The Pharma Innovation Journal 2023; 12(4): 1322-1328

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(4): 1322-1328

www.thepharmajournal.com
Received: 28-02-2023
Accepted: 30-03-2023

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Synergistic effect of medicinal plant extracts and antibiotics against bacterial pathogens

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Abstract
This *in-vitro* study gives us natural antibacterial agents which can be useful to increase the efficacy of antibiotics The experiment was carried out at MGM College of Agricultural Biotechnology, Gandheli during year 2018-19. The objective of the study was to find out suitable combination of medicinal plant extract and antibiotics. Five different medicinal plants *Zingiber officinale, Opuntia ficus indica, Bryophyllum pinnatum, Syzygium aromaticum* and *Curcuma longa* were selected for this study on the basis of their medicinal properties. The methanolic extract of these plant were mixed with solution of four different anibiotics. Total twenty treatment combinations were tested against three different bacterial pathogens *Escherichia coli, Pseudomonas syringae* and *Xanthomonas campestris.* These combinations were tested against pathogens using agar well diffusion assay. The experiment was carried out in Factorial Randomized Block Design (FRBD).The zone of inhibition was measured for each combination and compared with zone of inhibition of individual plant extract and antibiotics. It was observed that combination of *Opuntia ficus indica* + Gentamicin showed highest the zone of inhibition 38 mm compared to all other combinations. Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes.

Keywords: Antibacterial agents, medicinal plant extract, antibiotics, agar well diffusion assay, synergistic effect

Introduction
Medicinal plants are the richest bio-resource of drugs for traditional systems of medicine, modern medicine, neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. In more than 80% of developed countries, plants have been used as traditional medicine as they are the good source of compound derivation. Many plants have been used for their antimicrobial traits, which are chiefly due to the synthesis of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids (Al-Momani *et al*., 2007) [1] and their inhibitory effect against the growth of pathogens. Plants extracts have both phytochemical and antimicrobial properties and can be of great significance in therapeutic treatments (Nagesh *et al*., 2009) [11]. According to WHO, a medicinal plant is any plant which in one or more of its organs, contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drugs (Junaid *et al*., 2006) [10].

The most well-known member of Zingiber (Ginger) is *Zingiber offici*). Ginger is a member of the family Zingiberaceae; a small family with more than 45 genera, and 800 species; its scientific name is *Zingiber officinale* (*Z. officinale*). Ginger is truly a world domestic remedy. (Sharif *et al*., 2006) [17]. *Opuntia ficus indica* is a medicinal plant belonging to family Cactaceae. An opuntia fruit has highily medicinal values and was established to display many pharmacological properties such as anti-ulcerogenic, neuroprotective, antioxidant, hepatoprotective and anticancer activities. (Kannusamy *et al*., 2016) [7].

Bryophyllum pinnatum is a medicinal plant belonging to family Crassulaceae. The active components of *Bryophyllum pinnatum* posses antibacterial, anti tumorous, cancer preventive and insecticidal actions. From the upper respiratory infections and cough to stomach ulcer and infections of the skin, eyes and ears; it is widely known as “miracle leaf”. *Syzygium aromaticum* (Cloves), a spice used in Ayurveda, is a source of anti-microbial agents against oral bacteria that are commonly associated with dental caries and periodontal disease. Turmeric (*Curcuma long*) is extensively used as spice, food preservative and as colouring material. Curcumin, the main yellow bioactive component of turmeric has been shown to have wide spectrum of biological uses. This includes its anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiulcer and hypotensive activities. For traditional Ayurvedics, turmeric plant was an excellent natural antiseptic, disinfectant, anti analgesic (Verma *et al*., 2018) [15].

Escherichia coli is a gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of genus Escherichia that is commonly found in lower intestine of warm-blooded organisms. *Pseudomonas syringae* is a rod-shaped, Gram-negative bacterium with polar flagella. As a plant pathogen, it can infect a wide range of species, and exists as over 50 different pathovars. *Xanthomonas campestris* is bacterial species that causes a variety of plant diseases, including "black rot" in Cruciferous vegetables and bacterial wilt of turf grass.

