In-Vitro Study of Anti-Biofilm Activity of Marketed Drugs and Phytochemical Extracts

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Abstract— Biofilm is a slime layer formed on the surface of any substance by the accumulation of one or more than one microorganism by the help of extracellular matrix which contains polysaccharide, structural protein, cell debris and nucleic acid which is called extracellular polymeric stuff (EPS). Which can be formed anywhere like households, industrial pipes, medical equipments like catheters, medical implants, plant and animal tissue etc. Microbial biofilm has very bad impact on human health i.e., tooth decay when develop in tooth, cystic fibrosis when develop in lungs. In non-medical areas bio fouling of water, spoiling of food etc., occurs due to the presence of biofilm. Due to the composition of their structure they make themselves protective and resistance with the anti-microbial compounds. In this present research, to degrade these biofilms, we treated it with various phytochemical extracts, marketed medicines and combined them to make it effective against the biofilm. Which can further be used for the remedy of biofilm in upcoming year.

Keywords: Biofilm, Spoiling of Food, Cell Debris, Cystic Fibrosis, Medical Equipments, Phytochemical Extracts, Marketed Medicines

I. INTRODUCTION

Biofilm is basically formed when one or more than one type of microorganisms grows together [1]. They exchange their substrate, enable distribution of metabolic products, also remove out toxic chemicals to enable other communities live together and support each other. This help them to protect from antimicrobial attack, shear forces and immune system. Biofilm formed by P. aeruginosa & S. epidermidis [2].

Biofilm start to form when freely living microorganisms start to grow on suitable surface. This attachment is reversible attachment. After that they form monolayer and produce "Slime" for their production. Extracellular matrix is consisting of polysaccharides, structure proteins, cell debris and nucleic acid. These bacteria increase their number that exhibits important growth and communication between bacteria like quorum sensing. Biofilm grows in 3D by irreversible attachment. At last the biofilm gets mature and the cells of mature biofilm detaches and move to new surfaces to start the new biofilm cycle.

Microorganism can sense their surrounding which enables them to maximise the use of substrate and protect them for stress conditions and by the help of quorum sensing they meet up with each other and colonise to form biofilm. Biofilm is formed due to the defence mechanism of microorganism, whenever there is a stress condition produced due to physical (washing action of saliva/blood flow) or chemical mechanism (decline in nutrient content/change in pH/oxygen radicles/disinfectant/antibiotics).[1][3]

Biofilm can be found on dental plaque (a slimy build-up of bacteria that forms on the surface of the teeth), on the pond scum, on household and industrial pipes, biomaterials (contact lenses, catheters, pacemakers, prosthetic joints), minerals and metal surfaces, underwater, underground, above the ground, on plants and animal tissues etc.[1].In health areas biofilm can cause serious and important problem in medical and non-medical areas . due to the growth of biofilm on industrial and drinking water causes change in colour, odour, because of the chemical compounds they release results in harmful effects like demolition in water quality inducing troughpout loss, decrease heat transport and exchange in membrane techniques. Microorganism responsible for this are E. coli.,K.pnuemonia , K.oxytoca , E.cloacae , E.aglomerans , Salmonella spp. Etc.

Due to hygienic problems bacteria grow as a biofilm in their normal habitat which contains food borne pathogens. The important factors for food borne diseases and infectionsare the growth of microorganism on solid surfaces. The most common microorganism in pharmaceutical and food industries is L.monocytogenes. the 2nd most common pathogen in Ready-To-Eat (RTE) foods was E.cloacae and in poultry farm is S.enteritidis that cause food poisoning in human world-wide.

The most common isolated from indwelling medical devices are E.faecalis, S.aureus, S.epidermis, S.viridans, E.coli,K.pneumoniae, P.mirabilis and P.aeruginosa. Among these microorganisms P.aeruginosa and S.epidermis are most commonly microorganism present on cardiovascular devices [4].

II. MATERIAL AND METHOD

A. Collection of Samples

Samples were collected from the respective places.

1) From plant extract:

S. No.	Samples	Scientific name	LocationofCollection
1.	Mulethi	Glycyrrhiza glabra	Local market, Lucknow
2.	Neem	Azadirachtaindica	Local market, Lucknow
3.	Bitter gourd and their seeds	Momordica charantia	Local market, Lucknow
4.	Gram	Cicer arietinum	Local market, Lucknow
5.	Moong dal	Vigna radiata	Local market, Lucknow
6.	Methi	T.foenum-graecum	Local market, Lucknow
7.	Soybeans	Glycine max	Local market, Lucknow
8.	Wheat	Triticum	Local market, Lucknow

Table 2.1.1: Samples taken for testing the antibiofilm activity against given pathogen.

