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A STUDY OF SYNERGISTIC EFFECT OF PLANT EXTRACT WITH AMPICILLIN AND GENTAMYCIN ON PATHOGENIC BACTERIA

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ABSTRACT

The emergence of multiple drug resistant pathogenic bacteria are of great concern to the human health.Plants and plant extracts has been proved to be very beneficial for human health. Plant extract can be used as antimicrobial, food preservative agents and in controlling food poisoning diseases. By the long term use of similar type of antimicrobial agents, pathogenic bacteria develop some resistant property and show multiple drug resistant activity against antimicrobial agents. As time changes antimicrobial agents also need to modify with some new compositions. Present study is aimed to developed effective antimicrobial agent by combining it with aqueous and methanolic plant extract. *Lantana camara* was used to study their antimicrobial effect on pathogenic bacteria viz., *E. coli*, *Staphylococcus aureus* and, *Pseudomonas aruginosa.* The synergistic effect of *Lantana camara* plant parts extract with ampicillin and gentamycin were studied on pathogenic bacteria. The antimicrobial property were determined using agar disc diffusion technique.

Keyword: plant extract, antimicrobial activity, pathogenic bacteria

INTRODUCTION

It is proven that naturally occurring substances such as plant extracts show good antimicrobial activity.Increasing the number of multi drug resistant pathogenic bacteria is the great concern in this era to combat against diseases. The problems faced in curing of microbial infections in present scenario tends to discover novel drugs. Plant extract and pure plant origin compound in combination with conventional antibiotics may prove promising in providing an effective antibiotic for treatment of microbial infections in human. Nowadays approaches such as developing new class of antibiotics, implementing different drug delivery systems and use of natural antibiotic compounds are a way to overcome from multi drug resistant pathogens (Abreuet al., 2012; Fazly Bazzazet al., 2018).

Ampicillin and Gentamicin are two conventional antibiotic used for treatment of microbial infections in human since a very long time. Ampicillin is an antibiotic of class of aminopenicillins having a beta-lactumring. Ampicillin is used to treat number of bacterial infections such as respiratory tract infections, urinary tract infections, meningitis, salmonellosis, and endocarditis.Ampicillin is able to penetrate Gram-positive and some Gram-negative bacteria. Ampicillin acts as an irreversible inhibitor of the enzyme transpeptidase, which is needed by bacteria to make the cell wall (Petri 2011). Gentamycin is an antibiotic of class of aminoglycoside antibiotics that inhibits protein synthesis in Gram negative bacteria. Gentamicin is being used to treat several type of infections including bone infections, endocarditis, pelvic inflammatory disease, meningitis, pneumonia, urinary tract infections (Asaduzzamanet al., 2019).Nowadays many pathogenic bacteria shows resistant against these antibiotics. This study is subjected to use plant extract in combination with ampicillin and gentamicin to see their antimicrobial impact on multidrug resistant bacteria.

MATERIALS AND METHODS

Three species of pathogenic bacteria *E. coli, Staphylococcus aureus* and *Pseudomonas aruginosa* were selected for the study and their stock cultures were obtained from MTCC Unit of IMTECH, Chandigarh in the form of a slant. For plant extract, leaf and bark of *Lantana camara* was procured from Bithoor area in Kanpur and tannery effluent polluted area of Banther in Unnao, Jajmau in Kanpur. Plants was identified at Botany Dept., D.B.S. College Kanpur.

Aqueous Extraction of Leaves and bark of plant grown on unpolluted and tannery effluent polluted area

Fresh leaves and bark of *Lantana camara* was collected from the region of normal unpolluted areas of Bithoor and tannery effluent polluted areas of Banther in Unnao and Jajmau in Kanpur. Leaves and bark were washed properly with running water to avoid dust and other impurities. Leaves and bark of *Lantana camara* plant under sequential process were dried and pulverized into a coarse powder separately. Grinded powders of each sample were taken in 10 gm amount in separate flask and mixed with 100 ml of distilled waterand left for 48 hours. After 2 days, macerated leaves and bark were filtered by Whatman no 1 filter paper and then filtrate was evaporated at 40°C.

