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Antimicrobial Activity Of Crude Extracts Of Alchemilla Vulgaris and Salvia Officinalis L. Against Some Bacterial Strains Causing Food Poisoning Diseases

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Abstract: *Antimicrobial activity of different extracts of leaves of Alchemilla Vulgaris and Salvia Officinalis L. were investigated against Bacillus cereus, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa using agar disc diffusion technique. the antimicrobial activity of leaves of Alchemilla Vulgaris and Salvia Officinalis L were screened by used different extracts[MeOH; MeOH 70% EtOH ; EtOH70%]. The Results showed that the methanolic extracts have the highest effective against S. aureus respectively E. coli, with MIC ranged from 1.25 to 3 mg/ml and MBC of 3.0 to 6.25 mg/ml, while the methanolic70% extracts have the highest effective against B. cereus, with MIC ranged from 2.25 to 4.5 mg/ml and MBC of 5.0 to 10.25mg/ml but P. aeruginosa was very resistant to all extracts expect methanolic and methanolic70% extracts of the leaves of Salvia Officinalis L. which was effective against P. In general, the different extracts of leaves of Salvia Officinalis L. were more effective against the tested bacterial than the different extracts of leaves of Alchemilla Vulgaris. These plant extracts which proved to be potentially effective can be used as natural alternative preventives to control food poisoning diseases and preserve food stuff avoiding healthy hazards of chemically antimicrobial agent applications.*

Keywords: *antimicrobial, Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Alchemilla Vulgaris, Salvia Officinalis L.*

INTRODUCTION

Food poisoning is considered as one of the most common cause of illness and death in developing countries[1.2]. Most of food poisoning reports are associated with bacterial contamination especially members of Gram negative bacteria like Salmonella typhi, Escherichia coli and Pseudomonas aeruginosa [3].Other Gram positive bacteria includingStaphylococcus aureus and Bacillus cereus have been also identified as the causal agents of food borne diseases or food spoilage [4]. Prevention of food spoilage and their etiological agent is traditionally achieved by the use of chemical preservatives[5]. Despite of the proven efficiency of these chemical preservative in prevention and outbreak control of food poisoning diseases, their repeated applications has resulted in the accumulation of chemical residues in food and feed chain, acquisition of microbial resistance to the applied chemicals and unpleasant side effects of these chemicals on human health[6].Because of such concern, efforts have been focused on developing a potentially effective, healthy safer and natural food preservatives.Within these contexts is the utilization of plant extracts as antimicrobial agents for food preservation [7,8].Acute food borne infections and intoxications are much more of a concern to government and the food industry today than a few decades ago. Some of the factors that have led to that include, the identification of new aetiological agents, increasing number of large outbreaks being reported, the impact of food-borne diseases on children, the aging population and the immunocompromised individuals [9]. The epidemiology of food borne diseases is changing and reports from different parts of the world indicate that strains of resistant food borne pathogens have emerged as public health problem. Over the last two decades for examples, bacterial infections caused by *Salmonella enteritidis, Staphylococcus aureus, , Escherichia coli* and newer food borne pathogens have become increasingly

resistant to empirical antimicrobial agents [10].The search for new antimicrobial natural products from plant materials is essential in order to curb the menace of multiple antibiotics resistant pathogens. Medicinal plants constitute an effective source of antimicrobial natural products. The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs [11].Plants have been used in traditional medicine for many centuries as arbotifacients, contraceptives, for menstrual regulation, fertility control, as well for the treatment of ailments of both microbial and non-microbial origins [12].

MATERIALS AND METHODS

2.1.Plant material:

The leaves of *Alchemilla Vulgaris*were collected form Hama Al Salamya in Syria, the leaves of *Salvia Officinalis L.* collected from Latakia Kasab in Syria..Identification of plants maternal was done by the dipartites inthe Agriculture faculty- Aleppo University.

The leaves were washed , and dried in shade at ambient temperature (25-30°C) for 15-20 days , then they were ground into fine powder**.**

2.2. Extracts Preparation:

3g from each dry parts of plants were extracted with 100ml of [MeOH, MeOH 70%, EtOH, EtOH 70%,], three times,then the extractscombiended vaporated by using a rotary evaporator under decreased pressure,at 40°C until obtain the crude extract.

