos* (L.) Corr.

Elavarasi Natarajan*, Bharathi Purushothaman, Anija, Asha Krishna, Gowri

Department of biotechnology, Karpaga vinayaga College of engineering and technology,
MadurantakamTaluk,Tamilnadu,India-603308
E-mail:elavarasijawahar@gmail.com
Phone: 91-044-27565486

[Received-02/05/2012, Accepted-07/07/2012]

ABSTRACT:

In this study we investigated the antimutagenic and antibacterial activity of *Aegle marmelos* , an important medicinal plant of India .The antimutagenic effect of hydrogen peroxide damaged DNA was studied using acetone and aqueous dried fruit extracts of *Aegle marmelos* by Comet assay .The bioactive components present in dried fruit extracted by acetone and water exhibited promising antimutagenic activity. The antibacterial effect was studied against gram positive and gram negative bacterial strains using petroleum ether, chloroform and aqueous leaf extracts of *Aegle marmelos* by agar diffusion method in which all the extracts showed antibacterial activity equivalent to the standard antibiotic streptomycin.

Keywords: *Aegle marmelos*, antimutagenic activity, comet assay, antibacterial activity, agar diffusion method.

INTRODUCTION:

Aegle marmelos (L.) Corr. belongs to family *Rutaceae*, commonly known as bael (Hindi) and golden apple (English). It is found throughout India and is known from pre-historic time. *Aegle marmelos* has been used from time immemorial in traditional systems of medicine for relieving constipation, diarrhoea, dysentery, peptic ulcer and respiratory infections [1]. Several studies on different parts of *Aegle marmelos* showed that the plant possesses antidiarrhoeal [2], antidiabetic [3], anti-inflammatory, antipyretic, analgesic [4], anticancer [5], radioprotective [6] and antimicrobial activities [7,8] .

The continued emergence or persistence of drug resistant organisms and the increasing evolutionary adaptations by pathogenic organisms to commonly used antimicrobials have

reduced the efficacy of antimicrobial agents currently in use [9]. Therefore, antimicrobial agent effective against multiresistant pathogens need to be developed. Thus the present study was carried out to investigate antimicrobial activity of *Aegle marmelos* against various bacterial species.

The single-cell gel assay (also termed comet assay) is a very useful microelectrophoretic technique for evaluation of DNA damage and repair in individual cells. The technique was developed to visualize the DNA damage induced by radiation in mammalian cells [10]. This method has many applications in radiation biology, in estimation of oxidative damages and DNA crosslinks, in apoptosis, and in genotoxicity induced by chemical compounds [11]. As *in vitro* antigenotoxicity/antimutagenicity studies may be useful for discovering chemopreventive phytochemicals, the present study was also planned to investigate antimutagenic activity of *A. marmelos* fruits extracted with different organic solvents by human blood lymphocytes in the Comet assay.

MATERIALS AND METHODS

The plant material was collected from nearby locality and certified as *Aegle marmelo*s (L.) Corr. (Family: Rutaceae) by Siddha Central Research Institute, Ministry of Health and family welfare, Govt.of India.

ANTIMUTAGENIC ACTIVITY

Materials Required

Aegle marmelos fruit powder, Human Peripheral Lymphocyte's (HPL'S), Phyto haemagglutinins (PHA), 0.2µm pore sized cellulose acetate filter, Whatman's filter paper, Methanol extract, Water extract, Acetone extract, Hisep , Hydrogen peroxide, Incubator, Fully frosted microscope slides , 0.75% Normal melting agarose , Low melting agarose , Lysis solution (2.5M NaCl,100mM Na_2EDTA, 10mM Tris (pH 10) and 1% SDS to which 10% DMSO and 1% TRITON X100), Electrophoresis buffer(300mM NaOH and 100mM Na_2EDTA (pH 13)) Neutralization buffer (0.4M Tris,pH 7.5), EtBr , C mount camera(Olympus), OLYMPUS CKX 41 Epifluorescent inverted microscope, Comet scoring software (Tritek Comet score V1.5 Software).