2) Marketed Medicine:

S. No.	Samples	Location of collection
1.	Ibuprofen	Local market, Lucknow
2.	Aspirin	Local market, Lucknow
3.	Naproxen	Local market, Lucknow
4.	Paracetamol	Local market, Lucknow
5.	Amoxicillin	Local market, Lucknow
6.	Ciprofloxacin	Local market, Lucknow
7.	Azithromycin	Local market, Lucknow
8.	Aceclofenac	Local market, Lucknow
9.	Diclofenac	Local market, Lucknow

Table 2.1.2: Marketed medicine sample which is used for testing antibiofilm activity against given pathogen.

3) For bacterial extract:

Water sample was taken from the sewage present near the Ram Manohar Lohia Hospital, Lucknow, India.

P. aeruginosa and L. Rhamnosus these are the pathogens which is provided by MRD Lifesciences, Lucknow, India

a) Sample Preparation from plant extract:

2gm of dried samples were collected and individually dipped in 5ml of7 different solvent i.e. acetone, methanol, propanol, petroleum ether, benzene, chloroform, distilled water, for 48hrs.The filtrate part was collected after 48hrs and stored at 40ºC in hot air oven.The extract was dissolved in DMSO and collected in microcentrifuge tubes for Antibiotic sensitivity test.

b) Sample preparation from marketed medicine:

All the medicine samples were crushed and mixed with distilled water and prepared with various concentration i.e., 100mg/ml, 200mg/ml & 300mg/ml and collected for their various tests.

c) Antibiotic sensitivity test (AST):

In this technique we employed agar well diffusion method in which nutrient agar was prepared after that 20µl of bacterial culture was spread and wells are prepared after that 40µl of samples was loaded and incubated at 37°C for 24-48hrs. after that zone of inhibition was observed against the sample inoculated.

d) Minimum inhibitory concentration (MIC):

MIC is a technique in which we determine the lowest concentration of an antimicrobial or antibacterial agent which can have the potency to inhibit the growth of microorganism. As we know that multi drug resistance has emerged in recent years and it is very important to determine the resistance by MIC assay for copying inhibitory test.MIC was performed using NB media. Media was prepared and transferred 3ml each into 6 Test Tube and autoclaved it. Serial dilution of the sample was performed with 500µl.20µl of pathogen was inoculated to each Test Tube except the last Test tube which is used as blank and incubated at 37°C for 24 hrs.O.D was taken at 620nm at colorimetric.

e) Biofilm Preparation:

MRS media was prepared by using Peptone 10g/l, Beef extract 10g/l, Yeast extract 5g/l, Dextrose 20g/l, Tween 1ml/l, Na_2HPO_4 2g/l, Sodium Acetate 5g/l, Tri Ammonium Citrate 2g/l, $MgSO_4$ 0.2g/l, $MnSO_4$ 0.2g/l, having pH 6.2-6.6. and transferred 3ml each it into Test Tube and autoclaved it. 20µl of pathogens (P. Aeruginosa and L. rhamnosus) was inoculated under sterile condition and incubated at 37°C for 1 week.

f) Biofilm staining:

Biofilm formed was initially washed with phosphate buffer saline (PBS). After that crystal violate is added to the biofilm and kept for 1min. then it was washed with PBS, and kept for drying.

g) Treatment of biofilm:

1) With alcoholic extract: -

Extract was prepared by dipping 2g of dried sample and added to 5ml of solvents and added in equal volume and incubated at 37°C.

2) With various medicines

Antibiotics, antipyretics and analgesics used are ciprofloxacin, amoxicillin, azithromycin, antipyretics, ibuprofen, paracetamol, naproxen, aspirin, diclofenac and aceclofenac,

The medicinal extract was prepared after crushing the tablets and made a fine powder of it and mixed with 1ml distilled water. And added to equal volume to the biofilm and incubated at 37°C at shaker incubator.

h) Treatment of biofilm with Bacillus cereus:

The bacterial strain was isolated and identified using various biochemical tests i.e., gram staining, catalase test, endospore staining, mannitol test,using Bergey's Manual which was showing antimicrobial activity against and L.rhamnosus. Which was further inoculated in equal proportion to the biofilm and incubated at 37°C in shaker incubator for 12 hrs. Biofilm was prepared, stained and washed with PBS buffer. Samples were prepared in a Test tube.Poured it into the Test tube containing biofilm so that the biofilm gets dipped. Incubated it at 37°C for 24hrs and check the activity.

i) Phytochemical tests:

These are the tests which are performed to determine the photochemical compound present in the sample.

1) Test for flavonoids:

This is the test which is used to determine the presence of Flavonoid compounds.1ml of sample was taken in a microfuge tube Dil. NaOH of pH 10 is added drop wise to obtain yellow colour. After the colour obtained add HCl dropwise. If the yellow colour of the sample vanishes it means the sample is flavonoid positive.

2) Test for Phlobatannins:

This is the test which is used to determine the presence of Phlobatannins compounds. 1ml of extract was dissolved with 2ml of D.W. and filtered. It was boiled the filter along with 2% HCl. Red coloured precipitate is formed then it is Phlobatannins positive.

