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Antibacterial Activities of Ethanolic Combination of Different Plant Extract

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Abstract

In this study, the antibacterial potential of ethanolic extract combination of three Indian plants *Azadirachta indica (A. indica)*, Garlic (*Allium sativum L.*) and *Psidium guajava* were investigated against four microorganisms namely, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa*. Each combination of ethanolic plant extract was taken in the ratio of 1:1:1, 2:1:1, 1:2:1 and 1:1:2 separately. Disc diffusion method was used to evaluate antibacterial activity. The results indicated that the plant ratio have different degrees of bacterial growth inhibition, depending on the strains and combination of plant extract used.

Keywords

Antibacterial activity, *Azadirachta indica (A. indica)*, Garlic (*Allium sativum L.*), *Psidium guajava*, Plant extract, *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.*

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INTRODUCTION

In the last few decades, antibacterial resistance becomes the emerging problem in India as well as all over the world. This has gain attention to search for finding new alternative natural antibacterial agents which will be safer and effective. Moreover, there is a growing demand among consumers for the use of natural preservative or additives in processed foods. Natural additives are mostly preferred in comparison to chemical or synthetic additives because, these are safe, flavor enhancer and devoid of any side effects. Various plant extracts are now becoming popular as natural antibacterial preservatives or additives. These are used in very small amount to impart flavor, color, taste and aroma in food preparation to improve their palatability. Spices are also being used for stabilizing several food items from deterioration. Several studies have been shown that spices are considered to be a richest source of bio-active antibacterial compounds[1]**.** *Azadirachta indica (A. indica)* belongs to the family *Meliaceae*, commonly known as Neem. *A. indica* (leaf, bark and seeds) are known to contain antibacterial and antifungal activities against different pathogenic microorganisms; in addition to antiviral activity against vaccinia, chikungunya, measles, and

Coxsackie B viruses[2]. Various parts of the neem tree have been used as traditional Ayurvedic medicine in India. Neem oil and the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, various skin infections, constipation and also as a general health promoter. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and phthisis[3]. Garlic (*Allium sativum* L.) is a plant from the family of Aliaceae. It is an herbaceous plant with height of 20-40 cm, a bulb of strong odour and pungent taste[4]. Its therapeutic uses beneficial effects on the cardiovascular system, antibacterial, anticancer, anti-inflammatory, hypoglycaemic and hormone-like effects[5]. Garlic is a strong antibacterial agent against both Gram-positive and Gram negative bacteria such as *E. coli, Salmonella spp., Streptococcus spp., Staphylococcus aureus, Klebsiella spp., Proteus mirabilis, Shigella senteriae, Pseudomonas aeroginosa* and *Helicobacter pylori*, also it's effective even against those strains that have become resistant to antibiotics[6]. Psidium guajava, a tropical fruit guava of the family Myrtaceae is widely recognized as a plant of many herbal medicines[7]. Guava leaves have several chemical constituents such as comarins, essential oils, flavonoids, triterpenes and ellagitannins which are known to have antimicrobial properties. Guava leaves, roots, and fruits have been used for the prevention and treatment of diarrhea and antibacterial activity against common food borne diarrhea causing bacteria such *as Staphylococcus* species*, Shigella* species*, Salmonella* species*, Bacillus* species*, E. coli, Clostridium* species and food spoilage bacteria such as *Pseudomonas* species[8].The many parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums,diabetes, hypertension, and obesity[9].

MATERIALS AND METHODS

The plant of Neem (*Azadirachta indica*), Garlic (*Allium sativum)* and Guava (*Psidium guajava)* was selected for study. Its seed of neem, leaves of *Allium sativum,* and *Psidium guajava* were collected from college campus. The collected seeds and leaves were identified with the help of morphology and microscopic characterization.

PREPRATION OF PLANT EXTRACT

The completely dried material was powdered and 5 gm were taken in a separate container. To this 100 ml of ethanol was added and kept for 24 hrs. with periodic shaking then filtered and the filtrate was collected. The filtrates were stored at 4°C in air tight bottle[10].

