CHAPTER – VI

ANTIMICROBIAL ACTIVITY

CONTENTS

6.1 ANTIMICROBIAL ACTIVITY

Disease in human is majority caused by infection. Skilled running of antimicrobial medicine is of the first significance. The word chemotherapy is utilized for the drug action of freeloading infections in that the parasites (bacteria, viruses, protozoa, worms and fungi) are smashed or eliminate without injuring the host.

Large number of substances that we are known to have therapeutic usefulness were first utilized in the distant past. The Ancient Greeks utilized male fern, and the Aztecs chenopodium, used as intestinal anthelmintics. The Ancient Hindus treated leprosy and chaulmoogra. For 100 years ago moulds have been given to cured wounds, but in spite of the beginning of mercury as a action for syphilis (16th century), and the application of cinchona bark against malaria (17th century), the history of recent rational chemotherapy did not begin until Paul Ehrlich produced the thought from his surveillance that aniline dyes selectively stained bacteria in tissue microscopic synthesis and could selectively kill them. He introduced the word ‘chemotherapy’ and in 1906 he wrote:

“In order to use chemotherapy successfully, we must search for substances which have an affinity for the cells of the parasites and a power of killing them greater than the damage such substances cause to the organism itsel. This means… we must learn to aim, learn to aim with chemical substances.”

The pamaquin, mepacrine and antimalerials were produced from dyes and in 1935 dye (Prontosil) linked sulphonamides was first given as a outcome of systematic analysis by Domagk. The data derived from sulphonamides in meningitis, pneumonia and puerperal sepsis were dramatic and caused a revolution in medical and scientific thought.

In 1928, Fleming fortuitously rediscovered the long-known capability of Penicillium fungi to stifle the escalation of bacterial cultures but put the judgment sideways as a curiosity.

In 1939, mainly as an academic work out, Florey and Chain undertook discovery of antibiotics, i.e. medicine formed by microorganisms which are antagonistic to the escalation or life of other microorganisms. They synthesized penicillin and established its significant lack of poisionicity.

When the synthesis was administered to a policeman by joining streptococcal septicemia and staphylococcal there was spectacular development; unluckily the produce of penicillin (in the local Pathology Laboratory) could not maintain pace with the necessities (it was obtained from the urine of patient and re-injected); it run out and the patient afterward succumbed to infection. Subsequent growth amply confirmed the significant therapeutic capability of penicillin.

6.2 CLASSIFICATION OF ANTIMICROBIAL DRUGS

On the basis of type of organism antimicrobial agents can be divided as follow:

- Anthelmintic drugs.
- Antibacterial drugs
- Antiviral drugs
- Antiprotozoal drugs
- Antifungal drugs

A minute antimicrobials have useful activity across various of these groups. Some examples are metronidazole inhibits necessitate anaerobic bacteria (like Clostridium perfringens) and few protozoa that rely on anarobolic pathways (like Trichomonas vaginalis).

Antimicrobial medicine mainly of two type as follow:

- Bacteriostatic: Drug which can inhibit the bacteria. For example chloramphenicol and sulphonamides, tetracyclines.
- Bactericidal, Drug which can kill the bacteria. For example rifampicin, cephalosporins, penicillins, isoniazide and aminoglycosides.

6.3 CLASSIFICATION OF ORGANISMS

- Staphylococcus aureus is species of schizomycetes class; having Eubacterials order, micrococeaceac family and staphylococcus genus.
- Escherichia coli is species of schizomycetes class; having Eubacterial order, Enterobacteriaceae family and Escherichia genus.

- Bacillus subtillis is species of schizomycetes class; having Eubacterials order, Bacteriodaceac family and fusobacterium streptobacillus and sphaerophorus genus.
- Pseudomonas aeruginosa is species of schizomycetes class; having pseudominodales order, pseudominadaceac family and pseudomonas genus.

6.4 EXPERIMENTAL

The microbial potency of drugs was found by disc plate process. The test discs possessing 50mg per disc of sample compound. The potency was exhibit vst gram +ve bacteria are *Bacillus megaterium* [MTCC (121)] and*Staphylococcus aureus* [MTCC(96)] and gram –ve bacteria and*Proteus vulgaris* [MTCC(1771)], *Escherichia coli* [MTCC(443)].

Preparation of Media:

Bacterial potency nutrient agar is applicable. Nutrient agar is made as below.