Fig:1 *Aegle marmelos* fruit and dried fruit powder

Methodology

Preparation of fruit extract

The shade dried fruit was powdered using a mechanical grinder and passed through 40 mesh sieve.Each100 gm of this powder was extracted with 50ml of water and acetone respectively. The extracted samples were heated at 60˚c for 90 min .The extract was then filtered using whatman's filter paper. This procedure was repeated twice for complete extraction. The extracts were maintained in vacuum to obtain a semisolid mass.

Lymphocyte culture preparation

Human Peripheral Lymphocyte's(HPL'S) were cultured in RPMI 1640 (Himedia) media, supplemented with 20% heat inactivated Foetal Bovine Serum, antibiotics (Pencillin and

streptomycin). Phytohaemagglutinin (PHA) was used as the stimulant for cell proliferation. The culture was prepared using 0.2µm pore sized cellulose acetate filter (Sartorios) in completely aseptic condition. Human peripheral lymphocyte separated using Hisep (Himedia) . To 5µl of each extract 10µl Hydrogen peroxide and HPL's were added and incubated for 72hrs and after incubation cells were collected by centrifugation and used for comet assay.

Comet assay

Fully frosted microscopic slides were precoated with 1ml of 0.75% normal melting agarose (NMA) and stored at 4 ºC. This layer was removed before use and 120µl of 0.75% NMA was pippeted onto the slides and covered with a cover slip. Cell suspensions($1*10^4$/5-10µl)were mixed with 10µl of low melting point agarose and pipetted over the first layer of agarose.After each step the slides were incubated at 4 ºC for 10mins and allow agarose to set. Slides were then placed in cold lysis solution(2.5 M NaCl,100mM Na_2EDTA, 10mM Tris pH 10 and 1% SDS, 10% DMSO and 1% TRITON X100) immediately prior to use for 1hr. After lysis slides were placed in electrophoresis buffer (300mM NaOH and 100mM Na_2EDTA (pH 13))for unwinding of DNA .Electrophoresis was conducted in the same buffer by applying an electric current of 0.8v/cm(300mA) for 20min using an electrophoresis supply. NMA(80µl) was used as a final protective layer.Finally slides were washed thrice in neutralisation buffer(0.4M Tris,pH 7.5) for 5mins ,each dried and stained with 50µl EtBr(20µg/ml) .The stained slides were observed in OLYMPUS CKX 41 Epifluorescent inverted microscope using green filter(Excitation filter BP480-550C) and the image were captured using C mount camera(Olympus).

Image analysis

The comet photograph were scored using Comet scoring software(Tritek Comet score V1.5 Software) and the values were expressed as %DNA tail, head and total comet area.

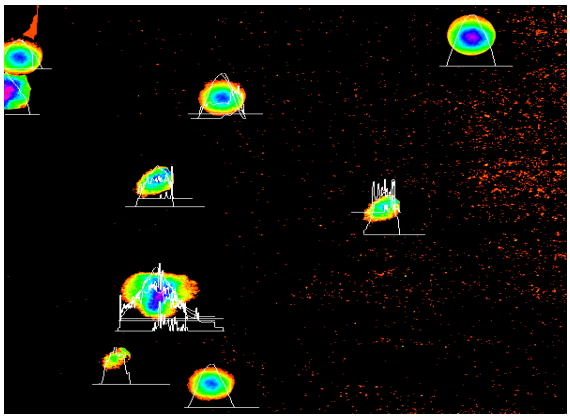


Fig:2 Model picture for comet assay

ANTIBACTERIAL ACTIVITY

Materials Required

Nutrient agar, *Aegle marmelos* leaves, Petroleum ether , Chloroform, Water, Streptomycin, E-coli, Peudomonas sp., Klebsiella sp., Bacillus sp., Staphylococcus sp.

