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Original Research Article

Antagonistic Effect of the Exotic Plant "*Prosopis juliflora*" Extract on Different Bacterial Pathogens

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A B S T R A C T

The methanolic crude extracts of fresh and dry leaves of *Prosopis juliflora* were assessed for their antibacterial property using well diffusion method on *Escherichia coli*, *Klebsiella sp.*, *Staphylococcus aureus*, *Streptococcus sp.*, and *Bacillus sp*. Results revealed that green leaves' extract has higher zone of inhibition compared with dry leaves with a diameter ranged between 10 and 22 against all tested bacteria and with more inhibition to Gram positives than Gram negatives. However, dry leaves extract inhibited *S. aureus* and *Streptococcus* sp. only with a zone of inhibition of 10 and 21 mm, respectively. Dilution experiments showed that the minimum inhibitory concentrations (MIC) of green leaves' extract was as follows: *S. aureus*-62.5 mg/ml, *Streptococcus sp*.-31.25 mg/ml, *Bacillus sp*.-16.125 mg/ml, *Klebsiella sp*.-125 mg/ml, and *E. coli* 500 mg/ml. However, MIC of dry leaves' extract was more noticeable on Gram positive bacteria with MIC value of 125 and 31.25 mg/ml against *S. aureus* and *Streptococcus sp*., respectively. Results indicated that the MIC of 250 mg/ml of green leaves' extract is equivalent to the effect of Gentamicin (30 µg) and Kanamycin (30 µg) against *E. coli* and *S. aureus*. It can be concluded that the *P. juliflora* tissue could be a potential source for anti-bacterial agents and probably a novel inhibitory metabolite from this plant stress its potential in medical applications.

Introduction

Research in herbal medicine has increased in developing countries as a way to rescue ancient traditions as well as an alternative solution to health problems. Therefore, with the increasing acceptance of traditional medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very important (Saadoun and Hamid, 1999).

At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total (Cowan, 1999). According to the statistics of year 2000 from World Health Organization (WHO), more than 60% of the world's population depends on traditional medicine for health care.

In United Arab Emirates (UAE), *Prosopis*

*julifloras*is a plant which is locally known as Al Ghwaif. *P. juliflora*, a member of family *Leguminocae*, is found in arid and semi-arid regions of India. It has been used as a folk remedy for catarrh, cold, diarrhea, dysentery, excrescences, flu, inflammation, measles, sore throat and in healing of wounds (Hartwell, 1971).

P. juliflora is a unique plant that occurs naturally in dry areas of northern South America and Central America, Mexico and southern USA. It has been introduced into many tropical areas, including northeastern Brazil, Africa, Australia, Southeast Asia and South Asia. Initial purpose of the introduction of this exotic species in sub-continent was sand dunes stabilization (Ahmad *et. al*., 1989). The value of the tree lies in its exceptional tolerance of drought and marginal soils. It tolerates strongly saline soils and seasonal water logging. It is sometimes said to dry out the soil and compete with grasses, particularly in dry areas; hence, in some areas it is considered a weed.

Invasive, exotic *Prosopis* species have been declared noxious weeds in Australia, South Africa, Sudan and Pakistan. The impact of *P. juliflora* in the UAE has been studied by Essa *et al*., (2006) that showed a great depressive effect on the number, density and frequency of the associated species, particularly its invasion to native vegetation *Prosopis cineraria*. It is being combated by many municipalities including the municipality of Dubai. (Kazmi *et al*., 2010)

Allelopathy is one of the interesting properties found in some plants as *Prosopis sp*. with a depressive effect on the associated flora attributed to the release of chemicals that affect other plants (Ridenour and Callaway 2001; Brewer, 2002; Bais*et al*., 2003). Moreover, the negative impact of the plant could be through light deprivation, competition for water and nutrient, or leaching of allelopathic compounds (Nobel 1989, Moro *et al*., 1997; Barnes and Archer 1999; Holmgren *et al*., 1997; Kitajima and Tilman 1996). In addition, several studies reported the presence of allelopathic compounds in *P. juliflora.* For example, Kaur *et al*. (2012) detected the L-tryptophan in leaf leachates of *P. juliflora.* Nakano *et al*. (2001) suggested that L-tryptophan may play an important role in allelopathy of *P. juliflora* leaves.

