e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:06/April-2024 Impact Factor- 7.868 www.irjmets.com

EVALUATION OF ANTAGONISM OF *AEGLE MARMELOS* AGAINST THE STANDARD *ENTEROCOCCUS FAECIUM* (MTCC 5695)

Sonal Agarwal*1, Jagriti Sharma*2

*1,2Department Of Biotechnology Raja Balwant Singh College, Khandari, Agra, India.

ABSTRACT

Aegle marmelos is used as medicine. Bael patra is another name of *Aegle marmelos*. Since ancient times, the *Aegle marmelos* plant has been used to treat a wide range of illnesses. The current study looks for *Aegle marmelos* leaf antimicrobial activity. Humans use the medications made from *Aegle marmelos* plants to treat a variety of illnesses. Using a Soxhlet apparatus, an extract of Bael leaves was prepared in various solvents, including ethanol and distilled water, in order to detect the antimicrobial activity of the leaves of *Aegle marmelos*. The Kirby-Bauer disc diffusion technique was used to test the antimicrobial susceptibility. In this case, Mueller-Hinton Agar media was employed. Spread across the petri plates with solidified MHA media containing the test organism *Enterococcus faecium* (MTCC 5695) was the nutrient broth. The sterile discs of Whatman's paper were left in a series of dilutions with varying concentrations of aqueous extract and ethanol. The disc that was dipped in distilled water served as the negative control, and the antibiotic gentamicin served as the positive control. The discs that had previously been prepared in various extracts were placed on Petri dishes that held MHA medium. We stored the plates for incubation. According to research, the clear zones that formed around the discs demonstrated how effective *Aegle marmelos* was at fighting microorganisms. According to the studies, ethanol extract of *Aegle marmelos* produced amazing results when compared to aqueous extract.

Keywords: *Aegle Marmelos*, Antimicrobial Activity, Aqueous Extract, Ethanol Extract.

I. INTRODUCTION

Aegle marmelos is a member of the Rutaceae family. *Aegle marmelos* is also referred to as Bengal quince, golden apple, stone apple, and Bael patra. It is a plant used as medicine. Since ancient times, the *Aegle marmelos* plant has been utilized as medicine to treat a variety of illnesses. Humans use the medications made from the *Aegle marmelos* plant to treat and alleviate both physical and mental illnesses **(Vlietinck, and Lindsay, 1995)**. *Aegle marmelos* therapeutic qualities suggest that it is a valuable source of numerous bioactive compounds. Because the *Aegle marmelos* plant can synthesize bioactive compounds with antimicrobial properties through secondary metabolism, it is a valuable source for pharmaceutical and therapeutic products**(Lis-Balchin. M and Deans S. G. 1997)**. Phytochemicals are the compounds that plants make. Certain phytochemicals are responsible for the antimicrobial activity of plants, which is why they are used in traditional medicines. Phytochemicals also help plants resist infections from bacteria, fungi, and plant viruses**(Chakravarti. And Gode et.al., 1985)**. All parts of the *Aegle marmelos* plant, including the fruits, stem, bark, and leaves, have therapeutic qualities and are used to treat a variety of skin and eye infections. *Aegle marmelos* leaves are thought to be one of the parts of the plant with the highest concentration of bioactive compounds**(Cowan. M. M. 1999)**.

II. MATERIAL AND METHOD

- **Glassware**: Petri dishes, test tubes, conical flasks, measuring cylinders, mortar and pestle.
- **Equipment**: Micropipettes, Soxhlet, Weighing Balance, Autoclave, Hot Air Oven, Hot Plate, Incubator, Laminar Air Flow, Microwave Oven
- **Test organisms**: Within the Enterococcus genus, Enterococcus faecium is a Gram-positive, gamma-hemolytic or non-hemolytic bacterium. Both humans and animals' gastrointestinal tracts may coexist with this organism. Enterococcus faecium was the strain of bacteria used in the investigation (MTCC- 5695).

