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Evaluation of antimicrobial activity of Senna (*Senna italica Mill*) plant and its synergistic effect with antibiotic drugs

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Abstract
Senna (*Senna italic* Mill) one of the famous medicinal plants in many Arabic countries. In this study, the antibacterial activity of Senna and its synergistic effect with antibiotics were investigated. Different bacterial groups were used in this study including: *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae (K. pneumonia), Acinetobacter baumannii* (*A. baumannii*), *Pseudomonas aeruginosa (P. aeruginosa), Staphylococcus aureus* (*S. aureus*) *and Salmonella spp.* The minimal inhibitory concentration (MIC) of the plant extracts against *E. coli, S. aureus* and *P. aeruginosa* were assessed using microdilution method. The synergistic effect between plants and extraction of antibiotics was evaluated using disk diffusion method. The results indicated that Senna plant has antibacterial properties against the tested bacteria with various degrees; this could be attributed to the plant active ingredients. Moreover, the zones of inhibition of all tested microorganisms resulted from plant extract-antibiotic combinations were greater than those resulted from antibiotics solely. The results also showed that methanolic extract of the plant has more inhibitory effect than that of the water extract. Further investigation of the plants extract to isolate and identify the active ingredients is recommended.

Keywords: medicinal plants, inhibition zone, ampicillin, *escherichia coli, staphylococcus aureus*

1. Introduction
An antimicrobial or antibiotic is a natural agent that kills microorganisms or inhibits their growth [1, 2]. Using of substances with antimicrobial properties is a common practice for at least 2000 years ago, as well as using plant extracts to treat infection. Today, numerous antimicrobial agents have been examined to treat a wide range of infectious diseases.

Many parts of medicinal plants are utilized in the extraction of raw drugs and possess varied medicinal properties [3, 4]. Rural people learned by trial and error to distinguish useful plants with beneficial effects by choosing appropriate combinations and processing methods to acquire consistent and optimal results [5].

Phytochemical contemplates have attracted the attention of botanists because of the advancement of new and modern systems. These strategies assumed a huge part in the look for extra assets of crude material for pharmaceutical industry [6].

Hail region is an essential part of the natural life in Saudi Arabia with rich floral diversity. Many plant species have been identified and classified into different families and orders in Hail district. The characteristic flora of Hail is ordinarily utilized in different ways such as source of food, herbal medicine and fodder for grazing animals.

The Senna is a typical shrub occurring mostly in the tropics and subtropics. It is a standout amongst the most popular indigenous medicinal plant of Saudi Arabia. Numerous types of Senna are generally utilized customarily to various sicknesses, for example, intestinal difficulties, haemomorphoids, circulatory system problems, calculi in the urinary system and sexually transmitted ailments [7]. The pods of *Senna* plant are rich source of bioactive compounds widely used as laxative [8]. Folk medicine practiced in some parts of the Kingdom of Saudi Arabia has helped people to prevent and cure many diseases such as stomach disturbances, diabetes, constipation, rheumatism and urinary disease. However, very little is known about folk medicine in Northern Saudi Arabia. Therefore the present study has been initiated to evaluate the antibacterial activity of Senna (*Senna italica* Mill) and its synergistic impact effect with antibiotic in Hail area.

2. Materials and methods
2.1 Collection of the plant and microorganisms samples
The plant materials used in this study consisted of *Senna italic* Mill (Senna) was collected from various sites in Hail region.

Antibiotics resistant bacteria used in the study included: *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae (K. pneumonia), Acinetobacter baumannii, Pseudomonas aeruginosa (P. aeruginosa) and Staphylococcus aureus* (*S. aureus*) *and Salmonella spp*. These microorganisms were obtained from the microbiology laboratory of the department of Biology, Faculty of Science, University of Hail, and also from King Khalid Hospital, Hail. The microorganisms were maintained on Brain Heart Infusion (BHI) agar medium (HiMedia) at 4 ºC for further experiments.

2.2 Preparation of plant extract
For aqueous extraction, 20 g of air-dried powder were added to150 ml of distilled water and boiled on slow heat for 2 hours. Then it was filtered through 8 layers of muslin cloth and centrifuged at 5000g for 10 min and the supernatant was collected. This procedure was repeated twice; after 6 hours, the supernatant was collected at an interval of 2 hours, pooled together and concentrated to make the final volume one-fourth of the original volume

2.3 Preparation of culture media, inoculums, reagents and antibiotics

Types of media in this study included: Brain Heart Infusion broth, Nutrient agar (bio-life) and Mueller-Hinton agar (HiMedia). Also methanol and water were used for extraction process. These media and the solvent were purchased from some company in Jeddah city.

