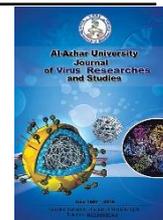




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Assessment of antibacterial activity of two medical plants *Azadirachta indica* (Neem) and *Senna alexandrina* against *Staphylococcus aureus*, *Enterococcus faecalis* and MRSA

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Abstract

Medicinal plants play a vital role in traditional folklore, as they can prevent and treat many diseases caused by microbes. In the present study, the antibacterial activities of ethanolic extracts of two medicinal plants Neem plant (*Azadirachta indica*) and *Senna alexandrina* were evaluated against Gram-positive bacteria *Staphylococcus aureus*, *Enterococcus faecalis*, and MRSA. Neem (*Azadirachta indica*) leaves ethanolic extracts produced significant antibacterial potential against Gram-positive bacteria. For *Enterococcus faecalis* at different concentrations of Neem ethanolic extracts (400, 300, 200, and 100 mg/ml) it gives different inhibition zones 12, 11, 10, and 5mm respectively, and for *Staphylococcus aureus* it gives different inhibition zones 13, 11, 9 and 3mm respectively, and against MRSA, it gives 12, 11, 9 and 0 mm respectively. MIC of Neem (*Azadirachta indica*) leaves ethanolic extracts against *Enterococcus faecalis* was at 150 mg/ml, MIC against *Staphylococcus aureus* was at 9.3 mg/ml, and for MRSA MIC was at 37.5mg/ml, but the antibacterial activity of *S. alexandrina* plant extract at different concentrations starting from (400, 300, 200 and 100 mg/ml) It has shown a great antibacterial potential against *Staphylococcus aureus* it gives different inhibition zones 22, 20, 18 and 13mm respectively. But it has shown no effect on Gram-positive bacteria *Enterococcus faecalis* and MRSA, since it did not give any growth inhibition zone with these bacteria.

Keywords: Medicinal plants, Antimicrobial activity, *Enterococcus faecalis*, Neem plant, *S. alexandria*.

1. Introduction

Antimicrobial resistance is one of the major problems facing global health today

[1], and it is a major source of morbidity and mortality globally [2]. Nowadays it is

becoming the leading cause of death globally [3]. A large number of bacteria have acquired and developed antimicrobial resistance mechanisms [2], which constitutes a burden on the global health system with the increasing financial cost [4]. With the increase in drug resistance, there are very few alternatives for patients, and as a result, the number of deaths associated with it has increased [5]. In America, there are 23,000 deaths annually related to drug resistance [2]. The emergence of infectious diseases and the development of antibiotic resistance in bacteria resulting in decreased action or failure of existing antibacterial agents [6], has resulted in an urgent need for the discovery of new, safe, and efficient antibacterial agents [7]. Compounds derived from plants can provide an essential source of new types of antibiotics. There are many types of phytochemicals of plant extract that can exert potential activity on sensitive and multidrug-resistant bacteria [6-8]. Medicinal plants play an important role in disease treatment in economically poor areas of the world, such as Africa. [9- 10]. The southern Mediterranean region, including Egypt, is a rich source of medical plants that have had many uses in alternative medicine throughout history. [11- 12]. Nowadays microbiologists doing research on plant products for preparing drugs against microbial diseases because plant products are decomposable and do not have any side effects, not harm human health [13]. *Senna alexandrina* is an all-season evergreen shrub 60-80 cm tall. The Senna plant has a lot of medicinal uses. These plants can be found in Asia, India, and Africa. [14]. The number of Senna species is approximately 260, but some authors trust the number of Senna species to be approximately 350. [15]. In Egypt, *Senna alexandrina* is a common medicinal plant, which is spread on both sides of the Nile Valley of the Nile River and the plant is used to treat stomachache and constipation due to its laxative effects [15-

