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RESEARCH ARTICLE

A COMPARATIVE STUDY OF THE ANTIBACTERIAL ACTIVITY OF *ARISTIDA PUNGENS DESF LEAVES*; ITS SYNERGIC EFFECT WITH SOME OF STANDARD ANTIMICROBS

[1]Hamza Bensaci, [1,*]Lakhdar Sekhri, [1]Mohamed Khaled Bachki, [2]Halima Benkina and [1]Abdelali Atmani

[1]Dynamics Laboratory Interactions and Reactivity of Systems, Process Engineering Department, Faculty of Applied Sciences, University Kasdi Merbah, Ouargla 30000, Algeria
[2]Colonel Chaabani Hospital, El-Meniaa W. Ghardaia, Algeria

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ABSTRACT

The present work is aimed mainly to investigate and compare the antibacterial activities of methanol, diethyl ether and ethyl acetate extracts of *Aristida pungens Desf. leaves*, and their synergic effect with some standard antibiotics on *Escherichia coli, Salmonella, Proteus mirabilis* and *Staphylococcus aureus* using well diffusion method. Results for antibacterial activity as obtained with *Aristida pungens Desf* plant revealed that the three different extracts tested showed weak bacterial activity against all the bacteria tested (*Escherichia coli, Salmonella, and Staphylococcus aureus*). The addition of all extracts to each of the standard antimicrobics, CZ, VA, and CN on the bacteria tested (*Escherichia coli, Salmonella, and Staphylococcus aureus*) showed either indifference or antagonism effects except the synergic effect of methanol extract on *Proteus mirabilis* and *Escherichia coli* with VA decreased respectively the area diffusion of bacteria from 100% to 99%. The results partially do not justify the claimed uses of the selected plant in the traditional system of medicine to treat various infectious diseases caused by the microbes. Further chemical and pharmacological investigations may be carried out to isolate and identify the chemical constituents in order to justify the claimed uses such as the treatment of anemia in the natural herbal treatments (folk medicine).

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INTRODUCTION

The use of medicinal plants increased significantly in the pharmaceutical fields and many researchers have focused their research on the study of those plants which are spread around the world. Medicinal plants are used for several therapeutic purposes, including diarrhea, fever, colds and other diseases (Gislene *et al*., 2000; El-Hilaly *et al*., 2003). Most bacterial infections are treated with antibiotics, but at present time the natural herbal treatments (folk medicine) has spread dramatically without resorting to drugs and synthetic materials. However, due to the appearance of new strains of the bacteria and the weakness of chemotherapeutics and antibiotic resistance exhibited by pathogens has led to the screening of several medicinal plants for their potential antimicrobial activity (NCCLS, 2004; NCCLS, 1985; Ishrak *et al*., 2000).

****Corresponding author: Lakhdar Sekhri,***
Dynamics Laboratory Interactions and Reactivity of Systems, Process Engineering Department, Faculty of Applied Sciences, University Kasdi Merbah, Ouargla 30000, Algeria.

In recent years there has been a flood of papers describing the synthesis of new antibacterial compounds and isolation of some natural products and study of their biological antimicrobial activities (Bensaci *et al*., 2016; Bassam *et al*., 2005; Sekhri *et al*., 2008; Babarbi *et al*., 2010; Thirupathaiah *et al*., 2008; Singh *et al*., 2007; Suresh Kumar *et al*., 2006). An increasing number of reports dealing with the assessment of antimicrobial effects of different extracts of various medicinal plants are frequently available (Chakra borty, 1996; Benkeblia, 2004; Babamer Zohra *et al*., 2012; Ashakkumar and Ramaswamy, 2013; Bindu *et al*., 2011; Quezel and Santa 1962). The urinary tract infection is the most common bacterial diseases in children, as it ranks second in terms of spreading infection after respiratory tract (Funfstuck *et al*., 1997; Lettgen, 1993; Strffon, 1974; Egorove, 1985; Rushton, 1997; Egorove, 1985). The urinary tract infection comes usually from attacking microorganisms urinary system that are mostly negative gram bacteria, from digestive system, as most of the infections at urinary system caused by bacteria intestinal Enterobacteriaceae including *Bacillus* colon *Escherichia coli*, which occupies a leading position among the races of this family (Rushton,

1997). As well as other pathogens include *Staphylococcus aureus* and *Streptococci* and sometimes as types fungus Candida fungal (Rushton, 1997). Therefore, we have chosen to study the leaves of medicinal plant, *Aristida pungens Desf*, a desert plant, because of the availability of year-round and represent the most of the plant size. This plant used in the treatment of anemia and some infectious diseases caused by the microbes in south of Algeria; its seeds used for manufacture of a type of bread, and the leaves are used as feed for livestock. The aim of this study was to evaluate the activity of aqueous and alcoholic, diethyl ether and ethyl acetate extracts; its synergic effect with some of standard antimicrobs against several Gram-positive and Gram-negative bacterial strains in vitro.

