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EFFECT OF SOME PLANT EXTRACTS ON PATHOGENIC BACTERIA .

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ABSTRACT

This study was carried out to determine the antibacterial activity of some plant extracts include : *Rosmarinus officinalis, Citrus sinensis, Eucalyptus globulus, Psidium guajava, Matricaria chamomilla, Allium cepa, Allium sativum* and *Nigella sativa* . These extracts prepared by four solvents (water – methanol – ethanol – acetone) at different concentrations (100 , 200 , 400 mg / ml) against pathogenic bacteria such as : *Escherichia coli, Salmonella* spp*, Shigella flexneri, Enterobacter sakazakii*, *Staphylococcus aureus* and *Bacillus cereus*. The antibacterial activity assessed by agar well diffusion method to evaluate inhibition of zone and also determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by using serial dilution method . The results showed that the extracts of *Rosmarinus officinalis, Citrus sinensis, Eucalyptus globulus, Psidium guajava* exhibited the higher zones of inhibition against all pathogenic bacteria while *Salmonella* spp was resistance to both the aqueous and ethanol extract of *Rosmarinus officinalis* and resistance to the aqueous extract of *Eucalyptus globulus* and *Psidium guajava*. *Matricaria chamomilla* showed moderate antibacterial effects except *Salmonella* spp was resistance to the ethanol extract while *Allium cepa* and *Allium sativum* showed lower antibacterial activity. *Nigella sativa* showed no antibacterial activity against all pathogenic bacteria. Respect to MIC and MBC of the *Rosmarinus officinalis*, the aqueous extract was 25 mg / ml for all bacteria, the methanol extract showed MIC (6.25 mg / ml) and MBC (12.5 mg / ml) for *Escherichia coli, Salmonella* spp*, Staphylococcus aureus and Bacillus cereus* also MIC was (12.5 mg /ml) and MBC (25 mg / ml) for *Enterobacter sakazakii* and *Shigella flexneri*. Ethanol extract showed MIC (6.25 mg / ml) and MBC (12.5 mg / ml) for *Escherichia coli* and *Staphylococcus aureus* while MIC was (12.5 mg / ml) and MBC was (25 mg / ml) for *Shigella flexneri* and *Bacillus cereus* but MIC and MBC were (< 25 mg / ml) for *Enterobacter sakazakii* . The acetone extract showed MIC at (6.25 mg / ml) and MBC at (12.5 mg / ml) for *Salmonella* spp and *Enterobacter sakazakii* respectively. MIC was (12.5 mg / ml) and MBC was (25 mg / ml) for *Escherichia coli, Staphylococcus aureus* and *Shigella flexneri* while MIC was (25 mg / ml) and MBC was (< 25 mg / ml). The other plant extracts showed MIC and MBC (< 25 mg / ml) for all tested pathogenic bacteria .