Methanolic Extraction of Leaves and bark of plant grown on unpolluted and tannery effluent polluted land

Fresh leaves and bark of *Lantana camara* was collected from the region of normal unpolluted areas of Bithoor and tannery effluent polluted areas of Banther in Unnao and Jajmau in Kanpur. Leaves and bark were washed properly with water to avoid dust and other impurities. Separately leaves and bark of *Lantana camara* plant under sequential process were dried, pulverized into a coarse powder. 10 gm of each finely grinded sample were taken in separate flask and mixed with 100 ml methanol and left for 48 hours. After 2 days, macerated leaves and bark were filtered by Whatman no 1 filter paper and then filtrate was evaporated at room temperature. This was treated as methanolic extract of leaves and bark of plant.

Inoculums preparation

Each bacterial strain was subcultured overnight at 35°C in Mueller-Hilton agar slants. The bacterial growth was harvested using 5 ml of sterile saline water, its absorbance was adjusted at 580 nm and diluted to attain viable cell count of 10^6 CFU/ml using spectrophotometer.

Screening for the antibacterial potential of the plant extracts and phytochemicals

Brain Heart Infusion liquid medium was prepared for the growth of bacterial culture. After 6 hours of growthat 37°C, microorganism with the concentration of 10^6cells/mL, was inoculated on the surface of agar plates. Filter paper discs (6 mm in diameter) loaded with either extracts or phytochemicals (50 µL) were subsequently mounted on the surface of each inoculated plate. Every extract (50 µL) was injected simultaneously into a well made of new plates to determine the efficiency of the technique. The plates were incubated at 37°C for 24 h to observe the zone of inhibition. Overall, cultured bacteria with halos equal to or greater than 7 mm wereconsidered susceptible to either the tested extract orphytochemical. DMSO and Tween 80 to 2% were used todissolve the extracts in the culture media when necessary. Thecontrols were the solvents used for each extract and thephytochemicals and they showed no inhibitions in preliminary studies. To evaluate the minimum inhibitory concentration (MIC) for each bacterial sample, the extracts and phytochemicals that indicated antibacterial activity were further examined. All the three bacterial samples [*E. coli, Staphylococcus aureus and Pseudomonas aruginosa*] were grown in nutrient broth for 6 hour. After,100 µL of 10^6 cells/mL was inoculated in tubes with nutrientbroth supplemented with different concentrations (10 – 500µL) of the extracts and phytochemicals, respectively. Afterwards 24 hours at 37°C, the MIC of each sample was determined (Bauer 1966 ; Bayer et al., 1966), (Nascimento et al.,2000).

Evaluation of the synergistic effect of antibiotics and plant extracts or phytochemicals on resistant bacterial samples

This evaluation was done according to (Muroi and Kubo 1996). Aliquots of 100 µL of resistant bacterial cultures (10^6 cells/mL) grown in 10 mL of nutrient broth for 6 hours were inoculated in nutrient broth supplemented with the respective antibiotics (50 µg/mL) with different concentrations of plant extracts. The concentration for plant extracts/phytochemicals ranged from 10-500 µg/mL based on MIC values that had

previously been evaluated. Antibiotics (Ampicilin, Gentamicin) were used at the sub-inhibitory concentration (50µg/mL). The growth conditions were the same as previously mentioned. After 48 hours, the optical density of each sample was documented and compared to those of MIC to verify any synergistic effect among the tested compounds.

RESULTS AND DISCUSSION

Yield of Extract (Leaves and Bark)

Leaves and bark of *Lantana camara* was crushed after thorough washing and drying and their yield has been listed in table 1.