Experimental

MATERIALS AND METHODS

Fresh plant/plant parts: ***Aristida pungens Desf. l*** plant was collected randomly from the El-Meniaa desert W. Ghardaia, south of Algeria in April 2016. The medicinal plant was deposited at Laboratoire de Dynamique Interaction et Réactivité des Systèmes, Department of Process engeneering, Faculty of Applied Sciences, University of Kasdi Merbah-Ouargla. Fresh stalks *plant* material was washed under running tap water, air dried under dark and then homogenized to fine powder using an electrical mixer "Panasonic Type" for 20 minutes, and stored in closed container away from light and moisture.

Preliminary Phytochemical Analysis

The preliminary phytochemical analysis of the crude powder of ***Aristida pungens Desf. l*** plant collected showed that this plant contains many active ingredients : *Coumarins, tannins,*and *terpenes*, one of the antioxidants of the bacteria responsible for the effect of microbs, also contains *flavonoids* including *glycosides* antioxidant, *phenols* and *saponins.* As for the nature of the extracts were characterized by clear viscous dark green color, due to the emergence of green chlorophyll pigment and material xanthine, also contains vegetarian jelly and glues (Singh *et al*., 2007).

Extraction of plant material

Each extract was prepared by soaking 200 g of the plant powder in a mixture of MeOH/H_2O (70/30) evaporated under reduced pressure. The second extract was prepared by soaking 200 g in diethyl ether, and the third extract was prepared by soaking 200g in ethyl acetate. Each of the resulting extracts was diluted with distilled water and left overnight. The methanol filtrates were subjected to extraction by various solvents with increasing polarity. All organic phases were separated and evaporated. The resulting residue was stored at 4°C.

Microorganisms

All bacterial standard strains: *Escherichia coli sp6504752*, *Staphylococcus aureus*, *Proteus mirabilis* and *Salmonella* were obtained from Colonel Chaabani Hospital, El-Meniaa, W. Ghardaia. ALGERIA.

Preparation of the bacterial culture media

3.7 of Muller Hilton agar were mixed with hot distilled water and autoclaved at 121°C and 2 atm for 15 minutes. After autoclaving, it was allowed to cool to 45°C in a water bath. Then the medium was poured into sterilized Petri dishes with a uniform depth of approximately 5 mm (Harbone, 1973).

Preparation of plant extract impregnated discs

Whatman N°1 filter paper was used to prepare discs of 5 mm in diameter. They were sterilized by autoclaving and then dried during the autoclaving cycle. The discs were then impregnated with extract of the plants (Swarnamoni, 2013).

Disc diffusion method

Disc diffusion method for antimicrobial susceptibility test was carried out according to the standard method by Kirby-Bauer to assess the presence of antibacterial activities of plant extracts (Harbone, 1984). A bacterial suspension adjusted to 0.5 McFarland standard ($1.5x10^8$ CFU/ml) was used to inoculate Mueller Hinton agar plates evenly using a sterile swab. The discs impregnated with the plant extracts were placed individually on the Mueller Hinton agar surface. The discs were spaced far enough to avoid both reflection waves from the edges of the Petri discs and overlapping rings of inhibition. The plate was then incubated at 37°C for 18 hours in inverted position to look for zones of inhibition. Zones of inhibitions produced by the sensitive organisms were demarcated by a circular area of clearing around the plant extract impregnated discs. The diameter of the zone of inhibition through the center of the disc was measured to the nearest millimeter. The resulting residue of all extracts stored at 4°C was tested at concentrations 10^{-1} g/ml and were prepared in DMSO.

