Preparation ,Diagnosis and Evaluation of the Effectiveness of a Mixture of Alcoholic Extracts of Cumin and Dill Against Bacteria and Fungi

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ABSTRACT

This study aims to investigate the biological and antimicrobial activity of extracts of two types of plants, which are *cuminum cyminum* (cumin) leaves and *Anethum graveolens* (dill) leaves, and these are types of the well-known medicinal plants in Iraq that are having curative properties in folk medicine. Extraction was performed to plants powders by cold process using a vibrating incubator at 40°C. The antimicrobial activity was studied by diffusion by digging - agar at concentrations (0.1, 0.5, 1, 2, 3) % and (0.1, 0.3, 0.5, 0.75, 1) % for cumin and dill respectively. The results of the tests showed that cumin had the highest effective ability when using a concentration of 3%, while the highest effectiveness of dill was at a concentration of 1% of the concentrations used, while it was the least inhibitory ability when using concentrations of (0.1) % from both the two plants against bacteria and fungi used in the research. The inhibition effectiveness of a combination of 2% cumin and 1% dill was determined, which was measured and found to be (21,22,19,18,15) mm and (21,20,20,17,15) mm and (22, 19, 18, 16, 16) mm against Escherichia *coli, Staphylococcus aureus,* and *Streptococcus mutans* were obtained, while it gave effectiveness ranged between (20, 20, 21, 21, 16) mm and (21, 20, 20, 17, 17) mm towards *Staphylococcus epidermis and Candida albicans*, with concentrations (100, 75, 50, 25, 12.5)%, respectively.

The active substances available in the alcoholic extract of cumin and dill plants were investigated by chemical methods, as the tests showed that they contain compounds of alkaloids, flavonoids, glycosides, saponins and tannins, which are the substances to which the antimicrobial destructive effect was attributed against various types of bacteria and fungi.

Keywords: Cumin, Dill, Antimicrobial activity, Alcoholic extract.

INTRODUCTION

Plants are one of the important sources in the manufacture of medicinal drugs, because they contain some biologically effective chemicals, and this has been employed in the preparation of several medicines from plant sources [1]. Medicinal plants have drawn the attention of scientists for a long time after their use as a means of treatment for many medical conditions, especially in recent years, when it became clear that they have an effect in the preparation of many medicines due to their biological pharmacological efficacy and the speed of their therapeutic effects, as well as the lack of side effects if compared. Chemically manufactured drugs, therefore, it has become one of the best treatment methods for many diseases that affect the human, because it contains chemical compounds and has a clear biological activity against various pathogenic bacteria and fungi [2]

Cumin is *Cuminum Cyminum*, which is from the tent family and one of the well-known and very ancient plants, as the part used is the fruit, and it is an herbal plant of winter crops [3].

A rough analysis of cumin seeds revealed that

they constituted of fixed oil, volatile oils, acids, essential oils and other elements. Also, cumin also contains some important components such as pinene, thymol, cumonaldehyde, terpene, simene and others, which have shown their effectiveness against many different diseases [4].

Also, this type of cumin showed high levels of petrosilinic fatty acids (an isomer of oleic acid) as well as sterols. Studies conducted on this type also showed high levels of proteins, phenols, sugars and fiber. It is worth noting that these residues revealed good and interesting antioxidant activity It is worth noting that cumin also has broad-spectrum antibacterial properties against both Gram-positive and Gram-negative bacteria. It is also an aromatic plant used in medical preparations, food industries and as a flavoring for foods [5, 6].

Dill is an annual plant in the family Anethum graveolens. It is considered among the spices, which include anise, parsley, caraway, coriander, cumin and fennel. The Mediterranean is the original home of dill, and Turkey is the original home of this plant, and its cultivation spread in ancient Greece. Dill seeds and fruits are brown and contain five long veins and taste. The aromatic in green leaves and seeds, but the seeds are more used for commercial and marketing purposes as a spice. The leaves are also eaten raw, added to salads, or cooked with soups [7]. Also, the fruits of dill contain multiple active ingredients such as the volatile oil, which is called dill oil, which is found in a ratio ranging between (3-4%) of the weight of the fruits, and its components resemble caraway oil, and the most important substance in its components is Carvone, which constitutes more than half the amount of the oil in this plant, the oil also contains limonene. Dill oil is light yellow in color and has a pungent aromatic smell. The essential oil of dill was common in use and had a distinctive smell. Also, the seeds of this plant contain viscous mucus, resinous materials and nitrogenous materials. The seeds also contain components and materials that resist cancerous tumors [8]. Dill was previously used as a treatment for diseases as it is useful for the stomach and heart. It also helps sleep, expels gases, and is useful in diaphragm spasm. Its ashes are also used after burning in dressing wounds, and as a diuretic and diuretic for lactating women. The leaves of dill are rich in vitamin (A) present in the form of carotenoids and vitamin (C) which considered as antioxidant vitamins. Its leaves also contain dietary fiber that stimulates the intestines and regulates the oxidation.