This allelopathic interference has been argued to be one of the mechanisms by which exotic may become successful invaders (Inderjit *et al*., 2008; Stinson *et al*., 2006). Therefore we have anticipated the presence of some biocative metabolites in the tissue of *P. juliflora* (Al Ghwaif) tree that could be active against some pathogenic bacteria. In a recent study by Saadoun *et al*. (2014), the effect of the organic and aqueous extracts of the invasive plant *P. juliflora* and the exotic *P. cineraria* were evaluated on the growth of soil microbiota, different Gram-positive and Gram-negative bacteria, and on seed germination of two desert plants. Since few reports showed the effect of the *P. juliflora* metabolites against antibiotic-resistant bacteria has not been previously studied, we investigated the antibacterial activity of its extract.

The allelopathic effect of *P. juliflora* (Al Ghwaif) tree in UAE has attracted us to study and evaluate the effects of the organic extracts of its green and dried leaves on growth of different Gram- positive and Gram-negative antibiotic resistant bacteria, which become a major clinical and public health problem within the lifetime of most people living today.

Materials and Methods

Sample collection

Green and dry plant leaves samples of the exotic plant (Al Ghwaif) were collected from camel race area, Ajman-UAE. Samples were collected in plastic bags and then transported to the lab and stored at room temperature for later use.

Samples treatment and processing

All green and dried leaves were separated then dried by spreading them out in the chemical hood at room temperature. After drying, the leaves were grinded by blender, then the grinded leaves were soaked in 1:5 (v/w) methanol. 100 g of leaves were soaked in 500 ml of methanol, and then incubated for 24h at room temperature with gentle shaking. The organic extracts were filtered through a muslin cloth to remove large plant tissue and then rotary evaporated at 60˚C for fast evaporation of methanol with a rotary evaporator (RE100, Heidolph, Germany). The final dried crude extracts were weighed and dissolved in distilled water, then; the extracts were centrifuged to make sure all the debris precipitates out of solution. The supernatants of both dry and green leaves were filtered through vacuum filtration using polystyrene non-pyrogenic Corning 500 ml Filter unit system 0.22 µm (Corning-USA), and then stored in the refrigerator.

Bacterial cultures

The stock for bacterial culture such as *Escherichia coli, Klebsiella sp., Stayphylococcus aureus, Streptococcus sp.,* and *Bacillus sp.* were provided by Department of Applied Biology, University of Sharjah, Sharjah. All the microbes were streaked onto individual nutrient agar plates and incubated at 37˚C for 24 h prior to the assay.

Antibacterial assay

a. Well – diffusion method

One ml of each bacterial culture aseptically was spread on Mueller-Hinton agar plates by using a sterile glass spreader. Holes in the agar were made by cutting with a sterile serological tube (6 mm) diameter. Each well was filled with 30 µl filter sterilized crude extracts of green and dry leaves. Sterile distilled water was used as a control.

b. MICs method

To determine the minimum inhibitory concentration (MIC) of the crude extracts of both green and dried leaves serial dilutions were prepared. The methanolic crude extracts of both green and dried leaves were serially diluted to final concentrations (mg/ml) of 250, 125, 62.5, 31.25 and 16. Several wells were made in each plate which was filled with 30 µl of different concentrations. Plates were incubated at 37˚C for 24 hrs. The sensitivity of the test organisms to the crude extract and the standard antibiotics is indicated by clear zone around wells. The inhibition measured with a transparent ruler and expressed as the degree of sensitivity (NCCLS, 1993) after measuring the diameter of inhibitory zones in mm formed around each hole. All plates were incubated in the incubator at37˚C for 24h.