Keywords : pathogenic bacteria – plant extracts – antibacterial activity

INTRODUCTION

Pathogenic bacterial infections have become a major health problem in worldwide, now the research increased with the growing number of food-borne illness outbreaks caused by pathogens such as *Staphylococcus aureus*, *Salmonella* sp, *Clostridium perfringens*, *Bacillus cereus* and enteropathogenic *E. coli* (Wilson and Droby 2000 ; Friedman *et.al*., 2002). These bacteria cause over 90 % of all cases of food poisoning . Infectious diseases an important cause of morbidity and mortality in developing countries so that pharmaceutical industries have produced a number of new antimicrobial drug, the emergence of multiple drug resistant bacteria has increased because these bacteria have the genetic ability to transmit and acquire resistance to antibiotics drugs used (Nascimento *et.al*., 2000) therefore we need develop alternative antimicrobial drugs for the treatment of infectious diseases from other sources (Cordell 2000). The increasing depend on drug from natural source gas led to extraction and development of many drugs and chemotherapeutic agents from traditional herbs (Falodun *et.al.,* 2006). Plant remedies are very important low cost alternative to industrially produced antibiotics which are not available because of their high price whereas these plants have great potential as antimicrobial compounds against microorganism so that they can be used in the treatment of infectious diseases caused by pathogenic bacteria such as *Salmonella* which causes salmonellosis, *E. coli* causes several death, *Staphylococcus* causes a variety of suppurative infections and toxinoses, pneumonia, mastitis, meningitis and urinary tract infections, *E. coli* and *B. subtilis* causes food poisoning (Sapkota *et.al*., 2012). Plants are rich a wide various of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids. These substances have antimicrobial properties (Bouzada Maria *et..al*., 2009) the medicinal properties of various plants and extracts have been recognized since the beginning of the 5 th century (Kay 1986). Plant extracts have been used in folk medical practices for the treatment of various ailment, the antimicrobial activities of plant extracts and oils are used in many applications including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies. There have been some studies on the antifungal activity of plant extract, inhibitory effects of aqueous extracts of garlic and onion. Garlic possess dietary and medicinal properties , many studies reported that garlic has antimicrobial effects . It inhibits the growth bacteria, molds and yeasts (Ross *et.al*., 2001). Garlic contain allicin which is found to exhibite antibacterial activity against a wide range of bacteria and antifungal activity and include the powerful antioxidants. Onion contain substances have antimicrobial activity against a wide microorganisms, its antibacterial power is effective against preventing numerous intestinal problem (Azu and Onyeagba 2007). Rosemary is herb and oil are used as spice and flavoring agent in food processing for its desirable flavor , high antioxidant and antimicrobial activities (Lo *et.al*., 2002 ; Quattara *et.al*., 1997) the main constituents are phenolic diterepens include carnosol , carnosic acid , methyl carnosate and phenolic acids such as rosmarinic and caffeic acid (Cuvelier *et.al*., 1996). Chamomile has been widely used in folk and traditional medicines for its multi therapeutic, cosmetic and nutritional values (Issac 1989). Guava leaves used for the treatment of gastrointestinal disease. (Barbahlo *et.al*., 2012) reported that all partes of guava widely use in curing diseases. Guava leaf extract have biological activities such as antibacterial, analgesic, anti-inflammatory, antimicrobial, phytotoxic, heptaprotection and hyperglycaemic and anticancer activities. Camphor has antimicrobial activity against some bacteria such as *Stophylococcus aureus* (Mahboobi *et.al*., 2007). Camphor essential oils contain two or three major components so that it used against bacteria and fungi, camphor was used as potential natural agents for food preservation as pharmaceutical products because of antimicrobial activities and chemical composition (Rota *et.al*., 2011). Black seed have medicinal importance and exhibit many pharmacological effects such as antiparasitic, antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory activities (Ali and Blunden 2003). Fruit peels are used in treatment of skin infections, urine infections, cancer, throat infections and allergies and it possess antimicrobial, antifungal, anti-inflammatory, antibacterial properties (Sagarika 2012)). Orange peel extract has been studied in several clinical trials, it inhibits the way cancer cells divide and grow. The chemical compositions used in herbal medicine for the treatment of many diseases (McGarvey and Croteau 1995).

This study aimed to determine the antibacterial activity of different solvents (water , methanol , rthanol , acetone) of *Rosmarinus officinalis, Citrus sinensis, Eucalyptus globulus, Psidium guajava, Matricaria chamomilla, Allium cepa*, *Allium sativum* and *Nigella sativa* against pathogenic bacteria such as (*Escherichia coli, Salmonella spp, Shigella Flexneri, Enterobacter sakazakii, Staphylococcus aureus* and *Bacillus cereus*) to evaluate diameter of inhibition zone and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of these plant extracts .

MATERIALS AND METHODS

Microorganisms : Six pathogenic bacteria which used in this study include *Salmonella* spp, *Shigella flexneri, Enterobacter sakazakii, Staphylococcus aureus and Bacillus cereus* were obtained from Faculty of Medicine. Mansoura University and *Escherichia coli* was obtained from Agricultural Research Center , Giza , Egypt .

Plant materials : *Rosmarinus officinalis* (rosemary), *Citrus sinensis* (orange), *Eucalyptus globulus* (camphor), *Psidium guajava* (guava), *Matricaria chamomilla* (chamomile), *Allium cepa* (onion), *Allium sativum* (garlic) and *Nigella sativa* (black seed) were purchased from the market of Mansoura City. The dried material was ground to a fine powder by using a blender and kept untile further use .

Solvents : Four solvents were used in this study (water , methanol , ethanol and acetone) were purchased from medicines company of Mansoura City .

Preparation of plant extracts : The dried (peels , leaves , bulbs , flowers , seeds and cloves) were prepared by soaking 50 g of the material in 200 ml of different solvents separately for 72 h. The mixture was filtered with Whatmann filter paper no. 1 the filtrate was evaporated in a rotary evaporator to concentrated. The dry residue stored at 5^0 C in an airtight bottle untile further use. The extracts redissolved in each solvent above to obtaine different concentrations (400 – 200 – 100 mg / ml).