Methodology

Preparation of leaf extract

The shade dried leaves were powdered using a mechanical grinder and passed through 40 mesh sieve. Each 100mg of this powder was extracted with 50ml of petroleum ether, chloroform and water respectively. The extracted samples were heated at 60˚c in for 90 min.The extract was then filtered using whatman's filter paper.This procedure was repeated for 2 times for getting the complete extract. The extracts were kept under vacuum to a get a semisolid mass.

Figure: 3 *Aegle marmelos* leaves and dried leaf powder

Agar diffusion method

The antibacterial activity of the extracts were studied by agar diffusion method against various bacteria such as Escherichia coli, Pseudomonas aeruginosa, Klebsiella (gram negative),Staphylococcus aureus and Bacillus subtilis (gram positive).The pure cultures of bacteria were subcultured on nutrient agar medium. Each strain was swabbed uniformly onto individual plates using sterile cotton swabs. Wells of 5mm diameter were made on nutrient agar plates using gel puncture.Using a micropipette, 25µl of each extracts were added onto each well on all plates. 25µl of Streptomycin solution (1µg/ml) was used as positive control and the respective solvents were used as negative control. After incubation at 37ºc for 24 hours, the diameters of zone of inhibition were measured.

RESULTS AND DISCUSSIONS

ANTIMUTAGENIC ACTIVITY

The antimutagenic activity of *Aegle marmelos* fruit extracts was demonstrated by comet assay. Hydrogen peroxide was used as the mutant for inducing DNA damage.(Fig 5). The extract alone did not cause any mutagenicity. The DNA damage induced by H_2O_2 was reduced effectively by both aqueous and acetone extracts (Fig 6&7) which was evident from values of tail length, tail area and percentage DNA in tail (Table 3&4).

Fig: 4 Comet assay 1 (Lymphocytes)

ANTIBACTERIAL ACTIVITY

The antibacterial activity of water , chloroform and petroleum ether extracts of *Aegle marmelos* leaf were studied by agar diffusion method. All the extracts showed good antibacterial activity against both gram positive and gram negative bacteria. The chloroform extract was found to have better activity than water and petroleum ether extract which was evident from the diameter of the zone of inhibition(Fig 8,9,10 & table 5,6,7).

The *Aegle marmelos* leaf extracts showed maximum activity against *Klebsiella* species, which was evident from the diameter of maximum zone of inhibition (32 mm for water extract,30mm for Petroleum extract and 32 mm for Chloroform extract).The antibacterial activity was minimum against *Pseudomonas* species (10mm for Water extract,14 mm for Petroleum ether extract and 12 mm for Chloroform extract-Fig 8,9,10).

CONCLUSION

The antimutagenic activity exerted by the polyphenolic compounds present in the *Aegle marmelos* fruit against reactive oxygen species induced by hydrogen peroxide (H_2O_2) in isolated human peripheral blood lymphocytes (PBLs) was demonstrated by comet assay using acetone, and water extracts and concluded that both the extracts showed very good antimutagenic effect.

The antibacterial activity exerted by cuminaldehyde and eugenol present in the *Aegle marmelos* leaf against gram positive and gram negative bacteria was demonstrated by agar diffusion method using aqueous, petroleum ether and chloroform extracts and concluded that all the extracts exhibited antibacterial activity comparable with the standard antibiotic streptomycin.

REFERENCES

1. Nadkarni A. K.: Indian Materia Medica, 3rd edn., p. 45, Popular Prakashan Private Ltd., Mumbai 2000.

2. Shobha F.G., Thomas M.: J. Ethnopharmacol. 76, 73 (2001).
3. Veerappan A., Shigeru M., Renganathan D.: J. Ethonopharmacol. 96, 159 (2005).
4. Lotufu L.V., Khan M.T., Ather A., Wilke D.V., Simenez P.C. et al.: J. Ethonopharmacol. 99, 21 (2005).
5. Jagetia G.C., Venkatesh P., Balinga M.S.: Int. J. Radiat. Biol. 80, 281 (2004).
6. Rana B.K., Jain A.K.: J. Ethnopharmacol. 57, 29 (1997).
7. Rani P., Khullar N.: Phytother. Res. 18**,** 670 (2004).
8. Trease G.,E., Evans W.C.: Textbook of Pharmacognosy, 12th edn. Balliere, Tindall, London 1989.
9. WHO Traditional medicine: Growing needs and potential, WHO Policy perspectives on medicines. p. 1, World Healh Organisation, Geneva 2002.
10. Ostling O, Johanson KJ Microelectrophoretic study of radiation- induced DNA damages in individual mammalian cells. Biochem Biophys Res Commun ,123:291–298(1984).
11. McKelvey–Martin VJ, Green MHL, Schemezer P, Pool–Zobel BL, De Méo MP, Collins A The single cell gel electrophoresis assay (comet assay): a European review. Mutation Res 288:47–63(1993).