Results and Discussion

Well-diffusion test for *P. juliflora* methanolic crude extract on two tested Gram negative bacteria (*E. coli* and *Klebsiella sp.*) and three Gram positive bacteria (*Staphylococcus aureus, Bacillus sp.,* and *Streptococcus sp.*) indicated inhibition of all tested bacteria (Table 1). Green leaves showed the widest zone of inhibition on all

tested bacteria with 22 and 19 mm zone of inhibition against *Streptococcus sp.,* and *Bacillus sp.,* respectively, whereas dry leaves revealed intermediate inhibition. The tests on Gram positive bacteria (*S. aureus, Streptococcus sp.,* and *Bacillus sp.)* had higher sensitivity than Gram negative bacteria (*E. coli* and *Klebsiella sp*.) (Table 1). Overall, *P. juliflora* methanolic crude extract of green leaves succeeded to inhibit growth of all tested bacteria.

For the determination of the inhibitory effect of methanolic crude extract of green leaves when compared to different standard antibiotics, data indicated clear zone of inhibition against all tested bacteria (Fig. 1). Minimum inhibitory concentration (MIC) activity of the crude extract of green leaves on *S. aureus, Streptococcus sp.,* and *Bacillus sp.* was 62.5, 31.25, and 16.125 mg/ml, respectively (Fig. 2a, 2b, and 2c). However, on *E. coli* and *Klebsiella sp.*, results showed less sensitivity with MIC activity of 500 and 125 mg/ml, respectively (Table 1) and Figures (2d, and 2e). *E. coli* showed the lowest sensitivity compared to other bacteria (Fig. 2d). Also, zone of inhibition against *Bacillus sp.* was 19 mm at 500 mg/ml concentration and the MIC was showed at concentration of 16.125 mg/ml (Fig. 2c). MIC of the crude extracts of dried leaves showed activity only against *Streptococcus sp.* and *S. aureus* (Table 1). For *S. aureus* the inhibition stops at the concentration of 125 mg/ml, while for *Streptococcus sp.*, the inhibition stops at the concentration of 31.25 mg/ml. However, MIC activity of methanolic crude extract of dry leaves against *E. coli* and *Klebsiella sp*. was not clear.

Comparing the concentration obtained from crude extracts of both green and dried leaves and the activity of different standard antibiotics [TE_{10}: Tetracycline (10 µg); E_{15}: Erythromycin (15 µg); K_{30}: Kanamycin (30 µg); Gen $_{30}$: Gentamicin (30 µg); Met $_{30}$: Methicillin (30 µg)], data indicated that the minimum inhibitory concentration (MIC) of *P. juliflora* green leaves' extract was as follows: *Staphylococcus aureus*-62.5 mg/ml, *Streptococcus sp*.-31.25 mg/ml, *Bacillus sp*.-16.125 mg/ml, *Klebsiella sp*.-125 mg/ml, and *E. coli* 500 mg/ml (Table 2). The MIC of 16.125 mg/ml against *Bacillus sp.* was close to Methicillin (30 µg), and the MIC of 31.25 mg/ml against *Streptococcus sp.* was relatively close to Erythromycin (15 µg) (Table 1). Moreover the MIC activity of 31.25 mg/ml against *Streptococcus sp.* was close to Erythromycin (15 µg) (Table 1).

In this work, exotic *Prosopis juliflora* was studied to determine the antagonistic effect of its metabolites on several antibiotic resistant bacteria. *P. juliflora* was collected from camel race area in Ajman UAE. To observe its antagonistic effects, the leaves (dry and green) were prepared for extraction. They were grinded to increase the surface area of extraction thereby increasing the efficiency of extraction. Both green and dry leaves were left for drying prior extraction, because drying decrease plant moisture content, prevent enzymatic activity and consequently preserve the product. Drying is the most common fundamental method for post-harvest preservation of medicinal plants, because it allows for quick conservation of the medicinal qualities of the plant material in an uncomplicated manner.