3) Test for tannins:

This is the test which is used to determine the presence of Tannin compounds. 1ml of sample was taken and added few drops of 1% lead acetate. Yellow precipitate is formed then the sample is Tannin positive.

III. RESULT

In this experiment we have performed antibiotic sensitivity test, minimum inhibitory test against microorganisms forming biofilm using various alcoholic extracts, medicinal extracts, bacterial stains and modified drug for the treatment of biofilm.

A. Prepared alcoholic extract:

Fig. 3.1: extract prepared by different solvents (A) Momordica charantia extracts (B) Momordica charantia seeds extracts (C) Glycyrrhiza glabra extracts (D) Azadirachtaindica extracts.

These are the extracts which is prepared by dipping 2gm of samples into seven different solvents i.e., Methanol, Petroleum Ether, Acetone, Propanol, Benzene, Chloroform, Distilled water after that the extract was collected in a bowl of weight 53.085gm and incubated at 40°-50° C and allowed the solvent to evaporate. Weight of the bowl after evaporation was 53.319gm. The extract prepared was of 234mg and dissolved in 1 ml DMSO and collected on a centrifuge tube.

B. Prepared biofilm:

these are the biofilm which are prepared after one-week incubation of P. aeruginosa and L. rhamnosusmicrobes in MRS media at 37°C.

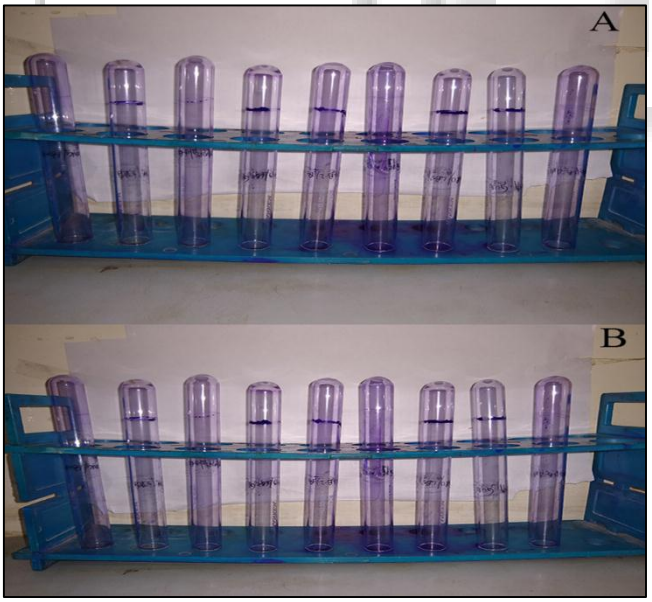


Fig. 3.2: Biofilm prepares and stained (A) P.aeruginosabiofilm, (B) L.rhamnosusbiofilm.

C. AST result of Alcoholic Extracts:

Different size of zone of inhibition was observed with different alcoholic extracts.

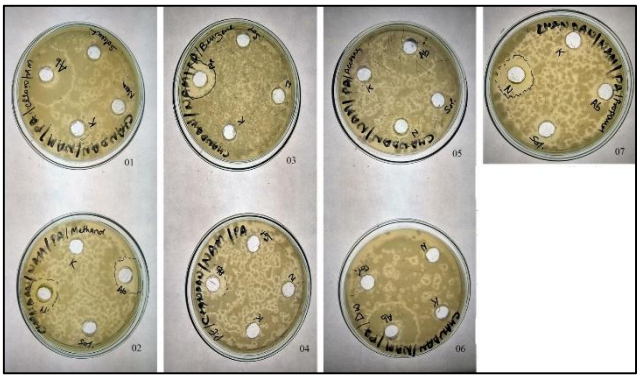


Fig. 3.3: AST performed in againstP.aeruginosawith different alcoholic extracts (1)M .charantia(K), A. indica(Neem) of chloroform, (2) M .charantia(K), A. indica(Neem) of methanol, (3) M .charantia(K), A. indica(Neem) of benzene ,(4) M .charantia(K), A. indica(Neem) of P.E.,(5) M .charantia(K), A. indica(Neem) of acetone, (6) M .charantia(K), A. indica(Neem) of D.W., (7) M .charantia(K), A. indica(Neem) of propan-2-ol.

Pathogen	Extract used	ZOI with following solvents						
		Benzene	Acetone	Methanol	P.E.	Propanol	Chloroform	D.W.
PA	M .charantia	0	0	0	0	0	0	0
	A. indica	0	0	16.5	0	19.5	0	0
	Antibiotic	14.5	0	18	16.5	0	0	0
	Solvent	0	0	0	0	0	0	0

Table 3.3.1: Zone of inhibition for the alcoholic extract of M .charantia, A. indica. Whereas first column is used for pathogen used, 2nd for the extract used and rest of the columns are used for the values of ZOI with different extracts.