Standard antimicrobs

All standard antimicrobs: Cefazolin (CZ), Vancomycin (VA) and Gentamicin (CN) were obtained from Italian company "*Liofilchem."*

RESULTS AND DISCUSSION

Two standard antimicrobs: Cefazolin (CZ), and Gentamicin (CN) exhibited a positive effect against all tested bacterial strains: Escherichia coli, Salmonella, Staphylococcus aureus and Proteus mirabilis. On the other hand Vancomycin (VA) was ineffective against Escherichia coli, Salmonella, and Proteus mirabilis. Moreover the solvent DMSO showed no effect against all tested bacterial strains. Table 1 and Table-2 summarized the microbial growth inhibition of these standard antibiotics.

Results for antibacterial activity as obtained with *Aristida pungens Desf.* revealed that the three different extracts tested (Methanolic, diethyl ether and ethyl acetate extracts) in vitro by agar disc diffusion were ineffective against 4 bacterial species: Escherichia coli, Salmonella, Staphylococcus aureus and Proteus mirabilis. The maximum antibacterial activity was recorded with methanol extract against Escherichia coli with an inhibition diameter of 06 mm at concentration 100 mg/ml g/ml. Table 3: summarizes the microbial growth inhibition of tested extracts of this plant.

Table 1. Conc. of some standard antibiotics with their antibacterial activity

Antibiotic	Conc.	Antibacterial activity
Cefazolin	30 mcg	Gram positive and negative bacteria
Vancomycin	30 mcg	Gram positive bacteria
Gentamicin	10 mcg	Gram positive and negative bacteria

Cefazolin: CZ; Vancomycin: VA; Gentamicin: CN

Table 2. Antibacterial activity of some standard antibiotics and DMSO

Bacteria / Antibiotic	*E. coli*	*Salmonella*	*Staphylococcus aureus*	*Proteus mirabilis*
Cefazolin	16	26	26	25
Vancomycin	-	-	22	-
Gentamicin	24	24	27	23
DMSO	-	-	-	-

Table 3. Antibacterial activity of methanolic, diethyl ether and ethyl acetate extracts of screened *Aristida pungens Desf.* Plant

Bacteria / Extract	*E. coli*	*Salmonella*	*Staphylococcus aureus*	*Proteus mirabilis*
Methanol	06	-	-	-
Diethyl ether	-	-	-	-
Ethyl acetate	-	-	-	-

Table 4. Summarizes the microbial growth inhibition of *Aristida pungens Desf.* & standard antimicrobics

Bacteria / Extract and antimicrob	*E. coli*	*Salmonella*	*Staphylococcus aureus*	*Proteus mirabilis*
E1 & (CZ 30 mcg)	17	23	23	21
E1 & (CN 30 mcg)	08	-	20	08
E1 & (CN 10 mcg)	24	18	25	22
E1 & (CZ 30 mcg)	16	25	25	22
E1 & (VA 30 mcg)	-	-	21	-
E1 & (CN 10 mcg)	24	19	26	20
E1 & (CZ 30 mcg)	16	24	22	21
E1 & (VA30 mcg)	-	-	20	-
E1 & (CZ 10 mcg)	22	20	26	19

E1: Methanol extract, E2: Diethyl ether extract, E3: Ethyl acetate extract

Table 5. The effect of combination between *Aristida pungens Desf.* Extract & standard antimicrobics on E. coli.

Synergism effect	Indifference effect	Antagonism effect
E1 with CZ CN effect increase 0.52%	E1 with CN CN effect still 0%	E3 with CN CN effect reduce 1.44%
E1 with VA VA effect increase 1%	E2 with CZ CZ effect still 0%	
	E2 with VA VA effect still 0%	
	E2 with CN CN effect still 0%	
	E3 with CZ CZ effect still 0%	
	E3 with VA VA effect still 0%	

E1: Methanol extract, **E2:** Diethyl ether extract, **E3:** Ethyl acetate extract