Methodology:

Preparation of Leaf extracts:

We have collected the *Aegle marmelos* leaves in September from Raja Balwant Singh College in Khandari, Agra. The gathered leaves were dried in the shade and cleaned under the running tap water. We used a mortar and pestle to grind these shade-dried leaves. Weighing balance was used to measure these crushed and powdered leaves. 15 grams of crushed leaves were weighed. Soxhlet apparatus was used to prepare the extract. Filter

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:06/April-2024 Impact Factor- 7.868 www.irjmets.com

paper was utilized to create a thimble. A thimble containing 15 grams of ground-up powdered leaves was placed in a Soxhlet extractor.

Distilled water (100 ml) and ethanol (100 ml) were the solvents utilized in the extraction process. The boiling points of the solvents were 100°C for distilled water and 78°C for ethanol. For a duration of approximately 48 hours or 24 cycles, or until the solvent in the extractor's siphon tube turned colourless, the solvent was heated to its boiling point. The Soxhlet apparatus consists of a flask with a circular bottom, an extractor, and a condenser. After the extract was made, it was refrigerated at 4°C to maintain its antibacterial properties.

Preparation of Mueller Hinton Agar plates : Utilizing a weighing balance to weigh the Mueller Hinton Agar. Put 7.5 grams of the medium in 250 millilitres of purified water. To fully dissolve the medium, boil for one minute while stirring frequently. Autoclave for fifteen minutes at 121°C. Using a level, horizontal surface, transfer the Mueller Hinton Agar to sterile petri plates, ensuring a consistent depth. Take out and let cool to room temperature.

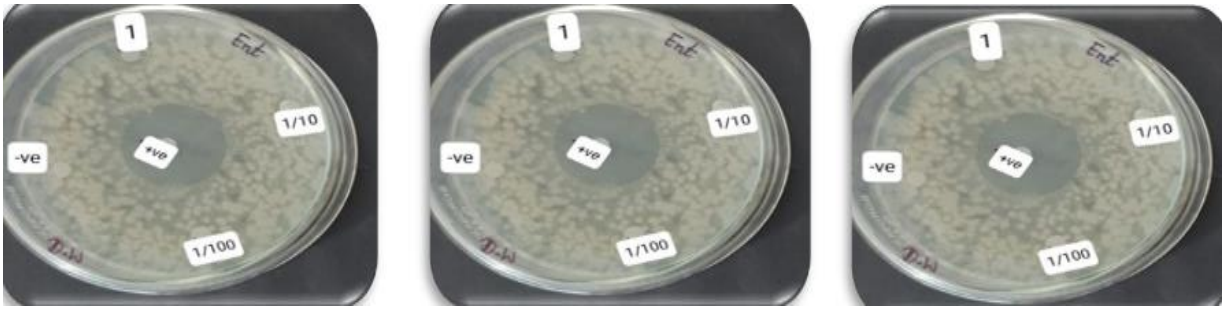
Antimicrobial activity of *Aegle marmelos* :The Kirby-Bauer disc diffusion technique was used to test the antimicrobial susceptibility. Whatman's sterile filter paper discs were used, and these discs were kept in serial dilutions of the two extracts with varying concentrations of aqueous extract and ethanol. The discs were left in a laminar air flow for drying. Over the solidified Muller Hilton agar-containing petri plates, the test organism *Enterococcus faecium* (MTCC 5695) was spread with its nutritional broth. The disc that was dipped in distilled water served as the negative control, and the antibiotic gentamicin served as the positive control. The discs that had previously been prepared in various extracts were placed on Petri dishes that held MHA medium. For a full day, these plates were stored in an incubator at 37°C.

RESULT: According to the studies, the clear zones that formed around the discs demonstrated how well *Aegle marmelos* inhibited microbes. The diameter of the zone of inhibition is used to calculate the extract's activity against a microorganism. Greater effectiveness of the extract against microbes is indicated by a larger diameter of the zone of inhibition. The absence of a zone of inhibition indicates that the test microorganisms were resistant to the extract.

According to the research, the ethanol extract displayed the maximum zone of inhibition, indicating a high level of maximum efficacy against *Enterococcus faecium*. The ethanol extract was found to be more effective than the aqueous extract.