The crude extracts were dissolved in DMF that did not affect bacterial activity [13]

2.3. Bacterial strains:

The antibacterial potency of each plant extract was evaluated using four bacterial strains causing food poisoning diseases. Two strains of Gram positive (Staphylococcus aureus and Bacillus cereus) and two strains of Gram negative (Escherichia coli, and Pseudomonas aeruginosa) bacteria. The bacterial strains

were provided from the microbiology laboratory, faculty of science, Aleppo university.

2.4. Inoculums preparation:

Each bacterial strain was subcultured overnight at 35°C in Mueller-Hilton agar slants. The bacterial growth was harvested using 5 ml of sterile saline water, its absorbance was adjusted at 580 um and diluted to attain viable cell count of 10^7 CFU/ml using spectrophotometer.

2.5. Antibacterial activity of plants extract:

The antibacterial activity of plant extract was preliminarily screened by well diffusion assay. LB agar plates were spread plated with 20 µL of bacterial strain ($1x10^7$cfu/ml). The wells of 6 mm diameter were made in the agar plates. Each plant extract was tested for antibacterial activity by adding 40 µL of extracts in different concentrations 5, 10 and 20 mg ml-1. The experiment was repeated thrice. The plates were incubated at 37oC for 24 hour. Subsequently, the plates were examined for zone of inhibition (ZOI) and diameter was measured in mm after subtracting well diameter [14].

To determine Minimum inhibitory concentration (MIC), required quantity of extract was added in to the LB broth of 4 ml to bring initial concentration of 20 mg ml-1. Then, two-fold serial dilution was done.

their respective concentrations are 20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078 mg ml-1. In each test tube 0.1 ml of standardized inoculum ($1x10^7$cfu/ml) was added. Two control tubes were maintained for each test batch namely extract control (tube containing plant extract and LB medium without inoculum) and organism control (tube containing LB medium and inoculum). The test tubes were incubated at 37oC for 24 hour. The lowest concentration (highest dilution) of plant extract that produced no visible growth (no turbidity) recorded as minimum inhibitory concentration. Minimum bactericidal concentration (MBC) was assed by sub-culturing test dilutions on to a drug free solid medium. The plates were incubated for 24 hours at 37. The lowest concentration of the antimicrobial at which no single colony observed after sub-culturing is regarded as Minimum bactericidal concentration [15].

RESULTS AND DISCUSSION

3.1. Antibacterial activity of plants extract:

Leaves of *Alchemilla Vulgaris* and *Salvia Officinalis* were investigated to evaluate their antibacterial activity against food poisoning bacteria including two strains of Gram positive bacteria (B. cereus and S. aureus) and two strains of Gram negative bacteria (E. coli, and P. aeruginosa) using disc diffusion method. Evaluation of antibacterial activity of these plant extracts was recorded in tables (1,3).

Table.1: Antimicrobial Activity of different extracts of leaves of *Alchemilla Vulgaris* against some bacterial strains of food poisoning diseases.

		Zone of inhibition (mm)			
extract	Concentration mg/ml	B. cereus	S. aureus	E. coli	P. aeruginosa
MeOH	5	8	9	11	-
	10	11	13	15	-
	20	14	17	20	7
MeOH 70%	5	10	-	8	-
	10	14	8	11	-
	20	18	12	15	-
EtOH	5	-	-	-	-
	10	-	8	9	-
	20	9	10	12	-
EtOH 70%	5	-	-	-	-
	10	8	9	11	-
	20	10	13	14	-

Table.2: MIC and MBC of different extracts of leaves of *Alchemilla Vulgaris.*

	MIC mg/ml				MBC mg/ml			
extract	B. cereus	S. aureus	E. coli	P. aeruginosa	B. cereus	S. aureus	E. coli	P. aeruginosa
MeOH	4.25	3.0	2.25	20	9.25	6.25	4.5	>20
MeOH 70%	4.5	8.6	5.0	>20	10.25	18.5	11.25	>20
EtOH	15.5	9.5	8.25	>20	>20	20.0	16.5	>20
EtOH 70%	9.25	8.5	7.5	>20	18.5	17.0	15.25	>20

