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Antibacterial Activity Investigation and Anti-Biotic Sensitive's for Different Solvents (Ethanol, propanol, DMSO and di Ethel ether) Extracts of Seeds, Leafs and Stems of (*Laurus azorica* and *Avena sterilis*) Plants

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A B S T R A C T

The antibacterial investigation of different extracts (Ethanol, propanol, DMSO and di Ethel either) was carried on different species of bacteria including Gram positive bacteria (*Staphylococcus aureus, Bacillus cereus and Streptococcus pneumoniae)* and Gram negative bacteria: (*Escherichia coli, Pseudomonas aeruginosa, Agnobacteriumsp,* and *Erwinia carotovora*).This study was carried out on leafs, stems and seeds of *Laurus azorica* and *Avena sterilis* plants, which growing at Al –Gabal Al –Akhder region, Libya.).The results recorded that, there are variations for antibacterial activities, also the results showed different effects on the selected microbial species which selected in this study, where some of extracts gave inhibition zones compared with those which no gave the effect on the studied bacteria. The results recorded different effects of the solvents extracts depending on the polarities and bacteria species. Also the anti-biotic sensitive studies of the plant extracts showed effects on the selected anti-biotic in this study.

Introduction

Plants typically produce several secondary metabolites such as phenols, flavonoids, quinones, tannins, alkaloids, saponins, and sterols, which are important sources of biocides and many other pharmaceuticals. Medicinal plants play an important role in pharmacological research and drug development. Plants have been cited as a rich source of drugs because they produce a wide range of biologically active molecules, most of which likely evolved as chemical defenses against predatory or infectious and antioxidant compounds (Cosimir *et al.,* 2008). Oxidative changes in the metabolic pathway causing disorders are oxidative changes in metabolism causing many diseases. Such bioactive compounds have gained special attention as they can protect the human body against the oxidative stress that can cause many diseases including cancer, cardiovascular disorders, aging and antimicrobial properties (Boussoussa *et al.,* 2014). Current evidence shows that several chronic diseases, including cardiovascular disorders and at least some kinds of cancer, are caused by free radical

oxidation of lipids, nucleic acids and proteins (El *et al.,* 2014). Many antioxidant compounds, including phenolics, carotenoids, anthocyanins, and tocopherols, can be found in plant Approximately 20% of known plants were used in pharmaceutical studies, with positive effects on the healthcare Plants that have beneficial phytochemicals can complement the needs of the human body by serving as natural antioxidants. Many studies have shown many plants to be rich sources of antioxidants as (vitamins A, C, E and phenolic compounds) they all act as plant-borne antioxidants, such as flavonoids, tannins and lignins (Altemimi *et al.,* 2017). The Arab medical tradition was found in the 10th century, expanded in the 11th and 12th centuries and reached its peak from the 13th to the 16th centuries and Declined from the seventeenth century to the nineteenth yet in the twentieth century, the use of traditional medicine, particularly herbal medicine, was still widespread throughout the Middle East, and it remains (Abu-Rabia, 2015). The use of such alternative medicines has become ever more common in the advanced world. Libya has an enormous wealth of medicinal plants scattered throughout a vast area, especially in the Al-Jabil Al-Akhtar region and these plants are used for their medicinal qualities in Libyan folk medicine Al-Jabal Al-Akhtar has a wide variety of plant species which display medicinal and economic importance plant derived pharmaceutical products are commonly used because they are cheaper, safer than synthetic drugs and easier to Iwu *et al.,* (1999).

Avena sterilis L its name in Arabic is (khafour or shufan,) and in language is English called (*Oats*). Most of active ingredients are: soluble fiber, proteins, polyunsaturated fatty acids, vitamins, niacin and phytochemicals. Felt tired Bran contains a range of B vitamins, proteins, fats, minerals and soluble fiber that are good for the heart, also called β-glucan. Such as magnesium, iron, copper, potassium, and selenium applications: nourishing, diuretic, laxative, sedative, sedative, antispasmodic and tonic.

Oral boiling water is administered to strengthen women after childbirth Increased milk while breastfeeding. Soaked seeds in water are used as a cosmetic product (Abu-Rabia, 2015). *Oats* are immune to a variety of major crop diseases and cultivated in crop rotation Limit the harmful effects of pathogen accumulation and alter soil microflora to suppress Pathogens Disease resistance of oats is mainly due to two major minor metabolite groups, flavonoids and saponins.

Sterilis avena L. Is *Oats* primary genetic relative (*Avena sativa L.), Avena sterilis* is common in regions throughout Africa, temperate Asia, India and Europe (Boczkowska *et al.,* 2016). *Oat* has many opportunities for development as food, industrial and pharmaceutical goods, all adding value to the *oat* crop *Oat* is mainly used for feeding animals and food for human health.

And also, a good food crop that supplies consumers with B-glucans and dietary fiber elements, high tocopherols and natural antioxidants. *Laurus (Lauraceae)* is a genus consisting of two species, *Laurus nobilis* and *L. azorica (Seub.) J. Franco*, The leafs *Nobilis* has been commonly used as a spice of flavor and has stimulant and narcotic properties, while its oil has fungicidal, bactericidal and insecticidal activity *L. Azorica* has been used as a stomach and antirheumatic agent.

Mechanisms of action Antimicrobial activities of phenolic compounds

With increased resistance to antibiotics, researchers began to look for alternative treatment or disease prevention so that medicinal plants were established extensively biological Resources of modern medicines (Derwich *et al.,* 2009).

Effect of different solvents on chemical compounds extraction

There are several extraction methods, depending not only on the extraction process but also on the solvent used for extraction. Polar and non-polar solvents. Polar solvents that have high electrical insulation Strong electrical solvents. The dielectric constant is known as the electrostatic law and it is an effect of energy on two charged ions and which are separated from each other. Polar solvents have high effectiveness in separation (blocking ions from each other), so the forces of attraction and repulsion are formed between the weak ions. Depending on a constant level in electrical insulation, the solvents produced by them are constant degrees of different color degrees at different temperatures. The polarity of the solvents depends on the dielectric constant value. Solvents that have an dielectric constant equal to 15 or higher are polar solvents and that have an dielectric constant less than 15 non-polar solvents. Also some of functional chemical groups are affecting on the polarity as presence of - OH, - C = O, - CHO and others. The difference in the polarity of the

extracting solvents can affect the solubility of chemical components in the sample and its extraction yield. Therefore, the selection of a suitable solvent system is one of the most important steps in optimizing the recovery of total phenolic compounds, total flavonoids, total condensed tannins and other biologically Different solvents, polarities may or may not be soluble. Polar solvents are commonly used for polyphenol recovery from plant matrices.

Aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate are the most suitable solvents. Ethanol was regarded as a strong solvent for extra polyphenol (Boussoussa *et al.,* 2014). The main aims of this to evaluate the effect of antibacterial activities of some plant extracts of two different (*Laurus azorica* and *Avena sterilis*) plants on positive and negative gram of some species of bacteria.