Preparation of inoculum : Stock cultures were maintained at 4^0 C on slopes of nutrient agar. Active cultures were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth that incubated at 37^0 C for 24 h .

Antibacterial activity test : The antibacterial activity of different plant extracts was determined by using the diffusion agar method (Kudi *et.al*., 1999). 1 ml of bacterial cultures was added to nutrient agar plate. A sterile cork-borer (9 mm diameter) was used to make well in nutrient agar plate then 100 µl of the different concentrations of the plant extracts and control were added to each well then the plates were incubated at 37^0 C for 24 h. The inhibition zone diameter was measured if a zone of growth inhibition around the well is present. The test was replicated three times .

Determination of minimum inhibitory concentration (MIC) : The MIC was tested by broth dilution method at concentrations (1.562 , 3.125 , 6.25 , 12.5 , 25 mg / ml) of plant extracts were added to test tubes. Each tube was inoculated with 0.1 ml of bacterial suspension and incubated at 37^0 C for 24 h. The MIC was the lowest concentration of the plant extracts that resulted in a clear tube .

Determination of bactericidal concentration (MBC) : The highest dilution of plant extracts not exhibiting bacterial growth was taken as the MIC . 100 µl of each tube were plated onto nutrient agar and incubated at 37^0 C for 24 h (Rota *et.al*., 2008) the highest dilution not exhibiting bacterial growth was reported as the MBC .

RESULTS AND DISCUSSION

Antibacterial activity of plant extracts :

The results of antibacterial activity of different solvent plant extracts to determine diameter of inhibition zone are shown in Table (1 – 7) .

Table 1 : Diameter of inhibition zone (mm) of *Rosmarinus officinalis* extracts against pathogenic bacteria:

Pathogenic bacteria	Concentrations mg / ml	Mean values of inhibition zone (mm)			
		Aqueous extract	Methanol extract	Ethanol extract	Acetone extract
Escherichia coli	100	9	16	17	17
	200	9	18	19	17
	400	11	19	20	20
	control	0	0	0	0
Salmonella spp	100	0	17	0	18
	200	0	20	0	20
	400	0	26	0	22
	control	0	0	0	0
Shigella flexneri	100	4	14	16	17
	200	5	16	17	17
	400	8	17	19	21
	control	0	0	0	0
*Enterobacter sakazaki*i	100	7	16	6	18
	200	8	17	7	19
	400	10	20	10	21
	control	0	0	0	0
Staphylococcus aureus	100	8	17	17	10
	200	9	18	17	17
	400	10	19	18	19
	control	0	0	0	0
Bacillus cereus	100	5	18	16	15
	200	5	20	17	15
	400	9	23	17	20
	control	0	0	0	0

0 = no effect 0

The obtained results in Table (1) showed that the methanol, ethanol and acetone extracts of rosemary exhibited high active on all bacteria but ethanol and aqueous extracts showed no effect on *Salmonela* spp , the aqueous extract showed low effect on all tested bacteria .

Table 2 : Diameter of inhibition zone (mm) of *Citrus sinensis* extracts against pathogenic bacteria:

Pathogenic bacteria	Concentrations mg / ml	Mean values of inhibition zone (mm)			
		Aqueous extract	Methanol extract	Ethanol extract	Acetone extract
Escherichia coli	100	10	11	18	11
	200	15	15	20	18
	400	16	17	22	19
	control	0	0	0	0
Salmonella spp	100	0	15	14	8
	200	0	19	22	10
	400	0	20	27	12
	control	0	0	0	0
Shigella flexneri	100	7	15	16	15
	200	10	18	18	18
	400	14	21	20	20
	control	0	0	0	0
Enterobacter sakazakii	100	10	13	14	9
	200	13	16	18	16
	400	18	21	20	20
	control	0	0	0	0
Staphylococcus aureus	100	8	13	14	9
	200	10	20	18	12
	400	19	26	20	22
	control	0	0	0	0
Bacillus cereus	100	4	12	13	13
	200	6	15	18	17
	400	10	18	20	20
	control	0	0	0	0

0 = no effect .

Data recorded in Table (2) showed that the all different extracts of orange peels showed high effect against all bacteria while the aqueous extract showed no effect on *Salmonella* spp .