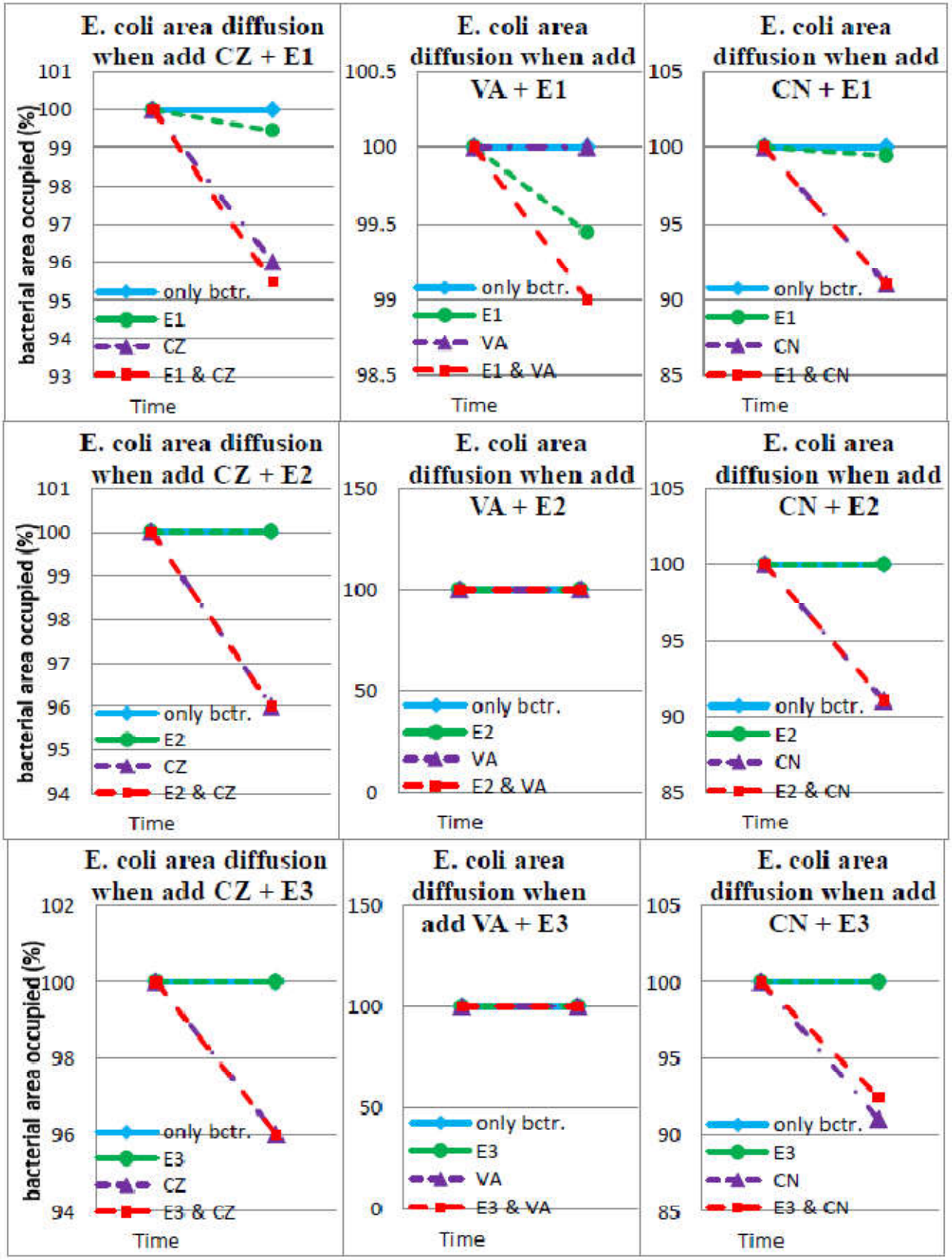
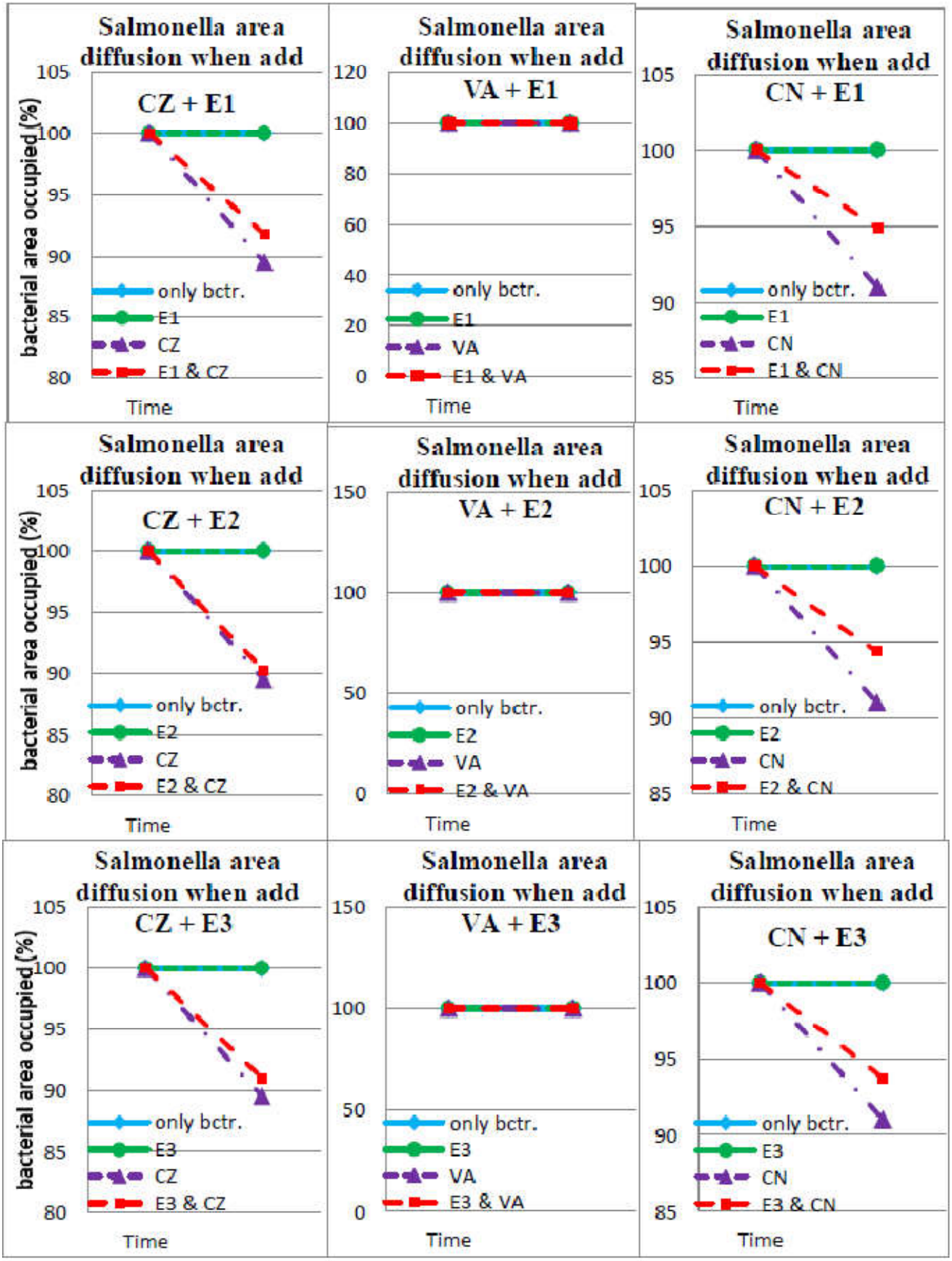