Experimental Part

The studied plants

Due to the importance of many plants which used at AL-Gabal AL-Khder region (Libya), this study was designed to select two different plants (*Laurus azorica* and *Avena sterilis*). The samples were collected from Al-Gabel Al – Kadar region during spring season of (2022).Stems, seeds and leafs of every species of the selected plants were separated then dried in open air and grinded by mortar then kept until analysis. (Figures 1 and 2).

Taxonomical investigation

The collected samples were identified in *Seliphium herbarium*, Botany Department, Faculty of Science, Omar Al Mukhtar University**.** In this study the antibacterial investigation of the studied samples was expressed as the following numbers, Table (1).

Antimicrobial activity

Preparation of extracts

Fresh of *Larae's azorica* and *Avena sterilis* washed two times tap water and subjected to shade drying at room temperature the dried plant material was powdered using a mechanical grinder (Akinpelu *et al.,* 2008). The seed, leafs and stems were separated, then dried again (Hamad *et al.,* 2014).

Solvent extraction

The powdered materials of *Larae's azorica* and *Avena sterilis* were extracted with different solvents, where 10 grams of each plant powders were added to 150 ml of aqueous and non-aqueous solvents (Ethanol, propanol, DMSO and di Ethyl ether). Crude extract was evaporated at 65 ^{0}C, with the rotary evaporator the extracts were collected and stored at 4^{0}C until further use (Hasan *et al.,* 2011). The extracts of solvents were expressed as (E, P, DO and DI) in this study.

Where: E: Ethanol, P: Propanol, DI, Di ethyl ether and DO: DMSO

Microorganisms

The extracts were individually tested against pathogenic bacteria, the following bacteria were tested:

Bacterial strains

Gram Positive bacteria

Three species of Gram positive (*Staphylococcus aureus, Bacillus cereus and Streptococcus pneumoniae)*.

Gram Negative bacteria

Two species of Gram negative (*Escherichia coli, Pseudomonas aeruginosa, Agnobacteriumsp,* and *Erwinia carotovora).*

Agar well diffusion method

Agar well diffusion method was followed to determine the antibacterial activity, mueller-Hintion ager (MH), Plates were swabbed (sterile cotton swabs) with pathogenic bacteria, well4 mm diameter were made in each of these plates using sterile cork borer, about100µl of different organic solvents. Were added by sterile syring into wells. The plates were incubated at37 °C for 18 – 24 h. zone of inhibition were measured using a meter rule as described by Mukherjee *et al.,* (1995) and Zeyaullah *et al.,* (2009).

Antibiotic Sensitivity Tests

In vitro antimicrobial susceptibility to nine antibiotics (Table, 2). The inoculums were prepared by adding

isolated colonies of the microorganism from an overnight nutrient agar plate into 2ml tryptone soya broth (TSB). *A sterile* cotton swab was dipped into the adjusted suspension.

The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab. The swab was streaked over the entire surface of the sterile Mueller Hinton Agar plate. This procedure was repeated by streaking two more times, rotating the plate approximately each time to ensure an even distribution of inoculums.

Plates were allowed to dry for 5 minutes and then the antimicrobial disks were dispensed onto the surface of inoculated agar plates using an Oxoid antibiotic. Plates were then incubated at 37 °C for 18-24 hours.

The diameters of the zones of inhibition are measured to the nearest mm using a venier calipers (junior), zones diameters were interpreted as being susceptible Sensitive (S) or Resistant (R) according to (NCCLS, 2001), Table (2).

Results and Discussion

Antibacterial studies

The Antibacterial activity studies were carried out on the solvent extracts for the studied plants (leafs, seeds and stems) against the selected species by applying the agar diffusion method and after incubation, the inhibition zones were recorded in mm, The results were described as following:

Gram positive bacteria

Staphylococcus aureus

According to Figures (1 -3), the results showed that *Staphylococcus aureus* was sensitive to all extracted ethanol except *Laurus azorica* (seeds), however propanol extract was the best bacterial growth inhibitory effect in all cases, while isolate was sensitive to Di Ethel extracts of *Laurus azorica* (leafs and stems) (had the least effect on inhibitory bacterial growth), more have the DOMS extract showed that *isolate* was only sensitive to *Laurus azorica* extracts (seeds, leafs and stems).

Bacillus cereus

The findings showed that *Bacillus cereus* was sensitive to ethanol extract of *Aveane sterilis* (seeds) and *Laurus azorica* (seed, leafs and stems), Whereas the extract of propanol showed that isolate was sensitive to *Laurus azorica* (seed, leafs and stem) but Di Ethel either revealed that(weakest effect inhibition on bacterial growth) isolate as sensitive only *to Laurus azorica* (stems), while DOMS showed that isolate was sensitive To*, Laurus azorica* (seeds and leafs) show Figures (4- 6).

Streptococcus pneumoniae

The results showed that *Streptococcus pneumoniae* is susceptible to ethanol extract as the best effect on growth inhibition is considered, with *Aveane sterilis* (stems) 18 mm being the highest growth inhibitor whereas isolate was sensitive to propanol extracted of *Aveane sterilis* (seed) and *Laurus azorica* (seed and stems), but Di Ethel either had the weakest inhibition of bacterial growth only isolate is sensitive *to Laurus azorica* (stems) also revealed that isolate was sensitive to DOMS extracted to *Laurus azorica* (seed)recorded on (Figures 7-9). Similar results observed by Ahmad (2010).

Gram negative bacteria

Escherichia coli

The results revealed that *Escherichia coli* was sensitive to ethanol extract of *Aveane sterilis* (leaves) and *Laurus azorica* (seeds, leaves and stems), while isolate it was sensitive to *Laurus azorica* extracts (seeds, leaves, stems), isolate It was also sensitive s to the extraction of Di ethyl ether *Aveane sterilis* (leaves and stems) however it showed sensitivity to DOMS extracts as it showed the best inhibition of bacterial growth, *Aveane sterilis* (leafs) and *Laurus azorica* (seeds, leaves and stems), Figures (10-12). Similar results observed by Ahmad (2010) and Al- Amiery, (2010).

Pseudomonas aeruginosa

The results showed the *Pseudomonas aeruginosa* sensitivity to propanol extracted Aveane *steriles* (leafs) and *Laurus azorica* (stems) also Di Ethel either showed sensitivity to *Laurus azorica* extract (stems), Figures (13 -15).

Table.1 The samples numbers in this study.

Sample No.	Sample Type
1	*Avena sterilis* seeds
2	*Avena sterilis* leafs
3	*Avena sterilis* stems
4	*Laurus azorica* seeds
5	*Laurus azorica* leafs
6	*Laurus azorica* stems

Table.2 Antibiotic sensitivity testing

Antibiotic	Symbol	Concentration
Sulphamethoxazole/trimethoprim	SXT	25 mg/ml
Gentamicin	CN	10 mg/ml
Tetracycline	TE	30 mg/ml
Vancomycin	CTX	30 mg/ml
Oxacillin	OX	1 mg/ml
Ampicillin	APX	30 mg/ml
Clindamycin	DA	2 mg/ml
Amoxicillin	AMX	10 mg/ml

Table.3 Antibiotic Sensitivity tests

Antibiotic	Symbol	Organism						
		S. aureus	***B. cereus***	***S pneumoniae***	***E.coil***	***P. aeruginosa***	***Agnobacterium sp***	***Erwinia carotovora***
Sulphamethoxazole /trimethoprim	**SXT**	S	R	R	R	R	R	R
Vancomycin	**CIP**	S	S	S	R	R	R	R
Gentamicin	**CN**	S	S	S	S	S	S	S
Oxacillin	**OX**	R	R	R	R	R	R	R
Ampicillin	**AMP**	R	R	R	R	R	R	R
Tetracycline	**TE**	S	S	S	S	S	S	S
Amoxicillin	**AMX**	R	S	R	S	R	R	S
Clindamycin	**DA**	S	S	S	S	R	R	R

*S-Sensitive; **R-Resistant.