The antibiotic kills the bacteria by causing the cell wall to disintegrate. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environment impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey., 1999) [18]. The rising prevalence of antibiotics resistant pathogenic in the last decades raises the demand for finding new alternative antimicrobial agents. The current study aim was to evaluate the antimicrobial activity of some local natural plants which have potential of treating diseases. The screening of crude extracts for synergistic interaction with standard antibiotics against resistant bacteria as this would pave the way for possible isolation of antibiotic resistance inhibitors. An effect arising between two or more agents, entities, factors or substances that produce an effect greater than the sum of their individual effects is Synergistic effect. Synergy is the creation of a whole that is greater than the sum of the separate effects. Efficacy is defined as the maximum effect a drug can produce regardless of the dose. Rapidly emerging resistant bacteria threaten the extraordinary health benefits that have been achieved with antibiotics.

2. Material and Methods

2.1 Experimental site

All the experimental studies were conducted in MGM college of Agricultural Biotechnology, Gandheli, Aurangabad (M.S.) during summer session of 2018-19.

2.2 Experimental Details

The work was undertaken to study the antibacterial activity of the methanolic extracts of *Zingiber officinale, Opuntia ficus indica, Bryophyllum pinnatum, Syzygium aromaticum*and *Curcuma longa*. The extracts are added with antibiotics such as Tetracycline, Gentamicin, Ampicillin and Streptomycin in order to improve the effect of antibiotics against the pathogens: *Escherichia coli, Pseudomonas syringae and Xanthomonas campestris.*

2.3 Statistical design

FRBD (Factorial Randomized Block Design)

2.3.1 Treatment Details

Total No. of Treatments: 20 (Twenty treatment combinations of 5 different medicinal plants, 4 different antibiotics)
Total No. of Replications: 03

Table 1: List of Medicinal plants used for study

Sr. No.	Symbol	Name of Plant	Part used
1.	M_1	*Zingiber officinale*	Rhizome
2.	M_2	*Opuntia ficus indica*	Stem
3.	M_3	*Bryophyllum pinnatum*	Leaves
4.	M_4	*Syzygium aromaticum*	Clove
5.	M_5	*Curcuma longa*	Rhizome

Table 2: List of Antibiotics used for study

Sr. No.	Symbol	Name of Antibiotic
1.	A_1	Tetracycline
2.	A_2	Gentamicin
3.	A_3	Ampicillin
4.	A_4	Streptomycine

Table 3: List of Bacteria:

Sr. No	Symbol	Name of Bacteria
1.	B_1	*Escherichia coli*
2.	B_2	*Pseudomonas syringae*
3.	B_3	*Xanthomonas campestris*

2.4 Media Preparation

Mueller Hinton Agar which is required for the growth of microorganisms was prepared. The medium was prepared by adding the 2 gm Beef extract, 17.5 casein hydrolysate, 1.5 gm starch, 17 gm Agar is dissolved in 1000 ml of distilled water in conical flasks. The required petri plates, glassware and media are autoclaved at 121 °C for 20 minutes at 15 psi pressure and the media was poured into sterile petri plates under aseptic conditions which are used for further use (Kamba *et al.*, 2010) [6].

2.5 Culture selection and Inoculation

The bacterial cultures of *Escherichia coli, Pseudomonas syringae* and *Xanthomonas campestris* are collected from MGM college of Agricultural Biotechnology, Gandheli, Aurangabad. The bacterial cultures of *Escherichia coli, Pseudomonas syringae*, and *Xanthomonas campestris* are sub cultured on Mueller Hinton Agar media and the culture plates were maintained at 37 °C for 24 hrs (Kurhekar 2006) [9].

