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Antimicrobial Activity of Some Antibiotics and Emblica Officinalis (Amla) Leaf Extract

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Abstract: ***The present study reveals to investigate the antimicrobial activity of aqueous and methonolic extract of Amla (Emblica officinalis) was used to determine against bacteria E.Coli and other is B.subtilis which is obtain milk and other products by well diffusion method and some was done by the combination effect of extract of some antibiotics. The both combination of leaf extract and antibiotics show the massive result for the combination of 6% of amla extract and 6%of ofloxin concentration. so the study shows that the combination of plants extract and antibiotic at low concentration shows high efficiency against pathogens By this study we can establish the conclusion that this alternative way of treatment can serve an important platform for the development of inexpensive, safe and effective way of treatment.***

Keywords: Plant extract, ofloxin, antimicrobial activity

1. Introduction

For a long time plant have been a good source of natural product for maintaining human health As we consider that extract of Amla show large antimicrobial potential .Amla belongs to the family Euphorbiaceae anti viral, anti cancer, antiinflimatory treatment. In Indian traditional medicines, all part of plants including leaves, bark, seeds, flower used as various extract preparation. So researcher paying their attention folk medicines knowing for new lead to improve better drugs against microbial infections. The aim of study was to research in vitro the possible existence of synergy between methanolic ex tact of amla, water and commonly used antibiotics (ofloxacin, amoxicillin, teacycline etc.

2. Materials and Methods

2.1 Plant material collection

The plant materials collections generally obtained commercially dried ground leaves of Amla near about 50mg leaves were extracted with water and methanol. The extraction deduction process done by the one part of the dried powder part and five part of sterilized water boiled for 16 minutes. After boiling the water the extract filter from what man filter papper, and then undergo autoclaving process at 120 degree calicoes for 15 minutes and then stored for further used at 5 degree temperature. The methanolic extract was prepared by using 0.5gm product and 5ml methanol, both shocked in a round bottom flask for 24hrs at room temperature, extract filter and stand for further used.

2.2 Microorganisms

The microorganisms used for antimicrobial test were E.coli and B.Subtilis obtained from milk on selected media like Mac Conkeys Agar medium and trypticase Soy Agar medium. These medium confirmed by Gram staining and biochemical testing.

2.3 Preparation of inoculums

Colony method was used for preparation of bacterial suspension. Colonies were taken directly from the plates and suspended into 5ml to 6ml of sterile 0.65% to0.85% saline. When the suspension adjusted to the turbidity of Mac Farland standard the bacteria contain about 10 colony forming unit

2.4 Well diffusion and disc diffusion method used for antimicrobial activity

To determine the antimicrobial activity of Emblica officinalis by using cup diffusion method and also in the combination with antibiotics like gentamicine, ciprofloxacin, kanamyicin, ofloxacin, amoxicillin.

2.5 Minimum inhibitory concentration determination (MIC)

For determination of Mac both medium were taken about 1ml in 10 test tubes for each bacterium. Combined plant extract and antibiotics will be incorporated into the broth and the tubes will be then inoculated by using inoculums medium with0.1ml respective bacteria and kept at 35 to 37 degree celceous temperature. We determine that MIC will be lowest concentration of antibiotics or extract shows in complete inhibition of visible growth.

Result

The total no of 150 samples of milk. Out of which 90 to 93 were positive. Among of them which70 to 72 were positive for the *E. coli* and19 to 21 for *B. subtilis*. After the isolation and identification of above isolated organisms the antibiotic testing was done by using well diffusion method for three concentration 5%, 3% and 2% and were compared with the standards provided by the CLSI chart.