LIST OF TABLES

TABLE NUMBER	TITLE
1.	Comet assay 1 (Lymphocytes)
2.	Comet assay 2 (Lymphocyte + H_2O_2 mutant)
3.	Comet assay 2 (Lymphocyte + H_2O_2 mutant+Acetone extract)
4.	Comet assay 2 (Lymphocyte + H_2O_2 mutant+aqueous extract)
5.	Diameter of zone of inhibition for water extract
6.	Diameter of zone of inhibition for chloroform extract
7.	Diameter of zone of inhibition for petroleum ether extract

LIST OF FIGURES

FIGURE NUMBER	TITLE
1.	*Aegle marmelos* fruit and dried fruit powder
2.	Model picture for comet assay
3.	*Aegle marmelos* leaves and dried leaf powder
4.	Comet assay 1 (Lymphocytes)
5.	Comet assay 2 (Lymphocyte + H_2O_2 mutant)
6.	Comet assay 2 (Lymphocyte + H_2O_2 mutant+Acetone extract)
7.	Comet assay 2 (Lymphocyte + H_2O_2 mutant+aqueous extract)
8.	Antibacterial activity of water extract
9.	Antibacterial activity of chloroform extract
10.	Antibacterial activity of petroleum ether extract

LIST OF ABBREVIATIONS

LIST	ABBREVIATION
DMSO	Dimethyl sulfoxide
EtBr	Ethidium bromide
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HPL's	Human peripheral lymphocyte's
LMA	Low melting agarose
Na_2EDTA	Disodium ethylene diamine tetra acetic acid
NaCl	Sodium chloride
NMA	Normal melting agarose
PBS	Phosphate buffer saline
PCR	Polymerase Chain Reaction
Triton X100	Polyethyleneglycol-4-tetraoctylphenolether

Fig: 4 Comet assay 1 (Lymphocytes)

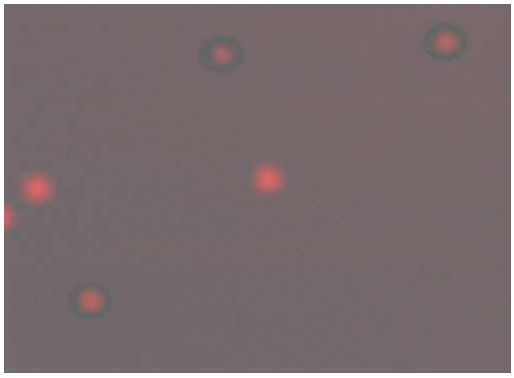


Table: 1 Comet assay 1 (Lymphocytes)

Comet Length (px)	Comet Height (px)	Comet Area (px)	Head diameter(px)	Tail Length (px)	Tail Area (px)	%DNA in Tail
219.000000	207.000000	45333.000000	207.000000	2.000000	1.000000	1.717608
116.000000	190.000000	22040.000000	190.000000	0.000000	1.000000	1.669435
224.000000	206.000000	46144.000000	206.000000	1.000000	1.000000	0.008314
212.000000	195.000000	39030.000000	212.000000	0.000000	21.000000	0.000209
210.000000	224.000000	38740.000000	204.000000	0.000000	1.000000	0.106277
194.000000	165.000000	29156.000000	152.000000	0.000000	1.000000	0.008314

