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Ankita Gautam
Warner College of Dairy Technology SHUATS Allahabad, Uttar Pradesh, India

Sangeeta Shukla
Warner College of Dairy Technology SHUATS Allahabad, Uttar Pradesh, India

Correspondence
Ankita Gautam
Warner College of Dairy Technology SHUATS Allahabad, Uttar Pradesh, India

Emblica officinalis (Amla) leaf extract potentiates antibacterial activity of some antibiotics

Ankita Gautam and Sangeeta Shukla

Abstract
The present study was undertaken to investigate the antibacterial activity of the plant extract potentiating the same for some antibiotics in combination. The antibacterial activity of aqueous and methanolic extract of Amla (Emblica officinalis) was determined against E.coli and B.subtilis isolated from milk and different dairy products by well diffusion methods and disc diffusion methods and the same was done for the combined effect of extracts and some antibiotics. The combination of extracts and antibiotics gives the effective result for the combination of 5% amla extract and ofloxacin (5%) concentration.
Our study therefore shows the combination of plant extract and antibiotics at low concentration are also effective 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: Amla (Emblica officinalis), plant extract, antibacterial activity, treatment, ofloxacin

1. Introduction
Plant produces a wide array of bioactive molecules or photochemical which probably evolved as chemical defense against predation or infection. Emblica officinalis is a deciduous tree, commonly known as Indian gooseberry or Amla (Kanthimathi and Soranam 2013) [10]. Amla belongs to the family Euphorbiaceae possesses antiviral, antibacterial, anticancer, (Jadon and Dixit 2014) [9]. Embilica officinalis enjoys a hallowed position in Ayurveda, an indigenous system of medicine in India. The major principle in Embilica officinalis active against microbes includes flavanoids, ascorbic acid, Gallic acid, alkaloids and hydrolysable tannins (Varghese *et al* 2013) [15]. In traditional Indian medicines, all parts of plant including the fruits, seed, leaves, roots, bark and flowers are used in various preparations. The use of amla in cold, anemia, dysentery, fever, gravel, sores. Leaves have been used for anti-inflammatory and antipyretic treatments. Today the pathogenic bacteria have developed resistance against existing antibiotics due to indiscriminate use of antimicrobial drugs to treat the infectious diseases. So some of the active compounds inhibit growth of the disease causing microbes either singly or in combinations (Maji *et al* 2010) [11]. For a long period of time plants have been a valuable source of natural products for maintaing the human health, especially in last decades with more intensive studies for natural therapies. There is a continuous and urgent need to discover the new antimicrobials compounds with the diverse chemical structure and novel mechanisms of action for new and re emerging infectious diseases. So researchers are increasingly turning their attention to folk medicines, looking for new leads to develop better drugs against microbial infections. Considering that extracts of amla (Emblica officinalis) show broad spectrum antimicrobial activity the aim of the study was to research in vitro the possible existence of synergy between water and methanolic extract of Embilica officinalis and commonly used antibiotics (Gentamicine, tetracycline, ciprofloxacin, kanamycin, Ofloxacin and amoxicillin).

2. Materials and Methods
2.1 Collection of plant materials
Embilica officinalis leaves were obtained commercially dried; ground leaves (50 gm for each extract) were extracted with water and methanolic. For aqueous extracted decoction process is done by boiling the one part of dried powder plant and 5 part of sterilized water will be taken in boiling water flask and is boiled for 15 min after boiling, the extract is filtered through What man filter paper no.1, autoclaved at 121 °C for 15 min and stored at 4°C for further use (Jhonson *et al* 2011). Similarly methanol extract was prepared by taking 0.5 gm of dried powder shocked in 5 ml methanol in round bottom flask at room temperature for 24 hrs on rotar shaker. The extract is then filtered with what man filter paper no. 1 and stored for further use.

2.2 Microorganisms
The bacteria used for the antibacterial test were *E. coli* and *B. subtilis* isolated from raw milk and various dairy products on selected media (Mac Conkeys Agar and Trypticase Soy Agar) which were confirmed by Gram Staining and Biochemical testings.

2.3 Inoculum preparation
Bacterial suspensions were prepared from overnight cultures by the direct colony method. Colonies were taken directly from the plates and suspended into 5 ml of sterile, 0.85% saline. The turbidity of initial suspension was adjusted comparing with 0.5 Mac Farland standards. When adjusted to the turbidity of a Mac Farland standard as suspension of bacteria contains about 10^8 colony forming units CFU/ml.

2.4 Antibacterial activity assay using well diffusion method and disc diffusion method.
Antibacterial activity of Emblica officinalis is determined by the cup diffusion method and disc diffusion method as mentioned by (Adwan and Mhanna 2008) [1] and (Ahmad *et al* 2010) [2] alone and in combination with antibiotics *i.e* (Gentamicine, tetracycline, ciprofloxacin, kanamycin, ofloxacin and amoxicillin).