Table: Antimicrobial activity of Aqueous extract of *Aegle marmelos*:

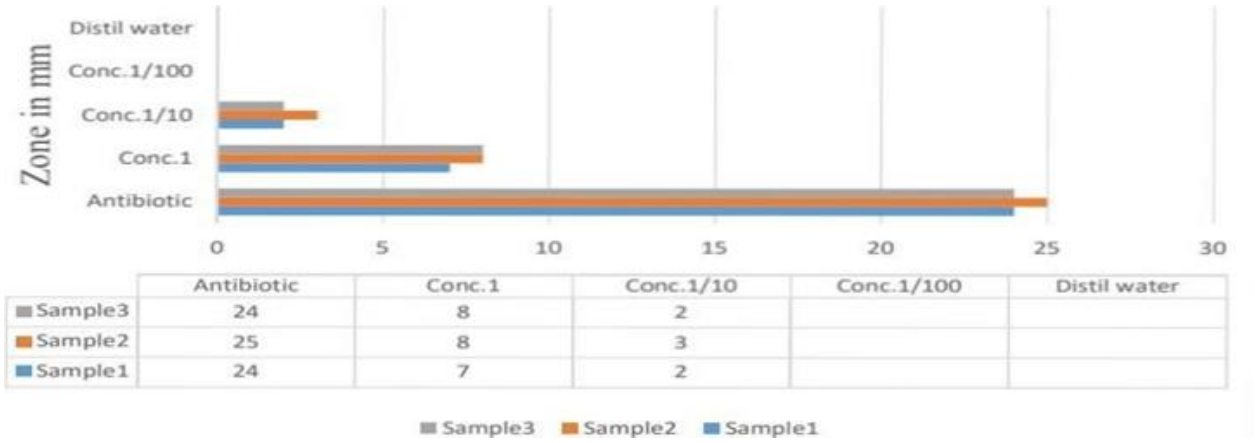
Aqueous			
	Sample1	Sample2	Sample3
Antibiotic	24mm	25mm	24mm
Conc.1	7mm	8mm	8mm
Conc.1/10	2mm	3mm	2mm
Conc.1/100	-	-	-
Distil water	-	-	-



Sample 1. **Sample 2.** **Sample 3.**

Fig: Antimicrobial activity of Aqueous extract of *Aegle marmelos* Sample (1,2,3)

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:06/April-2024 Impact Factor- 7.868 www.irjmets.com



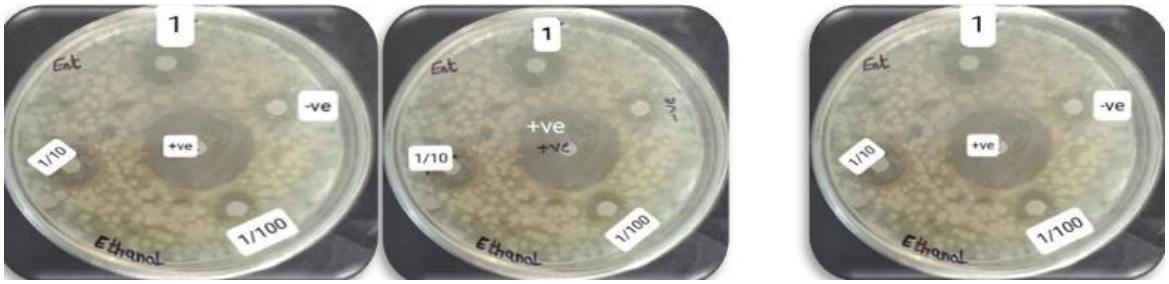
	Antibiotic	Conc.1	Conc.1/10	Conc.1/100	Distil water
Sample3	24	8	2		
Sample2	25	8	3		
Sample1	24	7	2		

Fig: Graphical representation of zone of inhibition of Aqueous extract of *Aegle marmelos*