Fig:5 Comet assay 2 (Lymphocyte + H_2O_2 mutant)

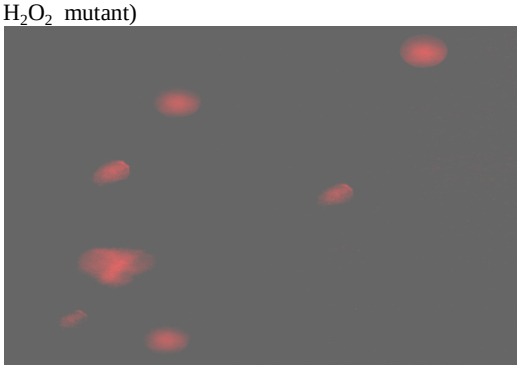


Table: 2 Comet assay 2 (Lymphocyte + H_2O_2 mutant)

Comet Length (px)	Comet Height (px)	Comet Area (px)	Head diameter(px)	Tail Length (px)	Tail Area (px)	%DNA in Tail
78.000000	76.000000	2500.000000	76.000000	2.000000	104.000000	11.029384
55.000000	62.000000	2240.000000	55.000000	10.000000	321.000000	12.777106
66.000000	58.000000	1409.000000	66.000000	4.000000	12.000000	0.183352
66.000000	63.000000	1248.000000	62.000000	18.000000	8.000000	0.014700
77.000000	72.000000	4534.000000	90.000000	7.000000	4.000000	0.026624
116.000000	89.000000	4665.000000	82.000000	34.000000	831.000000	22.703545

Fig: 6 Comet assay 3(Lymphocytes + H2O2 mutant + Acetone extract)

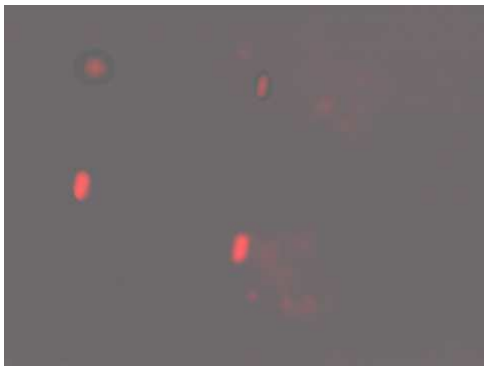


Table: 3 Comet assay 3(Lymphocytes + H2O2 mutant + Acetone extract)

Comet Length (px)	Comet Height (px)	Comet Area (px)	Head diameter(px)	Tail Length (px)	Tail Area (px)	%DNA in Tail
35.000000	53.000000	1855.000000	53.000000	0.000000	1.000000	0.219027
53.000000	46.000000	2438.000000	46.000000	7.000000	44.000000	10.096213
26.000000	28.000000	728.000000	28.000000	0.000000	1.000000	0.399106
36.000000	38.000000	1368.000000	38.000000	0.000000	1.000000	0.627383
34.000000	44.000000	1496.000000	44.000000	0.000000	1.000000	0.730731
45.000000	44.000000	1980.000000	44.000000	1.000000	1.000000	15.986383

Fig: 7 Comet assay 4 (Lymphocytes + H_2O_2 mutant + Aqueous extract)