Figure.1 Antibacterial activity of different solvent of studied plant extracted against *Staphylococcus aureus*.

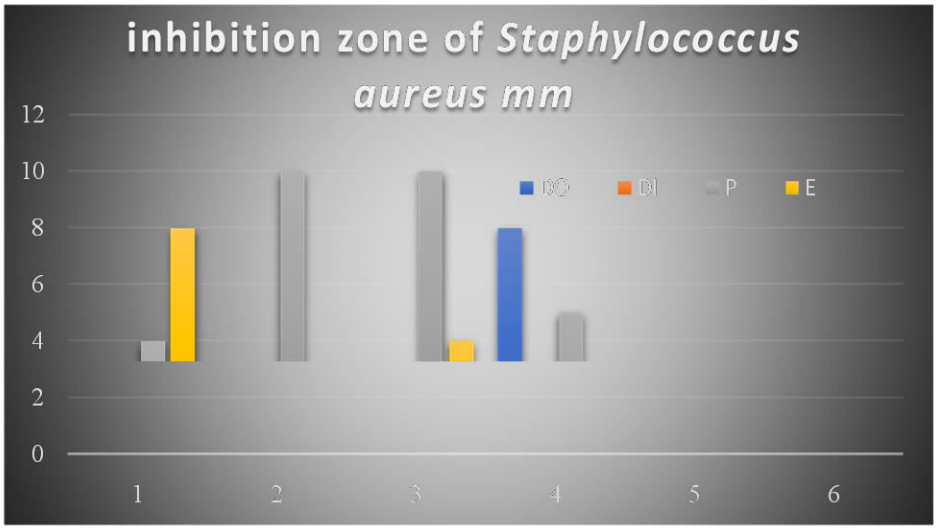


Figure.2 Effect of seeds, leafs and stem extracts of *Avena sterilis* against *Staphylococcus aureus*

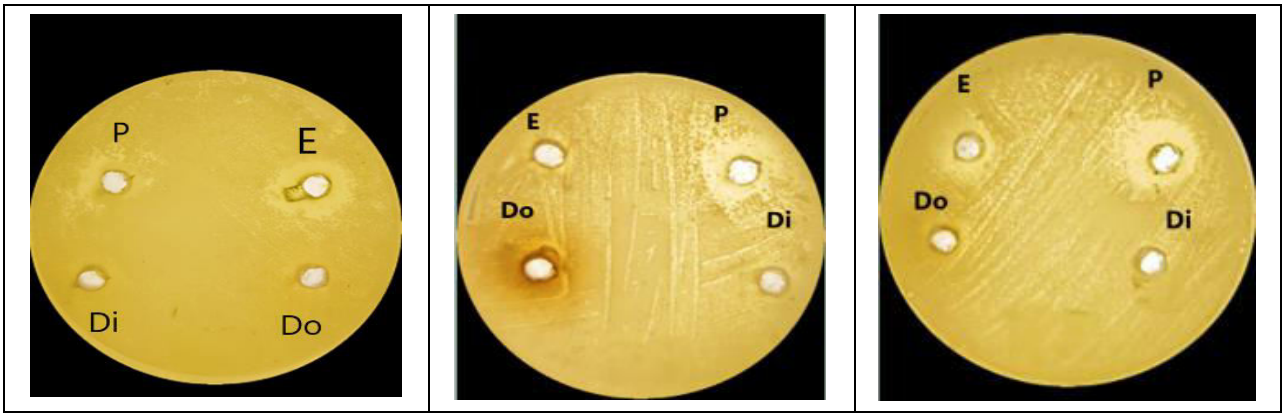


Figure.3 Effect of seeds, leafs and stem extracts of *Laurus azorica* against *Staphylococcus aureus*

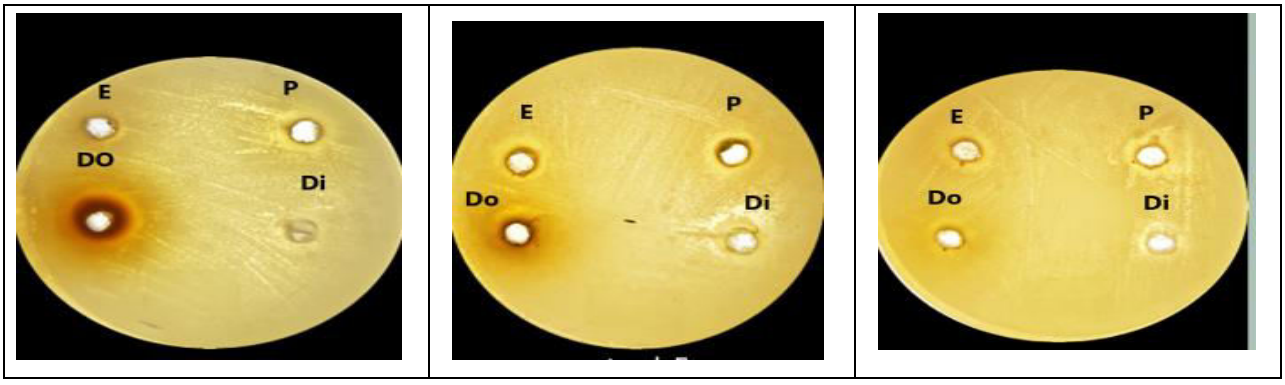


Figure.4 Antibacterial activity of different solvents of studied plant extracts against *Bacillus cereus*

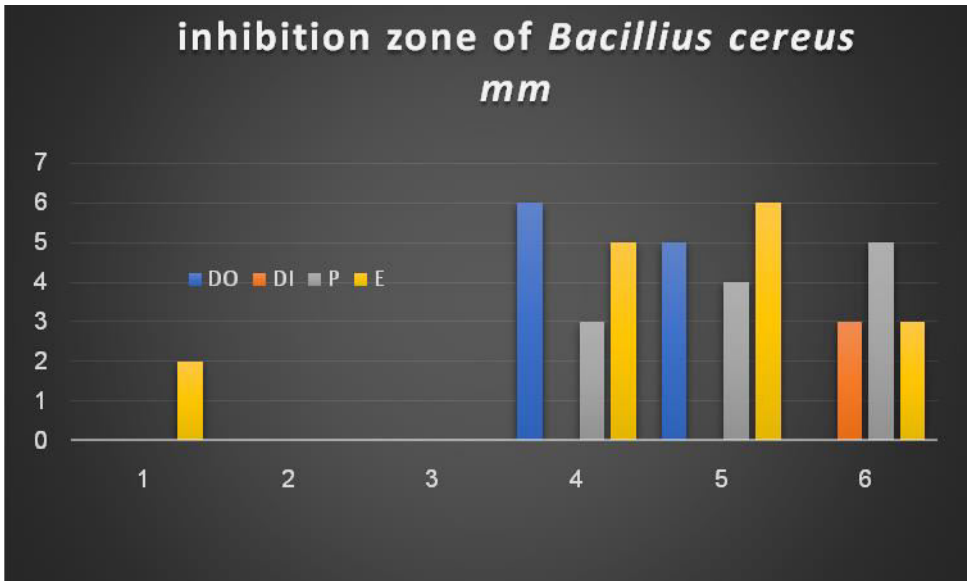


Figure.5 Effect of seeds, leafs and stem extracts of *Avena sterilis* against *Bacillus cereus*

