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Studying the Effect of Some Medicinal Plants Extracts and Antibiotics on some Pathogenic Isolates Bacterial

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Abstract. The inhibitory effect of black seed extract, Roselle flowers, cloves, eucalyptus leaves and mashed garlic cloves was studied on five isolated pathogenic bacteria, which included: Salmonella typhimurium, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Staphylococcus epidor. It appeared that the clove flowers extract was significantly excelled its inhibitory effect on the rest of the plant extracts of the studied bacteria, where it gave a general average of the lengths of the inhibitors diameters of 57 mm for S.typhimurium and E. coli were 41 mm, and S.epidermidis was 33 mm, while S. aureus was less. The types of bacteria studied were sensitive to the extract, amounting to 20 mm. The average effect of clove extract was 25.5 mm for all concentrations and for all studied bacteria. Clove extract is followed in the average lengths of inhibitors diameters is garlic and Roselle extract, where they were given an average of (18.11, 17.95) mm for each, respectively, for all concentrations and for all bacteria types, followed by an extract of the black seed which gave an average of 15.2 mm, while eucalyptus extract gave the lowest averages for the length of inhibitors diameter reached 10.5 mm. As for the inhibitory activity of antibiotics, the antibiotic Nalid acid exceeded with a general average of 23 mm, followed by Gentamcin reached 22.40 mm. Ampicillin gave the lowest mean in the inhibition diameters of 10 mm. From the above, we note the difference between the effectiveness of plant extracts and antibiotics. The extracts combined gave a general average inhibition diameter for all types of bacteria amounting to 17.45 mm, while combined antibiotics gave a general average inhibiting diameter for all types of bacteria reached 17 mm.

Keywords. Inhibitory effect, Plant extracts, Antibiotics, Inhibition diameters, Pathogenic Isolated bacteria.

1. Introduction

The utilization of medicinal plants in healthcare has been a practice of considerable antiquity. Medicinal herbs have crucial functions in the prevention of diseases, and their endorsement and utilization align with all currently established preventative techniques. The study and use of herbal medicine in illness treatment are seeing continuous growth. therapeutic plants provide as a significant reservoir of naturally occurring chemicals that possess therapeutic qualities [1,2]. Medicinal plants has



significant use in the treatment of human ailments and have a crucial position in the process of healing, mostly owing to the presence of various phytochemical elements. Medicinal plants, with inherent therapeutic properties, are employed for the treatment of several disorders and serve as a source of economic sustenance [3,4]. The research indicated that plant extracts have a clear inhibiting effect against pathogens due to effective compounds such as glycosides, Alkaloids, Soaps, etc. In addition to the lack of its side effects on the one hand, and on the other hand, its potency is effective against pathogens compared to known antibiotics and their ease of access and the inability of pathogens to resist them [5]. Other research has confirmed the inability of modern medicine to eliminate some microbes and consequently not to control the diseases it causes compared to its treatment with plant extracts because of its high effectiveness and it is inexpensive and has no side effects [6] and [7]. confirmed [8] emphasized that it contains many therapeutic compounds. Among the factors that determine the efficacy of the plant extract is the method of its extraction, the type of solvent used and the resistance of the pathogen to it [9]. For example, when treating bacterial and fungal infections with the antibiotics Ampicillin, Tetracycline and Amoxicillin, many problems cause the emergence of bacterial or fungal strains that are resistant to the antibiotic used and this resistance is either natural or acquired. The Gram-negative bacteria have a natural resistance because they contain Lipo Poly Sachrriideshg in the cell wall, which prevents the inhibitors from entering them, As for acquired, it increases rapidly and significantly [10], which limits the use of .The black seed plant (*Negill sativa* L.) sometimes called black cumin [11], and its seeds contain 40% of its weight, fixed oil and 1.5% volatile oil, which works antidiuretic in keeping blood sugar and as an antioxidant, which helps protect the body from the impact of free radicals that destroy the tissue [12], In addition to being an antifungal [13] and [14]. The black seed is an effective antidote to Gram-the positive and negative bacteria[15]. It strengthens the immune system, antiseptic and anti-worms, in addition to containing tannin, which is a toxic glycoside, and Nigella, which is a bitter glycoside, which is antibodies to Gram-the positive and negative bacteria and fungi [16]. As for cloves, it is one of the medically important plants where its flowers were used as an antibiotic and analgesic for the teeth and gums [17]. Studies have confirmed that the extract of leaves and stems of cloves has a severe effect on the bacteria *Streptococcus*. Sp responsible for dental caries [18]. As for garlic, its aqueous extract contains allicin present in its high-efficiency pilot oil against Gram-the positive and negative bacteria. In addition, it reduces cholesterol in the blood [19]. As for Roselle plant, the active compounds are found in the plant leaves, including Anthocyanins, Yaniding -3-Sambubioside, Delphinidin-3-Sambubiosid, Delphinidin -3-Glucose, which penetrate the walls of bacteria cells and weaken them and then kill them [20], In addition to containing some important organic acids, including Malic, Tartaric, Hibiscus and Ascorbic acid) in addition to that it lowers blood pressure and increases the speed of its circulation and movement and limits cancerous tumours and it is a diuretic and it limits the growth of pathogenic microorganisms in the human body in general and especially those in the digestive system [21]. As for the Eucalyptus plant, its leaves and flowers are used in the medical field [22], where its aqueous, alcoholic and oily extracts were used as an inhibitor against Gram-the positive and negative bacteria, as well as , the researcher [23] explained that the effectiveness of Eucalyptus due to that its leaves contain the basic compound which is 1,8-cineoled in its volatile oil and has an effective effect "on the plasma membrane of bacteria and then kill them, and it has an inhibitory activity against bacteria, viruses and fungi [24].