16]. The pods (fruits) are high in glycosides, including the anthraquinones, as well as sennosides A, B, C, and D [17-18]. The major phytochemical compounds found are Phenolic compounds globally in plants (about 10,000 compounds) and are responsible for the biological activity of plant extracts. [19- 20]. Research on natural products in plants is closely related to the presence of phenolic compounds as they have antimicrobial and antioxidant activities [21- 22]. Extracts of these plants showed antimicrobial activity against Gram-positive bacteria-*Staphylococcus aureus* [23]. *Azadirachta indica* (*A. indica*) is one of the *Meliaceae* families known as Neem. It has been used in traditional medicine since ancient times to treat a range of human diseases [24]. The leaves, seeds, and roots of Neem contain antibacterial and antifungal agents [25-26]. This biological activity of Neem stems from many bioactive compounds that are structurally and chemically diverse, with more than 140 compounds found in different parts of the plant [27]. Several types of biological compounds are extracted from Neem, including ketones, carotenoids, flavonoids, steroids, and phenolic compounds [28]. The antibacterial activity of *A. indica* leaves extract has been documented on different bacterial species including gram-positive bacteria such as *Staphylococcus* species [29 – 30]. The aim of this study is to an assessment of antibacterial activity of two medical plants neem plants against *staphylococcus aureus* and MRSA.

2. Materials and Methods

2.1 Bacterial isolates

Laboratory stock of pathogenic Gram-positive bacteria such as *Enterofaecalis*, *Staphylococcus aureus*, and MRSA were previously isolated and identified by cultural characteristics, biochemical tests, and PCR assays.

2.2 Sampling strategy, Collection of the medical plants

The plant leaves were collected from Jeddah - Saudi Arabia. The collected samples were packed in plastic containers and were transported to the Department of Microbiology, Faculty of Science, King Abdul-Aziz University, Jeddah - Saudi Arabia for the preparation of leaf extract and extensive studies.

2.3 Preparations of ethanolic leaf extract (Ethanolic extraction)

Fifty grams of dried powder of grained leaves were soaked in 500 ml 80% ethanol for 48 hours and kept in a rocking shaker at 75 rpm at room temperature 25°C the mixture was allowed to stand for 30min. Then filtered with filter paper. Rotatories evaporate was used at 45-55°C to evaporate excess water for 2-3 hours. After complete evaporation the residue obtained was semi-liquid, then decant into Petri dishes to complete dryness for one day then the residue was collected, weighed, and dissolved in di-methyl-sulfoxide (DMSO) 5% to obtain starting concentration of 500 mg/ml. [31].

2.4 Detection of the antimicrobial effect of the ethanolic plant extracts on Gram-positive bacteria

2.4.1 Agar well diffusion method (determination of inhibition zone diameter)

The molten Mueller Hinton agar was poured into each of the sterile petri plates 20 ml and allowed to solidify, bacteria strains were spread with 0.5 McFarland using a sterile cotton swab and allowed to stand for 10 minutes four open wells were made in each plate with sterile cork bore, add 100 µl of the ethanolic plant extracts in each well from different concentration 400,300,200,100mg/ml and leave it for 24 hours at 37 °c then start to take the result

and measure the diameter of inhibition zone.

2.4.2 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts against Gram-positive bacteria

Microplate (96 polystyrene well) was used for the preparation of different concentrations of plant extracts by serial dilution. Bacterial strains were taken after 24 hours of incubation at 35°C. The turbidity of bacteria suspension was adjusted to 0.5 McFarland equivalent to a concentration of $(1-2 \times 10^8)$ CFU /ml 10 µL aliquot of bacterial suspension in supplemented MH broth .100 µl of the medium was dispensed in each well of 96 well micro-plates that started from concentration 500mg/ml for Neem plant extract and 300 mg/ml for *S.alexandrina* plant extract. Row 2 was used as a negative control with 200 µl of media Row1was used as a positive control with 100 µl of media and +100 µl of bacteria suspension, the micro-plates were sealed in a plastic bag and were incubated for 24 hours at 37°C. MIC was determined as the lowest concentration of each plant extract that inhibited the bacteria growth. The minimum bactericidal concentration (MBC) was obtained by subculturing from each well of the microplate onto a nutrient agar plate. The well containing the lowest concentration of the extract that failed to show growth on subculture was considered as MBC for that test strain. Phenol red indicator was also employed as a growth indicator at the end of the incubation period. [32] & [33].