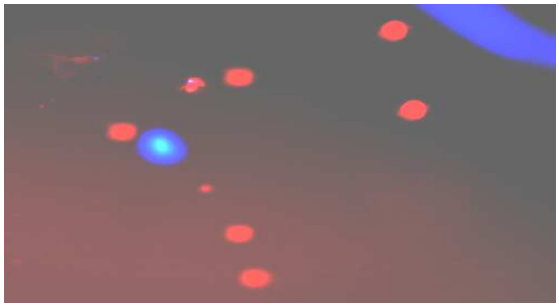
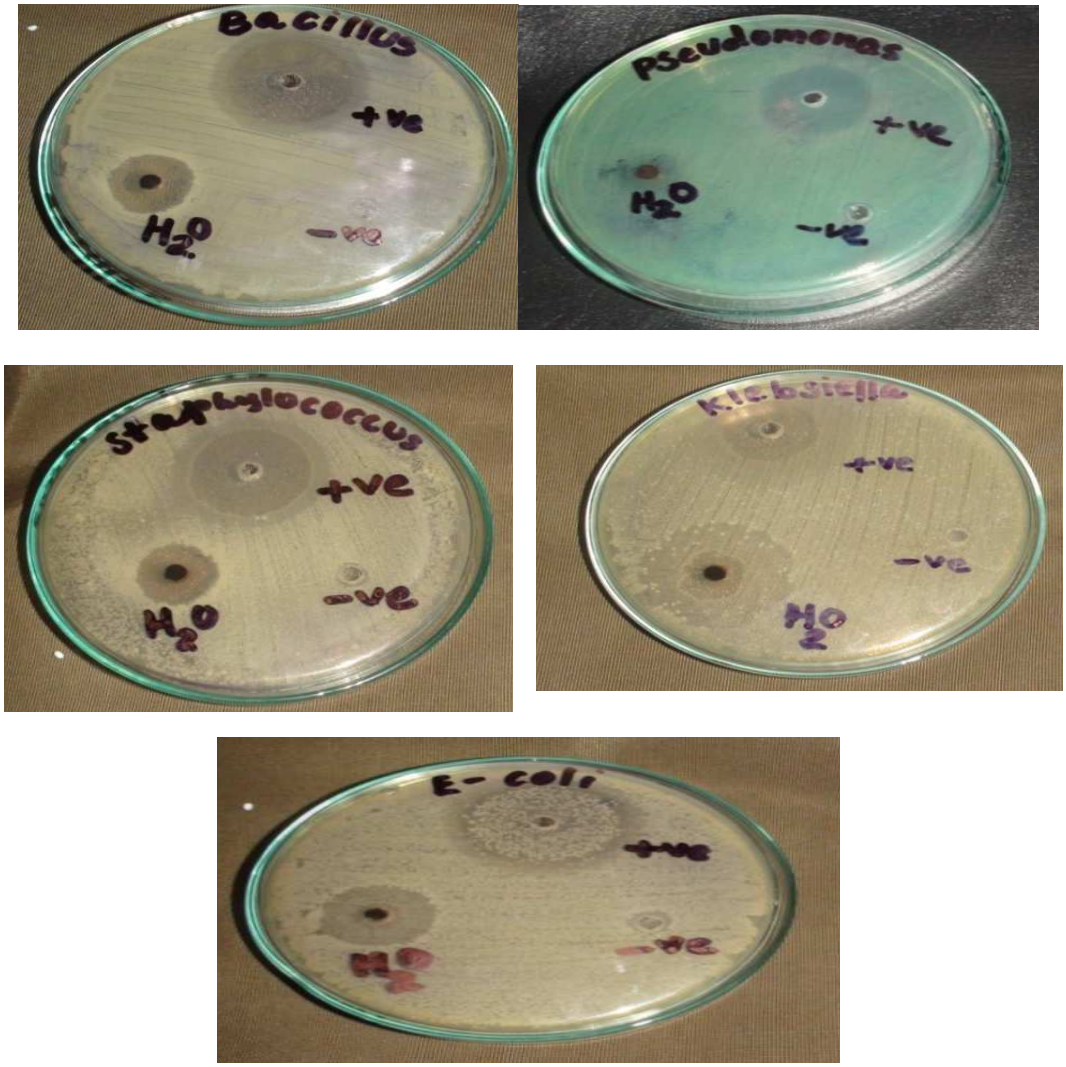


Table: 4 Comet assay 4 (Lymphocyte + H_2O_2 mutant + Aqueous extract)