2.6 Preparation of methanolic Extracts

Medicinal plant parts (Rhizomes of *Zingiber officinale*, Stem of *Opuntia ficus indica*, Leaves of *Bryophyllum pinnatum*, Cloves of *Syzygium aromaticum* and Rhizomes of *Curcuma longa*) are collected from local area and market. The plant parts were washed under tap water and air dried at room temperature. Dried plant parts 10 gm by quantity was ground to produce fine homogenous mixture. The mixture was soaked in 40 ml of 95% methanol at room temperature for 72 hours in dark. The solution was then filtered through Whatmann filter paper. The filtered medicinal plant extract was stored at – 20 °C which can be used for further use (Kamba *et al*., 2010) [6].

2.7 Preparation of antibiotics solution

100 mg of antibiotic was dissolved in 1 ml of distilled water making it as a stock solution. 100 µl of antibiotic stock solution was diluted to1 ml with double distilled water making it as a working solution of 10 mg/ml (Kamba *et al*., 2010) [6].

2.8 Preparation of treatment solution (medicinal plant extract and antibiotic solution)

The medicinal plant extracts and antibiotics solution was prepared as per the treatment levels. The treatment levels include according to the four wells in each petri plate (Kamba *et al*., 2010) [6].

Well Number	Treatment solution details
1.	10 µl extract + 90 µl distilled water
2.	10 µl antibiotic working solution + 90 µl distilled water
3.	5 µl extract +5 µl antibiotic working solution + 90 µldistilled water

2.9 Measurement of zone of Inhibition

The zone of inhibition around the wells for the activity responded by medicinal plant extract or antibiotics or the interaction between both the antibiotics and medicinal plant extracts are measured by the ruler to measure the diameter of the clear area around the wells (Doughari *et al*., 2008) [19].

2.10 Analysis of data

The data obtained on various observations is analyzed by "Analysis of variance (ANOVA)" method (Panse and Sukhatme., 1967) [13].

3. Results and Discussion

The study was carried out to study the antibacterial activities of methanolic extracts from different medicinal plants and to find out the interaction effect of plant extracts and antibiotics against pathogens.

3.1 Zone of inhibition of interaction of antibiotics and medicinal plants against *E. coli*

Table 1: Zone of inhibition (mm) of interaction of antibiotics and medicinal plants extracts against *E.coli*

Medicinal plants	Antibiotics			
	A_1	A_2	A_3	A_4
M_1	33.667	35.667	20.000	19.667
M_2	30.667	37.333	18.667	18.000
M_3	33.667	33.667	17.667	21.333
M_4	33.000	34.333	18.000	20.333
M_5	30.333	35.667	20.000	20.000