2.5 Determination of MIC (Minimum inhibitory concentration)
For determination of MIC, 1ml of broth medium will be taken into 10 test tubes for each bacterium. Combined antibiotic and plant extracts will be incorporated into the broth and the tubes will be then inoculated with 0.1ml of inoculums of respective bacteria (10^5 CFU ml^{-1}) and kept at 37 °C for 24 hrs. MICs will be the lowest concentrations of the antibiotic or extract resulting in complete inhibition of visible growth of the test organism (Vijayasanthi *et al* 2012) [16].

3. Results
A total of 150 samples of milk and different dairy products were studied. Out of which 93 were positive. Among which 72 were positive for the *E. coli* and 21 for *B. subtilis*. After the isolation and identification of above isolated organisms the antibiotic susceptibility 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

S. No.	Microorganisms	Zone of inhibition by antibiotics			
		Well Diffusion Method (Zone of inhibition in mm)			
		Antibiotics used	Concentrations used		
			5%	3%	2%
1.	*E. coli*	Gentamycin	23mm	21mm	18mm
		Tetracycline	30mm	25mm	21mm
		Ciprofloxacin	31mm	25mm	21mm
		Kanamycin	25mm	18mm	16mm
		Ofloxacin	28mm	26mm	22mm
		Amoxcycillin	24mm	22mm	20mm
2.	*B. subtilis*	Gentamycin	34mm	30mm	26mm
		Tetracycline	31mm	28mm	25mm
		Ciprofloxacin	33mm	31mm	29mm
		Kanamycin	34mm	30mm	28mm
		Ofloxacin	40mm	38mm	32mm
		Amoxcycillin	32mm	28mm	25mm

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 (31mm) 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. The zone of inhibition was observed and compared with the standard provided by Indu *et al.,* (2006) [7].

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

S. No.	Micro-organisms	Plant Extract (AMLA)					
		Well Diffusion Method (Zone of inhibition in mm)					
		Aqueous plant Extract			Methanol Plant Extract		
		5%	3%	2%	5%	3%	2%
1.	*E. coli*	24mm	22mm	20mm	15mm	13mm	10mm
2.	*B. subtilis*	34mm	30mm	26mm	23mm	22mm	19mm

A varying 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. Highest 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* (34mm) followed by *E. coli* (24mm) 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.

The combination of aqueous amla and ofloxacin were found to be most effective against the *B. subtilis* with 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 4).

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

S. No.	Micro-organisms	Amla Extracts at 5% + Different Antibiotics at 5% concentration					
		Well Diffusion Method					
		Gent	Tetr	Kan	Cipr	Offlo	Ammo
1.	*E. coli*	15	12	10	9	18	8
2.	*B. subtilis*	26	20	24	31	38	32

Table 4: Synergistic effect of methanol Amla Extract at 5% and Antibiotics

S. No.	Micro-organisms	Amla Extracts at 5% + Different Antibiotics at 5% concentration					
		Well Diffusion Method					
		Gent	Tetra	Kana	Cipr	Offlo	Ammo
1.	*E. coli*	8	15	15	12	16	9.5
2.	*B. subtilis*	26	22	22	33	38	30

MIC value for the combination of 5% antibiotic and 5% amla extract for the *E. coli* and *B. subtilis* lies between the range of 1.224 to 0.138 mg/ml. This signifies that the lowest MIC range for the combination of amla extract and ofloxacin at 5% concentaration was observed against the *B. subtilis* (0.138 mg/ml). So the present study shows the synergistic effect of amla extract and ofloxacin agaist the *B. subtilis* (Table 5).

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

S. No.	Organisms	5% Antibiotic used with combination of 5% Amla extract					
		Gen	Tet	Kana	Cipro	Oflox	Amox
1.	*E. coli*	1.210	1.186	1.018	1.232	0.161	1.114
2.	*B. subtilis*	1.224	1.158	1.038	1.220	0.138	1.112

4. Discussion

The present study describes the effectiveness of Amla plant extracts against, different locally isolated pathogenic organisms *E. coli* and *B. subtilis*. Similarly also done the comparative study of antimicrobial and antioxidant activity of amla, tulsi and neem plant extract. Also determined the antimicrobial activity of crude powder, aqueous as well metanolic extract of fruit and leaf of Emblica officinalis. In the favour of present study Saeed and Tariq 2007, [14] Mehrotra *et al* 2010, [12] Jadon and Dixit 2014 [9] and Varghese *et al* 2013 [15] also worked for the evaluation of in vitro antibacterial activity of Emblica officinalis against *E. coli* and *Bacillus* sp. encluding other pathogens also. Kanthimathi and Soranam 2013 [10] also showed the antibacterial effects of Emblica officinalis against bacterial pathogens. Bole *et al* 2010 [3] also demonstrated the antibacterial activities of amla plant extract in their study. The synergistic effect was also been studied in the present study which is in favour with the previous study of Hijleh *et al* 2009 [5]. Similarly Rakholiya and Chanda 2012 [4] also worked in in vitro interaction of certain antimicrobial agent in combination with some plant extracts against some pathogenic bacteria strain. Of also evaluated the combined antimicrobial activity of leaf extract of Phyllantus mellerianus with ciprofloxacin which supports the synergistic effect studied in the present study.

5. Conclusion

The present study has revealed the importace 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.

6. References

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