The Antibiotics used in the study which included: Vancomycin, Tetracyclines, Chloramphenicol and Ampicillin were purchased from pharmacies in Hail city.

2.4 Preparation of plant extracts standard concentasrations

One gram of each aqueous extract and alcohol pre-prepared (each separately) were taken and the aqueous extract was dissolved in 5 grams sterile distilled water, while alcoholic extracts was dissolved in 5 ml of DiMethylSulphoxide (DMSO). Thus 200 mg / ml of stock were obtained as a standard concentration of aqueous and methanol extracts. Aqueous extracts were sterilized and methanol extract was pasteurized for 15 minutes at temperature 62 ºC.

2.5 Preparation of inocula

Stock cultures were maintained at 4°C on nutrient agar slants for bacteria. Active cultures for experiments will be prepared by transferring a loopful of culture to 5 ml of Brain Heart Infusion broth and incubated at 37 °C for 24 hours.

2.6 Antibiotics activity assay

Antibiotic discs were placed on the surface of a Mueller-Hinton agar that had been inoculated with test microorganisms. During incubation, the antibiotics diffuse outward from the discs creating a concentration gradient. After 18-24 hours, the zone diameter of inhibition was measured and reference tables were used to determine if the bacteria are Sensitive (S), Intermediate (I) or Resistant (R) to the antibiotic.

2.7 Plant extracts activity assay

The paper disk diffusion assay was used to assess the plant antibacterial activity according to Kumar *et al*. [9]. A suspension of testing microorganisms was spread on Muller Hinton Agar (MHA) medium. The filter paper discs (5mm in diameter) will be placed on the agar plates which were inoculated with the tested microorganisms and then impregnating with 20µl of plant extract (concentration 200 mg/ml). The plates were subsequently incubated at 37°C for 24 Hrs. After incubation the growth inhibition zone was quantified by measuring the diameter of the zone of inhibition in mm.

2.8 Determination of MIC of plant extract by Microdilution Method

The 96-well plates were prepared by dispensing 50 µl of Mueller–Hinton broth for bacteria, into each well. A 50 µl from the stock solution of tested extracts (concentration of 200 mg/ml) were added into the first row of the plate. Then, two fold; serial dilutions will be performed by using a micropipette. The obtained concentration range was from 100 to 0.1953 mg/ml, and then 10 µl of inocula was added to each well except a positive control inocula was adjusted to contain approximately 1.5X108 CFU/mL. Plant extract with media were used as a positive control and inoculum with media were used as a negative control. The test plates were incubated at 37 °C for 18 h. After 18 h 50 µl of a 0.01% solution of 2, 3, 5-triphenyl tetrazolium chloride (TTC) was added to the wells and the plates were incubated for another hour. Since the colorless tetrazolium salt was reduced to red colored product by biological active bacteria, the inhibition of growth was detected when the solution in the well remained clear after incubation with TTC. MIC was defined as the lowest sample concentration showing no color change (clear) and exhibited complete the inhibition of growth [10, 11].

2.9 Synergism between plant extract and antibiotics

The bacterial cultures were grown in BHI broth at 37º C. After 4 h of growth, each bacterium was inoculated on the surface of Mueller-Hinton agar plates. Subsequently, the antibiotic disk (diameter =5mm) were placed on the surface of each inoculated plate and then added 20 µl of plant extract, to identify synergies effect between the plant extract at a concentration of 200mg/ml) and antibiotics. The plates were incubated at 37º C for 24 h. The diameters of clearing zones will be measured.