Table 1: Yield of Leaves and bark of *Lantana camara* grown on normal land and tannery effluent polluted land

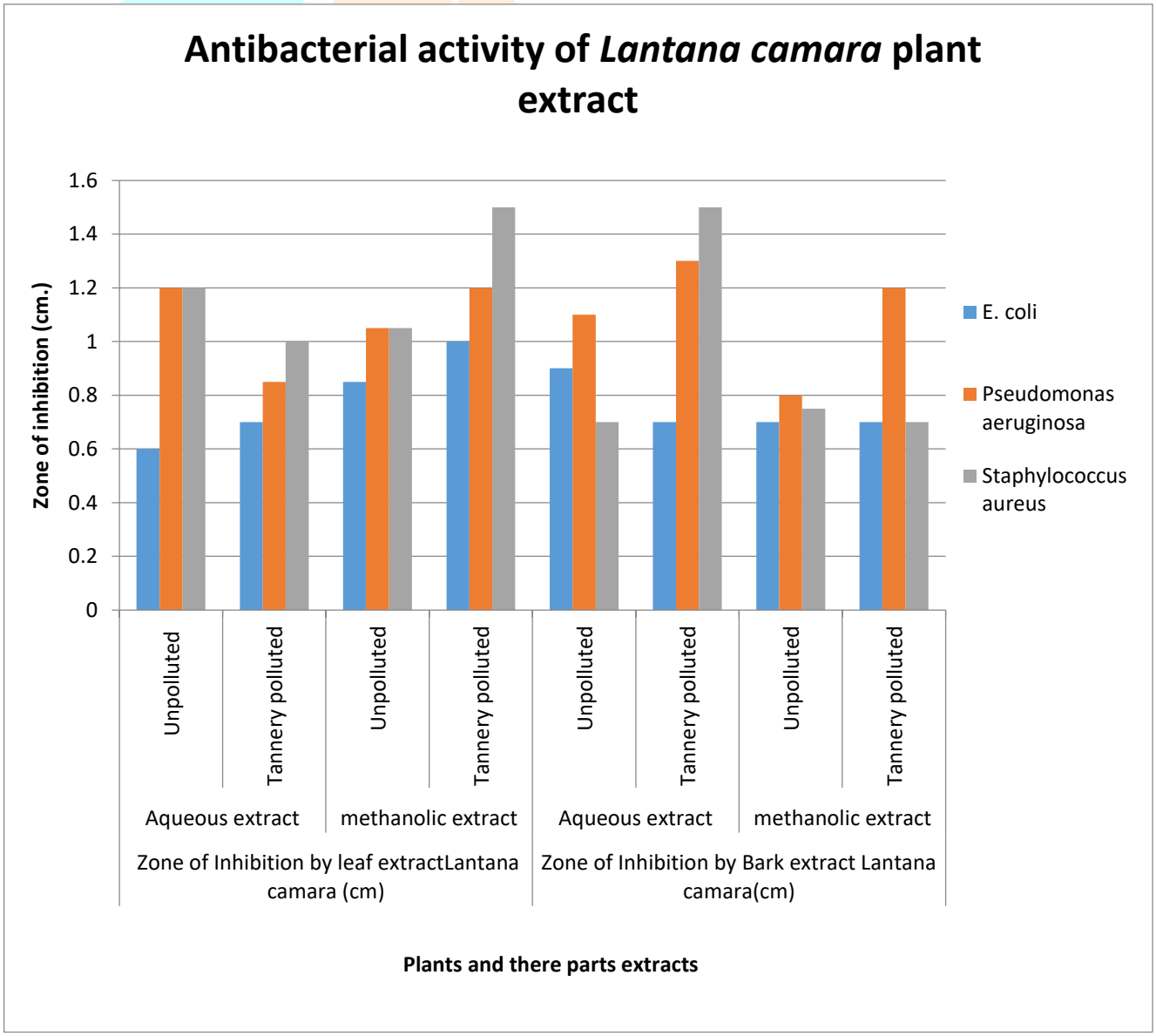
Plant	Wt. of Fresh leaves (gm.)	Wt. of Dried leaves (gm.)	Wt. of Fresh bark (gm.)	Wt. of Dried bark (gm.)
Lantana camara grown on normal land	20.10	7.16	22.97	4.67
Lantana camara grown on tannery effluent polluted land	31.06	10.10	17.82	5.69