Table 1: Zone of inhibition of antibiotics by well diffusion method at different percentage on different isolated pathogens

No. Microorganisms Zone of inhibition by antibiotics Well Diffusion Method (Zone of inhibition in mm)				
S.No	Antibiotics Used	Concentrations used		
		5%	3%	2%
1. E. coli	Gentamycin	20mm	19mm	17mm
	Tetracycline	28mm	24mm	21mm
	Ciprofloxacin	30mm	25mm	21mm
	Kanamycin	25mm	18mm	12mm
	Ofloxacin	26mm	21mm	19mm
	Amoxcycillin	24mm	22mm	20mm
2. *B. subtilis*	Gentamycin	30mm	28mm	20mm
	Tetracycline	28mm	18mm	15mm
	Ciprofloxacin	33mm	30mm	29mm
	Kanamycin	34mm	29mm	24mm
	Ofloxacin	40mm	36mm	31mm
	Amoxcycillin	32mm	25mm	20mm

The result for the antibiotic susceptibility predicts that the isolated *E. coli* and *B. subtilis* strain are sensitive to the selected antibiotics and show highest zone of inhibition at 5% concentration for each. In which the *E. coli* was found to be most sensitive against Ciprofloxacin (30mm) and *B. subtilis* was most sensitive against the ofloxacin with 40mm zone of inhibition (Table 1). Antibacterial activity of Amla plant extract was evaluated by well diffusion method for both aqueous and methanol plant extracts for 5%, 3%, and 2% concentration.

A various degree of response was observed with respect to different solvent of the amla. The isolated microorganisms were found to be sensitive and intermediate for the aqueous and methanol extract of amla. The High zone of inhibition was observed for the 5% aqueous extract. Among which it was observed that the aqueous extract of amla gives the highest zone of inhibition for the *B. subtilis* (38mm) followed by *E.coli* (25mm) respectively (Table 2). Similarly the antibacterial activity was observed for the combination of 5% aqueous extract with the 5% concentration of different antibiotics by using well diffusion method. The synergistic effect was seen among the extract and antibiotics as the combination prepared gives the high zone of inhibition against the isolated organisms when compared with zones observed with the extract and antibiotic alone

Table 2: Effect of Amla extract on isolated pathogens by well diffusion

Microorganisms Plant Extract (AMLA) Well Diffusion Method (Zone of inhibition in mm)							
S. No	Microorganisms	aqueous plant extract			methanolic plant extract		
		5%	3%	2%	5%	3%	2%
1	E.coli	25mm	20mm	18mm	15mm	11mm	10mm
2	B.subtilis	38mm	30mm	25mm	23mm	20mm	15mm

The aqueous combination of amla and ofloxacin were found to be most effective against the *B. subtilis* with their highest zone of inhibition of 38mm whereas similar result was observed for the methanol extract and ofloxacin having the highest zone of inhibition against the *B. subtilis* (38mm) respectively (Table 3). The above result shows that the combination of amla extract and ofloxacin at 5% concentration is most effective against the *B. subtilis.*

Table 3: Synergistic effect of methanol Amla Extract at 5% and Antibiotics

S.No	Microorganism	Amla Extracts at 5% + Different Antibiotics at 5% concentration					
		Well Diffusion Method					
1	E.coli	Gent	Tetr	Kan	Cipr	Offlo	Ammo
		20	12	10	10	9	8
2	B. subtilis	30	26	20	24	38	32

Value for MIC the combination of 5% antibiotic and 5% amla extract for the *E. coli* and *B. subtilis* lies between the rang of 1.220 to 0.135 mg/ml. This signifies that the lowest MIC range for the combination of amla extract and ofloxacin at 5% concentration was observed against the *B. subtilis* (0.135 mg/ml). So the present study shows the synergistic effect of amla extract and ofloxacin against the *B. subtilis.*

Table 4: MIC for 5% Antibiotic used with combination of 5% Amla extract

S.No	Organisms	5% Antibiotic used with combination of 5% Amla extract					
1 2	E. coli	Gen	Tet	Kana	Cipro	Oflox	Amox
		1.210	1.185	1.0171	0.122	0.151	1.115
	B. subtilis	1.114	1.156	1.035	1.021	0.136	1.102

3. Conclusion

The present study has revealed the importance of natural products to control antibiotic resistant bacteria and this alternative way of treatment can serve as an important platform for the development of inexpensive, safe and effective medicines.

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