TEST MICROORGANISMS

The Microbial Strains *Staphylococcus aureus, Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* were used. The strains were maintained on nutrient agar slants at 4°C.

PREPRATION OF CULTURE MEDIA

Mueller Hinton Agar (MHA) media was prepared by suspending 38 g in 1000 ml of distilled water. The media was sterilized by autoclaving at 121°C for 15 min and poured into sterile Petri plates at around 50°C. pH was adjusted by adding 0.1M HCL or 0.1M NaOH into the media[11].

ANTIBACTERIAL ASSAY

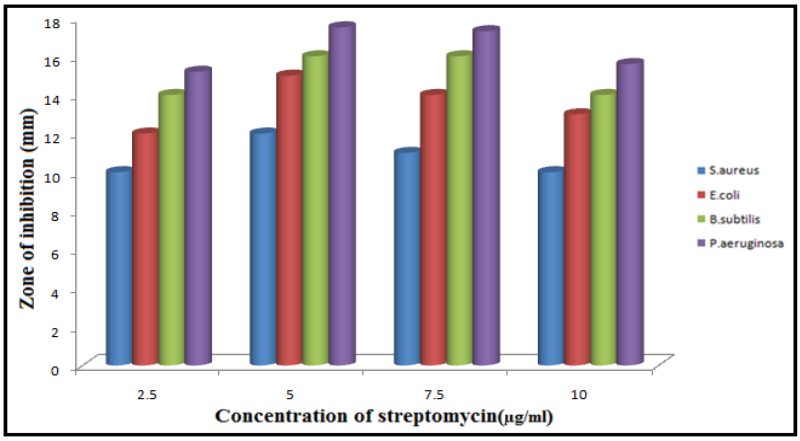
The antibacterial activity of ethanol extracts of *P. guajava, A. indica* and *Allium sativum* was done using the agar well diffusion assay as described. The zone of inhibition of bacterial growth around each well is measured and the susceptibility is determined. Medium-Muller Hinton Agar (3.8 gm/100 ml of distilled water) was prepared, autoclaved at 121^0C for 15minutes at 15lbs and poured in sterile petri plates up to a uniform thickness of approximately 5-6mm and the agar was allowed to set at ambient temperature and used. Wells of 6 mm in diameter were punched off with the help of sterile borer in MHA plates. Wells were then filled with the plant extract solution in the combinations. Petri plates were placed for 30 min in refrigerator for diffusion of extracts and then incubated at temperatures 37°C for 24 hours. At the end of the incubation period, the zone of inhibition was measured.[11,12]

RESULTS AND DISCUSSION

The results of antibacterial activity of streptomycin of various concentration (2.5mg/ml, 5mg/ml, 7.5mg/ml and 10mg/ml) against *Staphylococcus aureus* (*SA*), *Escherichia coli* (EC), *Bacillus subtilis* (*BS*) and *Pseudomonas aeruginosa* (PA) are given in Table.1. The highest ZOI (in mm) against *SA*, *EC, BS* and *PA* is found to be 15.2 mm, 17.5 mm, 17.3 mm and 15.6 mm respectively for streptomycin concentration of 10mg/ml.

Table.1: Antibacterial activity of various concentrations (2.5mg/ml, 5mg/ml, 7.5mg/ml and 10mg/ml) of Streptomycin against *SA, EC, BS* and *PA.*

Microorganisms	Concentration of Streptomycin (µg/ml)	Zone of inhibition(mm)
Staphylococcus aureus	2.5	10
	5	12
	7.5	14
	10	15.2
Escherichia coli	2.5	12
	5	15
	7.5	16
	10	17.5
Bacillus subtilis	2.5	11
	5	14
	7.5	16
	10	17.3
Pseudomonas aeruginosa	2.5	10
	5	13
	7.5	14
	10	15.6