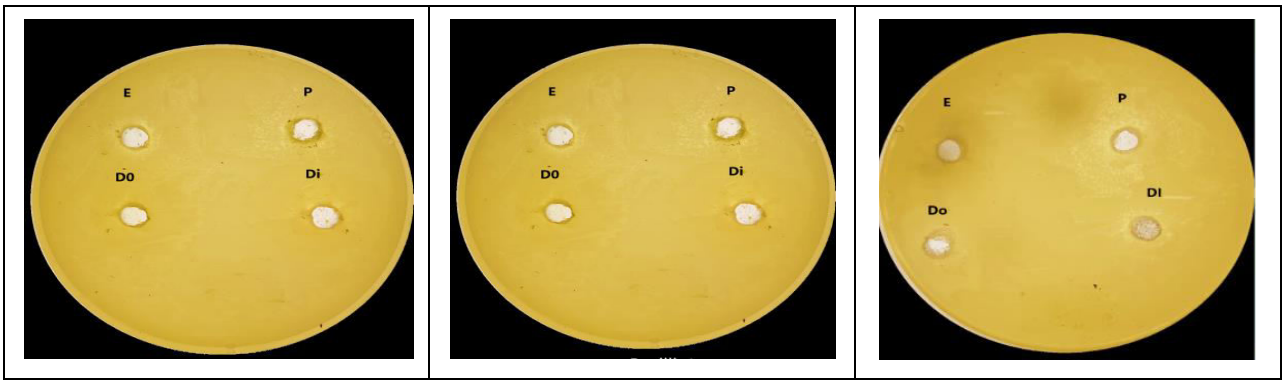


Figure.6 Effect of seeds, leafs and stem extracts of *Laurus azorica* against *Bacillus cereus*

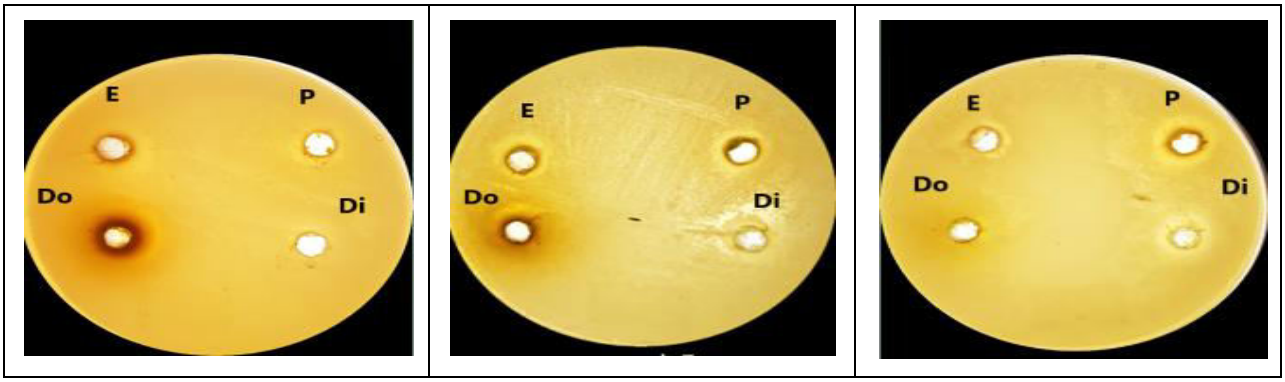


Figure.7 Antibacterial activity of different solvent of studied plant extracted against *Streptococcus pneumoniae*.

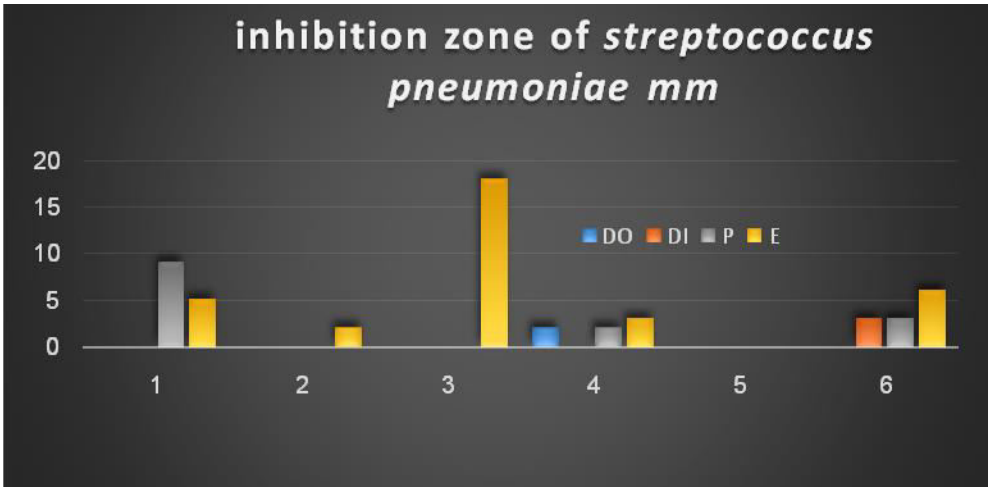


Figure.8 Effect of seeds, leafs and stem extracts of *Avena sterilis* against *Streptococcus pneumoniae*

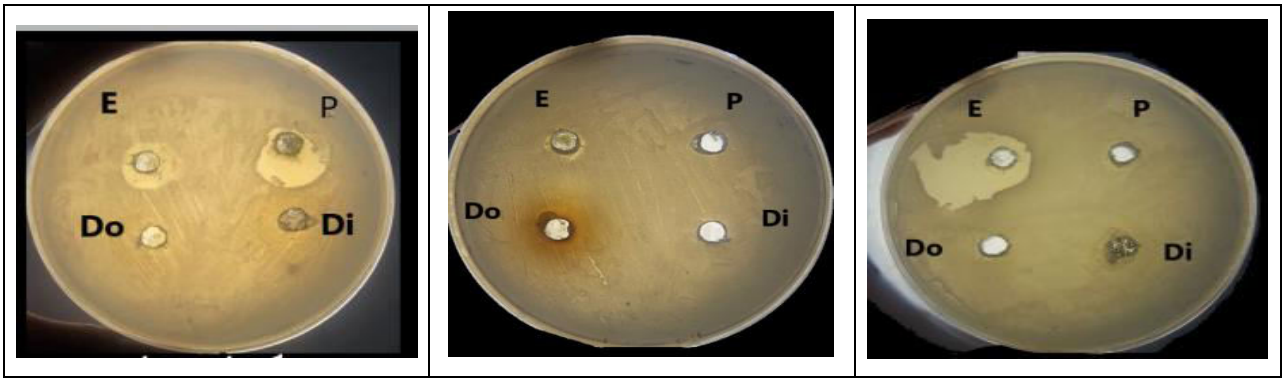


Figure.9 Effect of seeds, leafs and stem extracts of *Laurus azorica* against *Streptococcus pneumoniae*