3. Results

3.1 Agar well diffusion method

3.1.1 Effect of *Azadirachta indica* ethanolic extract on Gram-positive bacteria

Azadirachta indica leaves ethanolic extracts produced significant antibacterial potential against Gram-positive bacteria. For *Enterococcus faecalis* at different concentrations of ethanolic extracts of Neem plant (400, 300,200, and 100 mg/ml) it gives different inhibition zones

12, 11, 10, and 5mm respectively, and for *Staphylococcus aureus* it gives different inhibition zones 13,11,9 and 3mm respectively, and against MRSA, it gives 12,11,9and 0 mm respectively as shown in Fig. 1 &2. Table 1.

Table (1): Effect of *Azadirachta indica* ethanolic extract on Gram +ve bacteria.

Gram-positive bacteria	Zone of inhibition in mm at different concentrations (mg/ml)			
	400mg/ml	300mg/ml	200mg/ml	100mg/ml
<i>Enterococcus faecalis</i>	12mm	11mm	10mm	5mm
<i>Staphylococcus aureus</i>	13mm	11mm	9mm	3mm
MRSA	12mm	11 mm	9mm	-ve

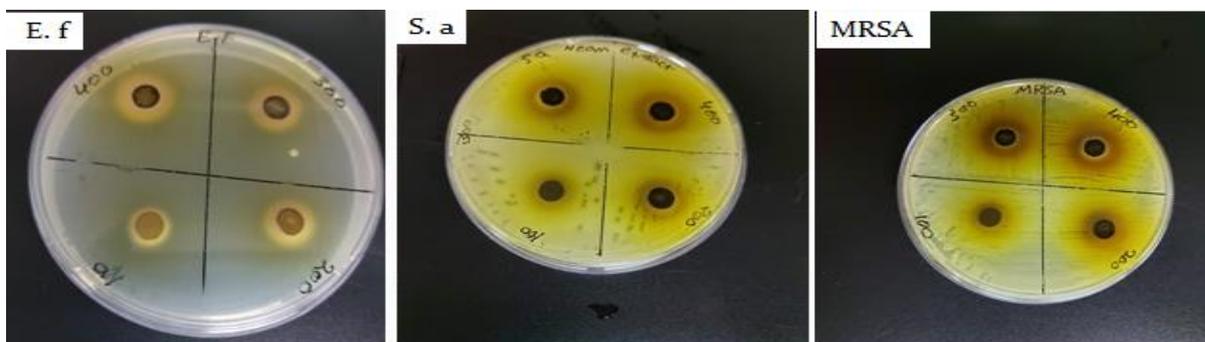


Figure (1): Effect of *Azadirachta indica* ethanolic extract at different concentrations starting from 400,300, 200, and 100 mg/ml on Gram-positive bacteria:

Enterococcus faecalis (E. f) gives different inhibition zones 12,11,10 and 5mm, respectively.

Staphylococcus aureus (S. a) gives different inhibition zones 13,11,9 and 3mm, respectively.

MRSA gives different inhibition zones 12,11,9and 0 mm respectively.