1) 5 g Peptone
2) 3 g Meat Extract
3) 5 g Sodium chloride :
4) 15 g Agar Agar

These four compounds are mixed well and made solution 1 liter by distilled water. 15 pound pressure was used in autoclave for stabilization at 125 ^{o} C temperature for 25 min. Then entire medium frozen at 45^{o}C and 20 ml poured sterilized Petri-dish was added. P^{H} of medium keeps 7.0 to 7.5

The civilization of bacteria was made in nutrient and dd water used for dilution. The composition of nutrient broth below:

1) 10 g Beef extract
2) 5 g Sodium chloride
3) 10 g Peptone

This media was utilized for the culture function. The civilization was cooled at 37^{o}C using incubator. Culture was spread on agar plates using swab under particular condition

5 mm diameter paper discs were made and above sterilized using autoclave. These discs were dried out to eliminate the solvent. Sterile test drug coated by discs were reserved in Petri dish possessing culture media.

6.4.1 ANTIMICROBIAL ACTIVITY OF COMPOUNDS B1-B19

Table 6.1 Experimental data of Compounds B1-B19

Samples	*S.aureus* (+Ve)	*B.megaterium* (+Ve)	*E.coli* (-Ve)	*P.vulgaris* (-Ve)
B1	4	9	7	4
B2	5	9	8	8
B3	7	11	6	4
B4	9	10	6	9
B5	7	8	9	12
B6	8	8	4	8
B7	8	9	9	6
B8	10	12	12	9
B9	12	10	11	12
B10	12	10	7	12
B11	5	8	8	12
B12	7	6	10	10
B13	6	8	4	6
B14	3	3	7	7
B15	11	10	6	4
B16	6	9	10	5
B17	6	8	4	10
B18	9	9	6	12
B19	11	11	6	7
Ampicillin	**15**	**14**	**17**	**19**
Gentamycin	**16**	**15**	**14**	**16**