The results in tables (1,2) showed that the different extracts of the leaves of *Alchemilla Vulgaris* have a good effect against the bacterial test except *Pseudomonas aeruginosa* which was very resistance to the different extracts, also the results showed that the MeOH extract have the highest effect against *Staphylococcus aureus* with MIC =3.0mg/ml ,MBC =6.25 mg/ml and *Escherichia coli* with MIC =2.25 mg/ml ,MBC =4.5 mg/ml while the MeOH70% extract have the highest effect against *Bacillus cereus* with MIC =4.5 mg/ml ,MBC =10.25 mg/ml, compared to other extracts, as shown in figure 1.

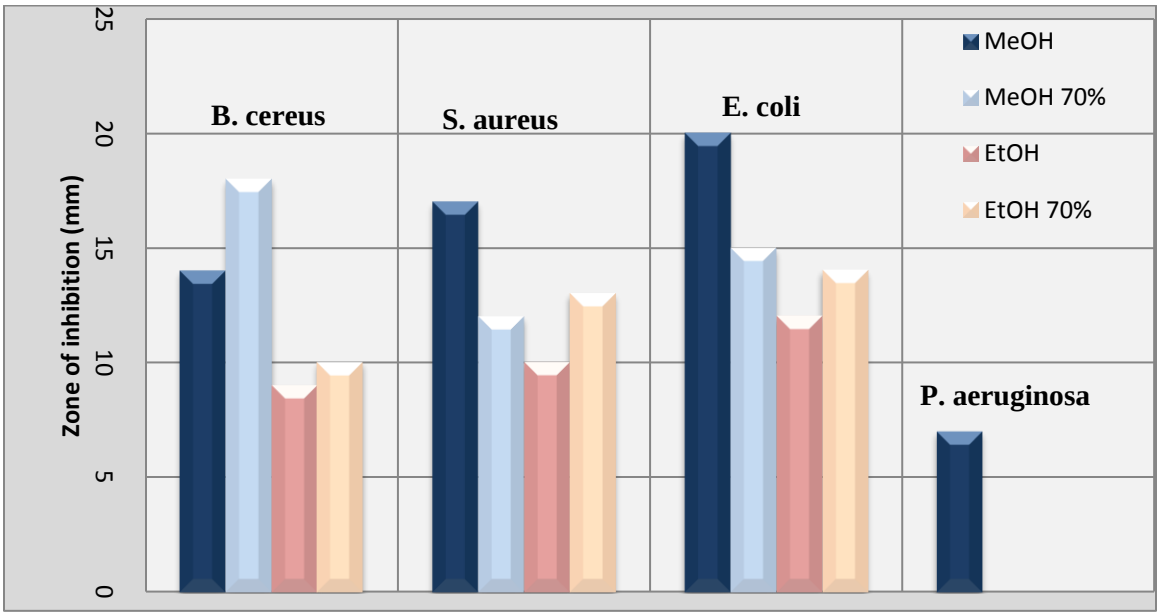

Figure.1: Antimicrobial Activity of different extracts of leaves of *Alchemilla Vulgaris* at concentration (20mg/ml) against some bacterial strains of food poisoning diseases.

Table.3: Antimicrobial Activity of different extracts of leaves of Salvia Officinalis against some bacterial strains of food poisoning diseases.

	Zone of inhibition (mm)				
Extract	Concentration mg/ml	B. cereus	S. aureus	E. coli	P. aeruginosa
MeOH	5	10	12	11	8
	10	17	19	17	14
	20	21	28	25	18
MeOH 70%	5	11	9	8	8
	10	19	17	14	11
	20	27	23	19	16
EtOH	5	-	-	9	-
	10	10	8	12	-
	20	12	11	16	-
EtOH 70%	5	7	-	10	-
	10	11	9	12	-
	20	13	12	17	7

Table.4: MIC and MBC of different extracts of leaves of Salvia Officinalis.