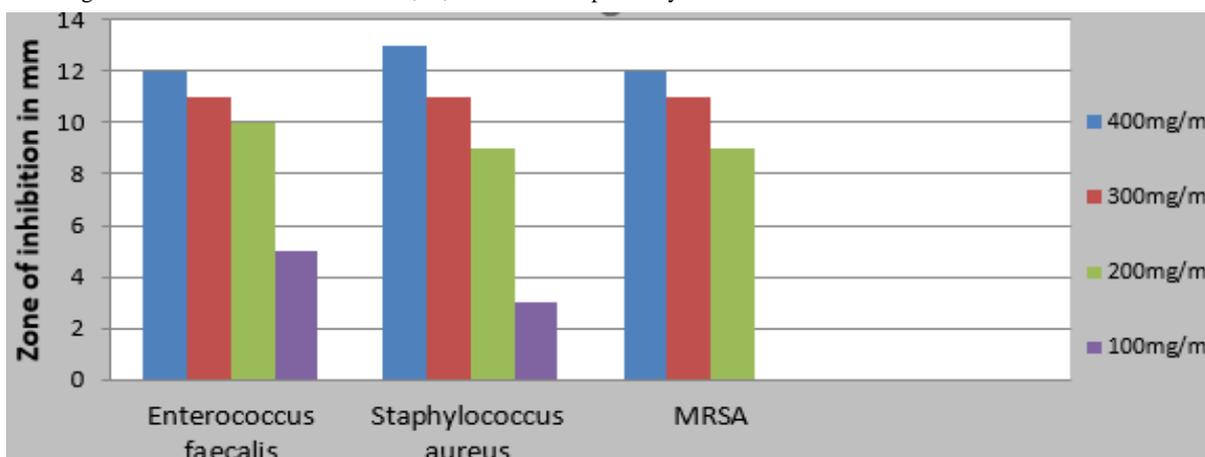


Figure (2): Effect of *Azadirachta indica* ethanolic extract on Gram -positive bacteria.

3.1.2 Effect of *S. alexandrina* ethanolic extract on Gram-positive bacteria

The antibacterial activity of *S. alexandrina* leaves plant extract at different concentrations starting from (400,300,200 and 100mg/ml) showed great antibacterial

potential against *Staphylococcus aureus* it gives different inhibition zones 22, 20, 18, and 13mm, respectively Fig.3& 5, Table 2. But it has shown no antimicrobial activity on Gram-positive bacteria *Enterococcus faecalis* and MRSA since it did not give any growth inhibition zone with these bacteria Fig .4

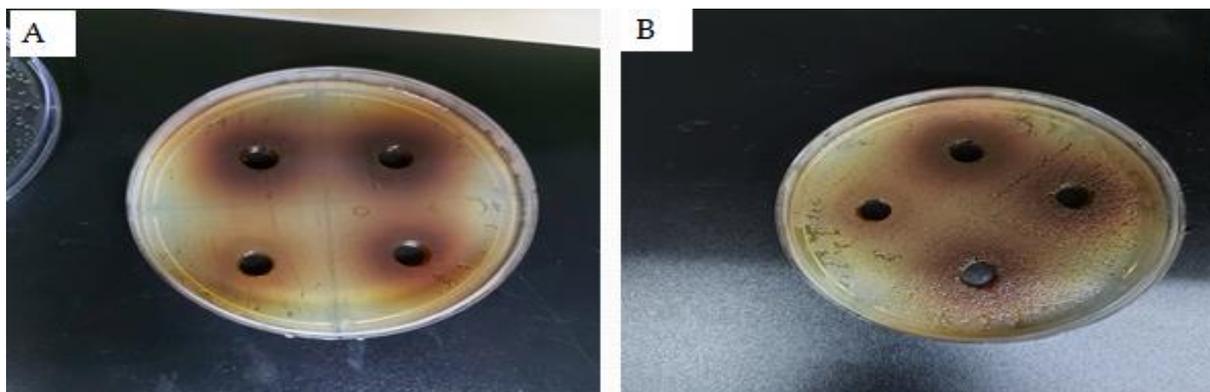


Figure (3): Effect of *Senna alexandrina* ethanolic extract at different concentrations starting from 400,300,200 and100 mg/ml does not give inhibition zone with Gram –positive bacteria the bacteria. A: *Enterococcus faecalis* (E.f :) B: MRSA.



Figure (4): Effect of *Senna alexandrina* ethanolic extract on *Staphylococcus aureus* at different concentrations starting from 400,300,200 and100 mg/ml and it gives different inhibition zones 22, 20, 18, and 13mm respectively.

Table (2): Effect of *Senna alexandrina* ethanolic extract on Gram-positive bacteria.