Antibacterial Activity of Leaves and Bark Extract of Selected Plants:

Two types of extract were prepared to get an idea of antibacterial activity of each plant i.e. aqueous and methanolic extraction. Both the extract were prepared and applied in disc diffusion method to get inhibition zone. Antibacterial activity of *Lantana camara* plants grown on normal as well as tannery effluent affected area/soil for all the bacterial species was carried out by evaluating zone of inhibition (in centimetre) by applying 50 µl extraction on whatman paper disc. The antimicrobial activity of aqueous and methanolic extract of leaves and bark of selected plants are listed in table 2 and graph 1.

Table 2: Antibacterial activity of *Lantana camara* plant extract

Microorganism	Zone of Inhibition by leaf extract *Lantana camara* (cm)				Zone of Inhibition by Bark extract *Lantana camara*(cm)			
	Aqueous extract		methanolic extract		Aqueous extract		methanolic extract	
	Unpolluted	Tannery polluted	Unpolluted	Tannery polluted	Unpolluted	Tannery polluted	Unpolluted	Tannery polluted
E. coli	0.6	0.7	0.85	1.0	0.9	0.7	0.7	0.7
Pseudomonas aeruginosa	1.2	0.85	1.05	1.2	1.1	1.3	0.8	1.2
Staphylococcus aureus	1.2	1.0	1.05	1.5	0.7	1.5	0.75	0.7



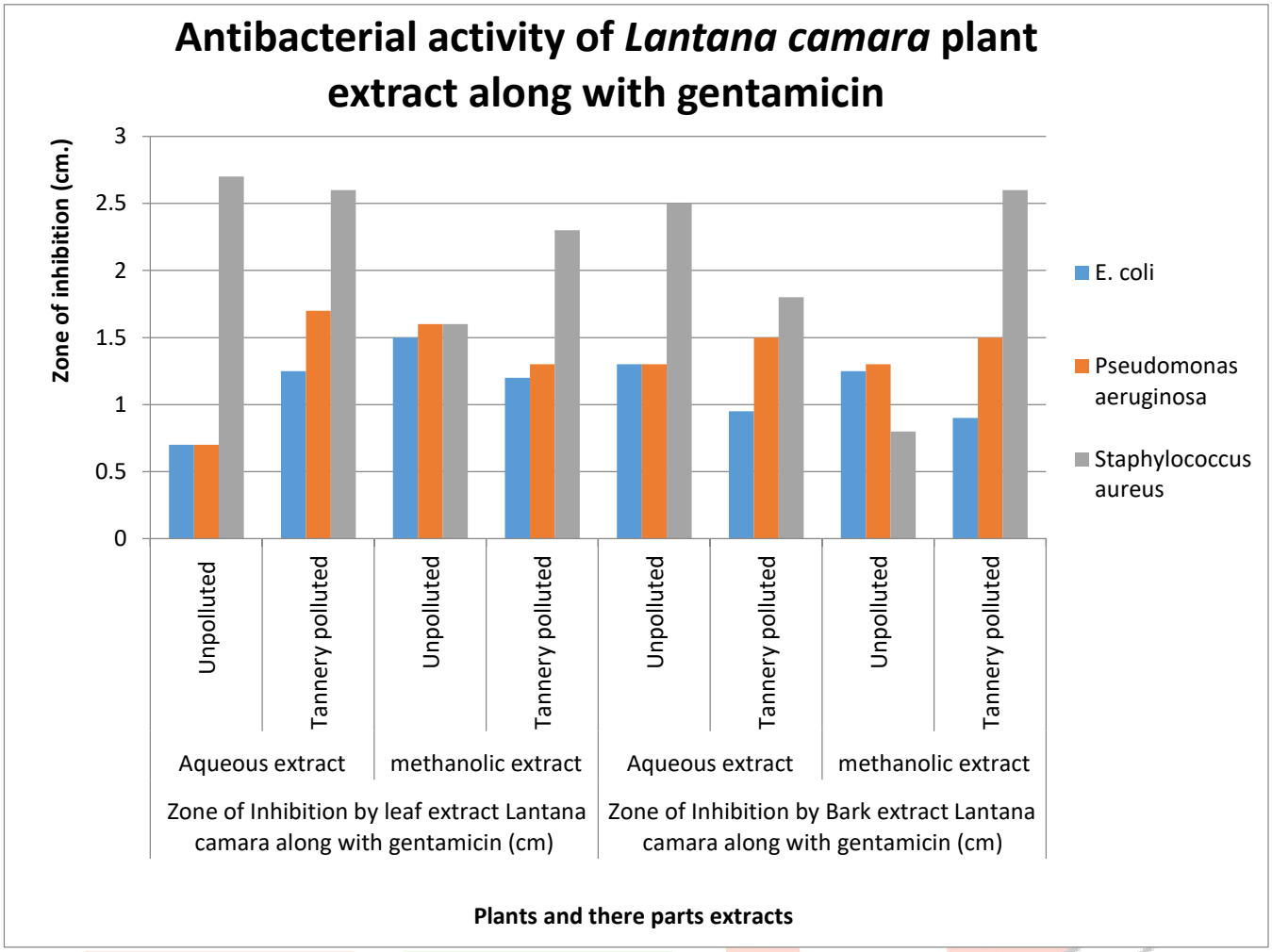
Graph 1: Antibacterial activity of *Lantana camara* plant extract