The obtained results in Table (3) cleared showed that all extracts of camphor showed high effects against all bacteria but not effect on *Salmonella* spp

Table 3 : Diameter of inhibition zone (mm) of *Eucalyptus globulus* extracts against pathogenic bacteria:

Pathogenic bacteria	Concentrations mg / ml	Mean values of inhibition zone (mm)			
		Aqueous extract	Methanol extract	Ethanol extract	Acetone extract
Escherichia coli	100	13	8	14	10
	200	17	13	15	10
	400	19	19	16	12
	control	0	0	0	0
Salmonella spp	100	0	0	0	0
	200	0	0	0	0
	400	0	0	0	0
	control	0	0	0	0
Shigella flexneri	100	10	13	7	10
	200	13	15	9	11
	400	20	17	15	14
	control	0	0	0	0
Enterobacter sakazakii	100	10	14	12	8
	200	15	15	14	12
	400	17	22	17	16
	control	0	0	0	0
Staphylococcus aureus	100	8	8	9	12
	200	11	16	11	14
	400	19	20	13	15
	control	0	0	0	0
Bacillus cereus	100	6	9	8	7
	200	10	13	10	11
	400	12	18	17	14
	control	0	0	0	0

0 = no effect

From the summarized results exhibited in Table (4) showed that the methanol , acetone, ethanol and aqueous extracts of guava was high effect against all bacteria except *Salmonella* spp exhibited resistance to the aqueous extract .

Table 4 : Diameter of inhibition zone (mm) of *Psidium guajava* extracts against pathogenic bacteria:

Pathogenic bacteria	Concentrations mg / ml	Mean values of inhibition zone (mm)			
		Aqueous extract	Methanol extract	Ethanol extract	Acetone extract
Escherichia coli	100	13	15	9	10
	200	16	16	10	14
	400	18	19	16	15
	control	0	0	0	0
Salmonella spp	100	0	14	17	8
	200	0	15	17	10
	400	0	17	22	11
	control	0	0	0	0
Shigella flexneri	100	11	10	13	10
	200	16	13	14	13
	400	16	13	18	13
	control	0	0	0	0
Enterobacter sakazakii	100	14	13	11	8
	200	15	15	12	13
	400	18	15	16	14
	control	0	0	0	0
Staphylococcus aureus	100	11	12	12	11
	200	14	16	12	12
	400	17	17	15	14
	control	0	0	0	0
Bacillus cereus	100	8	14	9	9
	200	10	15	13	12
	400	14	17	15	15
	control	0	0	0	0

0 = no effect .

The obtained results in Table (5) showed that the methanol, ethanol and acetone extracts of chamomile showed moderate active against all bacteria but ethanol extract showed no effect on *Salmonella* spp , the aqueous extract showed low effect on all bacteria at high concentration .

Table 5 : Diameter of inhibition zone (mm) of *Matricaria chamomilla* extracts against pathogenic bacteria:

Pathogenic bacteria	Concentrations mg / ml	Mean values of inhibition zone (mm)			
		Aqueous extract	Methanol extract	Ethanol extract	Acetone extract
Escherichia coli	100	0	7	11	11
	200	0	13	12	14
	400	5	14	15	15
	control	0	0	0	0
Salmonella spp	100	2	11	0	9
	200	3	15	0	10
	400	7	16	0	12
	control	0	0	0	0
Shigella flexneri	100	0	10	8	7
	200	2	11	8	12
	400	5	13	9	13
	control	0	0	0	0
Enterobacter sakazakii	100	4	12	11	13
	200	4	15	12	15
	400	8	16	12	17
	control	0	0	0	0
Staphylococcus aureus	100	0	14	9	12
	200	0	17	10	16
	400	5	18	14	17
	control	0	0	0	0
Bacillus cereus	100	0	11	8	8
	200	0	12	10	10
	400	1	14	13	12
	control	0	0	0	0

0 = no effect

The obtained results in Table (6) clearly showed that the methanol , ethanol and acetone extracts of onion showed low effect on all bacteria but the aqueous extract showed no effect on all bacteria .