Gram-positive bacteria	Zone of inhibition in mm at different concentrations (mg/ml)			
	400 mg/ml	300mg/ml	200mg/ml	100mg/ml
<i>Enterococcus faecalis</i>	-ve	-ve	-ve	-ve
<i>Staphylococcus aureus</i>	22 mm	20 mm	18 mm	13 mm
MRSA	-ve	-ve	-ve	-ve

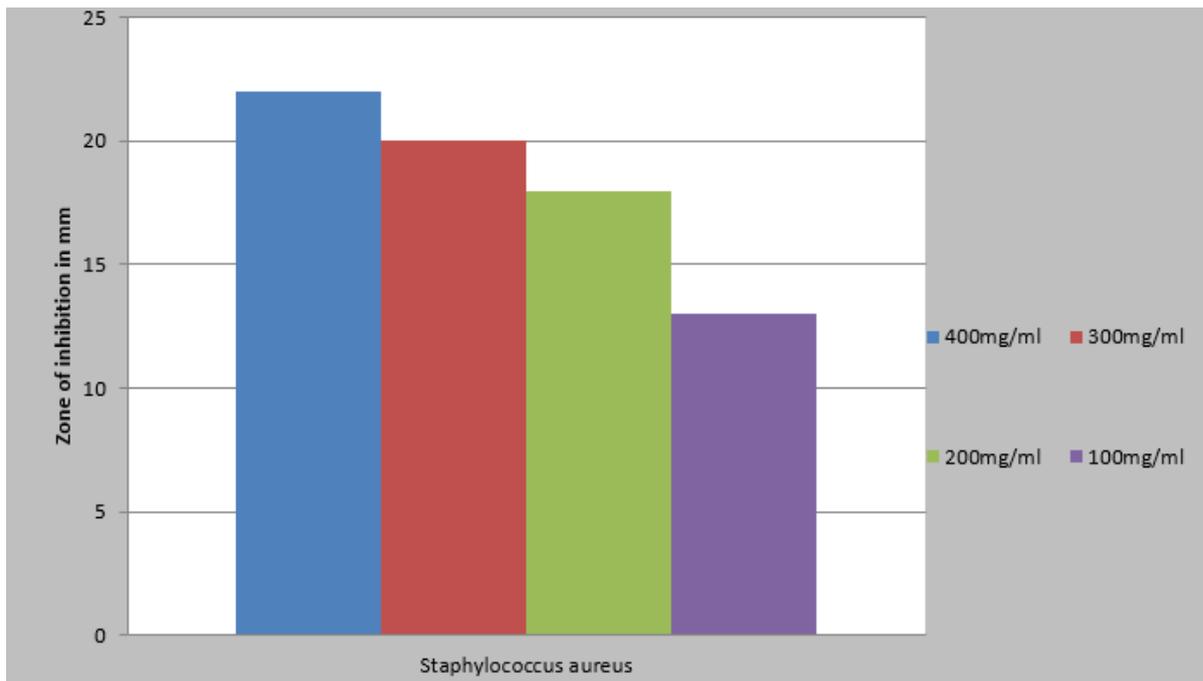


Figure (5): Effect of *Senna alexandrina* ethanolic extract on Gram positive bacteria. *S.aureus*.

3.2 Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of plant extracts against Gram-positive bacteria

MIC of ethanolic extracts of Neem plant against *Enterococcus faecalis* was at 150 mg/ml, MIC against *Staphylococcus aureus* was at 9.3 mg/ml and for MRSA MIC was at 37.5mg/ml as shown in fig.6& 7, Table 3.