Synergistic effect of plant extract along with gentamicin

To study the synergistic effect of plant extract along with gentamicin all the plant extract were used in combination with gentamicin (50µg/ml) to evaluate the antimicrobial property. The results of synergistic effect of plant extract along with gentamicin are mentioned in table 3 and graph 2.

Table 3: Antibacterial activity of *Lantana camara* plant extract along with gentamicin

Microorganism	**Zone of Inhibition by leaf extract *Lantana camara* along with gentamicin (cm)**				**Zone of Inhibition by Bark extract *Lantana camara* along with gentamicin (cm)**			
	Aqueous extract		**methanolic extract**		**Aqueous extract**		**methanolic extract**	
	Unpolluted	Tannery polluted	Unpolluted	Tannery polluted	Unpolluted	Tannery polluted	Unpolluted	Tannery polluted
E. coli	0.7	1.25	1.5	1.2	1.3	0.95	1.25	0.9
Pseudomonas aeruginosa	0.7	1.7	1.6	1.3	1.3	1.5	1.3	1.5
Staphylococcus aureus	2.7	2.6	1.6	2.3	2.5	1.8	0.8	2.6



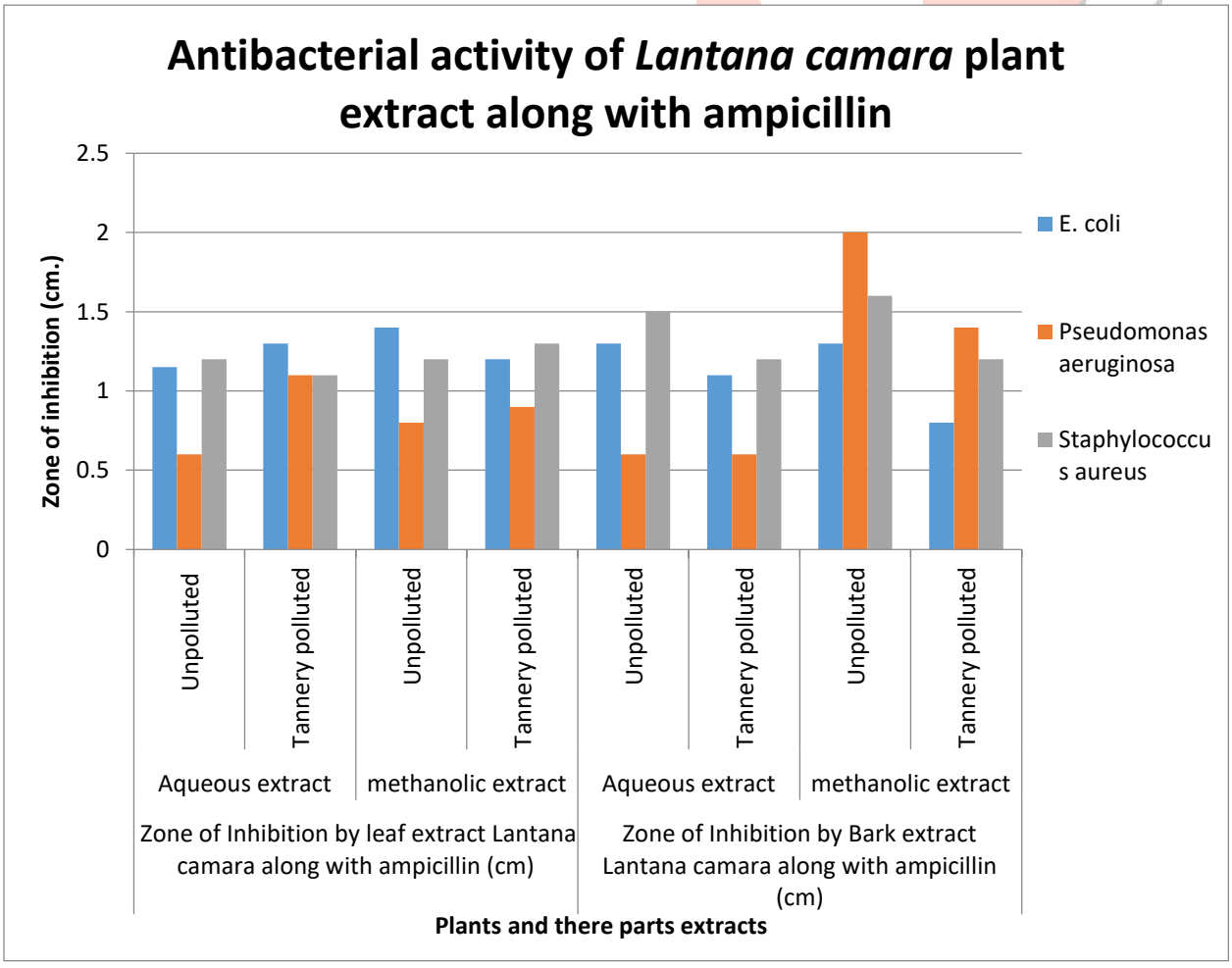
Graph 2: Antibacterial activity of *Lantana camara* plant extract along with gentamicin

Synergistic effect of plant extract along with ampicillin

To study the synergistic effect of plant extract along with ampicillin all the plant extract were used in combination with ampicillin (50µg/ml) to evaluate the antimicrobial property. The results of synergistic effect of plant extract along with ampicillin are mentioned in table 4 and graph 3.

Table 4: Antibacterial activity of *Lantana camara* plant extract along with ampicillin

Microorganism	Zone of Inhibition by leaf extract *Lantana camara* along with ampicillin (cm)				Zone of Inhibition by Bark extract *Lantana camara* along with ampicillin (cm)			
	Aqueous extract		methanolic extract		Aqueous extract		methanolic extract	
	Unpolluted	Tannery polluted	Unpolluted	Tannery polluted	Unpolluted	Tannery polluted	Unpolluted	Tannery polluted
E. coli	1.15	1.3	1.4	1.2	1.3	1.1	1.3	0.8
Pseudomonas aeruginosa	0.6	1.1	0.8	0.9	0.6	0.6	2.0	1.4
Staphylococcus aureus	1.2	1.1	1.2	1.3	1.5	1.2	1.6	1.2



Graph 3: Antibacterial activity of *Lantana camara* plant extract along with ampicillin

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