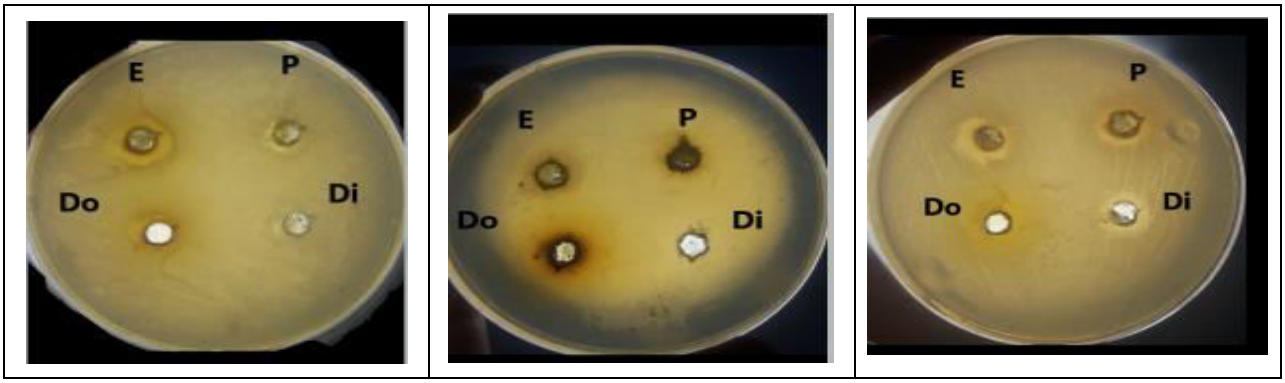


Figure.10 Antibacterial activity of different solvents of studied plant extracts against *Escherichia coli*.

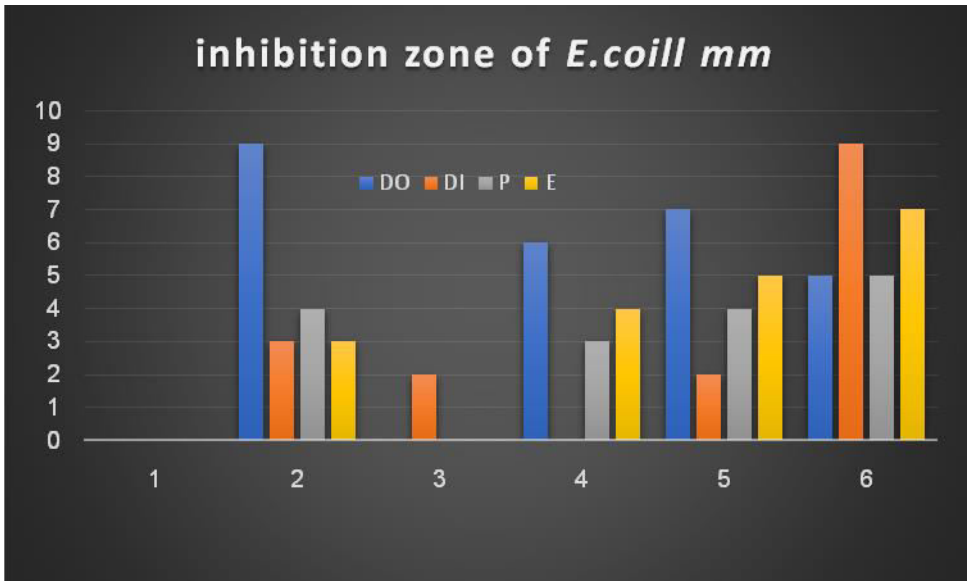


Figure.11 Effect of seeds, leafs and stem extracts of *Avena sterilis* against *Escherichia coli*

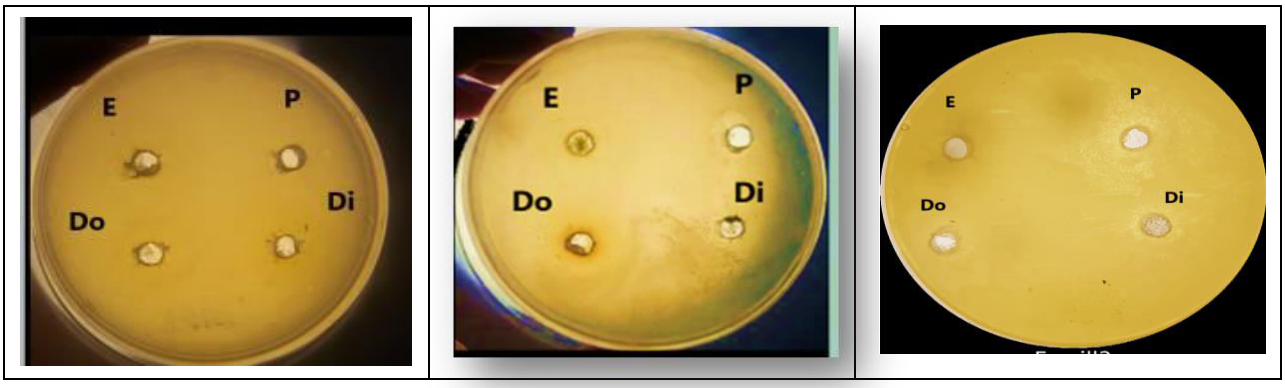


Figure.12 Effect of seeds, leafs and stem extracts of *Laurus azorica* against *Escherichia coli*

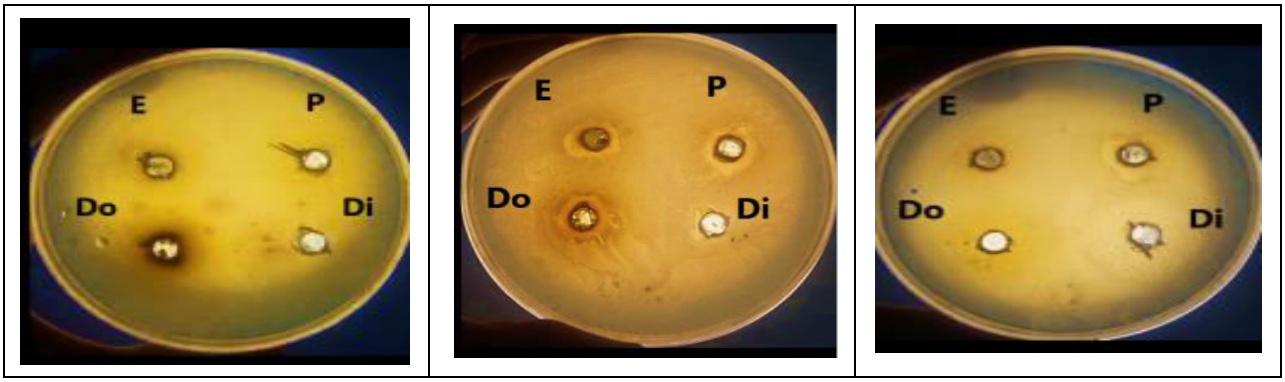


Figure.13 Antibacterial activity of different solvents of studied plant extracts against *Pseudomonas aeruginosa.*