The study aims to:

- Diagnosis of pathogenic bacteria from various sources.
- Measurement of the Inhibition activity of plant extracts (garlic, cloves, black seed, Roselle plant and eucalyptus) on pathogenic bacterial isolates.
- Measurement of the Inhibition activity of antibiotics used on isolated pathogenic bacteria.

2. Materials and Methods

2.1. *The Following Types of Bacteria were Used in the Experiment*

(*Salmonella typhimurium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Escherichia coli*), Antibiotics used (Amoxicillin, Gentamicin, Tetracycline, Nalidixic acid, Ampicillin), Medicinal plants used in the experiment (Black seed (seeds), Roselle plant (flowers), cloves plant (flowers), Eucalyptus (leaves), garlic (cloves)).

2.2. *Preparing the Plant Parts used in the Experiment*

The seeds of black seed and flower leaves of the Roselle plant were obtained from my previous research on the two plants. As for the eucalyptus leaves, they were collected from the gardens of AL-Musiab Technical College and the garlic and clove flowers plant were obtained from local markets. The required plant parts (except garlic cloves) were collected, as they were classified in the Seed Inspection and Certification Department in Babylon and in the Field Crops Department of the Faculty of Agriculture, Al-Qasim Green University in Babylon. The samples were washed well with water for the purpose of getting rid of impurities and dust, then they have dried away from direct sunlight after being placed on filter paper with continuous lubrication to ensure that they do not rot or fungal infection and were crushed and turned into powder and stored in paper bags and stored until use in a place where it reduces temperature and humidity.

As for the garlic cloves, they were cleaned, peeled and crushed with a blender well, preserved with nylon bags and preserved until use, as well as away from high temperature and humidity.

2.3. *Prepare Plant Extracts*

Plant extracts were prepared according to the method reported [25]. By taking 100 g of the plant portion prepared from the previous step and placing it in a beaker containing 100 g of distilled and sterile water, they were mixed with the electric mixer and left the mixture for 24 hours at a temperature of 37 degrees C. Then the mixture was filtered using a sterile medical gauze to get rid of plankton.

2.4. *Isolation and Diagnosis of Pathogenic Bacteria*

Samples were taken from the patients in Al-Hilla Teaching Hospital and Marjan Medical City Hospital for the period from 3/24/2019 to 2/4/2020 using a cotton swab method (according to the method [26]). The swabs were distributed within 15 minutes from the moment the sample was taken to the blood agar and by a method of plating these media. Then the dishes were incubated fortified with a temperature of 37 m for a period of 24 hours. It was also used for this nutritional media to activate and diagnose isolates, as well as the use of pigment and reagents to study the colony's appearance, colour, and degree of transparency, as well as the shape and arrangement of cells and the extent of their interaction with the pigment of Gram negatively and positively in addition to biochemical tests.