Table: Antimicrobial activity of Ethanol extract of *Aegle marmelos*

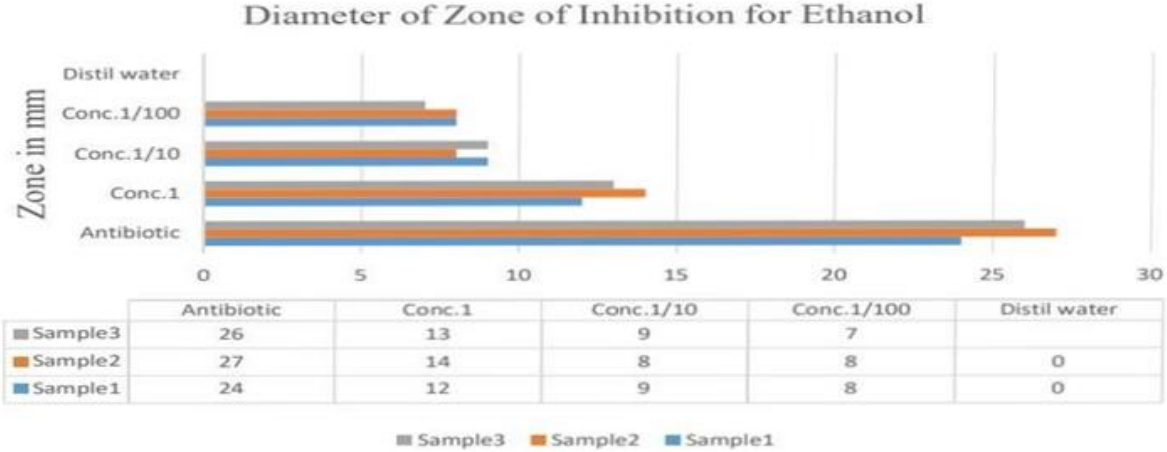
Ethanol

	Sample1	Sample2	Sample3
Antibiotic	24mm	27mm	26mm
Conc.1	12mm	14mm	13mm
Conc.1/10	9mm	8mm	9mm
Conc.1/100	8mm	8mm	7mm
Distil water	-	-	-



Sample 1. **Sample 2.** **Sample 3.**

Fig: Antimicrobial activity of Ethanol extract of *Aegle marmelos* Sample (1,2,3)



	Antibiotic	Conc.1	Conc.1/10	Conc.1/100	Distil water
Sample3	26	13	9	7	
Sample2	27	14	8	8	0
Sample1	24	12	9	8	0

Fig: Graphical representation of zone of inhibition of Ethanol extract of *Aegle marmelos*

According to this study, *Aegle marmelos* leaves have the strongest antibacterial activity against *Enterococcus faecium*. The *Aegle marmelos* leaves' ethanol extract produced the best results when compared to the Aqueous

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:06/April-2024 Impact Factor- 7.868 www.irjmets.com

extract, while the distilled water extract showed the least amount of inhibition. The analysis of *Aegle marmelos* antimicrobial activity revealed that, when compared to Aqueous extract, the maximum zones of inhibition against *Enterococcus faecium* were seen in the Ethanol extract.

Statistical study:

Mean: A means is quantity that has a value which is intermediate to the extreme values of a set of numbers. Mean serves to summarize a given group of data, often to better understand the overall value of a given data set.

$$\text{Mean} = \frac{\text{Sum of all values}}{\text{No. of all values}}$$

Table: The mean values of Aqueous extract of all the Sample of *Aegle marmelos*

	Antibiotic	Conc.1	Conc.1/10	Conc1/100	Distil water
Sample 1	24	7	2		
Sample 2	25	8	3		
Sample 3	24	8	2		
Mean	24.333333	7.666667	2.333333		

For the Antibacterial Activity of Aqueous Extract of *Aegle marmelos* the value of mean for samples 1, 2, 3 are 24.33, 7.667, 2.33.