MXA	
SE ±	0.570
CD 1%	1.507

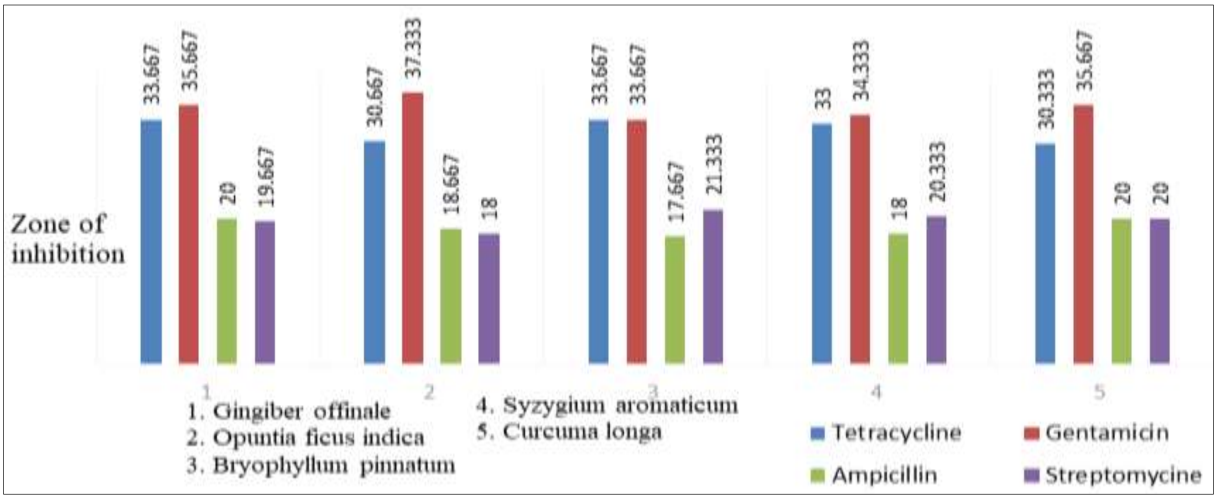

Fig 1: Zone of inhibition (mm) of interaction of medicinal plant extracts and antibiotics against *E.coli*

The data presented in Table.3, depicted in Fig.3, Treatment M_2A_2 (*Opuntia ficus indica* + Gentamicin) shows maximum zone of inhibition of 37.333 mm which is significantly superior over M_1A_2 (*Z. officinale* + Gentamicin) and M_5A_2 (*C. longa* + Gentamicin) having zone of inhibition 35.667 mm and 35.667 mm. Where M_1A_2, M_5A_2 and M_4A_2 are at par.

3.2 Zone of inhibition (mm) of interaction effect of antibiotics and medicinal plants against *P. syringae*

Table 2: Zone of inhibition (mm) of interaction effect of antibiotics and medicinal plants against *P. syringae*

Medicinal plants	Antibiotics			
	A_1	A_2	A_3	A_4
M_1	29.213	31.293	13.323	12.667
M_2	25.000	23.667	13.333	12.333
M_3	23.667	28.553	13.667	14.000
M_4	26.617	27.667	12.000	12.667
M_5	12.333	26.000	14.667	12.667

M X A	
SE ±	0.373
CD 1%	1.157

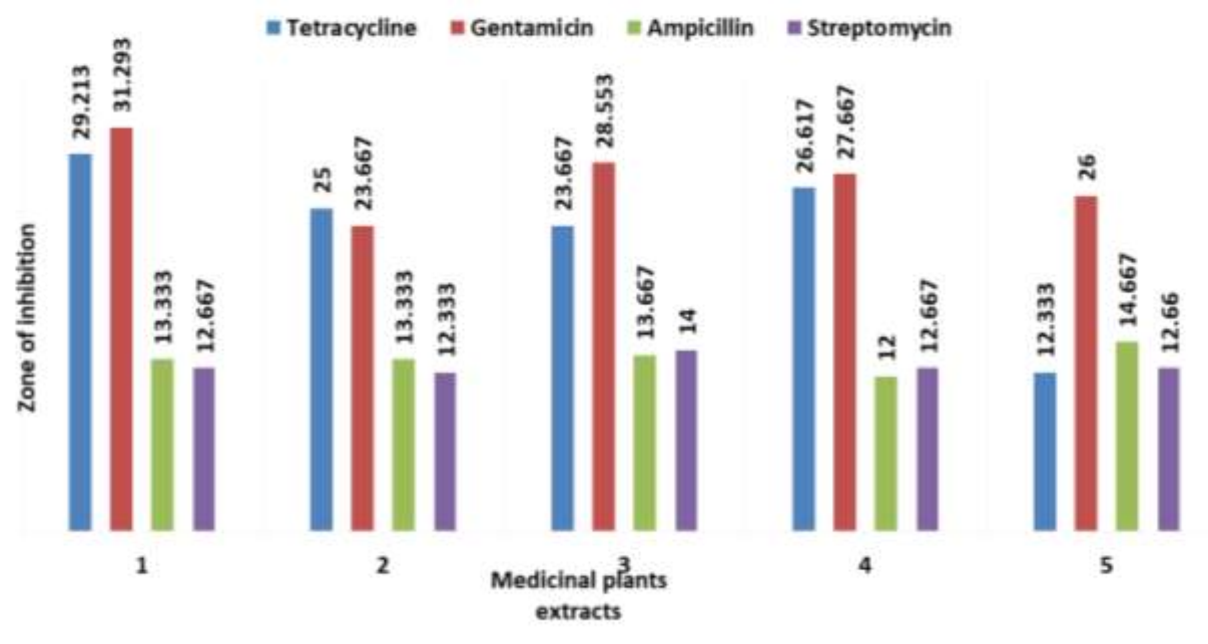

Fig 2: Zone of inhibition (mm) of interaction medicinal plant extracts and antibiotics against *P. syringae*