2.5. *Testing for Bacterial Isolation Sensitivity with Plant Extracts*

Plant concentrations were prepared with concentrations (25, 50, 75, 100%) and the Agar well diffusion method was used [27] in testing the bacterial sensitivity to plant extracts. As 0.1 ml of bacterial tracers were spread to each species on Hinton's solid medium using the Cork borer, where five pits were made with a diameter of 5 mm for each pit and added 0.1 ml of plant extract and concentrations above and add distilled water in the fifth pit for comparison. The plates were left for 15 minutes, then received 37 ° C for 24 hours, after which the damping diameter of the ruler was measured [28].

2.6. *Testing the Sensitivity of Bacteria to Antibiotics*

A disk diffusion method [29] was followed, where 5 pure single colonies were taken from the surface of the blood vessels with a sterile Loop bacterial carrier and placed in a test tube containing 5 ml of Nutrient broth with good shaking until darkness formed, After 2 hours, the Petri dishes containing

Nueller Hinton ager were cultured with the prepared stuck bacterial, where the surface of the Ager was cultured with a sterile Cotton swab, Then the Agar was planned from all sides to distribute the quantity evenly , The dishes were left to dry for 25 minutes, after which the antibiotic tablets were placed with sterile forceps on the surface of the cultures Agar, Where the 5 discs were used for each dish separated by equal distances between the discs and the dishes were placed in the incubator at a temperature of 37 m for a period of 24-72 hours according to the growth period for each type of bacteria then the diameter of the inhibition area is measured by the ruler.

2.7. Chemical Detection of the Active Secondary Compounds used in the Study

- Alkaloids: Alkaloids were used for this Mayer reagent leaving a white precipitate, as well as a Wanger reagent brown precipitate [30].
- Phenols: phenols. Ferric Chlorid [31] and Fohn reagent [32] are used for this.
- Flavonoids: Flavonoids were used for this KoH alcoholic reagent [33].
- Detection of resins: Resin was detected using method [31] where a gel precipitate appeared.
- Detection of Saponins by using HgCl₂ and showing a red precipitate [32].
- Glycosides detection: It was used for this Benedict reagent [33].
- Detection of Tainates: It was detected by using 1% Lead Acetate where a gelatinous white precipitate appeared.
- Detecting Volatile Oil when adding a little clear extract on the filter paper to the degree of saturation and exposure to ultraviolet light, leading to the appearance of a gray color indicating the presence of volatile oil.

Table 1. The active substances in plant extracts used in the study.

Plant / compounds	Cloves plant	Eucalyptus	Roselle plant	Black seed	Garlic
Alkaloids	+	+	+	+	-
Phenols	+	+	+	+	+
Flavonoids	+	+	+	-	+
Resins	+	+	+	+	+
Tannin	+	+	+	+	+
Saponins	+	+	+	+	+
Glycosides	+	+	+	+	-
Volatile Oil	+	+	-	+	+

3. Results and Discussion

Table (1) showed that there are some secondary active substances in one plant and not in another plant. We notice that garlic extract is free of alkaloids and Glycosides while Eucalyptus and Clove plant extract contains all the active substances. While the black seed lacks flavonoids, while the extracts of the leaves and flower of the Roselle plant are lacking in the volatile oils. This is due to their genetic and physiological composition. As for Table (2), it shows the effect of plant extracts on the studied bacterial species. We notice the black seed extract clearly affecting the types of bacteria studied with different inhibition rates in different concentrations of the extract. We notice at the concentration of 25% the most sensitive bacteria of the black seed was *S. typhimurium*, where the inhibition diameter was 14 ml, while *S. sepidermidis* was the most resistant bacterial to the extract so that it was not affected at all, so the inhibition diameter was equal to zero. The highest inhibitory activity of the black seed extract was for *S.typhimurium* bacteria was 33 ml at 100% concentration. The inhibitory activity of black seed can be explained by the fact that it contains the compound Thymoquinone, which is a derivative of the compound Thymohydroquinon, and the latter is a strong inhibitor of bacteria that are Gram-the positive bacteria [34,35] As for, the presence of alkaloids, including Nigellimine, Nigellicine and N-Oxide, are responsible for their antigenic efficacy, as well as their anti-oxidant glycoside, Saponins, sulfates and phenols with inhibitory efficacy on the Gram-the positive bacteria [36]. The most common bacteria that are resistant to black seed extract are *S. aureus* and *S. epidormidi*. They were given the lowest inhibition rate of 20 ml for both species. As for garlic extract, it gave the highest rates of inhibiting diameters against isolated pathogenic bacteria.