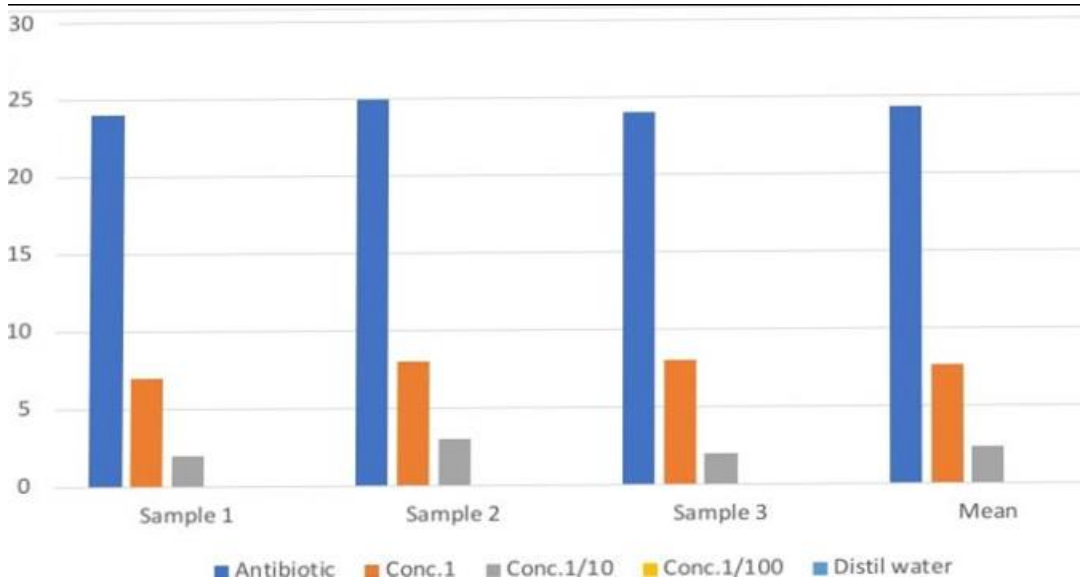


Fig: Graphical representation of mean of Aqueous extract of *Aegle marmelos*

Table: The mean values of Ethanol extract of all the Sample of *Aegle marmelos*

	Antibiotic	Conc.1	Conc.1/10	Conc.1/100	Distil water
Sample1	24	12	9	8	0
Sample 2	27	24	8	8	0
Sample 3	26	13	9	7	0
Mean	25.66	13	8.66	7.66	

For the Antibacterial Activity of Ethanol extract of *Aegle marmelos* the values of Mean for sample 1,2,3 are 25.667, 13, 8.667, 7.667.

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:06/April-2024 Impact Factor- 7.868 www.irjmets.com

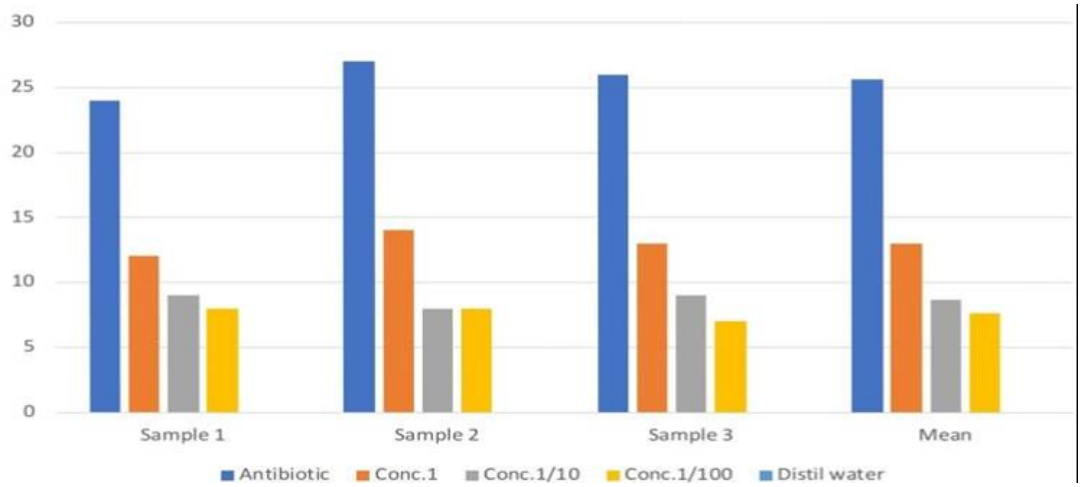


Fig: Graphical representation of mean of Aqueous extract of *Aegle marmelos*

Standard deviation:

Standard deviation is a statistic that measures the dispersion of a dataset relative to it's Mean.