Green and dry leaves extracts were tested for the antagonistic effect on different Gram positive and Gram negative bacteria (*Staphylococcus aureus, Streptococcus sp., Bacillus sp., E. coli,* and *Klebsiella sp.).* Mueller-Hinton agar plates were seeded with the bacterial cultures, and then a primary test was carried out using well diffusion method. Current available

screening methods for the detection of antimicrobial activity of natural products fall into several groups, including diffusion and dilution methods. The diffusion method is known as qualitative technique since this method will only give an idea of the presence or absence of substances with antimicrobial activity. On the other hand, dilution methods are considered quantitative assays once they determine the minimal inhibitory concentration (Vanden Berghe and Vlietink, 1991).

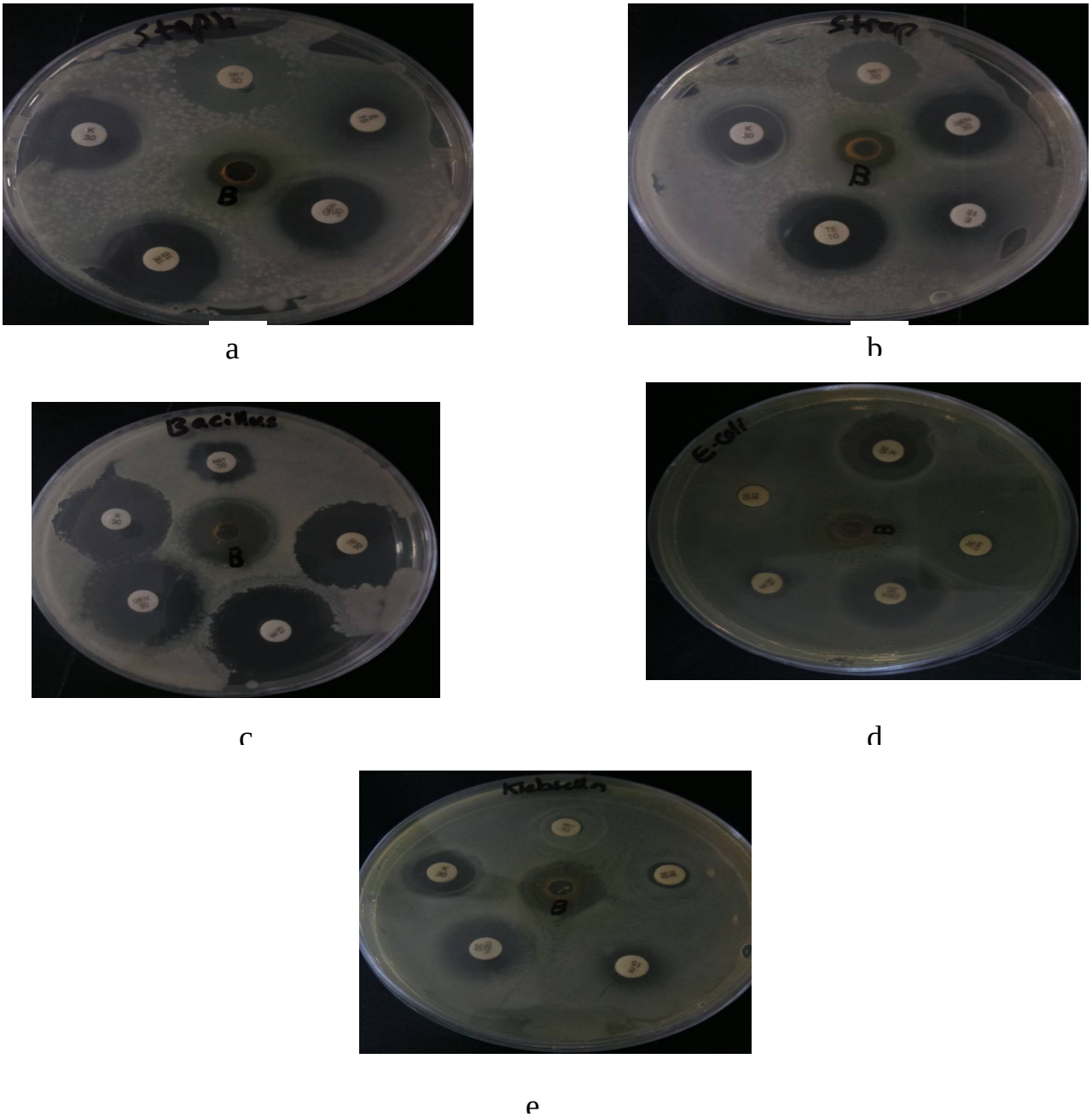
The antibacterial activity of methanolic crude extract of both dry and green leaves of *P. juliflora* as tested by agar diffusion method and employing two different types of reservoir wells filled with the extract, is shown in Figure (1). Both dry and green leaves' extract were effective with a higher effect on Gram positive bacteria (*S. aureus*, *Streptococcus sp.*, and *Bacillus sp.)* than Gram negative bacteria (*E. coli*, and *Klebsiella sp.).* These results are consistent with the bacterial structure. The difference in sensitivity between Gram negative and positive bacteria to inhibition by plant extracts is supported by other researchers (Saadoun and Hameed, 1999; Saadoun et al., 2008; Saadoun et al., 2014; Shelef, 1983). Overall, *P. juliflora* proved to have a strong antimicrobial activity and can be considered to have a broad spectrum of action.