Dill has biological the materials and equipment used properties including antimicrobial, antispasmodic as well as antihyperlipidemic effects. Natural antioxidants exist in dill have recently captivate great interest because of their potential to eliminate free radicals [9].

The aim of my research is to assess the antimicrobial effectiveness of extracts of dill and cumin mixtures of them and detect the responsible components for their action

Materials and methods

Materials

Cumin and dill were collected from Al-Shorja area in Baghdad, then they were dried in the shade and at room temperature in a well-ventilated environment and ground using an electric mill for the purpose of obtaining the powder of the two plants which is used later to prepare the alcoholic extract for both. Ethyl alcohol; sigma Aldrich, USA. Purified water was obtained from Milli-Q purifying.

Preparation of the extracts

: To prepare the alcoholic extract of the two plants (50) gm of cumin powder was weighed and placed in a funnel of filter paper (Thimble), then it was placed in a 250 ml Soxhlet device after adding 200 ml of absolute Ethanol to it and left for 3 hours to complete the extraction process. Then the solution was filtered using a Whatman type filter paper, then the extract was concentrated using a rotary evaporator until a very dense liquid was obtained. 4 pm) until use. Also, in the same way, dill extract was obtained. Detection of effective compounds in cumin and dill powder [10]

A- Detection of tannins: (1ml) of aqueous lead acetate (1%) was added to (1ml) of the alcoholic extract, when a white precipitate as an indication of the existence of tannins

B- Carbohydrate test: The test was carried out using Molish's reagent, where (1 ml) of alcoholic extract was mixed with (5) drops of phenphthol alcohol in a test tube, shaken well, and then (2.5 ml) of sulfuric acid was added, when a blue ring was formed indicating the existence of carbohydrates.

C- Glycosides test: The glycosides were detected by means of Fehling reagent, and the appearance of a red precipitate indicates the presence of glycosides.

D- Phenols test: 0.1 gm of the extract was dissolved in (1 ml) of distilled water, add 1-2 drops of ferric chloride solution FeCl3, when blue or green color appears indicating the presence of phenols.

E- Resins test: (1 ml) of lead acetate (1%) was added to (1 ml) of the extract, and when a white precipitate is formed indicates the presence of resins

F- Detection of flavonoids: - (1 ml) of alcoholic (5N) potassium hydroxide reagent was added to (1 ml) of the extract, and when a yellow precipitate appears, the result is positive, indicating the existence of flavonoids.

G- Detection of saponin:- (1 ml) of aqueous mercury chloride reagent (5%) was added to (1 ml) of the extract, and when a white precipitate is formed, the result is considered positive and indicates the presence of saponins.

H- Alkaloid test:- Detection of alkaloids using Wagners reagent by adding several drops of the reagent to (1 ml) of the extract, and when turbidity appears indicating the presence of alkaloids

I- Protein test: Detection of proteins using Bayuret detection, which consists of (80%) copper sulfate dissolved in distilled water and (1 ml) of (10%) of the reagent, and when the violet color is an indication of the presence of proteins

J- Coumarins test: Coumarins were detected by placing a quantity of the alcoholic extract of the plant in a test tube, then covered with a filter paper moistened with dilute sodium hydroxide solution, and heated in a boiling water bath for a few minutes, then exposed a filter paper to a source of ultraviolet rays, when the color of the paper in a bright yellow-green color indicates the Presence of coumarin.

K- Detection of terpenes and steroids: Mix (1) g of the extract in a little chloroform and add to it a drop of acetic anhydride and then a drop of concentrated sulfuric acid. Appearance of the brown color indicates that it contains the terpenes extract, but if it is after a period of dark blue color, it indicates that the extract contains steroids [11].

Test the effectiveness

The final concentrations of cumin powder (0.1, 0.5, 1, 2, 3) % and concentrations (0.1,0.3,0.5,0.75, 1) % were prepared for dill plant and the effectiveness was tested against (*Escherichia coli, Staphylococcus epidermidis, Staphylococcus aurues, Streptococcus mutans)* and *Candida albican* using the Agar-well diffusion method, as inoculation of the nutrient agar medium by (Sterile swab) of bacterial suspension and the tested yeast. Drills with a diameter of[6] mm were made on the surface of the culture medium by means of a cork drill. The prepared concentrations were placed at the rate of (0.1) ml for each hole, in addition to a hole containing the antibiotic gentamicin 10 micrograms for comparison, which was kept at 37 °C for 24 hours. The effectiveness of the disinfectant was determined by measuring the diameter of the damping area around each hole.