Comet Length (px)	Comet Height (px)	Comet Area (px)	Head diameter(px)	Tail Length (px)	Tail Area (px)	%DNA in Tail
89.000000	86.000000	1932.000000	86.000000	3.000000	4.000000	0.127587
49.000000	67.000000	1454.000000	49.000000	0.000000	1.000000	5.802017
74.000000	88.000000	1942.000000	74.000000	0.000000	1.000000	1.521148
75.000000	90.000000	1118.000000	66.000000	9.000000	6.000000	0.379072
39.000000	46.000000	621.000000	39.000000	0.000000	1.000000	0.003329
64.000000	60.000000	1578.000000	64.000000	0.000000	1.000000	0.260764

Fig: 8 Antibacterial activity of aqueous extract

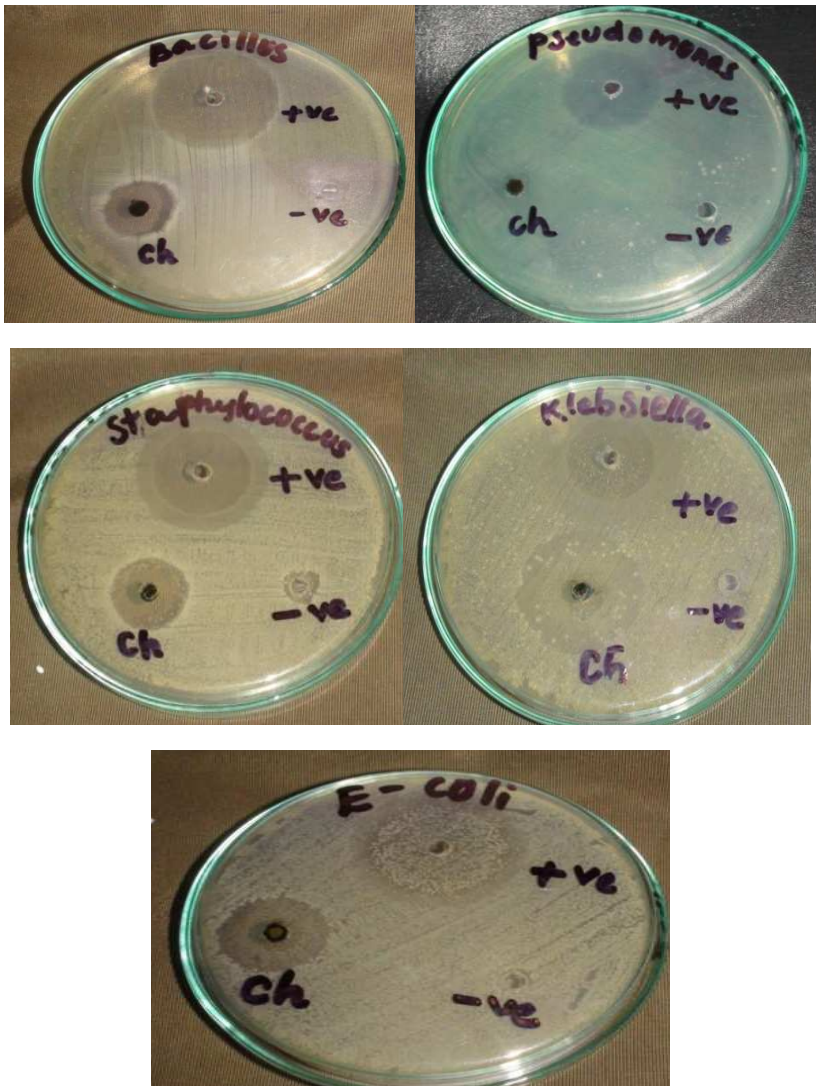


+ve➔ streptomycin, H2O➔ aqueous extract , -ve➔ water

Table: 5 Diameter of Zone of inhibition for Water extract

Microorganisms	**+ve control(streptomycin)in mm**	**-ve control(water)in mm**	**Water extract in mm**
E-coli	35	0	20
Klebsiella	40	0	32
Bacillus	25	0	10
Staphylococcus	35	0	17
Pseudomonas	35	0	16