Salmonella typhimurium was 27 mm. As for the most resistant bacteria, it was E-coli, which gave the lowest inhibition rates to 19 mm and that the effect of the extract for the rest of the bacteria was confined between the two rates (19-27) mm, and the inhibitory activity of the garlic clove extract is due to its active compounds including Allin, Scordinin-A and Phytonicidine [37]. This substance is the cause of the characteristic smell of garlic and converts Allin to sulfur organic compounds, including Diallyl, Diallyl disulphide trisulphide. The compounds resulting from the crushing of garlic cloves have an adverse effect on many types of Gram-positive and negative bacteria as well as on a wide spectrum of fungi [38,39]. As for the inhibition mechanism, the active substances contained in the mashed garlic cloves inhibit the formation of the cellular wall of bacteria. Cefotaxime is a "potent and effective" inhibitor of both Gram-positive and negative bacteria in addition to its inhibition of positive and negative air bacteria of a pigment -gram and anaerobic bacteria, As for clove extract, its inhibition ability excelled on the rest of the extracts for all studied bacteria. It gave the highest average of *S.typhimurium* inhibition diameter of 57 mm, followed by E-coli, where the inhibition diameter reached 41 mm. While the bacteria was the most resistant to the extract of *S. aureus*, the average inhibition diameter was 20 mm, due to the high inhibitory ability of cloves because it contains Eugenol, a phenolic compound that has an analgesic effect of gingivitis and is anti-microbial (14). It works to inhibit the action of the cell membrane and then inhibit the action of bacteria in general [40], as well as from the antioxidant compounds [41]. As for the extract of Eucalyptus leaves, it gave the highest average of inhibition diameter of *S. aureus* was 23 cm at high concentration while *S. typhimurium* was the most resistant or least sensitive bacteria direction of the extract reached 17 mm at 100% concentration. and can explain the high inhibitory activity of the extract is that it contains biologically active compounds such as Saponins, Tainates and alkaloids in addition to its base oil (Volatile Oil) that contains the compound 1.8 - Cineolled, which has a great impact on the cytoplasmic membrane of the bacteria and to which a large proportion of the high inhibitory activity of the eucalyptus extract is due [42] and [23], It also contains Cuminal compounds, Phellandrens and Aromadendral, in addition to flavonoids, Tainates and Saponins and all of these substances have antagonistic waders with positive and negative bacteria of Cram dye [43]. Some compounds are made as secondary metabolites that protect the plant from attacking bacteria, fungi and viruses [44]. The high inhibitory effect of the eucalyptus leaf extract on the elimination of Gram- positive and negative bacteria can be explained by the presence of its oil on the phenolic compounds: Carvacrol and Thymol in its volatile oil [45]. As for the extract of leaves of Roselle flowers, it achieved a high inhibition ability of bacteria. *S.aureus* followed by E-coli, giving an average inhibition diameter of 26 and 25 mm, respectively, at high concentration. The extract affected all concentrations on all studied bacteria and shared the same effect as garlic clove extract, We can due to the high inhibitory ability of Roselle plant to the fact that it contains many secondary metabolites products that have proven effective as: antibacterial Antioxidative and anticarcinogenic in addition to containing organic acids such as citric, malic and ascorbic [46]. Also, its inhibitory effect is due to the extract of its leaves containing Anthocyanins, Yaniding-3-Sambnbioside, delphinidin -3-sambnbiosid in addition to dephinidin-3-Glucose. These compounds attack the bacterial cell wall, causing it to be destroyed and completely destroyed, in addition to that it contains active compounds such as lollins And alkaloids that weaken and kill both Gram-positive and negative bacteria [20]. The variation of the inhibitor effect of plant extracts is due to the type of plant and consequently, the type of active compounds in it and the plant age and the storage conditions intended to the extent of preserving the active substance [2], Returning to Table (2) we note that there is a direct relationship between the intensity of the inhibition and the increase in the concentration of the extract, that is, the least diameters Inhibition was for low concentrations and the greater the concentration, the more inhibition the explanation for this increased concentration of inhibitor with increased concentration [25]. The aqueous extract of cloves was distinguished by obtaining the highest mean of inhibition doses when treated with isolated pathogenic bacterial straining followed by the effect of Roselle and then garlic.