DISCUSSION

The biological activity of the alcoholic cumin extract against bacteria *(Escherichia Coli* , Staphylococcus aurues, Streptococcus mutans, *Staphylococcus epidermidis and Candida albicans* was tested using the method of digging diffusion. Table (2) indicates the results of the inhibitory activity of cumin at the concentrations used (3,2, 1,0.5, 0.1%), which gave an inhibitory value (15,13,12,0, 0) and (18, 14, 11 ,0 ,0)

towards Bacterial strains. (*Escherichia.coli and Staphylococcus aurues)*, while it gave inhibitory diameters that ranged between (16, 14, 14, 0, 0) and (17,14,11,0 ,0) against *Streptococcus mutans* and *Staphylococcus epidermidis* respectively, while for *Canaida albicans* the diameters ranged Inhibitory between (16,13,11,0,0). For concentrations (3, 2, 1, 0.5, 0.1) % respectively.

Table (2): Results of the inhibitory activity of cumin at different concentrations

Concentrations / Microbes	3%	2%	1%	0.5%	0.1%	Gentamicin
Escherichia. coli	15	13	12	0	0	17
Staphylococcus aureus	18	14	11	0	0	20
Staphylococcus epidermis	17	14	11	0	0	17
Streptococcus mutans	16	14	14	0	0	17
Candida albicans	16	13	11	0	0	16

While the biological activity of the alcoholic dill extract against the bacteria (*Escherichia. Coli*, *Staphylococcus aureus, Streptococcus mutans*, *Staphylococcus epidermidis* and *Candida albicans*) was tested using the method of diffusion by digging, the results showed that the extract of alcoholic dill has good antibacterial properties (inhibitory properties). As a control factor, as Table (3) indicates the results of the inhibitory activity of dill at the concentrations used (0.1, 0.3, 0.5, 0.75, 1) % as it gave an inhibitory value (11, 11, 12, 14, 16) mm and (11, 10, 11, 15, 17) mm towards *Escherichia. coli and Staphylococcus aurues*), while it gave inhibitory diameters that ranged between (13, 14, 15, 15, 17) and (10, 10, 11, 11,12) against Staphylococcus *epidermidis and Streptococcus mutans)* respectively. As for *Canaida albicans*, the inhibitory diameters ranged between (11, 14, 18, 20, 20). For concentrations (0.1, 0.3, 0.5, 0.75, 1) %, respectively.

Table (3): The results of the inhibitory activity of dill at the concentrations used (0.1, 0.3, 0.5, 0.75, 1) %

Concentrations / Microbes	0.1 %	0.3 %	0.5%	0.75 %	1 %	Gentamicin
Escherichia. coli	11	11	12	14	16	17
Staphylococcus aureus	11	10	11	15	17	20
Staphylococcus epidermis	13	14	15	15	17	17
Streptococcus mutans	10	10	11	11	12	17
Candida albicans	11	14	18	20	20	16

The biological efficacy of a mixture of 2% cumin with 1% dill was evaluated, which is a concentration of 100% against *(Staphylococcus epidermidis, Streptococcus mutans, Staphylococcus aurues, Escherichia. Coli* and *Candida albicans)* using the diffusion method, the results showed that the alcoholic extract had a good inhibitory ability compared with the antibiotic (gentamicin) as a control agent. Table (4) indicates the results of the inhibitory efficacy of the combination with the concentrations used (100,75, 50, 25, 12.5) % as it gave an inhibitory value of (22,21,19,18, 15) mm and (21, 20, 20, 17,15) mm against *Escherichia. Coli* and *Staphylococcus aurues*), while it gave inhibitory diameters ranged between (20,20,21,21,16) mm and (22,19,18,16,16) mm against *Staphylococcus epidermidis* and *Streptococcus mutans* respectively, while on *Canaida albicans* the inhibitory diameters ranged Between (21,20,20, 17,17). For concentrations (100, 75, 50, 25, 12.5) % respectively.

Table (4): shows the inhibitory efficacy results of a mixture of 2% cumin with 1% dill.