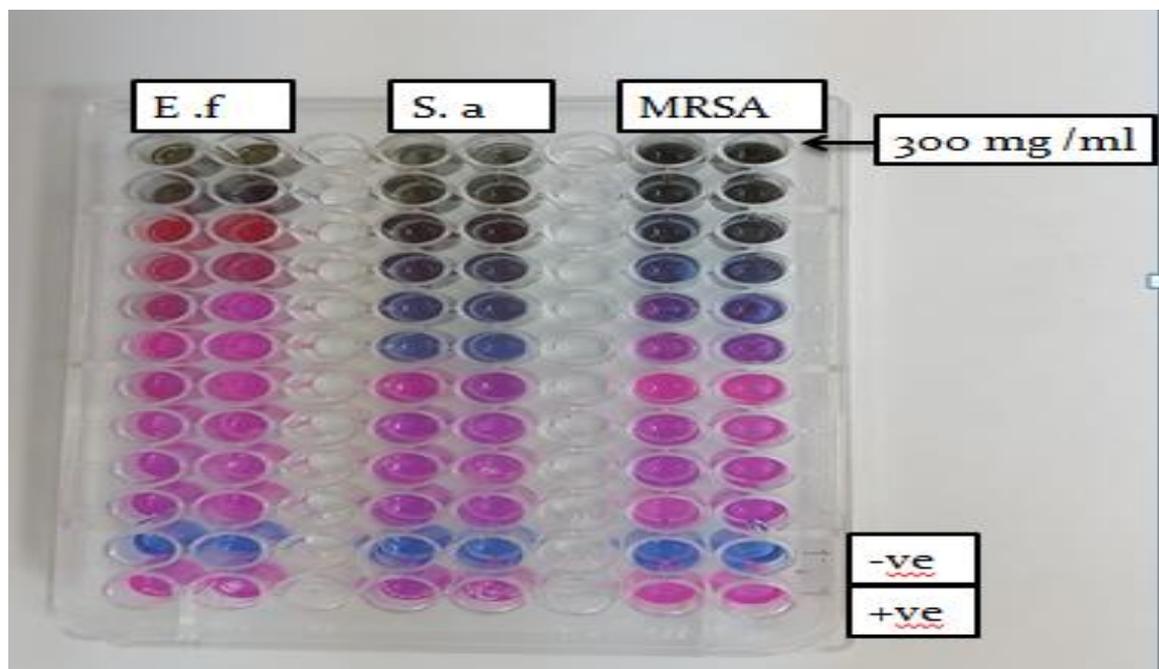
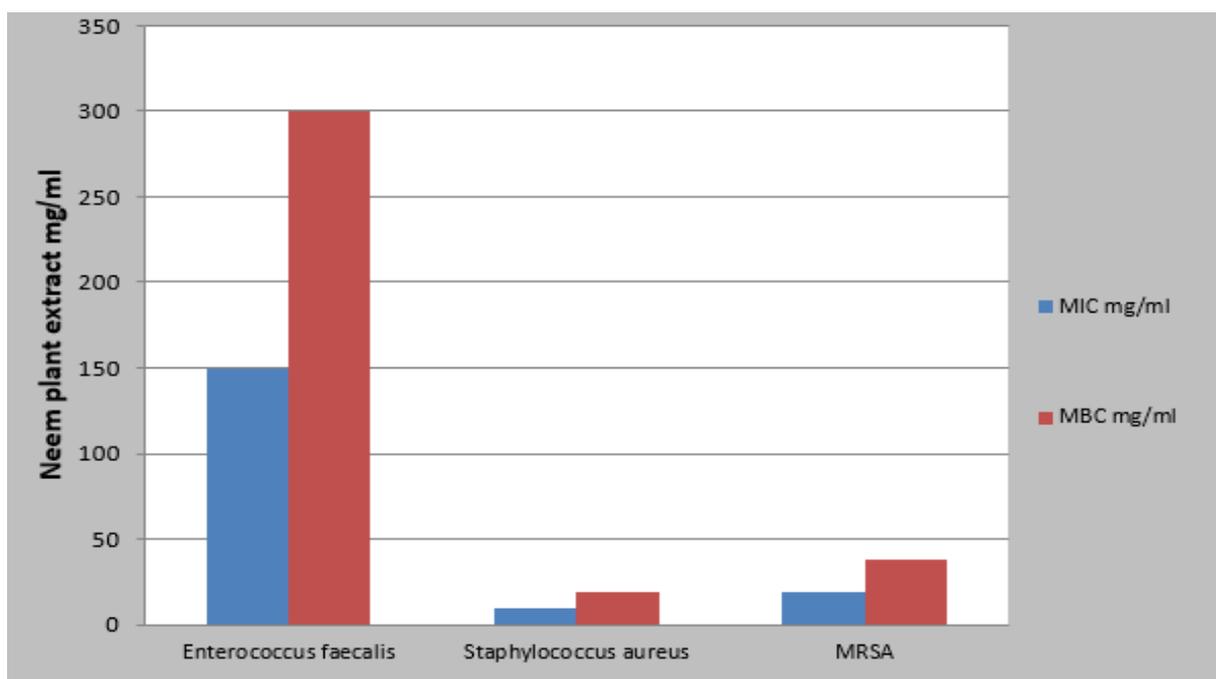