$$\sigma = \sqrt{\frac{\sum(x_i - \mu)^2}{N}}$$

σ = population standard deviation

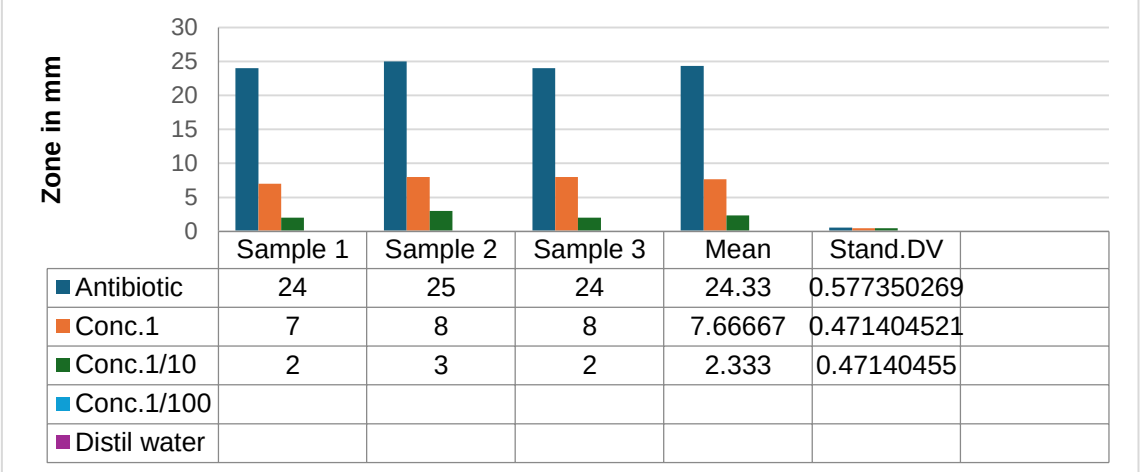
N = the size of the population

x_i = each value from the population

μ = the population mean

For the Antibacterial activity of Aqueous Extract of *Aegle marmelos* the value of mean and standard deviation for Sample 1,2,3 are as shown below in the graph.

Graphical representation of zone of inhibition of Aqueous extract of *Aegle marmelos* and the Mean and Standard deviation of values of Sample 1,2,3



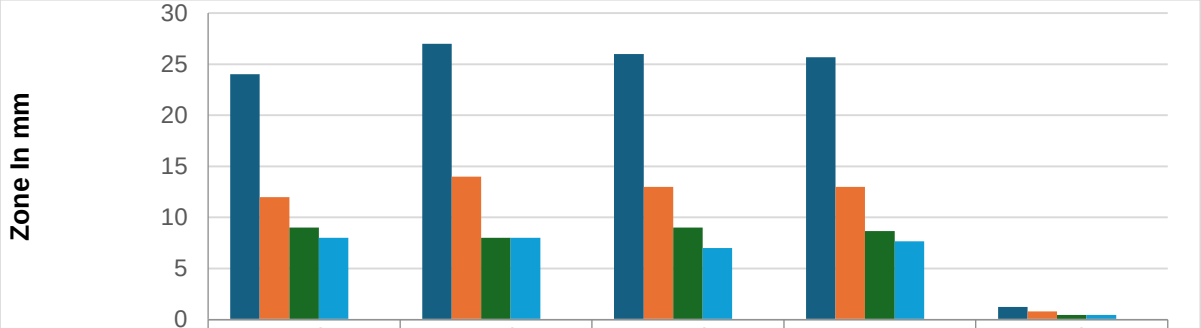
	Sample 1	Sample 2	Sample 3	Mean	Stand.DV	
Antibiotic	24	25	24	24.33	0.577350269	
Conc.1	7	8	8	7.66667	0.471404521	
Conc.1/10	2	3	2	2.333	0.47140455	
Conc.1/100						
Distil water						

The values of mean and standard deviation with the comparison of Antibiotic shows effective alteration at the level on the basis of statical calculations.

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:06/April-2024 Impact Factor- 7.868 www.irjmets.com

For the Antibacterial Activity of Ethanol extract of *Aegle marmelos* the values of Mean and Standard deviation for sample 1,2,3 are as shown in the graph.