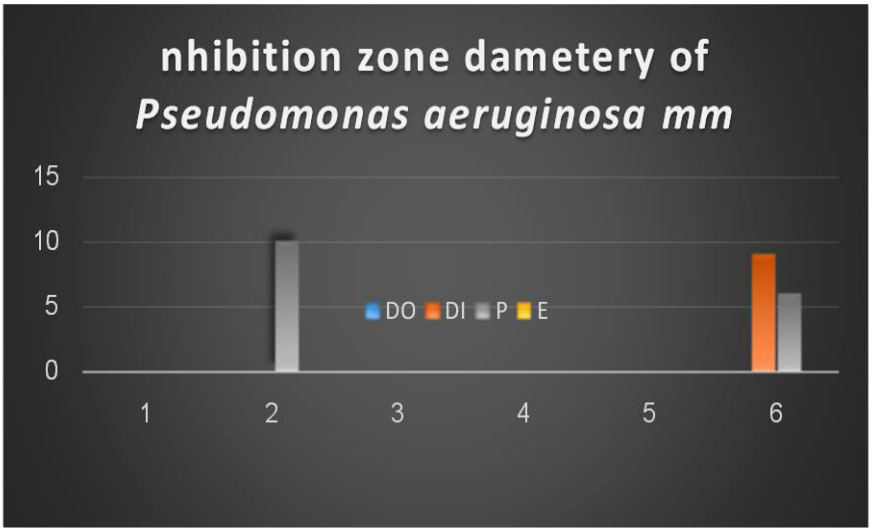


Figure.14 Effect of seeds, leafs and stem extracts of *Avena sterilis* against *Pseudomonas aeruginosa*

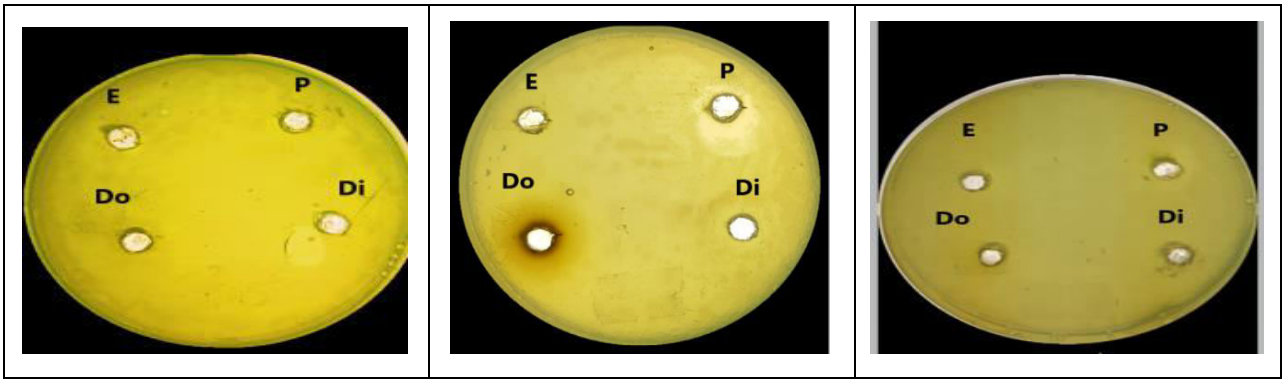


Figure.15 Effect of seeds, leafs and stem extracts of *Laurus azorica* against *Pseudomonas aeruginosa*

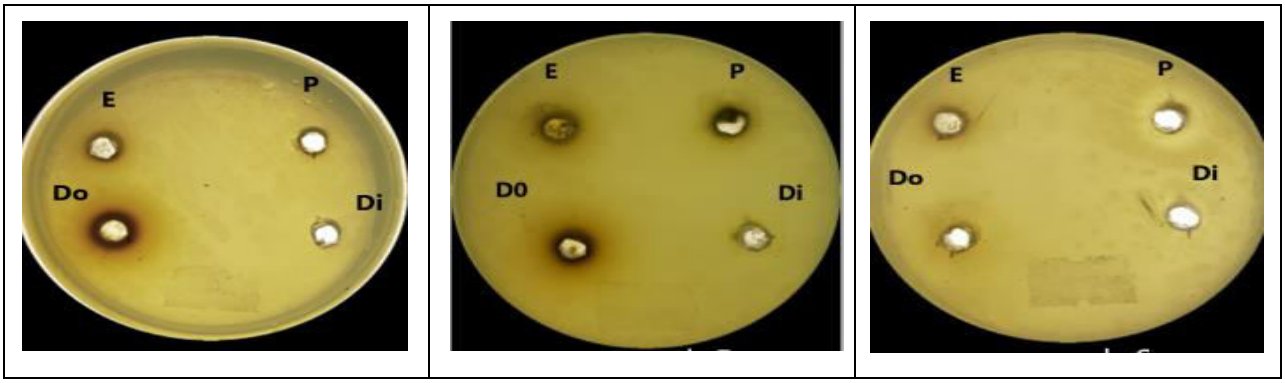


Figure.16 Antibacterial activity of different solvents of studied plant extracts against Inhibition Zone of *Agrobacterium* sp.

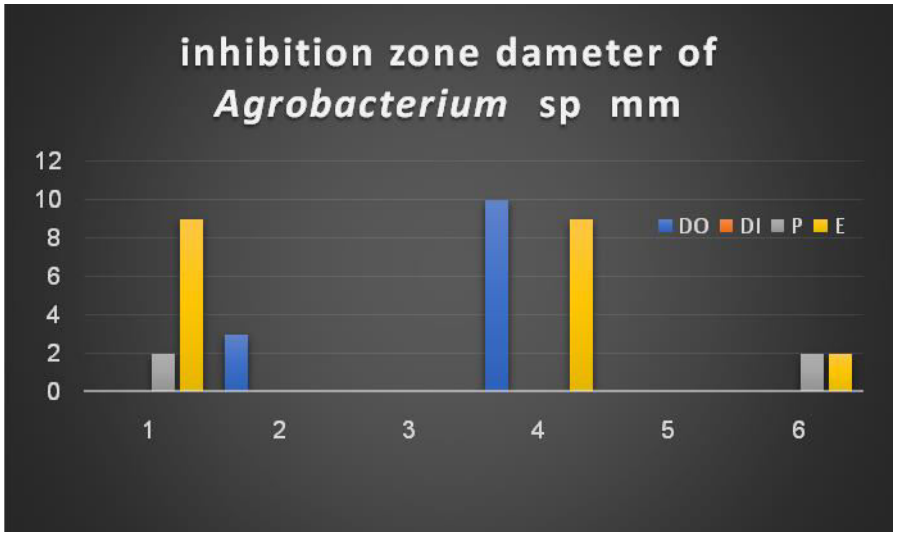


Figure.17 Effect of seeds, leafs and stem extracts of *Avena sterilis* against *Agrobacterium* sp