The data depicted in Table.5. Fig.5, treatment M_1A_2 (*Z. officinale* + Gentamicin) shows maximum zone of inhibition of 31.293 mm and significantly superior over M_1A_1 *(Z. officinale* + Tetracycline). M_1A_1 (29.213 mm), M_3A_2 (28.553 mm) are at par and M_4A_2 (27.667 mm) and M_4A_1 (26.617 mm) are at par. M_4A_1 is significantly superior over rest of all treatments.

3.3 Zone of inhibition (mm) of interaction of medicinal plant extracts and antibiotics against *X. campestris*

Table 3: Zone of inhibition (mm) of interaction of medicinal plant extracts and antibiotics against *X. campestri*

Medicinal plants	Antibiotics			
	A_1	A_2	A_3	A_4
M_1	27.667	26.667	19.667	14.333
M_2	25.333	28.333	19	16
M_3	26.667	27.667	17	12
M_4	25.667	28.667	19	16.667
M_5	28	28.667	14.667	13.667

MXA	
SE ±	0.452
CD 1%	1.358

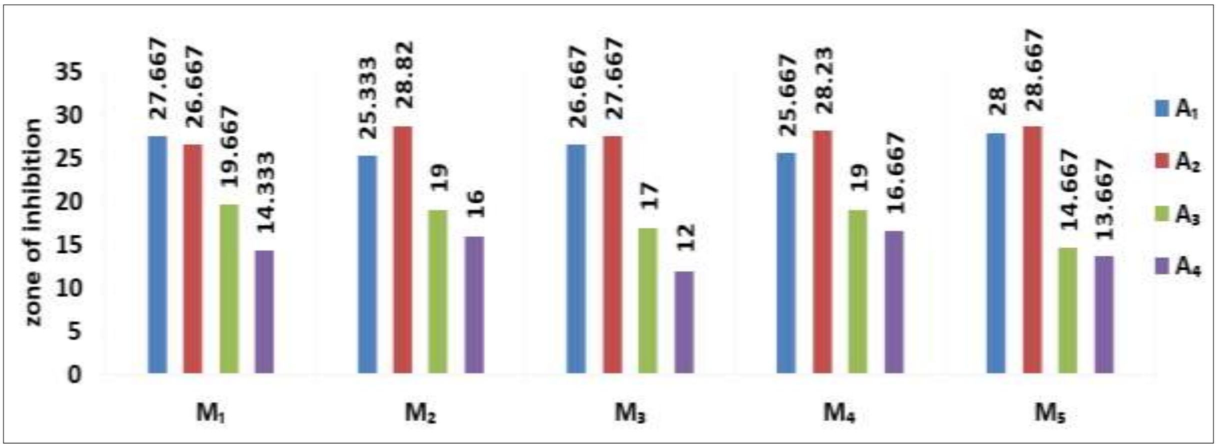

Fig 3: Zone of inhibition (mm) of medicinal plant extracts and antibiotics against *X.campestris*

The data depicted in Table.9 and Fig.9, shows the treatment M_4A_2 (28.667 mm) and M_5A_2 (28.667 mm) shows the maximum zone of inhibition, where treatment M_4A_2 (28.667 mm), M_5A_2 (28.667 mm) and M_2A_2 (28.333) are at par. M_5A_1 (28 mm) and M_1A_1 (27.667 mm) are at par, M_1A_2 (26.667 mm), M_3A_1 (26.667 mm) and M_4A_1 (25.667 mm) are at par and M_2A_1 (25.333 mm) is significantly superior over rest of all treatments.