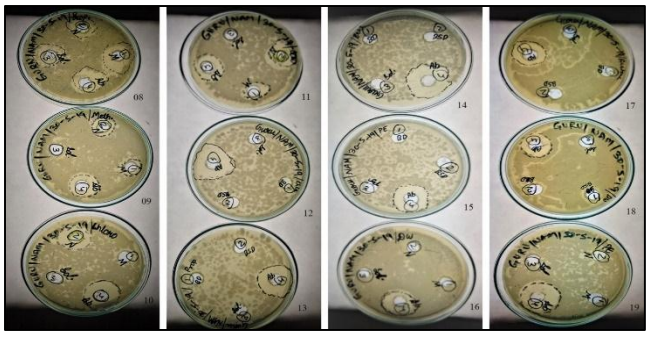


Fig. 3.2: (8) M & N of propan-2-ol, (9) M & N of methanol, (10) M & N of chloroform, (11) M & N of acetone, (12) BD & BSD of chloroform,(13) BD & BSD of propan-2-ol, (14) BD & BSD of benzene, (15) BD & BSD of P.E., (16) M & N of D.W., (17) BD & BSD of acetone, (18) BD & BSD of D.W., (19) M & N of P.E.,(M= Glycyrrhiza glabra, N= Azadirachta indica, BD= Momordica charantia charantia and BSD= Momordica charantia charantia seeds).

Pathogen	Extract used	ZOI with following solvents						
		Benzene	Acetone	Methanol	P.E.	Propanol	Chloroform	D.W.
PA	G. glabra	0	15.5	13	0	22.5	0	0
	A. indica	0	18	15.5	0	17.5	18.5	0
	Antibiotic	20.5	19	18	22	21.5	22	19
	Solvent	0	0	0	0	19.5	0	0
Pathogen	Extract used	ZOI with following solvents						
		Benzene	Acetone	Methanol	P.E.	Propanol	Chloroform	D.W.
PA	M. charantia	0	0	0	0	0	0	0
	M. charantia seed	0	0	0	0	0	0	0
	Antibiotic	24	18.5	21	19	22	25.5	17
	Solvent	0	0	0	0	0	0	0

Table 3.3.2: Zone of inhibition for the alcoholic extract of G. glabra, A. indica, M. charantia, M. charantia seeds. Whereas first column is used for pathogen used, 2nd for the extract used and rest of the columns are used for the values of ZOI with different extracts.

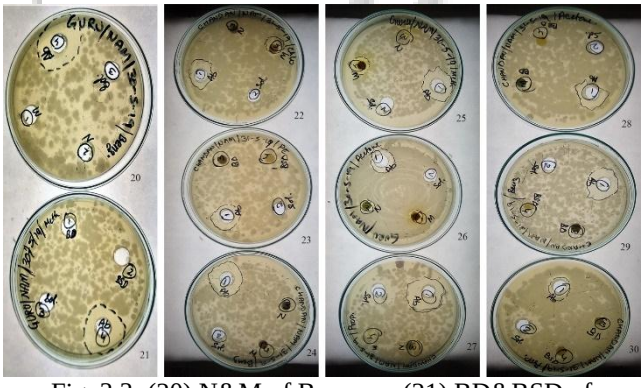


Fig. 3.3: (20) N&M of Benzene, (21) BD&BSD of Methanol, (22) N&M of Chloroform,(23) BD&BSD of P.E., (24) N&M of Benzene, (25) N&M of Methanol, (26) N&M of Acetone, (27) N&M of Propan-2-ol, (28) BD&BSD of Acetone, (29) BD&BSD of Benzene, (30) BD&BSD of Propan-2-ol. Test performed on plate no. 22 to 30 are from alcoholic extract discs.

Pathogen	Extract used	ZOI with following solvents					
		Benzene	Acetone	Methanol	P.E.	Propanol	Chloroform
PA	G. glabra discs	0	7.5	11	0	0	10.5
	A. indicadiscs	0	8	0	0	0	0
	Antibiotic	21	15	20	16	22.5	14
	Solvent	0	0	0	0	0	0
Pathogen	Extract used	ZOI with following solvents					
		Benzene	Acetone	Methanol	P.E.	Propanol	Chloroform
PA	M. charantiadiscs	0	0	16.5	0	0	11
	M. charantia seed discs	0	0	0	0	0	0
	Antibiotic	19.5	16	18	16.5	14	17.5
	Solvent	0	0	0	0	0	0

Table 3.3.3: Zone of inhibition for the alcoholic extract discs of G. glabra, A. indica, M. charantia, M. charantia seeds. Whereas first column is used for pathogen used, 2nd for the extract used and rest of the columns are used for the values of ZOI with different extracts.