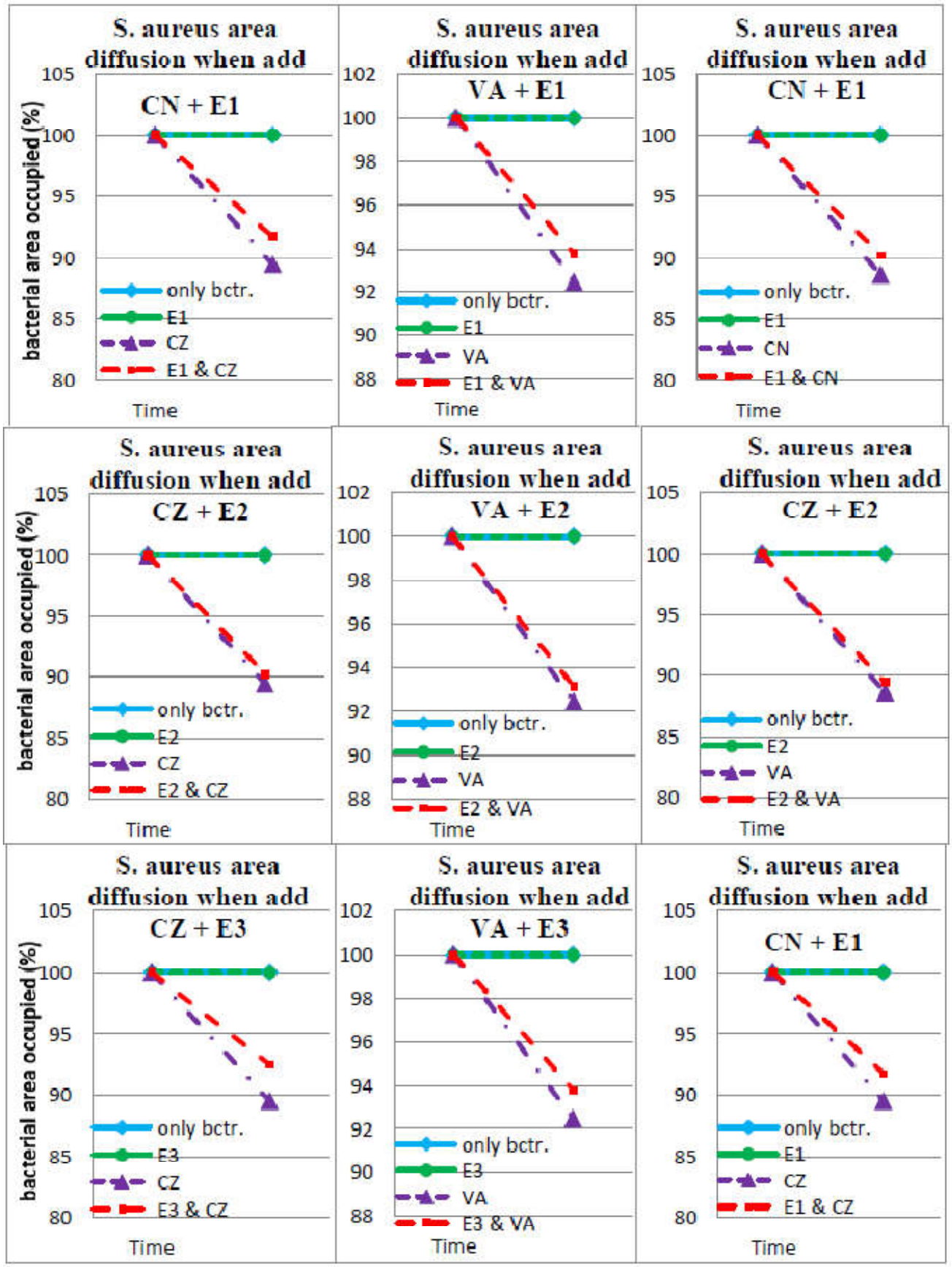