Table.1 MIC activity of *P. juliflora* crude fresh green and old dry leaves' extract as assayed by well diffusion method and compared to different standard antibiotics against different pathogenic bacteria.

	Green Leaves Extract Concentration (mg/ml)						Dried Leaves Extract Concentration (mg/ml)						Antibiotic[a]				
Bacteria	500	250	125	62.5	31.25	16.125	500	250	125	62.5	31.25	16.125	TE_{10}	E_{15}	K_{30}	Gen_{30}	Met_{30}
S. aureus	11	10	9	7	-	-	10	8	6	-	-	-	21	13	20	20	20
Streptococcus sp.	22	19	15	13	10	-	21	18	15	12	10	-	21	12	16	19	18
Bacillus sp.	19	17	15	14	12	10	-	-	-	-	-	-	25	26	25	25	15
E. coli	10	-	-	-	-	-	-	-	-	-	-	-	-	10	20	20	-
Klebsiella sp.	11	10	10	-	-	-	-	-	-	-	-	-	8	17	18	17	-

[a]TE_{10}: Tetracycline (10 µg); E_{15}: Erythromycin (15 µg); K_{30}: Kanamycin (30 µg); Gen_{30}: Gentamicin (30 µg); Met_{30}: Methicillin (30 µg).

Fig.1 The inhibitory effect of methanolic crude extract of fresh green leaves (B) against *S. aureus* (a), *Streptococcus sp*. (b), *Bacillus sp*. (c), *E. coli* (d), and *Klebsiella sp*. (e) compared to different standard antibiotics.