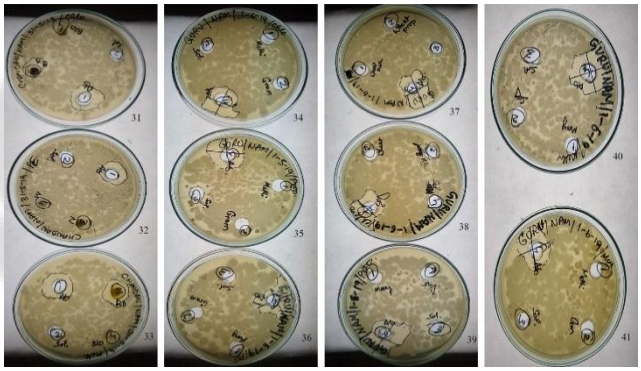


Fig. 3.3: (31) BD&BSD of chloroform, (32) N&M of P.E., (33) BD&BSD of methanol, (34) Methi& Gram of chloroform, (35) Methi& Gram of propan-2-ol, (36) Methi& Gram of P.E., (37) Wheat of methanol & propan-2-ol,(38) Wheat & Soy of chloroform, (39) Moong & Soy of propan-2-ol, (40) Moong & Soy of methanol, (41) Methi& Gram of methanol.

Pathogen	Extract used	ZOI with following solvents		
		Methanol	Propanol	Chloroform
PA	T. foenum-graecum	0	0	0
	C. arietinum	0	0	0
	Antibiotic	19.5	21	16.5
	Solvent	0	0	0
Pathogen	Extract used	ZOI with following solvents		
		Methanol	Propanol	

PA	Glycine max	0	0	
	Vigna radiata	0	0	
	Antibiotic	22	21.5	
	Solvent	0	0	
Pathogen	Extract used	ZOI with following solvents		
		Chloroform	Methanol	Propanol
PA	Glycine max	17	0	0
	Triticum	0	0	0
	Antibiotic	0	18.5	
	Solvent	0	0	0
Pathogen	Extract used	ZOI with following solvents		
		Chloroform		
PA	Vigna radiata	0		
	Cicer arietinum	0		
	Antibiotic	21.5		
	Solvent	0		

Table 3.3.4: Zone of inhibition for the alcoholic extract of C. arietinum, V. radiata, T. foenum-graecum, G. max, Triticum. Whereas first column is used for pathogen used, 2nd for the extract used and rest of the columns are used for the values of ZOI with different extracts.

D. AST result of bacterial extract:

Different size of zone of inhibition was observed with nine different bacterial strains isolated from sewage water sample.

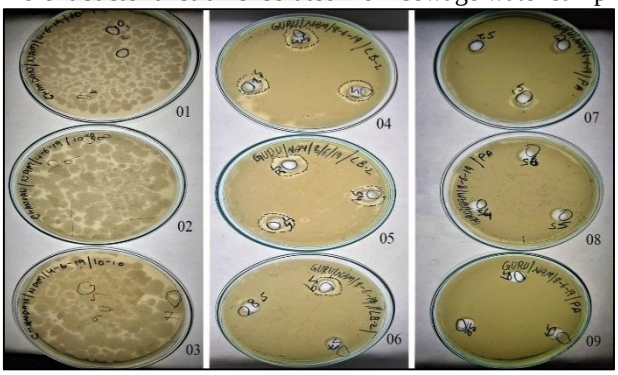


Fig. 3.4:Plate no.1,2,3 are the plates which contains the samples isolated from sewage water and the plates having no.4 to 9 are the plates having result of AST performed against given pathogens with bacterial strains obtained from sewage water sample.

Bacterial sample	ZOI on LB-2	ZOI on PA
S1	14.5	13.5
S2	14.5	0
S3	15	0
S4	14	0
S5	13.5	0
S6	13.5	0
S7	0	0
S8	0	0
S9	13.5	0

Table 3.4.1: Zone of inhibition for the samples extracted from the sewage water sample. Whereas 1st column is used for samples used, 2nd for the pathogens used for the test performed for antibiogram activity to form ZOI with different bacterial strains.

E. AST result of medicinal extract:

Different size of zone of inhibition was observed with different medicinal extracts.

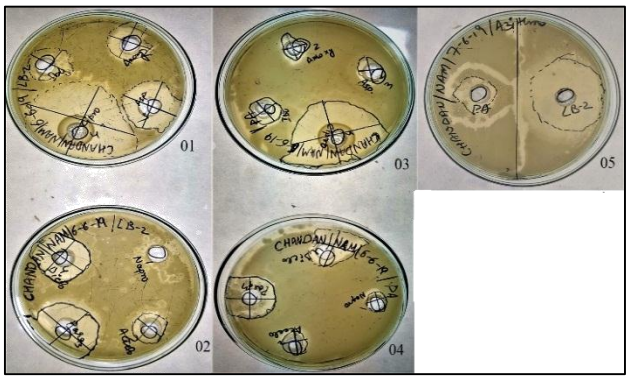


Fig. 3.5:AST result of medicinal extract(Ibuprofen, Aspirin, Naproxen, Paracetamol, Amoxicillin, Ciprofloxacin, Azithromycin, Aceclofenac, Aceclofenac) against provided pathogens. Plates having no.01 & 02 are the plates of L.rhamnosus and plates having no. 03 & 04 are the plates ofP.aeruginosaand the plate having no. o5 is of AzithromicinagainstP.aeruginosa&L.rhamnosus.