3. Results and discussion

3.1 Antibacterial activity of Senna plant

Plants are the biggest medication stores which create perpetual bioactive concoction mixes affecting animal and human health [12]. They are rich in secondary - metabolites and are potential source of medications. The present study investigated the impact of Senna water and methanol extracts on growth of the tested bacteria. The Inhibition zone diameters of the tested bacteria against the tested plant are shown in Table (2). The tested bacteria used in this study were: *E. coli, K. pneumonia, A. baumannii, P. aeruginosa, S. aureus*) *and Salmonella spp.*

From the results presented in Table (1) and Figures (1) it is clearly seen that water extracts of Senna did not inhibited growth of most of the tested bacteria with exception to *P. aeruginosa and A. baumannii* bacteria where the inhibition zone diameter was 2 mm for both microorganisms. On the other hand, the methanol extract of the plant inhibited all tested microorganisms with varying degrees, and the inhibition zone of *E. coli, K. pneumonia, A. baumannii, P. aeruginosa, S. aureus*) *and Salmonella spp.* was 8, 2, 2, 3, 5 and 4 mm, respectively. In general, the methanol extract of the tested medicinal plants has more inhibition effect against the tested bacteria in compare to the water extract as indicated in Figure.

Table 1: Inhibition zone diameters of *Senna italica* (Senna) against the tested bacteria

No.	Extract	Test organism	Inhibition zone (mm)	Control
1	W	*P. aeruginosa*	0	0
2	W	*K. pneumonia*	0	0
3	W	*E. coli*	0	0
4	W	*A. baumannii*	2	0
5	W	*S. aureus*	0	0
6	W	*Salmonella spp.*	0	0
6	M	*P. aeruginosa*	3	0
7	M	*K. pneumonia*	2	0
8	M	*E. coli*	8	0
9	M	*A. baumannii*	2	0
11	M	*S. aureus*	5	0
12	M	*Salmonella spp.*	4	0

W: Water extract; M: Methanol extract

Antibacterial activity of spices and different plants have been all around reported [12-14]. Vlietinek *et al.* [15] screened about 100 medicinal plants, utilized by conventional healers to infections in Rwanda, for their antimicrobial properties. Their study showed that 45% of the tested plant were active against *Staph aureus*, 2% against *E. coli*, 16 % against *Pseudomonas aeruginosa*. In Philippines the antibacterial activities of crude ethanol extracts of 12 Philippine medicinal plants were investigated by Demetrio *et al*., [16]. They found that favorable antagonistic activities were exhibited by the ethanol extracts of *Psidium guajava*, *Phyllanthus niruri*, *Ehretia microphylla* and *P. betle* which had the greatest potential value against both Gram-negative and Gram-positive multidrug-resistant bacteria. Abdalla and Abdallah [17] found that a total of 142 plant species belonging to 64 families in Sudan, showed antibacterial activities when extracted using different solvents (polar and non-polar) and tested against some gram negative or gram positive bacteria *in vitro*.

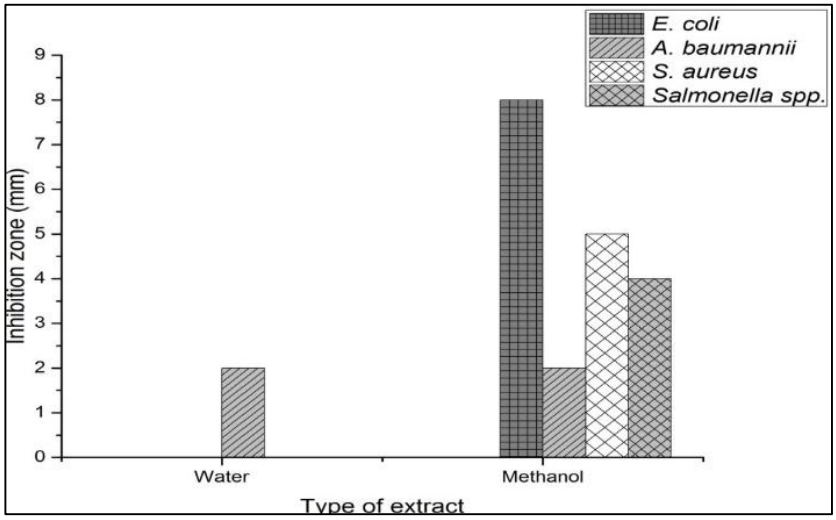

Fig 1: The inhibition zone of tested bacteria using Senna water and methanol extract

3.2 Synergism between the plant extracts and antibiotics

The synergism between Senna plant extract and antibiotics is presented in Table (2). It is found that the plants extracts inhibited the tested microorganisms by using antibiotics mixture, moreover, the inhibition zones diameters increased when combined with antibiotics mixture. This means the synergism between plant extracts and antibiotics resulted in inhibition of tested microorganisms with varying degrees. Moreover, the methanol extract was more effective than water extracts against all tested microorganisms. The most inhibited microorganism was *E. coli* when using water extract where the inhibition zone diameter was 13 mm and 10 mm for the combination of Senna extract plus antibiotic and for antibiotic only, respectively. However, the plant water extract had the same synergism with antibiotics against each of *Staphylococci aureus* and *Acinetobacter baumannii* where the inhibition zone diameter was 12 mm and 9 mm for the combination of Senna extract plus antibiotic and for antibiotic only. *K. pneumonia* was also inhibited by water extract where the inhibition zone diameter was 5 mm and 8 mm for the combination of Senna extract plus antibiotic and for antibiotic only.