Fig.1. The bacterial area occupied (%) of *Aristida pungens Desf* extracts and tested antibiotics on *Escherichia coli.*



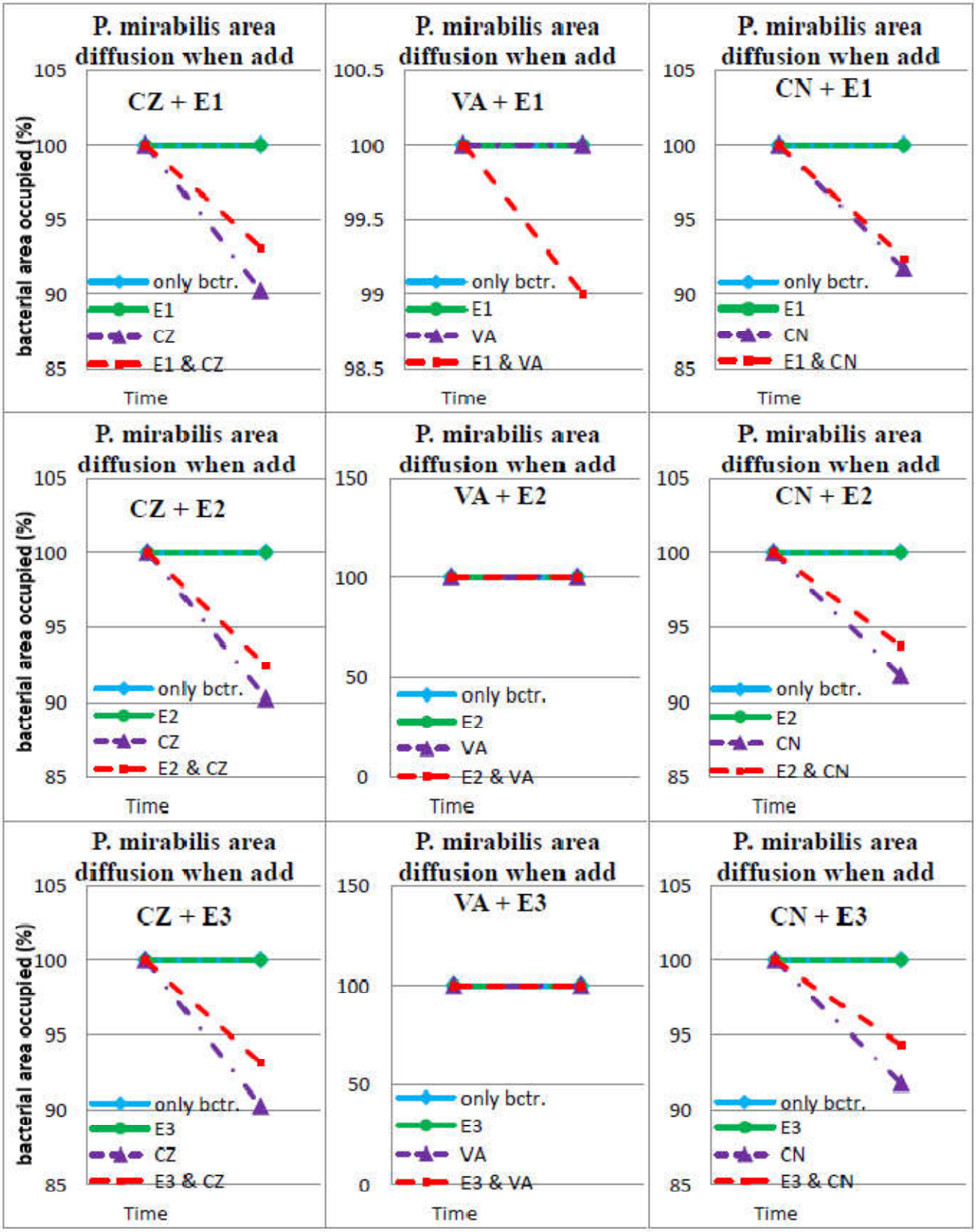
E1: Methanol extract, **E2:** Diethyl ether extract, **E3:** Ethyl acetate extract