Medicine sample	ZOI on LB-2	ZOI on PA
Ibuprofen	18.5	10.5
Aspirin	23	11
Naproxen	0	8.5
Paracetamol	24.5	20
Amoxicillin	18	11
Ciprofloxacin	41.5	32
Azithromycin	31.5	24
Aceclofenac	13	10
Diclofenac	21	16.5

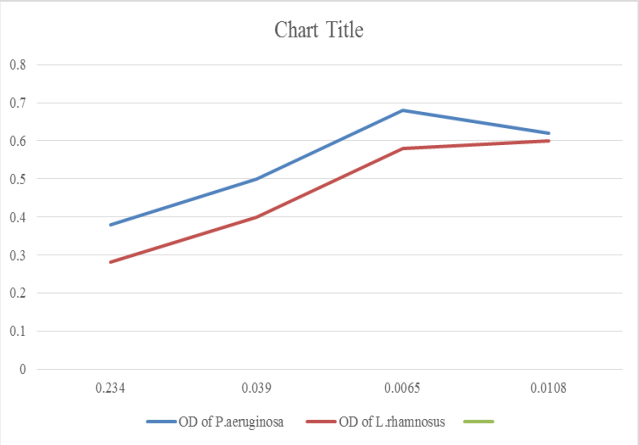
Table 3.5.1: Zone of inhibition for the marketed medicines. Whereas 1st column is used for samples used, 2nd for the pathogens used for the test performed for antibiogram activity to form ZOI with different bacterial strains.

F. MIC test result for alcoholic extract: -

MIC value of Mulethi extract against P.aeruginosa and L.rhamnosus

Concentration of extract	OD of P.aeruginosa	OD of L.rhamnosus
0.234	0.38	0.28
0.039	0.50	0.40
0.006	0.68	0.58
0.00108	0.62	0.60
0.00018	0.76	0.73

Table3.6.1: Minimum inhibitory concentration test performed against given pathogen P.aeruginosa and L.rhamnosus with Mulethi (G.glabra) extract.



Graph 3.6. 1: Chart for the MIC done of P. aeruginosa and L. rhamnosus.

G. AST result of modified medicine:

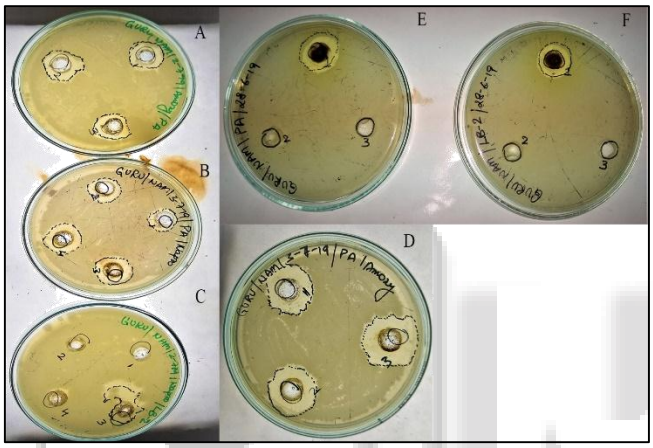


Fig. 3.6: AST result of medicines after the modification of Naproxen & Amoxycillin with G. glabra (Mulethi). Plates labeled E&F are the result of direct G.glabra (Mulethi) extract.

Modified medicine	ZOI on LB-2				ZOI on PA			
	20 mg	40 mg	60 mg	100 mg	20 mg	40 mg	60 mg	100 mg
Naproxen-G. glabra	0	0	18	0	12	13.5	15	16
Amoxycillin-G. glabra	11.5	14	12.5	N.A.	17	18.5	20.5	N.A.
G. glabra	14	0	0	N.A.	16	0	0	N.A.

Table 3.6.1: Zone of inhibition for the given pathogen P.aeruginosa and L.rhamnosus with the modified medicine of naproxen and amoxicillin with Mulethi (G.glabra) extract.Whereas first column is used for medicine used, restfor the pathogen P.aeruginosa and L.rhamnosususedfor the values of ZOI with different medicine.