There is a pressing need to control antimicrobial resistance by enhanced antimicrobial utilization and decrease of hospital cross infection. Be that as it may, the advancement of new antibiotic drugs ought to be proceeded as they are of prime significance to keep up the adequacy of antimicrobial treatment. In developing countries the WHO appraises that around 75% of the populace depends on plant based arrangements utilized as a part of their customary therapeutic framework and as the essential requirement for human essential medicinal services [18].

Table 2: The Synergism between plant extract and antibiotics

S. No.	Extract	Test organism	Inhibition zone (mm)	Control (mm)	Antibiotic Inhibition zone (mm)	Synergism Antibiotic + plant extract (mm)
1	W1	*S. aureus*	0	0	9	12
2	W2	*K. pneumonia*	0	0	5	8
3	W3	*E. coli*	0	0	10	13
4	W4	*A. baumannii*	0	0	9	12
5	W5	*Pseudomonas aeruginosa*	0	0	6	10
6	M1	*S. aureus*	3	0	9	13
7	M2	*K. pneumonia*	6	0	6	10
8	M3	*E. coli*	0	0	9	14
9	M4	*A. baumannii*	4	0	10	13
10	M5	*Pseudomonas aeruginosa*	3	0	8	12

W: Water extract; M: Methanol extract

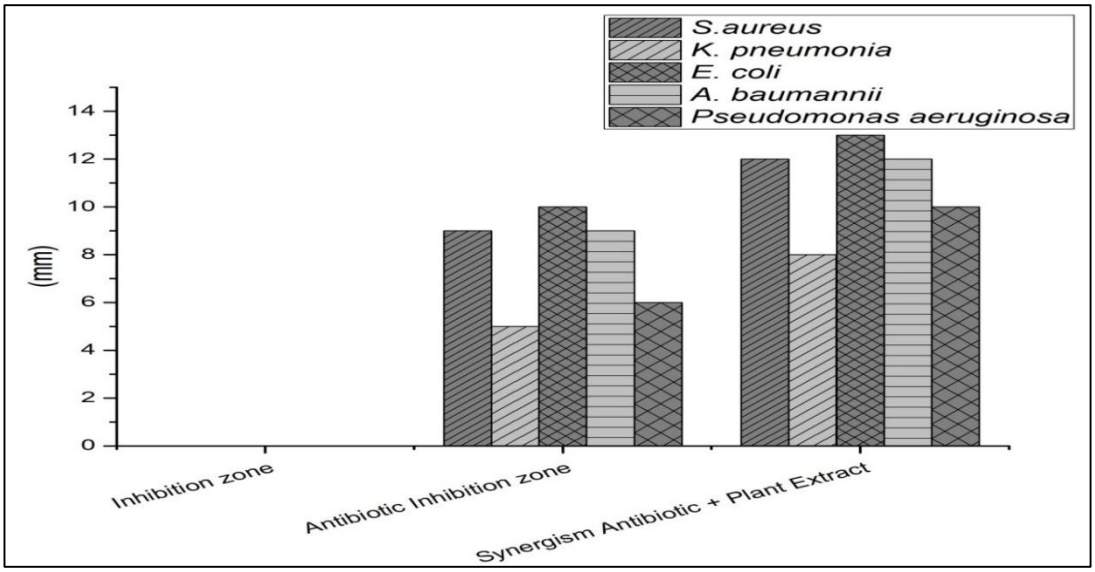

Fig 2: The synergism between Senna extract and antibiotic

Antibiotic resistance is a problem that continues to challenge the healthcare sector in a large part of the world in both developing and developed countries and the spread of multi-drug resistant bacteria in hospitals settings remains a challenging problem [19]. The findings of this study show the likelihood of utilizing these extracts in the treatment of bacterial diseases, and the aftereffects of this study was empowering, in spite of the requirement for clinical studies to decide of the genuine adequacy and potential harmful effects in vivo. These outcomes revealed the significance of plant extract when combined with antibiotic drugs.