Figure.18 Effect of seeds, leafs and stem extracts of *Laurus azorica* against *Agrobacterium* sp

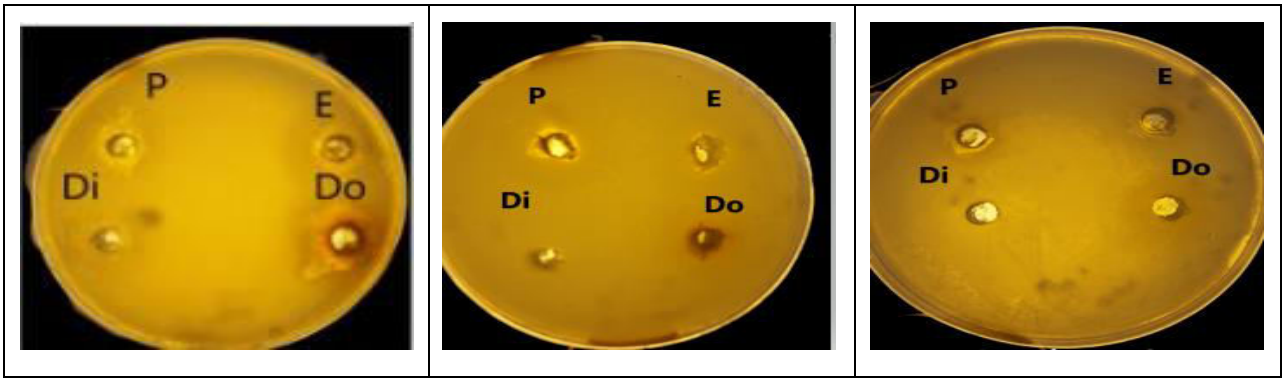


Figure.19 Antibacterial activity of different solvents of studied plant extracts (inhibition zone) of *Erwinia carotovora*.

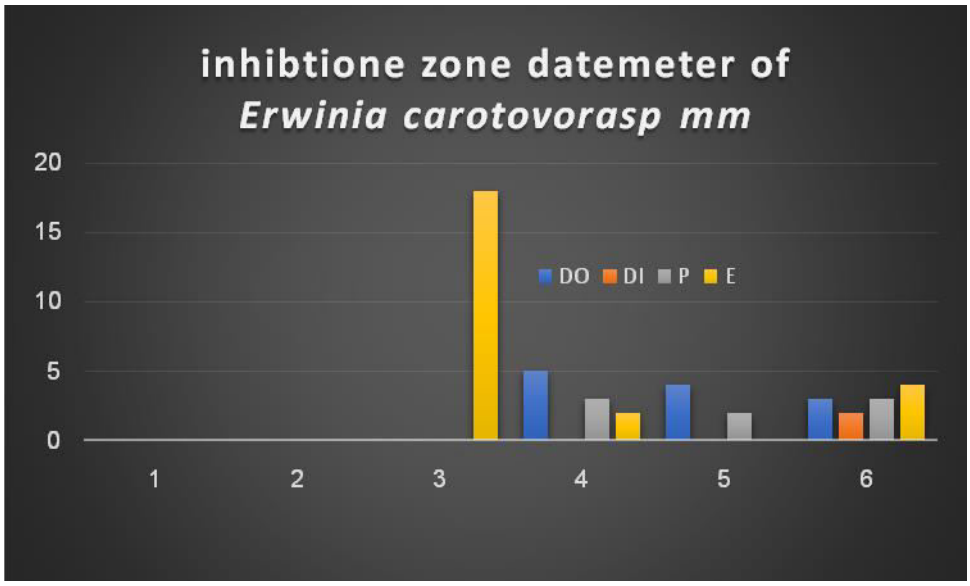


Figure.20 Effect of seeds, leafs and stem extracts of *Avena sterilis* against *Erwinia carotovora*

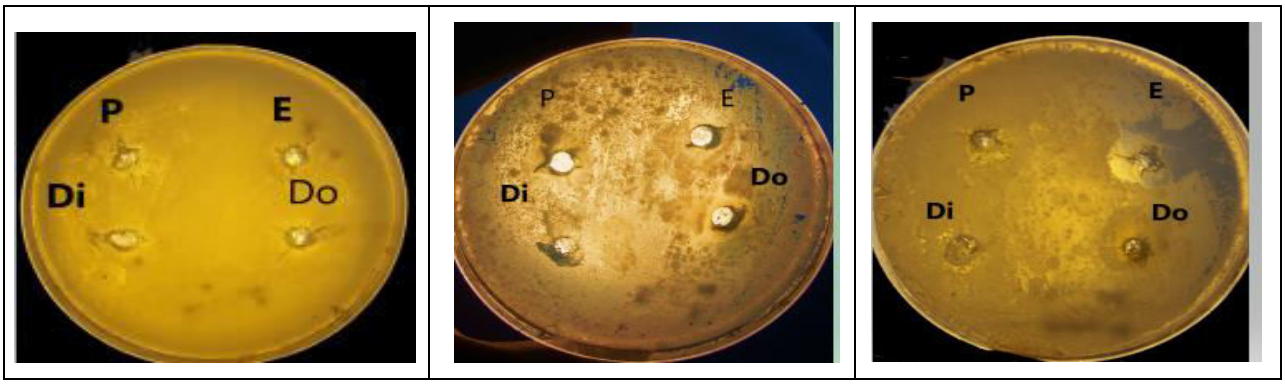


Figure.21 Effect of seeds, leafs and stem extracts of *Laurus azorica* against *Erwinia carotovora*