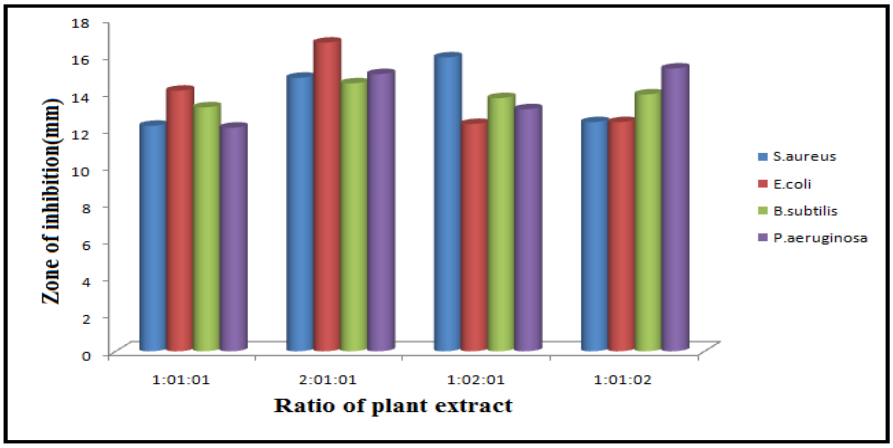


In the present study, different ratio of the ethanolic extracts (1:1:1, 2:1:1, 1:2:1 and 1:1:2) of the seed of *Azadirachta indica,* leave of *Allium sativum and* leaves *Psidium guajava*, showed antibacterial activities against all the bacterial pathogen (*Staphylococcus aureus, Escherichia coli, Bacillus subtilis,* and *Pseudomonas aeruginosa*). Plant extract ratio 2:1:1 inhibited the growth of *Escherichia coli* to maximum extent 16.7 mm show in Table.2 & Figure .1.

Table.2: Antibacterial activity of various combination (1:1:1, 2:1:1, 1:2:1 and 1:1:2) of Plant extract against *BS, SA, EC* and *PA.*

Combined plant extract used for microbial assay	Ratio of extract used for microbial assay	Zone of inhibition(mm)			
		Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonas aeruginosa*
Azadirachta indica : Allium sativum: Psidium guajava	**1:1:1**	**12.2**	**14.1**	**13.2**	**12.1**
	2:1:1	**14.8**	**16.7**	**14.5**	**15**
	1:2:1	**15.9**	**12.3**	**13.7**	**13.1**
	1:1:2	**12.4**	**12.4**	**13.9**	**15.3**

Fig.1. Antibacterial activity graph of various combination (1:1:1, 2:1:1, 1:2:1 and 1:1:2) of Plant extract against *BS*, *SA*, *EC* and *PA*.



The result was found that plant extract ratio 1:1:1 was shown maximum activity against *Escherichia coli* with zone of inhibition 14.1 mm and lowest activity against *Pseudomonas aeruginosa* with zone of inhibition 12.1 mm. Plant extract ratio 2:1:1 was shown maximum activity against *Escherichia coli* with zone of inhibition 16.7 mm and lowest activity *Bacillus subtilis* with zone of inhibition 14.5 mm. The result was found that Plant extract ratio 1:2:1 was shown maximum activity against *Staphylococcus aureus* with zone of inhibition 15.9 mm and lowest activity against *Escherichia coli* with zone of inhibition 12.3 mm. Plant extract ratio 1:1:2 was shown maximum activity against *Pseudomonas aeruginosa* with zone of inhibition 15.3 mm and lowest activity against *Staphylococcus aureus* and *Escherichia coli* with zone of inhibition 12.4 mm.

CONCLUSION

The present study has helped in demonstrating the potential bioactive compound of natural plant extracts that are eco-friendly, economical and available in bulk to the farmers with easy preparation protocols. The *Azadirachta indica (A. indica), Psidium guajava* Linn and Garlic (*Allium sativum* L.) Plant parts are used for the development of various industrial and pharmaceutical products. In this paper, attempt was made to medicinal properties of ratio 2:1:1 of ethnolic plant extract against human bacterial pathogens. The extracts ratio 2:1:1 were shown better results against *Escherichia coli* than other pathogens. From the results, it was concluded that plant extract of *Azadirachta indica (A. indica), Psidium guajava* Linn and Garlic (*Allium sativum* L.) was shown effective and efficient result against bacterial pathogen used and could serve as good source of antibacterial agents.

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