Concentrations / Microbes	100%	75%	50%	25%	12.5%	Gentamicin
Escherichia.coli	22	21	19	18	15	17
Staphylococcus	21	20	20	17	15	20

aureus						
Staphylococcus epidermis	20	20	21	21	16	17
Streptococcus mutans	22	19	18	16	16	17
Candida albicans	21	20	20	17	17	16

The positive effect of the alcoholic extract of cumin and dill plants is due to its containment of many effective compounds, as the results indicated that it contains many chemical compounds, which include alkaloids, tannins, saponins, flavonoids, carbohydrates, phenols, glycosides and other active substances as shown in Table (5).

Table (5): shows the presence of the active substances in cumin and dill using chemical methods

No.	Detection Type	Cumin	Dill
1	Tannins	+	+
2	Terpines	+	+
3	Glycosides	+	+
4	Phenols	+	+
5	Resins	+	+
6	Flavonoids	+	+
7	Saponins	+	+
8	Alkaloids	+	+
9	Coumarins	+	+
10	Steroids	+	+

The active substances in cumin and dill were diagnosed by spectroscopic methods. Figures (1, 2) by infrared spectroscopy with (FTIR) technology showed several distinct absorptions, in which the active sites found in cumin and dill plants were infrared, which are originally due to the chemical composition of the main components, including carbohydrates and their derivatives. Alkaloids, glycosides and other substances available in the two extracts, and it was observed that the values of some of them were close to each other, and the effective absorption sites appeared, represented by the structure (OH) that belongs to carboxylic acids, alcohol or water, and groups (C=O) that belong to esters, carboxylic acids, aldehydes, ketones, and a group (C=C) which refers to the aliphatic or aromatic structures present in the two extracts.

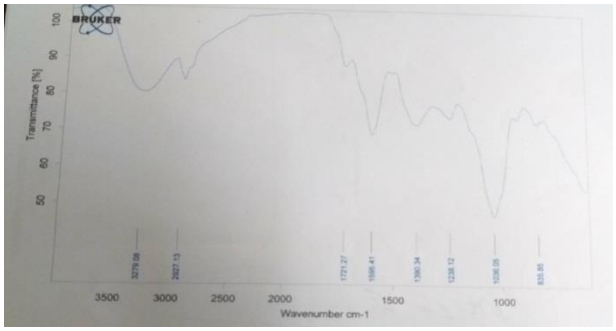

Figure (1): Infrared spectroscopy (FTIR) for cumin

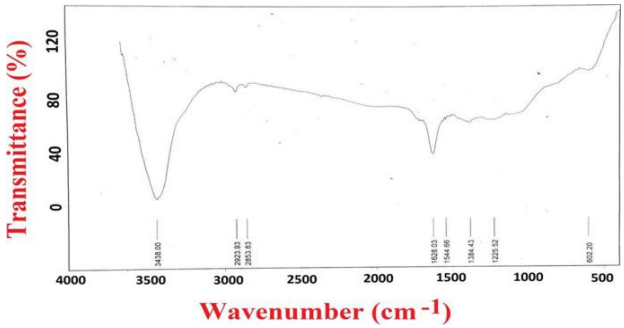

Figure (2): Infrared spectroscopy (FTIR) for dill

Figures (3, 4) show the results of the examination of cumin and dill extract using (UV spectrum) technique. The results indicated the appearance of three distinct peaks at wavelengths (202, 262.5 and 348) nm.

The compounds of cumin and dill extract were also diagnosed using the GC mass technique, as the results indicated the emergence of many compounds, which numbered about (54) compounds, the most important of which are phenolic acids such as (Caffeic, Gallic acid , palmitic acid and flavonoids such as (Kaempherol, Quercetin and rutin) added to alkaloids, ketones, aldehydes and glycosides with the presence of paraffin and Figures (5 , 6) show the chromatogram of two extracts using the gas chromatography technique [12].

The results of antibacterial activity in my research was effective as the results of Derakhshan S et *al* evaluated the activity of dill seeds essential oil based on the inhibition zone diameter and minimum

inhibitory concentration (MIC) against some important pathogenic bacteria including: Vibrio cholerae, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. Moreover, the impact of various concentrations of cumin alcoholic extract was assessed biofilm composing ability of K. pneumoniae. The biofilms were formed on semi-glass lamellas and investigated by a scanning electron microscope. Dill essential oil showed a reasonable to moderate activity against the employed strains. The highest antibacterial efficacy was detected against S. aureus (inhibition zone of 15 mm) and V. cholerae (inhibition zone of 14 mm) [13]. Kengne CI et *al*, characterized the antimicrobial activities of the extracts of Rumex abyssinicus and their combinations with ciprofloxacin and fluconazole employing the broth microdilution method by determining the minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC). The effects of the extracts on the bacterial cell membrane were measured [14].