Fig.2. The bacterial area occupied (%) of *Aristida pungens Desf.* extracts and tested antibiotics on *Salmonella*



E1: Methanol extract, E2: Diethyl ether extract, E3: Ethyl acetate extract

Fig.3. The bacterial area occupied (%) of *Aristida pungens Desf.* extracts and tested antibiotics on *Staphylococcus aureus*



E1: Methanol extract, E2: Diethyl ether extract, E3: Ethyl acetate extract

Fig.4. The bacterial area occupied (%) of *Aristida pungens Desf.* extracts and tested antibiotics on *Proteus mirabilis*

Table 6. The effect of combination between *Aristida pungens Desf.* Extract & standard antimicrobics on Salmonella

Synergism effect	Indifference effect	Antagonism effect
	E1 with VA VA effect still 0%	E1 with CZ CZ effect reduce 2.29%
	E2 with VA VA effect still 0%	E1 with CN CN effect reduce 3.94%
	E2 with VA VA effect still 0%	E2 with CZ CZ effect reduce 0.79%
		E2 with CN CN effect reduce 3.36%
		E2 with CZ CZ effect reduce 1.56%
		E3 with CN CN effect reduce 2.75%

E1: Methanol extract, E2: Diethyl ether extract, E3: Ethyl acetate extract

Table 7. The effect of combination between *Aristida pungens Desf.* Extract & standard antimicrobics on S. aureus

Synergism effect	Indifference effect	Antagonism effect
		E1 with CZ CZ effect reduce 2.29%
		E1 with VA CN effect reduce 1.31%
		E1 with CN CZ effect reduce 1.62%
		E2 with CZ CZ effect reduce 0.79%
		E2 with VA VA effect reduce 0.67%
		E2 with CN CN effect reduce 0.83%
		E3 with CZ CZ effect reduce 3%
		E3 with VA VA effect reduce 1.31%
		E3 with CN CN effect reduce 0.83%

E1: Methanol extract, E2: Diethyl ether extract, E3: Ethyl acetate extract

Table 8. The effect of combination between *Aristida pungens Desf.* Extract & standard antimicrobics on P. mirabilis

Synergism effect	Indifference effect	Antagonism effect
E1 with VA VA effect increase 1%	E2 with VA VA effect still 0%	E1 with CZ CZ effect reduce 2.88%
	E3 with VA VA effect still 0%	E1 with CN CN effect reduce 0.71%
		E2 with CZ CZ effect reduce 2.21%
		E2 with CN CN effect reduce 2.02%
		E3 with CZ CZ effect reduce 2.88%
		E3 with CN CN effect reduce 2.63%