Table 6 : Diameter of inhibition zone (mm) of *Allium cepa* extracts against pathogenic bacteria:

Pathogenic bacteria	Concentrations mg / ml	Mean values of inhibition zone (mm)			
		Aqueous extract	Methanol extract	Ethanol extract	Acetone extract
Escherichia coli	100	0	6	7	5
	200	0	8	8	6
	400	0	11	11	7
	control	0	0	0	0
Salmonella spp	100	0	3	0	6
	200	0	7	0	6
	400	0	11	3	8
	control	0	0	0	0
Shigella flexneri	100	0	6	4	7
	200	0	11	4	8
	400	0	12	7	11
	control	0	0	0	0
Enterobacter sakazakii	100	0	5	4	5
	200	0	7	6	7
	400	0	11	10	10
	control	0	0	0	0
Staphylococcus aureus	100	0	4	6	8
	200	0	7	7	9
	400	0	10	10	9
	control	0	0	0	0
Bacillus cereus	100	0	6	6	8
	200	0	8	9	9
	400	0	10	11	11
	control	0	0	0	0

0 = no effect

Data recorded in Table (7) showed that the methanol extract of garlic showed moderate effect on all bacteria while the ethanol and acetone extracts exhibited low effect on some bacteria , the acetone extract did not effect on *Salmonella* spp also the ethanol extract had not effect on both *Salmonella* spp, *Enterobacter sakazakii* and *Staphylococcus aureus* . The aqueous extract showed no effect on all bacteria .

Table 7 : Diameter of inhibition zone (mm) of *Allium sativum* extracts against pathogenic bacteria:

Pathogenic bacteria	Concentrations mg / ml	Mean values of inhibition zone (mm)			
		Aqueous extract	Methanol extract	Ethanol extract	Acetone extract
Escherichia coli	100	0	6	8	6
	200	0	10	9	8
	400	0	12	11	8
	control	0	0	0	0
Salmonella spp	100	0	0	0	0
	200	0	7	0	0
	400	0	15	0	0
	control	0	0	0	0
Shigella flexneri	100	0	8	2	5
	200	0	10	3	7
	400	0	11	3	7
	control	0	0	0	0
Enterobacter sakazakii	100	0	6	0	2
	200	0	6	0	4
	400	0	11	0	5
	control	0	0	0	0
Staphylococcus aureus	100	0	4	0	8
	200	0	8	0	10
	400	0	11	0	10
	control	0	0	0	0
Bacillus cereus	100	0	10	8	9
	200	0	11	10	9
	400	0	13	11	10
	control	0	0	0	0

0 = no effect .

The results confirmed that the plant extracts represent a rich potential source of alternative and environmentally acceptable control agents for infectious organism due to their antimicrobial properties. The plant extracts contain active substances. The methanol extract of almost plants showed highest effect than other extracts. Many studies reported that the differentiated effect of plant extracts on microorganisms based on their composition, the characteristics of plant material and concentration used. Rosemary and orange showed higher inhibitory effect followed by camphor and guava. Chamomile showed moderate effect, garlic and onion showed lowest effect, black seed showed no effect. Rosemary is valuable medicinal herbal and widely used in pharmaceutical because it have high antimicrobial activity due to carnosic acid and carnosol and is rich source of phenolic compounds (Del Campo *et.al*., 2000) , the antioxidant activity is due to these compounds. Fruit peels possess a quality of antimicrobial, anti-inflammatory, antibacterial and antifungal properties , it used in the treatment of skin tnfectious. Many studies has been used orange peel extract in treatment cancer whereas found that orange peels contain active compounds makes it useful in folk medicine to treat many diseases (Evanepoel 2001). The researches reported that the potential use of flavonoids in citrus as inhibitor s of neoplastic transformation and as free radical scavengers to prevent oxidative skin damage (McGravey and Croteau 1995). The results showed that the guava leaves extract have antimicrobial activity due to several chemical compounds such as comarins, essential oils, flavonoids, triterpenes, ellagitannins guajaverine and psydiolic acid. Guava leaves are useful medicinal plant and used in folk medicines to treat diarrhea causing bacteria such *Staphylococcus*, *Shigella*, *Salmonella, Bacillus, E. coli, Psedomonas* (Gran and Demello 1999). Guava leaf extracts have many biological activities such as antibacterial, anti-inflammatory, analgesic, antimicrobial, phytotoxic, hepatoprotection and antihyperglycaemic and anticancer activities (Barbalho *et.al*., 2012). The antimicrobial activity of eucalyptus due to the presence of two or three major compounds are terpenes and terpenoids as well as aromatic and aliphatic, the monoterpenes are constituting 90 % of essential oils and comprising a great variety of structure (Burt 2004). Essential oils have many therapeutic properties they are used for their antiseptic properties against infectious diseases of fungal and dermatophytes (Zrira *et.al*., 2005). Eucalyptus was used as potential natural agents for food preservation as pharmaceutical products (Rota *et.al*., 2011). Studies showed antibacterial and antiviral activity of myrtaceous in eucalyptus oil . The chamomile have antimicrobial activity, it has been widely used in folk traditional medicines for its multi therapeutic, cosmetic and nutritional values (Issac 1989). Chamomile flowers contain essential oil 0.4 -2 % which has antibacterial and antifungal properties and more than 120 chemical constituents. The antioxidant, inflammatory and sedative effects of chamomile and its major components such as (apigenin, azulene and bisabolol) were reported by (McKay and Blumberg 2006). From the results showed that garlic and onion exhibited low effect on bacteria although the garlic possess allicin which it powerful antibiotic and antifungal compound. Allicin which is released when garlic is crushed exhibit antibacterial activity against a wide of bacteria such as *E. coli.* Allicin effects through membranes may greatly inhancethe intra cellular interaction with thiols (Mirelman *et.al.*, 2000). This result may be related to the method of extraction because allicin is not a very stable compound, it degrades and destroyed by heat. Onion contain sulfur compounds showed anti-inflammatory, thiosulfinates components have antioxidant activity also contain flavonoids active against microorganisms and quercetin (Azu and Onyeagba 2007) and reported that garlic is more effective than onion and this may be attributed to the high molecular weight of the onion extract and the rate of diffusion is slowly reduced and it takes longer time. Black seed showed no antimicrobial activity on all tested bacteria although black seed extract or oil possess antimicrobial, antioxidant, antitumor activities due to contain fixed oil, protein, alkaloids, saponins and essential oils (Burtis and Bucar 2000). This result may be related to the solvent used in the extraction and concentration of extracts .