3.5 Enhanced efficacy of antibiotics

Zone of inhibition of only antibiotics and only medicinal plant extract were obtained against all three bacterial and compared with the combination. It was discovered that the medicinal plant extract and antibiotics shows synergistic effect to increase the increased efficacy of antibiotics against selected pathogens. The data of enhancement is as below

Table 4: Increased zone of inhibition of Antibiotics on *Escherichia coli*

Sr. No.	Zone of Inhibition of Medicinal plants (mm)	Zone of Inhibition of Antibiotics(mm)	Combined Zone of Inhibition (mm)
1.	*Zingiber officinale* (12)	Tetracycline (33 mm)	*Zingiber officinale* + Tetracycline (35 mm)
2.	*Opuntia ficus indica* (10 mm)	Tetracycline (27 mm)	*Opuntia ficus indica*+ Tetracycline (31 mm)
3.	*Syzygium aromaticum* (14 mm)	Tetracycline (28 mm)	*Syzygium aromaticum* + Tetracycline (31 mm)
4.	*Curcuma longa* (13 mm)	Tetracycline (28 mm)	*Curcuma longa* + Tetracycline (31 mm)
5.	*Zingiber officinale* (11 mm)	Gentamicin (32 mm)	*Zingiber officinale* + Gentamicin (36 mm)
6.	*Opuntia ficus indica* (12 mm)	Gentamicin (32 mm)	*Opuntia ficus indica* + Gentamicin (38 mm)
7.	*Syzygium aromaticum* (13 mm)	Gentamicin (32 mm)	*Syzygium aromaticum* + Gentamicin (35 mm)
8.	*Opuntia ficus indica* (12 mm)	Ampicillin (16 mm)	*Opuntia ficus indica* + Ampicillin (19 mm)
9.	*Syzygium aromaticum* (12 mm)	Ampicillin (15 mm)	*Syzygium aromaticum*+ Ampicillin (18 mm)
10.	*Curcuma longa* (13 mm)	Ampicillin (15 mm)	*Curcuma longa* + Ampicillin (20 mm)

Above table describes the data about increased or enhanced efficacy of antibiotics against *E. coli* when antibiotics are interacted with medicinal plant extracts. The combination of *Opuntia ficus indica* and gentamicin shows the highest zone of inhibition of 38 mm

Table 5: Increased efficacy of antibiotics on *Pseudomonas syringae*

Sr. No	Zone of inhibition of Medicinal plants (mm)	Zone of inhibition of Antibiotics(mm)	Combined zone of inhibition
1.	*Zingiber officinale* (14 mm)	Tetracycline (22 mm)	*Zingiber officinale* + Tetracycline (30 mm)
2.	*Opuntia ficus indica* (13 mm)	Tetracycline (19 mm)	*Opuntia ficus indica* + Tetracycline (25 mm)
3.	*Bryophyllum pinnatum* (12 mm)	Tetracycline (20 mm)	*Bryophyllum pinnatum* + Tetracycline (24 mm)
4.	*Syzygium romaticum* (16 mm)	Tetracycline (24 mm)	*Syzygium aromaticum* + Tetracycline (28 mm)
5.	*Gingiber officinale* (10 mm)	Gentamicin (28 mm)	*Gingiber officinale* + Gentamicin (32 mm)
6.	*Syzygium aromaticum* (14 mm)	Gentamicin (24 mm)	*Syzygium aromaticum* + Gentamicin (26 mm)
7.	*Curcuma longa* (0 mm)	Gentamicin (26 mm)	*Curcuma longa* + Gentamicin (30 mm)

The above table describes the data about increased or enhanced efficacy of antibiotics against *P. syringae,* when antibiotics are interacted with medicinal plants extracts. The combination of Gingiber officinale and gentamicin shows the highest zone of inhibition of 32 mm.