Fig:9 Antibacterial activity of chloroform extract

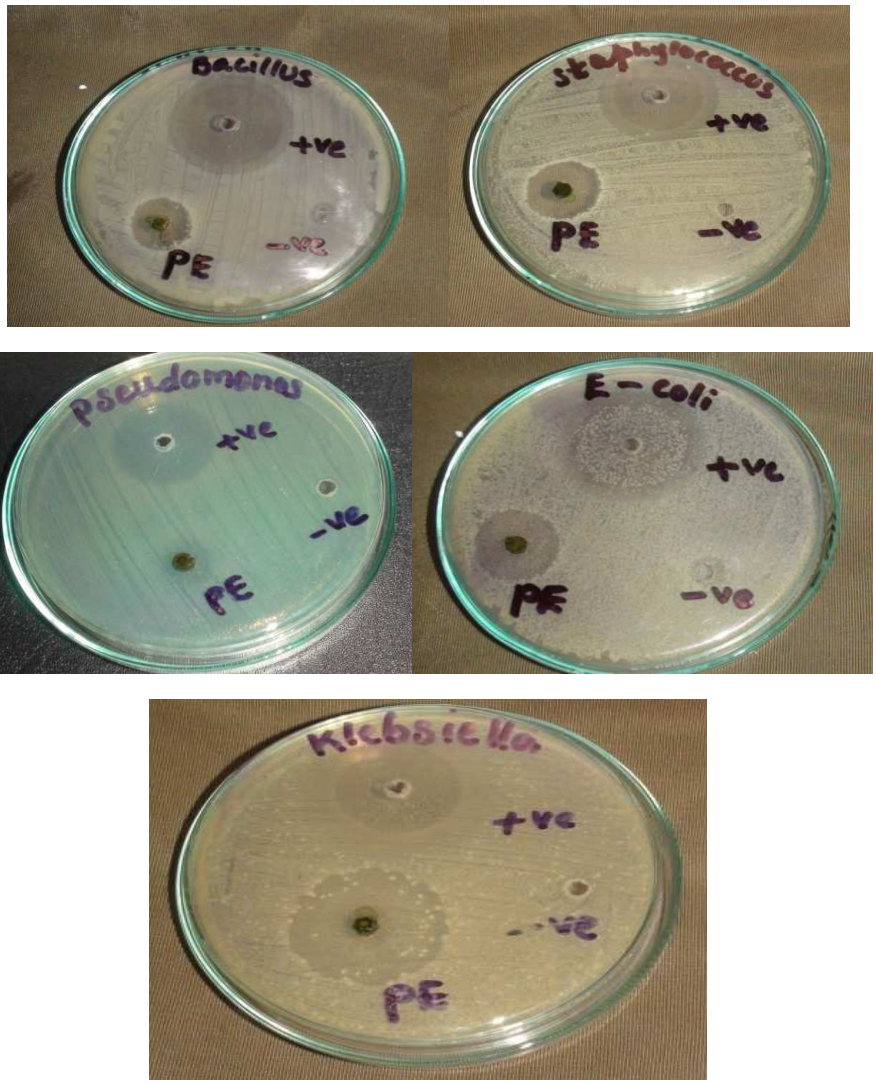


+ve➔ streptomycin, Ch➔ Chloroform extract , -ve➔ Chloroform

Table: 6 Diameter of Zone of inhibition for Chloroform extract

Microorganisms	**+ve control(streptomycin)in mm**	**-ve control(water)in mm**	**Chloroform extract in mm**
E-coli	35	0	22
Klebsiella	40	0	32
Bacillus	25	0	12
Staphylococcus	35	0	19
Pseudomonas	35	8	20

Fig: 10 Antibacterial activity of Petroleum ether extract



+ve➔streptomycin, PE➔ Petroleum ether extract , -ve➔ Petroleum ether

Table: 7 Diameter of Zone of inhibition for petroleum ether extract

Microorganisms	**+ve control (streptomycin) mm**	**-ve control (Petroleum ether) mm**	**Petroleum ether extract(mm)**
E-coli	35	0	20
Klebsiella	40	0	30
Bacillus	25	0	10
Staphylococcus	35	0	15
Pseudomonas	35	0	19