E1: Methanol extract, E2: Diethyl ether extract, E3: Ethyl acetate extract

The resulting residue of all extracts stored at 4°C were tested at concentrations of 100 mg/ml were prepared in DMSO .As far as the synergic effect is concerned the combination of methanol extract with each of the standard antimicrobics, CZ, VA, and CN were most active and showed high synergic effect. The maximum antibacterial activity was recorded with E1 & CZ against Escherichia coli, Salmonella, and Staphylococcus aureus. Moreover, the maximum antibacterial activity was recorded with E1 & CN against Proteus mirabilis, whereas E1 & VA showed no synergic effect against Escherichia coli, and Salmonella. The combinations of diethyl ether extract with each of the standard antimicrobics, CZ, VA, and CN were also most active and showed no synergic effect. The maximum antibacterial activity was recorded with E2 & CZ against Escherichia coli, Salmonella, and Staphylococcus aureus, whereas E2 & VA showed no synergic effect Salmonella. Similar results were recorded with E2 & CZ; E2 & VA. Table-4 Summarizes the microbial growth inhibition of *Aristida* & standard antimicrobics. From the Table-4, and for figuring the effect of combination between standard antimicrobics and *Aristida pungens Desf.* extracts, we drew Fig.1, Fig. 2, Fig. 3, and Fig. 4. The bacterial area occupied (%) of *Aristida pungens Desf.* extracts and tested antibiotics on the bacterial species: Escherichia coli, salmonella, Staphylococcus aureus and Proteus mirabilis are summarized in Fig.1, Fig. 2, Fig. 3, and Fig. 4. As far as the synergic effect is concerned the combination of all extracts with each of the standard antimicrobics, CZ, VA, and CN against Escherichia coli, showed indifference effects except:

- the synergic effect of methanol extract with CZ which decreased the area diffusion of bacteria from 96% to
- the synergic effect of methanol extract with VA which decreased the area diffusion of bacteria from 100% to 99%, (increase VA effect to 1%) as shown in Fig.1.

The addition of all extracts with each of the standard antimicrobics, CZ, VA, and CN on *Salmonella* gave either indifference or antagonism effects as shown in Fig. 2. The addition of all extracts with each of the standard antimicrobics, CZ, VA, and CN on *S. Aureus* were most active and showed either indifference or antagonism effects as shown in Fig. 3. The addition of all extracts with each of the standard antimicrobics, CZ, VA, and CN on *Proteus mirabilis* showed either indifference or antagonism effects except the synergic effect of methanol extract with VA which decreased the aria diffusion of bacteria from 100 to 99% as shown in Fig. 4. Generally, the three different extracts of this plant are ineffective towards the tested bacteria and methanol/H_2O extracts are more potent compared to ethyl acetate and ether extracts. This weak effect may be due to the extract effect on the permeability of the cell membrane and the function of the bacterial cell (Al-Abed, 2008).

Conclusion

This study underscored the antimicrobial activity of one chenopodiaceae species namely: *Aristida pungens Desf.* extracts using three different solvents: Diethyl ether, Ethyl acetate, and Methanol with increasing polarity against four bacteria strains. This medicinal plant averred to be ineffective against three types of gram negative bacteria: *Escherichia coli, Salmonella, and Proteus mirabilis* and one type of gram positive *Staphylococcus aureus*. The addition of all extracts to each of the standard antimicrobics, CZ, VA, and CN on the bacteria tested (*Escherichia coli, Salmonella, and Staphylococcus aureus*) showed either indifference or

antagonism effects except the synergic effect of methanol extract on *Proteus mirabilis* and Escherichia coli:

- The synergic effect of methanol extract with CZ on *E. Coli* which decreased the area diffusion of bacteria from 96% to 95.48%, (increase CZ effect to 0.52%) as shown in Fig. 1.
- The synergic effect of methanol extract with VA on *E. Coli* which decreased the area diffusion of bacteria from 100% to 99%, (increase VA effect to 1%) as shown in Fig. 1.
- The synergic effect of methanol extract with VA on *P. Mirabilis* which decreased the area diffusion of bacteria from 100% to 99%, (increase CZ effect to 1%) as shown in Fig. 4.

The results partially do not justify the claimed uses of the selected plant in the traditional system of medicine to treat various infectious diseases caused by the microbes. Further chemical and pharmacological investigations may be carried out to isolate and identify the chemical constituents in order to justify the claimed uses in the traditional system of medicine, the treatment of anemia.

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