Graphical representation of Zones of inhibition of Ethanol extract of *Aegle marmelos* and the Mean and Standard deviation of values of all the Samples(1,2,3)



	Sample 1	Sample 2	Sample 3	Mean	Stand.DV
Antibiotic	24	27	26	25.66	1.247223583
Conc.1	12	14	13	13	0.816496581
Conc.1/10	9	8	9	8.66	0.471416306
Conc.1/100	8	8	7	7.66	0.471416306
Distil water					

The values of Mean and Standard deviation with the comparison of Antibiotics shows effective alteration at the level on the basis of Statistical calculations.

III. DISCUSSION

The traditional Indian medical system is aware of the therapeutic benefits of plants. The use of ethno medicinal plants in India has been extensively studied. Plant phytochemical extracts have been proposed as sources of antiviral, antitumor, and antimicrobial agents in allopathic medicine **(Nair R. et al. 2005; Ramya S. et al. 2008)**. A huge number of researchers from all over the world have looked into how plant extracts affect bacteria.

There have been reports of antimicrobial, antifungal, and other properties in plants. Many researchers have clarified this **(Sasidharan VK. Et al. 1998)**, **(Ramya S et al. 2008)**, **(Sudharameshwari K. et al. 2007)**.

According to the current study, *Aegle marmelos* leaves have the strongest antibacterial activity against bacterial pathogens. The ethanolic extracts of *Aegle marmelos* leaves showed the best results when compared to all other solvents, while the distilled water extract showed the least amount of inhibition. The analysis of antimicrobial activity revealed that the maximum zone of inhibition against *Enterococcus faecium* was observed in the Ethanol extract when compared to the Aqueous extract.

Aegle marmelos was primarily found to contain tannins, phlobatannins, saponins, terpenoids, alkaloids, and polyphenols as antibacterial compounds.

IV. CONCLUSION

The purpose of the study was to Identify *Aegle marmelos* antimicrobial activity against bacteria. As a result, two different kinds of extracts—Aqueous and Ethanol were made from *Aegle marmelos* leaves. Kirby-Bauer method was utilized to ascertain the Antimicrobial Properties Disc Diffusion Method. The current study's findings showed that the bacteria are to Bael, which prevents them from growing.

As a result of this investigation, the bacteriostatic agent Bael (*Aegle marmelos*) is clearly depicted in the results. Aside from its possible antimicrobial properties. Given the well-known health benefits of bael, using the plant's medicinal qualities to improve health is safe. Ultimately, it can be said that Bael has antimicrobial qualities and may be used as a starting point for more studies. New properties will be investigated further in anticipation of humanity's promised future.

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:06/April-2024 Impact Factor- 7.868 www.irjmets.com

V. REFERENCE

[1] Chakravarti BK and Gode kd.(1985)*Isolation of epicatechin from Pterocarpus marsupium and It's pharmacological Action.* Planta Medica. ;1:56.

[2] Cowan. M. M.(1999) *Clinical microbiology review,* vol.12, no.4, pp.564-582.

[3] Lis-Balchin. M and Deans. S. G.(1997) *Jour. of. Appl. Micro.* Vol.82.no.6, pp.756-762.

[4] Nair R, Kalariya T and Chanda S.(2005) *Antibacterial Activity of some selected Indian Medicinal Flora.* Turk J Boil; 29:41-47.

[5] Ramya S, Govindaraji V, Kannan NK and Jayakumararaj R (2008) *Invitro Evaluation of Antibacterial Activity Using Crude Extracts of Catharanthus roseus* L.(G.) Don. *Ethnobotanical Leaflets.*a; 12:1013-18.

[6] Sasidharan Vk, Krishna Kumar T and Manjula CB. (1998) *Antimicrobial Activity of Nine Common Plants in Kerala, India.* PJS. ;127(1): 59-67.

[7] Sudharameshwari K and Radhika J.(2007) *Antibacterial screening of Aegle marmelos, Lawsonia inermis and Albizzia libbeck. Afr J. Traditional, Complementary and Alternative Medicines* ;4:205-10.

[8] Vlietinck, A. J and Lindsay S.(1995) *Journal of ethano pharmacol,* 31-47.