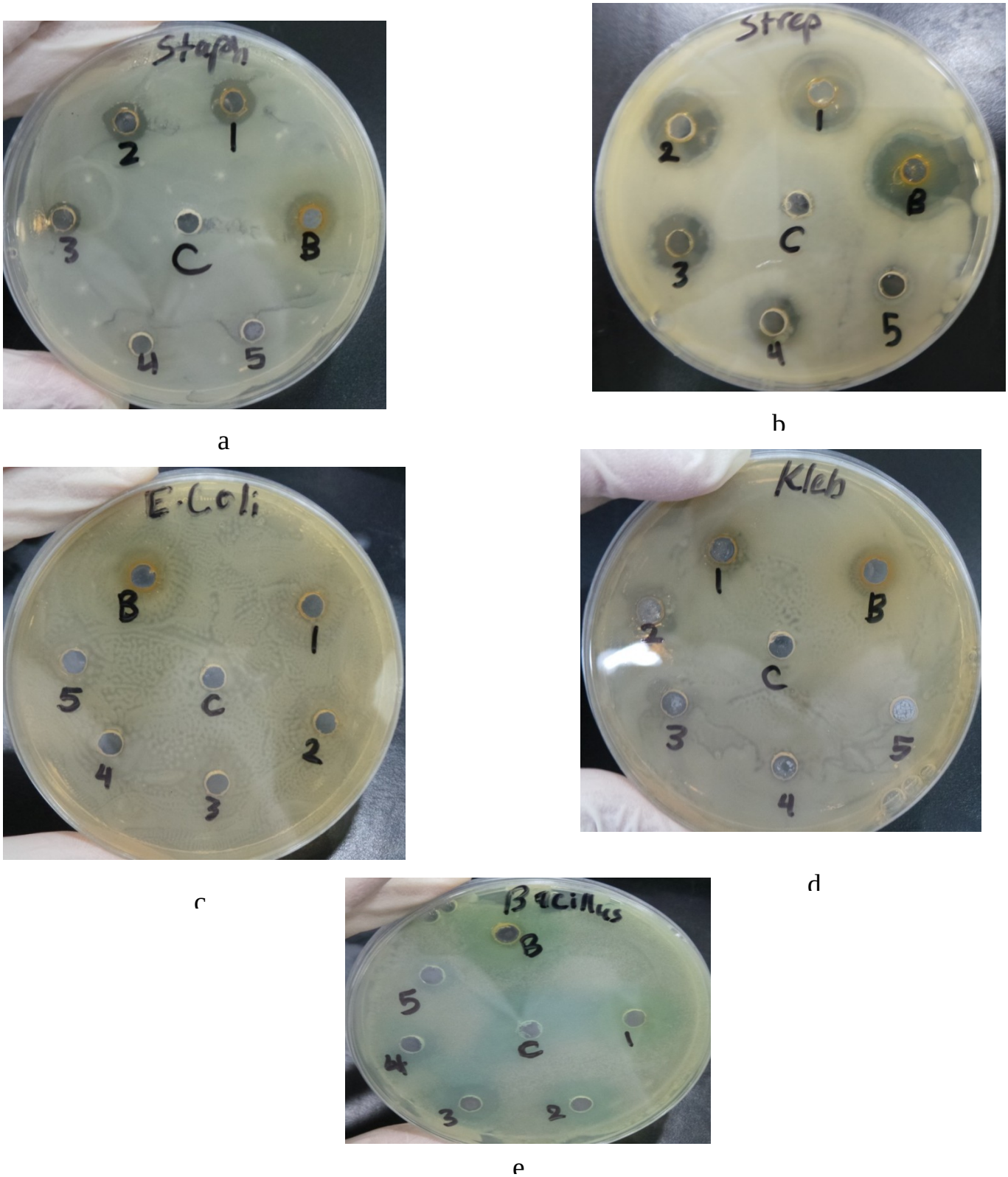


Fig.2 MIC activity of methanolic crude extract of fresh green leaves (B) against *S. aureus* (a), *Streptococcus sp*. (b), *Bacillus sp*. (c), *E. coli* (d), and *Klebsiella sp*. (e).

Furthermore, the minimum inhibitory concentration (MIC) effect of the crude plant extract on *Bacillus sp.*, *Streptococcus sp.*, and *S. aureus*, was 16.125, 31.25, 62.5 mg/ml, respectively. However, *E. coli* and *Klebsiella sp.* showed less sensitivity with MIC of 500 and 125 mg/ml, respectively (Table 2). These concentrations were compared to different standard antibiotics, and data indicated that the MIC of 16.125 mg/ml against *Bacillus sp.* was close to Methicillin (30 µg), and the MIC of 31.25 mg/ml against *Streptococcus sp.* was relatively close to Erythromycin (15 µg) (Table 1). Similarly, Swapnil and Verma, (2011) showed a comparable zone of inhibition when they compared zone of inhibitions created by alkaloid rich fractions of *P. juliflora* with that of standard antibiotics.

Many research efforts were carried out to find out the various phytochemicals of this invasive plant and the mechanisms of action as well as bioactivity of the various phytochemicals. Several alkaloids have been isolated from leaf extracts having pharmacological properties (Ahmed et al., 1989, Aqeel et al., 1989; Swapnil and Verma, 2011). Apart from alkaloids, other important compounds isolated from *P. juliflora* including flavone glycoside, Patulitrin, Prosogerin D, Procyanidin, ellagic acid, tannin and polystyrenes (Rastogi and Mehrotra, 1993).

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