Table 5: Increased efficacy of Antibiotics on *Xanthomonas campestris*

Sr. No	Zone of inhibition of Medicinal plants (mm)	Zone of inhibition of Antibiotics (mm)	Combined zone of inhibition (mm)
1.	*Zingiber officinale* (13 mm)	Tetracycline (24 mm)	*Zingiber officinale* + Tetracycline (28 mm)
2.	*Opuntia ficus indica* (12 mm)	Tetracycline (25 mm)	*Opuntia ficus indica* + Tetracycline (28 mm)
3.	*Syzygium aromaticum* (18 mm)	Tetracycline (23 mm)	*Syzygium aromaticum* + Tetracycline (26 mm)
4.	*Curcuma longa* (13 mm)	Tetracycline (24 mm)	*Curcuma longa* + Tetracycl-ine (28 mm)
5.	*Zingiber officinale* (12 mm)	Gentamicine (24 mm)	*Zingiber officinale* + Gentamicin (28 mm)
6.	*Opuntia ficus indica* (12 mm)	Gentamicin (23 mm)	*Opuntia ficus indica* + Gentamicin (28 mm)
7.	*Bryophyllum pinnatum* (13 mm)	Gentamicin (23 mm)	*Bryophyllum pinnatum* + Gentamicin (28 mm)
8.	*Syzygium aromaticum* (16 mm)	Gentamicin (24 mm)	*Syzygium aromaticum* + Gentamicin (29 mm)
9.	*Curcuma longa* (10 mm)	Gentamicin (22 mm)	*Curcuma longa* + Gentamicin (29 mm)
10.	*Syzygium aromaticum* (14 mm)	Streptomycine (12 mm)	*Syzygium aromaticum* + streptomycine (18 mm)

The above table describes the data about increased or enhanced efficacy of antibiotics against *X. campstris*, when antibiotics are interacted with medicinal plants extracts. The combination of *Syzygium aromaticum* and gentamicin, *Curcuma longa* and gentamicin shows the highest zone of inhibition of 29 mm.

This *in-vitro* study gives us natural antibacterial agents which can be useful to increase the efficacy of antibiotics. The data suggests that the different medicinal plant extracts has the capability to increase the effect of antibiotics over different bacterial pathogens.

Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. The practice of using spices as supplementary or alternative medicine in developing countries like India will not only reduce the clinical burden of drug resistance development but also the side effects and cost of the treatment with allopathic medicine. *Zingiber officinal, Syzygium aromaticum* and *Curcuma longa* in their spicy nature with free radical inhibitions index performs other toxic factors which of course responded to the antibacterial effect observed in the study. The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment.

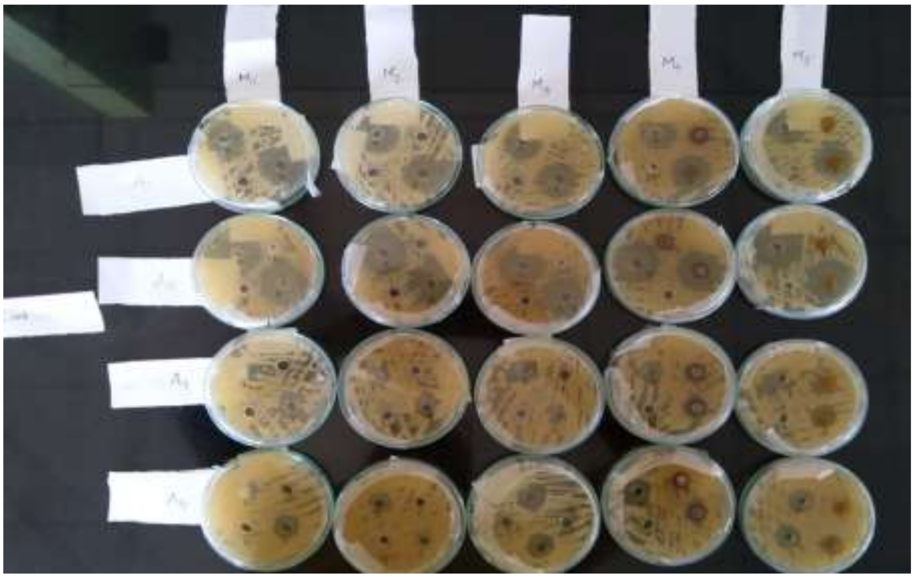

Plate 1: Agar well diffusion method. (Antibiotics X Medicinal plant extract X *Escherichia coli*)