H. Treatment of biofilm:

1) By mulethi (Glycyrrhiza glabra) extract:

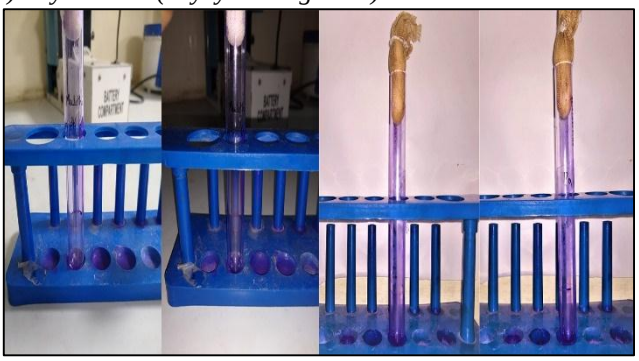


Fig. 3.8.a: Treatment of biofilm with the help of Mulethi (Glycyrrhiza glabra) extract. Whereas (A) was the biofilm ofP.aeruginosa, (B) was the biofilm ofL.rhamnosus, (C) is the treatment of P.aeruginosa biofilm and (D) is after treatment ofL.rhamnosusbiofilm.

2) By Bacillus cereus which was obtained by sewage water sample:

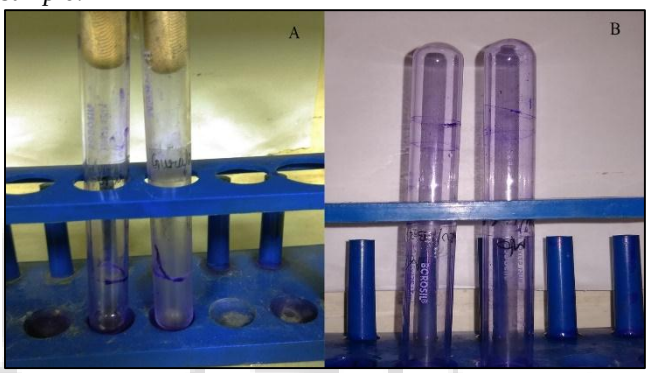


Fig. 3.8.b: Result of treatment of biofilm by the help of microorganism Bacillus cereus obtained from sewage water sample figA is without treatment and fig B is after the treatment.

I. By various medicines:

100mg of medicines are taken and poured it into the TT till the biofilm gets dipped and incubated at 37°C for overnight to check the activity of that medicines upon biofilm.

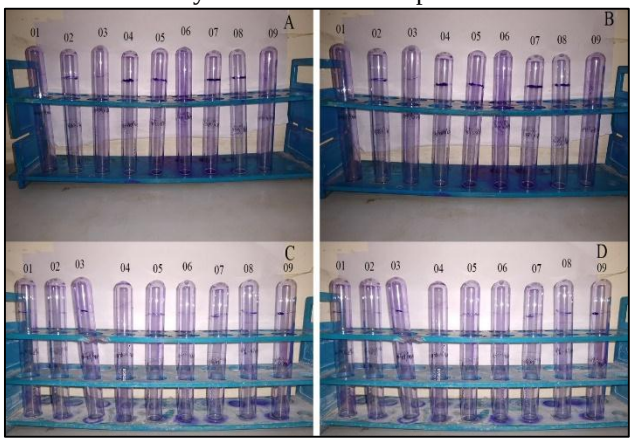


Fig. 3.9: Treatment of biofilm with medicinal extracts prepared by various medicines taken (A) and (C) are the biofilm ofL.rhamnosusand (B) and (D) are the biofilm ofP. aeruginosawhereas fig. having labelled (C)&(D) are the fig. after the treatment of biofilm (Medicine used in testubes (1)

Azithromycin, (2) Aspirin, (3) Naproxen, (4) Ciprofloxacin, (5) Diclofenac, (6) Aceclofenac, (7) Paracetamol, (8) Ibuprofen,(9) Amoxicillin.

J. By modified medicine:

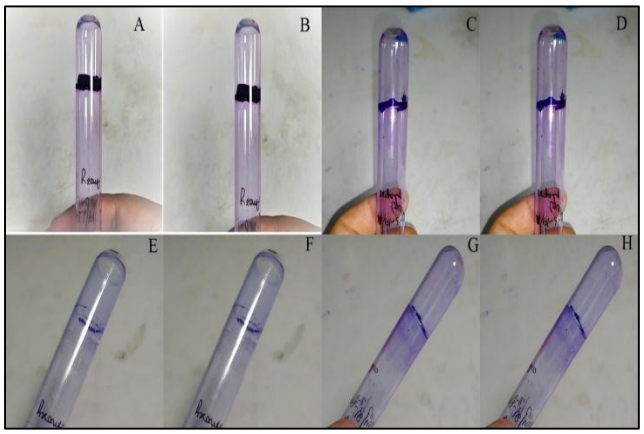


Fig. 3.10: Treatment of biofilm with the help of drug Amoxicillin & Naproxen with Mulethi (Glycyrrhiza glabra). (A) & (B) are the tubes treated with modified amoxicillin & (C) & (D) are the tubes treated with modified naproxen whereas E, F, G& H are treated respectively.