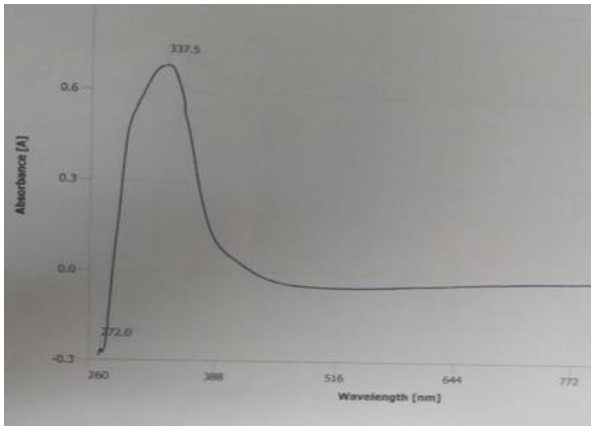

Figure (3): UV spectrum of cumin extract

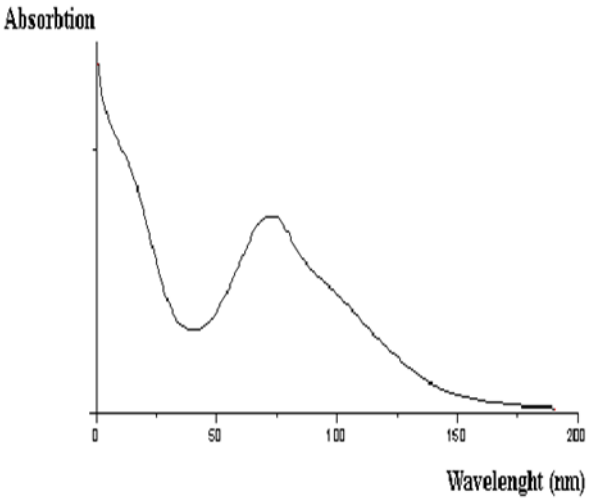

Figure (4) :UV of dill extract

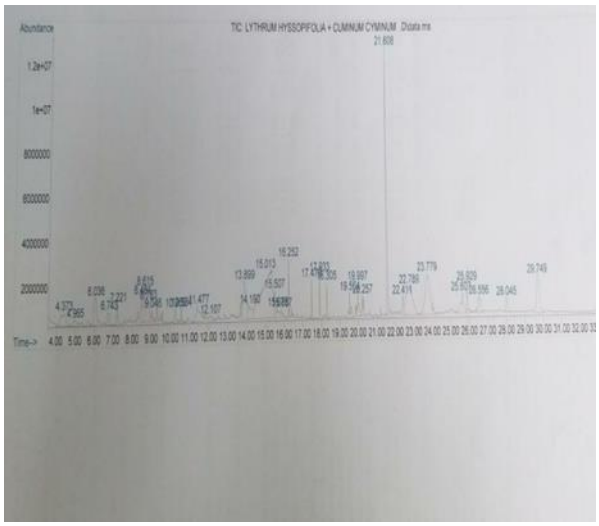

Figure (5) : GC mass of cumin using

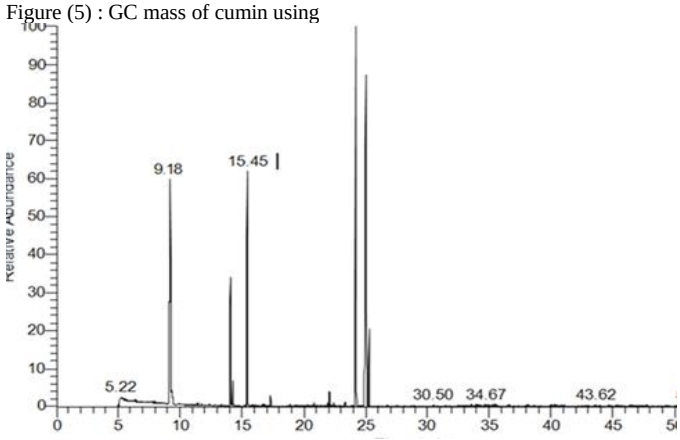

Figure (6): GC of dill

CONCLUSION

Cumin and dill has a therapeutic value and great activity against many bacteria. The effectiveness of

these cumin and dill extracts against bacteria was proved and attributed to the presence of effective groups that have a significant inhibition efficacy on various strains of bacteria. The results presented in this research confirmed antimicrobial properties of the extracts from the cumin and dill

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