Figure (6): MIC micro-plate starting from concentration 300 mg/ml, and it decreases descendently to half at each well. -ve (negative control) and +ve (positive control) **E. f:** MIC of *Enterococcus faecalis* at 150mg/ml **S. a:** MIC of *Staphylococcus aureus* at 9.3 mg/ml. **MRSA:** MIC of MRSA at 18.75 mg/ml.

Table (3): MIC and MBC of Neem plant (*A. indica*) against Gram-positive. bacteria.

Gram-positive bacteria	MIC (mg/ml)	MBC (mg/ml)
<i>Enterococcus faecalis</i>	150	300
<i>Staphylococcus aureus</i>	9.3	18.6
MRSA	18.75	37.5

**Figure (7):** MIC and MBC of *A. indica* against Gram +ve bacteria.

4. Discussion

The antibacterial properties of Neem leaves seem to be linked to their antioxidant and anti-inflammatory effects, which in turn, affect bacterial cell processes and inhibit cell growth [34 - 35]. The present study revealed that the ethanolic extract of Neem plant leaves has an antibacterial effect against Gram-positive bacteria *E. faecalis*, MRSA, and *S. aureus* that is similar to a study by Mistry [36] revealed that Neem extract was active against *E. faecalis* and *S. aureus*. A similar study conducted by

Parashar, Sutar, and Sanap [37] showed that the optimal effect on *S. aureus* can be achieved by the Neem extract from the plant leaves. Research shows that Neem is active against Gram-positive bacteria. A study by Rathod [38] examined the effects of ethanol and aqueous extracts of Neem leaves and bark on *S. aureus*. Also, the present results agree with the results of Sucheta, Sohini and Narayan [39] who revealed that ethanolic extracts of Neem (*Azadirachta indica*) leaves produced significant antibacterial potential against Gram positive *Staphylococcus aureus*, *Bacillus subtilis* and *Listeria monocytogenes*, and Gram-negative

Pseudomonas aeruginosa and *Escherichia coli* between 10-20 mg/ml concentration but failed to kill *Salmonella typhimurium* during *in vitro* study. Ethanolic extracts of shade dried and powdered *Senna alexandrina* leaves showed antibacterial activity against the tested Gram- positive bacteria *Staphylococcus aureus* only, but it has shown no effect on gram-positive bacteria *Enterococcus faecalis* and MRSA, since there is no growth inhibition zone with these bacteria. It is also clear that the chemical constituents of (ethanol, methanol, petroleum ether, and aqueous solutions) present in *Senna alexandrina* work against microbial diseases .these chemical constituents were extracted and the effectiveness of these chemical compounds was measured with the help of the disc diffusion method for following bacteria's Gram-positive bacteria-*Staphylococcus aureus*, [23] This result is consistent with the result of our research, where the Senna plant showed antibacterial activity against Gram-positive bacteria-*Staphylococcus aureus* at different concentrations starting from (400,300,200 and 100mg/ml) showed great antibacterial potential against *Staphylococcus aureus* it gives different inhibition zones 22, 20, 18 and 13mm ,respectively.

5. Conclusion

The focus of this particular study was to assess how Neem leaf extracts affect Gram-positive bacteria. The ethanolic extract of *A. indica* leaves possessed strong antibacterial activity against different types of Gram-positive bacteria *E. faecalis*, MRSA, and *S. aureus*. Also *Senna* is a very energetic plant and has important value in the market and traditional medicinal system as it has an antimicrobial effect against *S.aureus* .

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