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) :

The results from MIC and MBC of plant extracts were shown in Table (8).

The aqueous extract of rosemary showed MIC and MBC more than (25 mg / ml) against all bacteria and the methanol extract was MIC 12.5 mg / ml) and MBC (6.25 mg / ml) against *Escherichia coli* , *Staphylococcus aureus* and *Bacillus cereus* but MIC was (12.5 mg / ml) and MBC was (25 mg / ml) for *Shigella flexneri* and *Enterobacter sakazakii* . The ethanol extract possessed MIC at (6.25 mg / ml) and MBC at (12.5 mg / ml) against *E. coli* and *St. aureus* while MIC was (12.5 mg / ml) and MBC was (25 mg /ml) for *Shigella flexneri* and *Bacillus cereus* but MIC and MBC were more than (25 mg / ml) against *Enterobacter sakazakii* , while *Salmonella spp* was resistance to the ethanol extract . The acetone extract showed MIC at (6.25 mg / ml) and MBC at (12,5 mg / ml) for *Salmonella* spp and *Enterobacter sakazakii*. MIC was (12.5 mg / ml) and MBC was (25 mg / ml) for *Escherichia coli* , *Staphylococcus aureus , Shigella flexneri* but MIC was (25 mg / ml) and MBC was more than (25 mg / ml) for *Bacillus cereus*.

Other plant extracts of camphor, guava, orange, chamomil, onion, garlic and black seed showed that MIC and MBC were more than (25 mg / ml) for all bacteria.

From the results the MIC values for the extracts were lower than their MBC values this suggests that they were bacteriostatic at lower concentrations but bactericidal at high concentrations. From the results obtained the most sensitivity was observed in the plant extracts of rosemary .

Table 8 : Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Rosmarinus officinalis* extracts against pathogenic bacteria :

Pathogenic bacteria	Aqueous Extract mg /ml		Methanol Extract mg /ml		Ethanol Extract mg /ml		Acetone Extract mg /ml	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	< 25	< 25	6.25	12.5	6.25	12.5	12.5	25
Salmonella spp	< 25	< 25	6.25	12.5	-	-	6.25	12.5
Shigella flexneri	< 25	< 25	12.5	25	12.5	25	12.5	25
Ennterobacter sakazakii	< 25	< 25	12.5	25	< 25	< 25	6.25	12.5
Staphphylococcus aureus	< 25	< 25	6.25	12.5	6.25	12.5	12.5	25
Bacillus cereus	< 25	< 25	6.25	12.5	12.5	25	25	< 25

0 = no determine.