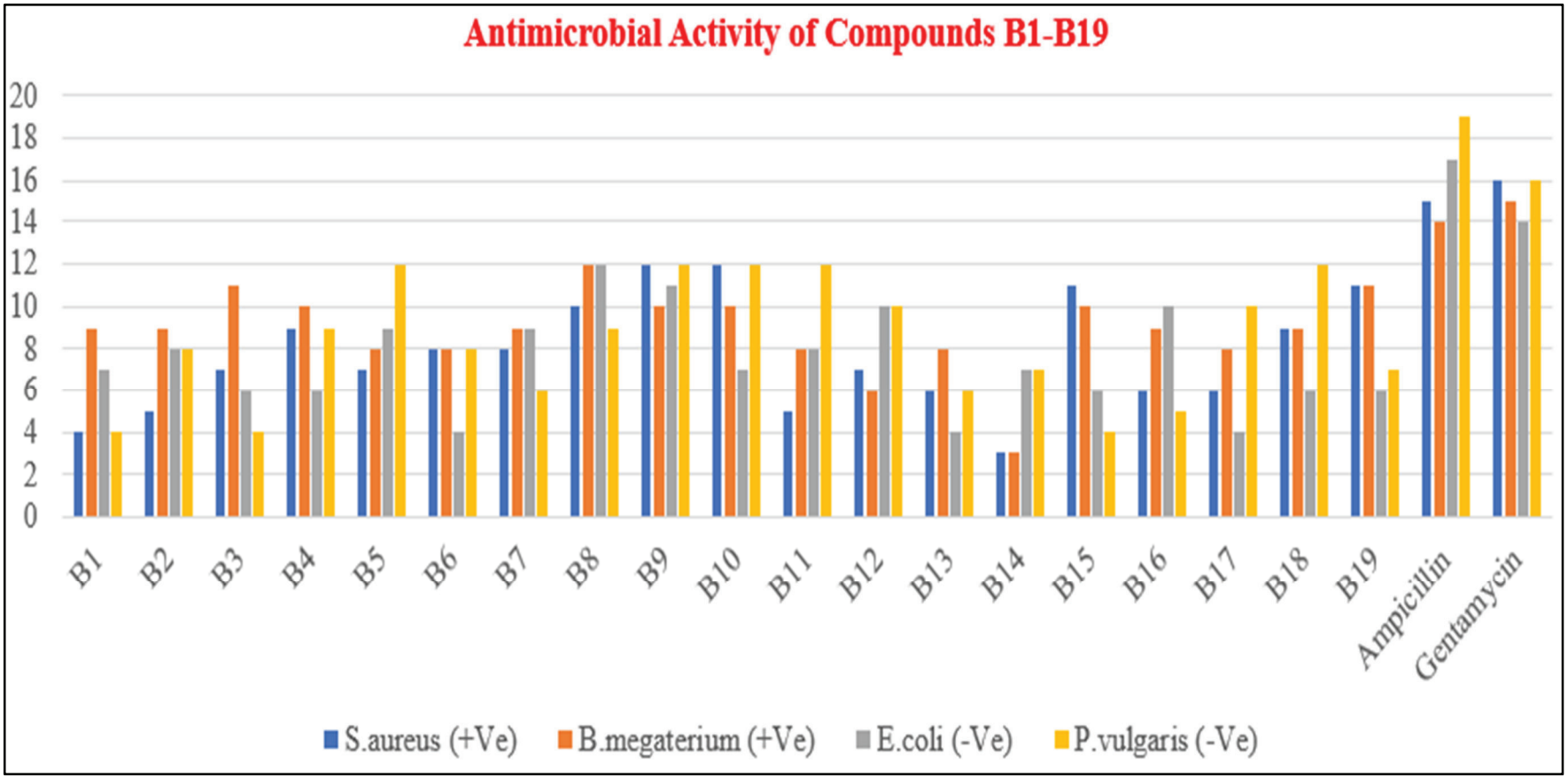

Fig. 6.1: Antimicrobial activity of Compounds B1-B19

A short review of results of antibacterial screening of the compounds is mentioned here:

(I) **Against *Staphylococcus aureus*:**

Maximum activity was found in compounds (B9 and B10) zone of inhibition-12.0 m.m. and minimum activity were found in compounds (B14) zone of inhibition -3.0 m.m

(II) **Against *Bacillus megaterium*:**

Maximum activity were found in compounds (B3, and B19) zone of inhibition -11.0 m.m whereas minimum activity were found in compound (B14) zone of inhibition -3.0 m.m.

(III) **Against *Escherichia coli*:**

Maximum activity were found in compounds (B8) zone of inhibition -12.0 m.m and minimum activity were found in compounds (B6, B13 and B17) zone of inhibition -4.0 m.m

(IV) **Against *Proteus vulgaris*:**

Maximum activity was found in compound (B5, B9-B11 and B18) zone of inhibition -12.0 m.m (near to standard drug) and minimum activity were found in compounds (B1, B3 and B15) zone of inhibition -4.0 m.m

6.4.2 ANTIMICROBIAL ACTIVITY OF CCOMPOUNDS C1-C19

Table 6.2 Experimental data of Compounds C1-C19

Samples	*S.aureus* (+Ve)	*B.megaterium* (+Ve)	*E.coli* (-Ve)	*P.vulgaris* (-Ve)
C1	9	4	5	4
C2	9	9	12	8
C3	7	12	10	10
C4	9	8	8	6
C5	7	6	8	7
C6	8	9	9	6
C7	8	12	12	5
C8	10	12	10	10
C9	12	12	10	12
C10	12	6	4	7
C11	7	7	7	6
C12	6	8	6	8
C13	8	6	3	10
C14	3	6	11	4
C15	10	9	6	7
C16	9	4	6	6
C17	8	9	9	10
C18	9	12	11	4
C19	11	11	4	6
Ampicillin	**15**	**14**	**17**	**19**
Gentamycin	**16**	**15**	**14**	**16**