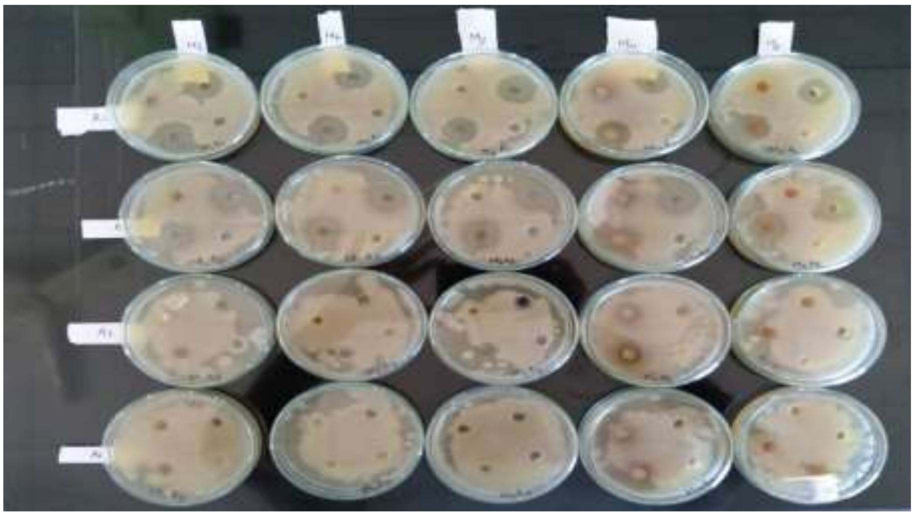

Plate 2: Agar well diffusion method (Antibiotics X Medicinal plant extract X *Xanthomonas campesteris*)

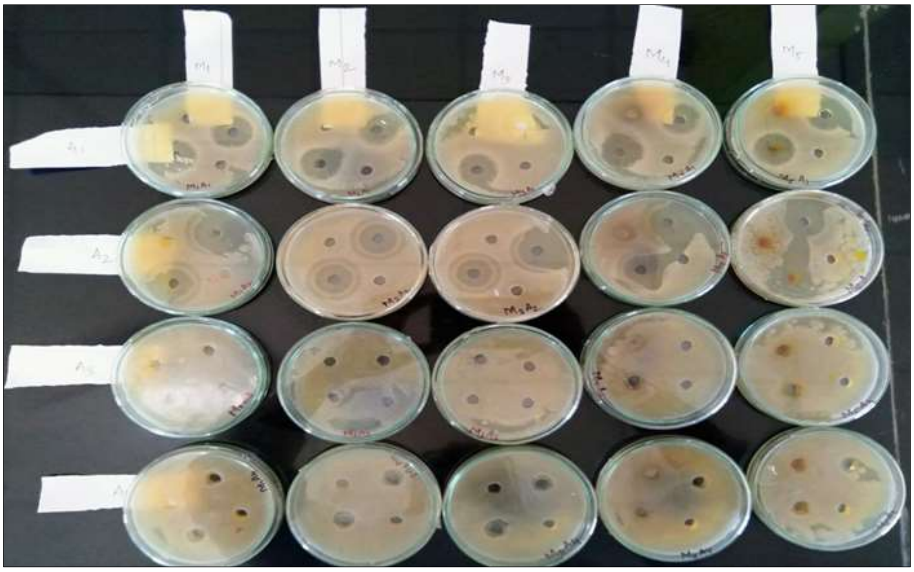

Plate 3: Agar well diffusion method. (Antibiotics X Medicinal plants X *Pseudomonas syringae*)

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