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تأثير بعض المستخلصات النباتية على البكتيريا الممرضة .

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أجريت هذه الدراسة لتقدير النشاط المضاد للبكتيريا لبعض المستخلصات النباتية مثل حصالبان , قشر البرتقال , الكافور , الجوافة , البابونج , البصل , الثوم , حبة البركة وحضرت المستخلصات بإستخدام 4 مذيبات (ماء –ميثانول – إيثانول – أسيتون) وتم عمل تركيزات مختلفة (100 – 200 – 400 مجم / مللى) لقياس قطر منطقة التثبيط للبكتيريا الممرضة وهى , *Escherichia coli* , *Salmonella* spp *Shigella flexneri, Enterobacter sakazakii, Staphylococcus aureus, Bacillus cereus* وذلك بإستخدام طريقة الإنتشار فى الأجار . أيضاً تم تقدير أقل تركيز مثبط وأقل تركيز قاتل للمستخلصات النباتية بإستخدام طريقة سلسلة التخفيفات وتم عمل تركيزات (56, 1- 175, 3 – 25, 6- 12,5 – 25 مجم / مللى) . وأظهرت النتائج أن مستخلصات حصالبان , قشر البرتقال , الكافور , الجوافة كانت أكثر تأثير على جميع البكتيريا فيما عدا بكتيريا *Salmonella spp* كانت مقاومة للمستخلص المائى والإيثانولى لحصالبان , المستخلص المائى للكافور والجوافة . أما البابونج أعطى تأثير متوسط على جميع البكتيريا فيما عدا *Salmonella* spp كانت مقاومة للمستخلص الإيثانولى . البصل والثوم أعطوا أقل تأثيرعلى البكتيريا وجميع الأنواع البكتيرية تحت الدراسة كانت مقاومة للمستخلص المائى لكل منهما . حبة البركة كانت غير مؤثرة على جميع البكتيريا الممرضة .

وبدراسة أقل تركيز مثبط وأقل تركيز قاتل أوضحت النتائج الأتى بالنسبة لمستخلصات حصالبان :

أولاً : المستخلص المائى كان أقل تركيز مثبط (MIC) وأقل تركيز قاتل (MBC) 25 مجم / مللى لجميع الميكروبات الممرضة .

ثانياً : المستخلص الميثانولى كان أقل تركيزمثبط 25, 6 مجم / مللى وأقل تركيز قاتل 5, 2 1 مجم / مللى لكل من *Salmonella* spp, *Staphylococcus aureus* , *Escherichia coli* and *Bacillus cereus* وكان أقل تركيز مثبط 5 , 12 مجم / مللى وأقل تركيز قاتل 25 مجم / مللى لكل من *Shigella flexneri* and *Enterobacter sakazakii* .

ثالثاً : المستخلص الإيثانولى كان أقل تركيز مثبط 25, 6 مجم / ماللى وأقل تركيزقاتل 5, 12 مجم / مللى لكل من *Escherichia coli* and *Staphylococcus aureus*. وكان أقل تركيز مثبط 5 , 12 مجم / مللى وأقل تركيز قاتل 25 مجم / مللى لكل من *Bacillus cereus* and *Shigella flexneri* وكان أقل تركيز مثبط وأقل تركيز قاتل أكثر من 25 مجم / مللى لبكتيريا Enterobacter sakazakii أما *Salmonella* spp كانت مقاومة لذات المستخلص

رابعاً : المستخلص الأسيتونى كان أقل تركيز مثبط 25, 6 مجم / مللى وأقل تركيز قاتل 5, 12 مجم / مللى لبكتيريا *Enterobacter sakazakii* وكان أقل تركيز مثبط 5 , 12 مجم / مللى وأقل تركيز قاتل 25 مجم / مللى لكل من *Staphylococcus aureus* , *Shigella flexneri* , *Escherichia coli* and وكان أقل تركيز مثبط 25 مجم / مللى وأقل تركيز قاتل أكثر من 25 مجم / مللى لبكتيريا *Bacillus cereus* .

وبالنسبة للمستخلصات النباتية الأخرى لكل من : الكافور , الجوافة , البابونج , البصل , الثوم , قشر البرتقال أوضحت النتائج أن أقل تركيز مثبط وأقل تركيز قاتل كان أكثر من 25 مجم / مللى لكل الميكروبات المستخدمة فى الدراسة .