	MIC mg/ml				MBC mg/ml			
extract	B. cereus	S. aureus	E. coli	P. aeruginosa	B. cereus	S. aureus	E. coli	P. aeruginosa
MeOH	3.5	1.25	1.5	4.5	7.5	3.0	4.25	10.5
MeOH 70%	2.25	4.25	4.5	5.0	5.25	5.5	11.5	13.5
EtOH	6.25	9.25	4.5	>20	10.25	20.0	9.25	>20
EtOH 70%	5.0	9.5	4.25	20	10.0	18.5	9.5	>20

The results in tables (3,4) showed that the different extracts of the leaves of *Salvia Officinalis* have a high effect against the bacterial test except *Pseudomonas aeruginosa* which was very resistance to the most extracts, expect the methanolic and methanolic70% extracts which have a good effective against *Pseudomonas aeruginosa* with MIC range 4.5 to 5.0mg/ml and MBC 10.5 to 13.5 mg/ml , also the results showed that the MeOH extract have the highest effect against *Staphylococcus aureus* with MIC =1.25 mg/ml ,MBC =3.0 mg/ml and *Escherichia coli* with MIC =1.5 mg/ml ,MBC =3.0 mg/ml while the MeOH70% extract have the highest effect against *Bacillus cereus* with MIC =2.25 mg/ml ,MBC =5.25 mg/ml, compared to other extracts, as shown in figure 2.

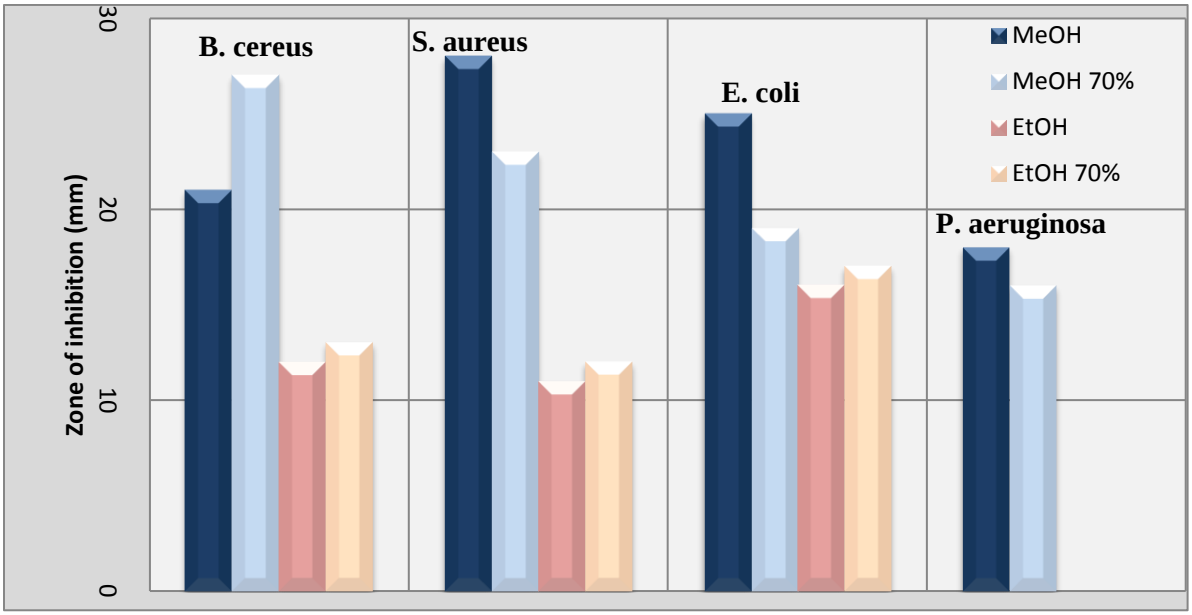

Figure 2. Antimicrobial Activity of different extracts of leaves of *Salvia Officinalis* at concentration (20mg/ml) against some bacterial strains of food poisoning diseases.

The results in the tables [1,3] showed that the leaves of *Salvia Officinalis* have higher effective against the test bacterial comparing with the leaves of *Alchemilla vulgaris.*

CONCLUSION

The plant extracts of *Alchemilla Vulgaris* and *Salvia Officinalis* which proved to be potentially effective as (*B. cereus, S. aureus, E. coli, P. aeruginosa*) can be used as natural alternative preventives to control food poisoning diseases and preserve food stuff avoiding healthy hazards of chemically antimicrobial agent applications.

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