K. Phytochemical tests:

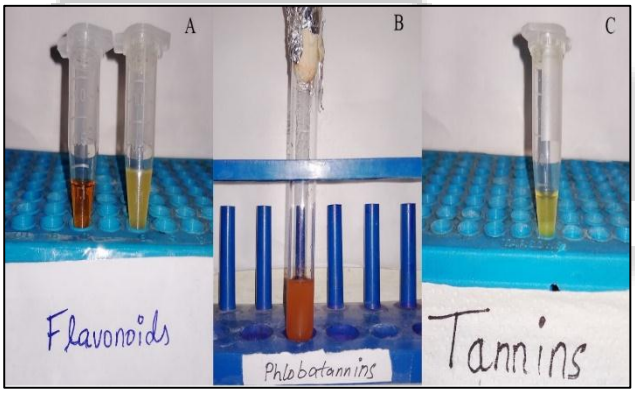


Fig. 3.11:Test performed with Mulethi (Glycyrrhiza glabra) extract to determine the phytochemical agent present on the sample. (A) for flavonoids, (B) for phlobatannins& (C) for Tannins.

Sr. no.	Phytochemical test	Result
01	Flavonoids	+ve
02	Phlobatannins	+ve
03	Tannins	-ve

Table 3.11.1: result of phytochemical tests performed for mulethi extract.

IV. Discussion

Perhaps microbial biofilm has serious impacts on human health and medical devices. Due to their complex structure they are becoming resistance to the antibiotics or antimicrobial compound and has become a major challenge for the researchers to degrade the biofilm. To overcome this problem various types of phytochemical extracts, marked medicines and bacterial strains were obtained and their microbial activity was tested by various tests like AST, MIC, etc., the AST was performed to check whether these samples obtained are able to show the antibiogram activity against the given pathogen. As a result, the propanolic extract of mulethi (G. glabra) gives the best result on AST among all of them. Then MIC was performed to check or to determine the minimum dose of the sample required to kill the pathogen. After gaining the best dose i.e., 234mg of the sample and that dose of the sample was used for the treatment the biofilm. Then, the treatment of biofilm was done with, various marketed medicine and obtained bacterial strain. From there, the obtained bacterial strain was also forming biofilm by combining with the pathogens and medicines amoxicillin and naproxen shows lesse rsults as P. aeruginosa has gained resistivity against these medicines. Then amoxicillin and naproxen were combined with mulethi (G. glabra) and formed 20mg medicine which is able to degrade the biofilm and shows positive result after the treatment of bacterial biofilm.

V. Conclusion

In this present study we checked the antibiofilm activity of the marketed drugs, phytochemical extracts and bacterial strains against one-week old biofilms of P. aeruginosa and L. rhamnosus. From there it has been concluded that the propanolic extract of mulethi (G. glabra) was able to degrade the biofilm and from marketed drugs naproxen and amoxicillin who have given few results on AST but they are not able to degrade the biofilm alone. But after the combination of the propanolic extract of mulethi (G. glabra) they start degrading the biofilm successfully. And these combinations can be used for the treatment of biofilm in future.

References

[1] Vidyanagar, A (2016) http://amp.livescience.com/57295-biofilms.html
[2] Hollmann, B., Perkins, M. and Walsh, D. Biofilms and their role in pathogenesis. BiteSized Immunology. Category: Pathogens and Diseases
[3] Jamal, M., Tasneem, U., Hussain, T. and Andleeb, S. (2015) Bacterial Biofilm: Its composition, formation and role in human infections. Research and Review: Journal of Microbiology and Biotechnology 5
[4] Tasneem, U., Yasin, N., Nisha, I., Shah, F., Rasheed, U., Momin, F., Zaman, S. and Qasim, M (2018) Biofilm producing bacteria: A serious threat to public health in developing countries. 1
[5] Hentzer, M., Riedel, K., Rasmussen, T. B., Heydorn, A., Andersen, J. B., Parsek, M. R., Rice, S. A., Eberl, L., Molin, S., Hoiby, N., Kjelleberg, S. and Givskov, M. (2002) Inhibition of quorum sensing in Pseudomonas aeruginosa biofilm bacteria by a halogenated furanone compound. Microbiology. 148, 87-102
[6] Kumari, R., Mishra, RC., Yadav, A. and Yadav, J. P. (2018) Screening of traditionally used medicinal plants for their antimicrobial efficacy against oral pathogens and GC-MS analysis of Acacia nilotika extract. IJTK. 18(1),162-168
[7] Huigens III, R. W., Richards, J. J., Parise, G., Ballard, T. E., Zeng, W., Deora, R. and Melander, C. (2007) Inhibition of Pseudomonas aeruginosa Biofilm

formation with Bromoageliferin Analogues. J. AM. CHEM. SOC. 129, 6966-6967

[8] Zarko, L. S., Dover, R. S., Brumfeld, V., Mangoni, M. L. and Shai, Y. (2015) Mechanism of Biofilm Inhibition and Degradation of Antimicrobial Peptides. Bio. Jour. Imm. Pub. 10.1042/BJ20141251