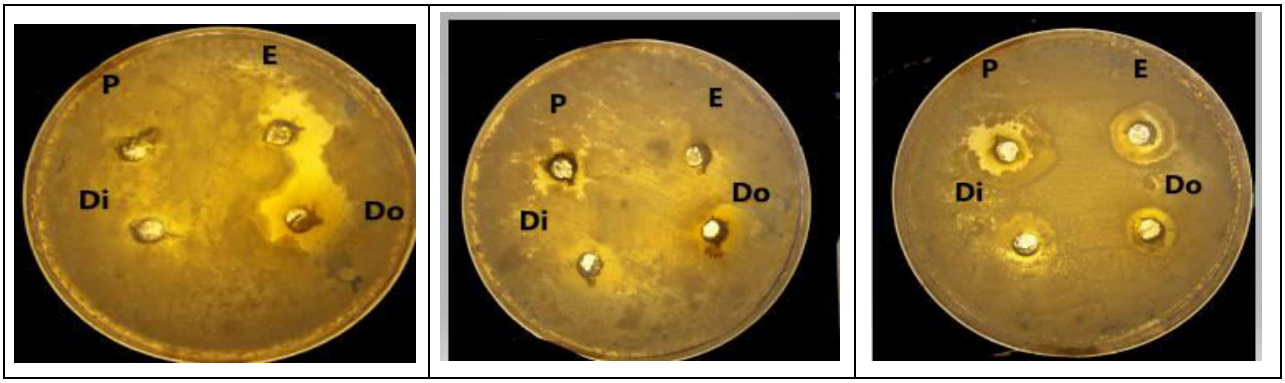


Figure.22 Antibiotic sensitivity testing of *Staphylococcus aureus.*

Figure.23 Antibiotic sensitivity testing of *Bacillus cereus*

Figure.24 Antibiotic sensitivity testing of *Streptococcus pneumoniae*

Figure.25 Antibiotic sensitivity testing of *Escherichia coli*

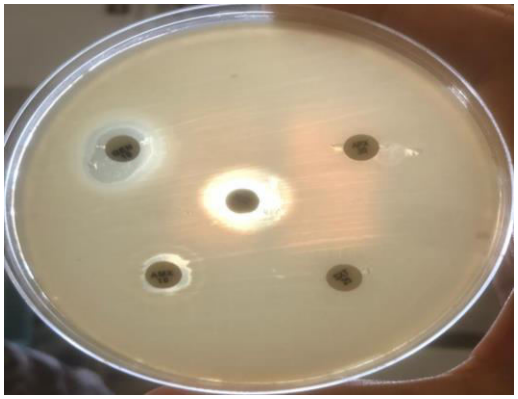


Figure.26 Antibiotic sensitivity testing of P*seudomonas aeruginosa*

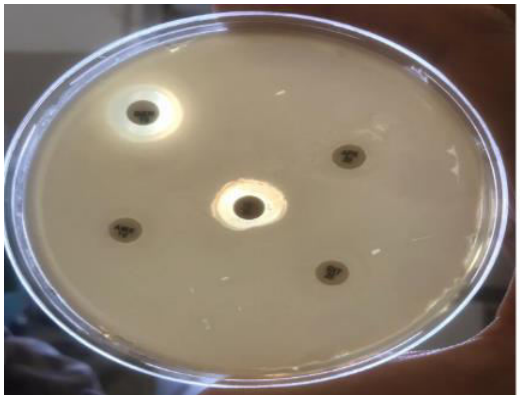


Figure.27 Antibiotic sensitivity testing of *Agrobacterium*

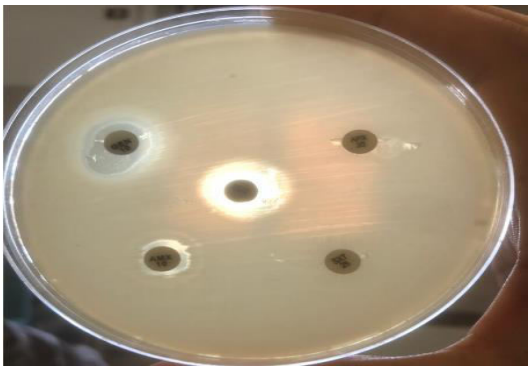
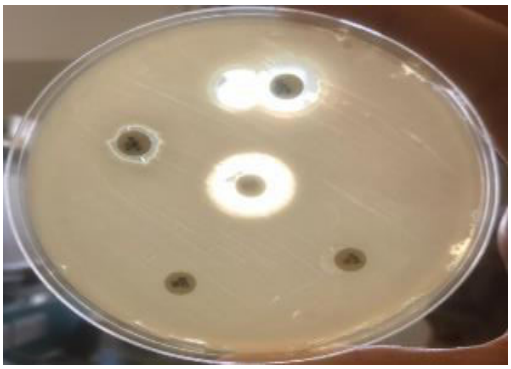


Figure.28 Antibiotic sensitivity testing of *Erwinia carotovora*



Agrobacterium sp

The results showed that *Agrobacterium* sp is sensitive to ethanol extracted from *Aveane sterilis* (seeds) and *Laurus azorica* (seeds and stems) while *Aveane sterilis* (seed) and *Laurus azorica* (stems) is sensitive to propanol isolates were also sensitive to *Aveane sterilis* (leafs) *Laurus azorica* (seeds) According to Figures (16-18).

Erwinia carotovora

The results show that *Erwinia carotovora* was sensitive to ethanol extracted *Aveane sterilis* (stems) *Laurus azorica* (seeds and stems), while isolate was sensitive to propanol extracted *Laurus azorica* (seed, leafs, seed) also isolate was sensitive to Di ethyl ether *Laurus azorica* extracted (seeds) and bacteria were sensitive to DOMS *Laurus azorica* extracted (seed, leafs and stems), Figures (19-21).

This study shows that the antimicrobial activity studies were carried out on alcoholic extracts for the studied plants in, *Laurus azorica* and *Aveane sterilis* (seeds, leafs, stems) against the selected bacteria have inhibitory effects on the growth of the studied bacterial, showed a difference in the effect of the extracts in the process of inhibiting bacterial growth. Due to different polarity in the solvent. It was also found that some of the extracts that showed the greatest inhibition against bacteria were similar in chemical composition as shown in the photochemical screening test, an example of this *Staphylococcus aureus*. 2p and 3p) *Bacillus cereus* (4E and 4Do) *Escherichia coli* (2DO and DI). The results were similar to some studies concluded the volatile

compounds from plants *Laurus nobilis*, reported that It has anti - bacterial activity (Al-marri *et al.,* 2014; Ramos, 2013 and Goudjil *et al.,* 2015). Also, the result agrees with Hussein *et al.,* (2019) has been reported that *Laurus nobilis* contain various phenolic compounds, such as alkaloids, saponins, tannins, anthraquinones, steroids, flavonoids, glycosides, and glucosides contents. Biochemical activities, including antibacterial activity Results revealed that the inhibitory activity of the plants against Gram positive and Gram-negative and, antioxidant, Moreover, the results of this study were consistent with some of the research reported that *Aveane stative* extract contains anti-toxin properties for pathogenic bacteria (Ahmed *et al.,* 2010 and Marmouzi *et al.,* 2017).

Antibiotic Sensitivity test

The results showed the antibiotic test CEN and TE showed an inhibitory effect for each of the studied bacterial species, while all the bacteria species showed resistance against APX and OX, while SXT showed sensitivity only to *Staphylococcus aureus*, however AMX, VA and DA were sensitive to 37.5%. Similarity was observed in sensitivity to the antibiotics also between *Escherichia coli* and *Pectobacterium*sp also between *Pseudomonas aeruginosa* and *Agnobacterium*sp. According to the results recorded in this study the different types of solvents for extract some chemical compounds of two selected plants of *Aveane sterilis* and *Laurus azorica* plants (seeds, leafs and stems), investigate their antibacterial activities on some selected species of bacteria the results showed that different effects on the selected pathogenic bacteria were observed, these different are mainly due to the effect of polarity of used solvents.

Author Contributions

Moftah Al Feture Moftah Aljamal: Investigation, formal analysis, writing—original draft. Hamad. M. Adress. Hasan: Validation, methodology, writing—reviewing. Huda. M. Al Sonosy:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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