Table 2. Shows the inhibitory effect of pathogenic bacteria isolated by antibiotics. Ampicillin was distinguished by its high inhibitory effect on all studied bacteria except for E-coli.

Bacteria / Plant	Black seed				Garlic				Cloves plant				Eucalyptus				Roselle plant			
S.typhimurium	2	50	75	10	2	50	75	10	2	25	50	75	2	5	10	10	2	25	50	75
S.epidormidis	1	18	23	33	1	19	23	27	1	35	43	49	1	-	-	12	1	11	15	17
E-coli	4	-	9	15	4	15	19	24	4	12	20	25	4	33	-	9	4	12	20	8
K.pneumoniae	-	9	15	20	-	15	19	24	-	12	20	25	-	33	-	9	-	12	20	8
S.aureus	6	11	16	22	6	14	17	19	6	28	13	37	6	41	-	8	6	14	20	10
average	1	12	18	21	1	17	20	24	1	6	11	17	1	23	-	7	1	11	17	12
Total	1	12	14	20	1	16	22	25	1	-	9	13	1	20	-	12	1	15	17	19
	4	62	86	11	4	81	10	11	4	81	11	17	4	17	-	3	4	98	97	56
	0	6	6	0	0	81	1	9	0	81	4	141	0	4	-	9	0	98	97	56
	8	12.	17.	23.	8	16.	20.	23.	8	16.	22.	28.	8	34.	-	7.	8	13.	19.	11.
	4	2	2	2	4	2	4	8	4	12	8	12	4	8	-	2	4	6	4	2

The highest inhibition rate for *S.typhimurium* was 20 mm, followed by *S.aureus*, while the inhibition rate for *E-coli* was zero.

As for the antiparticle Tetracyclin, the highest inhibition rate was 19 mm when treated with *S.epidormidis*, while the lowest inhibition diameter was 11 mm with *K. pneumoniae*. As for the rest of the bacteria, it was affected by the antibiotic mentioned between these two numbers (11-19)mm. As for the Gentacin antibiotic, it achieved the highest average inhibition diameter with *S.epidormidis* of 25 mm, followed by *S. aureus*, the inhibition diameter was 24 mm, while *S.typhimurium* gave the lowest inhibiting diameter, which is the lowest sensitivity of 18 mm. While the antibiotic Amoxillin gave the highest average of inhibitory activity when treated with bacteria *K.pneumoniae* reached 19 mm and the lowest diameter of the inhibitor was with bacteria *S. aureus* reached 11 mm and rates of inhibition diameter for the rest of the bacteria types ranged between these two numbers. The antibiotic Nalid acid was excelled to being the most modified in the inhibitory diameters of the bacteria used in the study, followed by Gentamicin, while the Ampicillin antibiotic was the least inhibiting effect on the studied bacteria.

Table 3. Inhibitory ability of antibiotics against isolated bacterial pathogens.

Bacteria / Antibiotics	Ampicillin	Nalid acid	Tetracyclin	Gentamicin	Amoxillin
<i>S.typhimurium</i>	20	20	16	18	15
<i>S.epidormidis</i>	10	23	19	25	12
E-Coli	-	21	13	22	16
<i>K.pneumoniae</i>	8	26	11	23	19
<i>S.aureus</i>	12	25	16	24	11
Average	10 mm	23mm	15	22.4	14.6
Total	50	115		112	73

Conclusion

Clove extract had a 25.5 mm average impact across all concentrations and microorganisms. Clove extract, garlic, and Roselle extract had average inhibitor diameters of 18.11, 17.95, and 15.2 mm, respectively, for all concentrations and bacteria types, followed by black seed extract and eucalyptus extract. Its lowest average was 10.5 mm. Antibiotic inhibition averaged 23 mm for Nalid acid and 22.40 mm for Gentamicin. Ampicillin had the lowest inhibition diameter mean of 10 mm. As shown above, plant extracts work differently than antibiotics. The extracts combined inhibited all bacteria at 17.45 mm, while the antibiotics combined inhibited them at 17 mm.

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