3.3 Minimum inhibitory concentration (MIC) of Senna plant

Senna plant extracts were evaluated for their Minimum Inhibitory Concentration (MIC) against *E. coli*, *S. aureus*, *K. pneumonia*, *P. areuginosa*, *A. baumannii* and *P. aeruginosa.* The results of MIC are presented in Table (3). It has been found that Senna plant extracts showed antibacterial activity against *E. coli*, *S. aureus*, *K. pneumonia*, *A. baumannii* and *P. aeruginosa* with MIC values ranging from 2.25-50 mg/ml. The tested extracts showed different levels of antimicrobial activity depending on tested bacterial species. Suggesting that very small amount of the extracts are required to inhibit the growth of the bacteria.

The results also indicated that that there is a reduction in MIC in case of water extract of the plant against *P. aeruginosa* (6.25 mg/ml), and the methanol extract against *K. pneumonia* (5.125 mg/ml). MIC values of the tested plant extracts against *E. coli* were 50 and 25 mg/ml for water extract and methanolic extract, respectively. While MIC of water extract and methanolic extract against *S. aureus*, *K. pneumonia*, *E. coli*, *A. baumannii* were 50 and 25, 12.25 and 5.125, 25 and 12.5, 12.5 and 12.5, 12.5 and 6.25 mg/ml, respectively. Nkuo-Akenji *et al.* [20] used methanol extracts of plant parts commonly used in Cameroon for the treatment of typhoid fever for antibacterial activity against Salmonella typhi, S. paratyphi and S. typhimurium, he found that plant extracts with low MIC (1 mg/ml and lower) may contain compounds with therapeutic activity.

So, the results indicate the possibility of using Senna extracts in the treatment of bacterial infections. The result of this study was empowering, in spite of the requirement for clinical reviews to decide of the genuine viability and potential lethal effects *in vivo*. These results were revealed the importance of plant extracts when associated with antibiotic drugs in control of bacteria.

Table 3: Minimal inhibitory concentrations (MIC) of Senna extracts against the tested microorganisms.

S. No.	Extract	Test organism	MIC value (mg/ml)
1	W1	*S. aureus*	25
2	W2	*K. pneumonia*	12.5
3	W3	*E. coli*	25
4	W4	*A. baumannii*	12.5
5	W5	*P. aeruginosa*	12.5
6	M1	*S.aureus*	25
7	M2	*K. pneumonia*	5.125
8	M3	*E. coli*	12.5
9	M4	*A.baumannii*	12.5
10	M5	*P. aeruginosa*	6.25

W: Water extract; M: Methanol extract

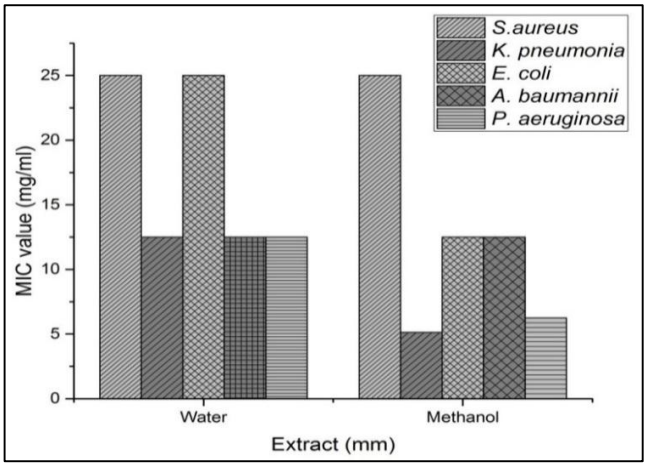

Fig 3: Minimum inhibitory concentration (MIC) of Senna plant water and methanol extract

4. Conclusion

On the basis of the antibacterial assay of the present study *E. coli* has been found susceptible to the employed Senna plant extracts than other tested microorganisms.

Based on the results, it can be concluded that the *Senna* (*Senna italica* Mill) plant extracts have great potential as antibacterial components against microorganisms and they can be used in the treatment of infectious diseases caused by resistant microorganisms. Future work is expected to segregate the secondary metabolites from the extracts contemplated with a specific end goal to test particular antibacterial activity and the hidden mechanisms.

5. Acknowledgements

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