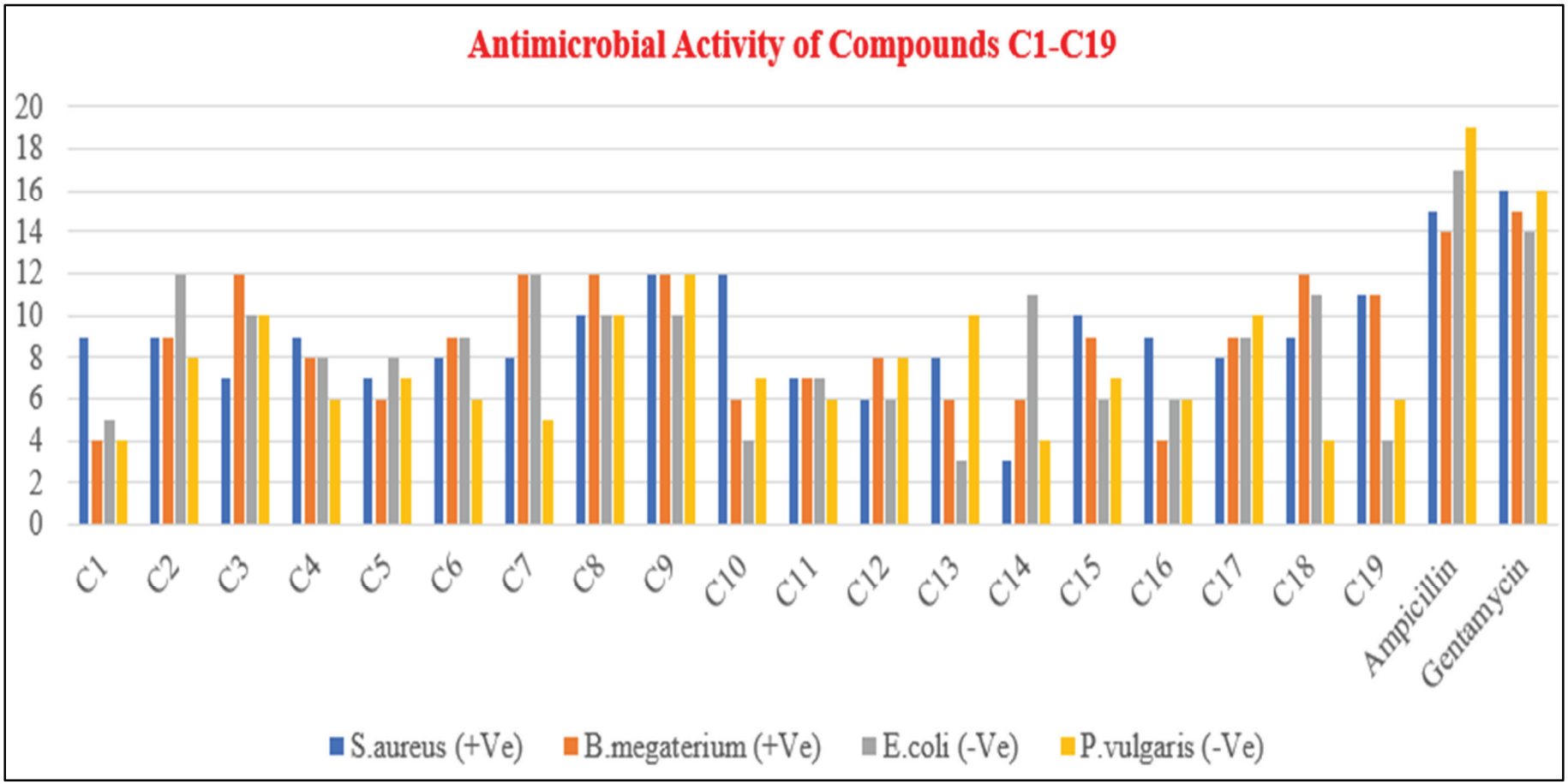

Fig. 6.2: Antimicrobial activity of Compounds C1-C19

A short review of results of antibacterial screening of the compounds is mentioned here:

(I) Against *Staphylococcus aureus*:

Maximum activity were found in compounds (C9 and C10) zone of inhibition -12.0 m.m whereas minimum activity was found in compound (C14) zone of inhibition -3.0 m.m.

(II) **Against *Bacillus megaterium*:**

Maximum activity was found in compound (C3, C7-C10 and C18) zone of inhibition -12.0 m.m (near to standard drug) and minimum activity were found in compounds (C1 and C16) zone of inhibition -4.0 m.m.

(III) **Against *Escherichia coli*:**

Maximum activity was found in compounds (C2 and C7) zone of inhibition-12.0 m.m. and minimum activity was found in compound (C13) zone of inhibition -3.0 m.m.

(IV) **Against *Proteus vulgaris*:**

Maximum activity was found in compound (C9) zone of inhibition -12.0 m.m and minimum activity were found in compounds (C1, C14 and C17) zone of inhibition -4.0 m.m.

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