An Extraction, Characterization and Evaluation of Antimicrobial Effectiveness of Raphanus Sativus Extracts

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ABSTRACT
This study research aimed to prepare radish seeds extracts (aqueous and alcoholic) by maceration method and study the effect of both extracts on the enteric bacteria were lived in the intestine such as Escherichia coli bacteria and another types of Bacteria to show the effectiveness of both extracts. Chemically revealed to investigate the presence of active groups in both extracts containing tannins, glycosides, flavonoids, resins, saponins, and alkaloids which attributed the main effect to giving the effectiveness for anti-growth microorganisms. The biological effectiveness of the extracts was tested of E. coli bacteria and a number of bacterial species. The results showed that alcoholic extract was the stronger effectiveness than the aqueous extract and the standard gentamicin antibiotic 100% was the best concentration, the inhibition zone reached (32) mm for the E. coli bacteria compared to gentamicin antibiotic which inhibition zone reached just (17) mm. its showed also the effectiveness from another types of Bacteria (in the same concentration 100%), inhibition zone ranged between (28-32) mm compared to gentamicin that reached just (17) mm. it also showed the effectiveness for yeasts such as Candida albicans. Inhibition zone reached (21) mm compared to standard antibiotic nystatin which reached just (16) mm. As for the aqueous extract showed the effectiveness of concentration 100% about E. coli bacteria which inhibition zone reached (21) mm compared to gentamicin antibiotic which reached just (17) mm, also showed the effectiveness against Streptococcus mutans bacteria, the inhibition zone reached (20) mm compared to gentamicin that reached just (17) mm. the aqueous extract did not show any effect about types of another bacteria and yeasts.

INTRODUCTION

In many of the nations that produce them, medicinal plants enjoy tremendous attention and hold a prominent place in agricultural and industrial output. Pharmaceutical Studies have also shown that medicinal plants have a physiological and pharmacological effect in treating diseases, and they are safe to use, with few side effects compared to synthetic drugs. [1] The radish, Raphanus sativus, belongs to the Magnoliphyta cultivar of the Brassicaceae family. It is a widely distributed plant and is characterized by its ease of cultivation and reaching maturity quickly within (30) days. [2, 3] The importance of radish is because it contains Raphanin, Raphaiol, and these are all considered substances that inhibit the growth of bacteria. The radish has many benefits, including an appetite suppressant, gas repellent, expectorant, anti-scurvy and useful for joint pain. Radish seed juice has a pungent taste due to the presence of Glogosinolates and Myrosinase. [4] The intestinal family includes a large group of bacteria that live in the human intestine and are gram-negative rod-shaped, motile, non-motile, aerobic, or anaerobic, and cause many diseases in humans. urinary tract, and Salmonella sp. Which causes typhoid and Shigella sp. Which causes dysentery

and Viberio bacteria that cause cholera and other types.[5] The aim of this study is to determine the effect of the aqueous and alcoholic extract of the radish seeds extracts on the bacteria causing skin infections and to study the biological effectiveness of these extracts on a number of pathogenic bacteria.

MATERIALS AND METHODS

After being bought from the neighborhood market, radish plant seeds were identified as Raphanus sativum by the University of Baghdad's College of Science, Department of Life Sciences herbarium.

PREPARATION OF PLANT EXTRACTS

Aqueous extract (soaking): (50) gm of radish seeds was taken, crushed with a grinder, and placed in a (1) liter glass beaker; It was mixed with 600 milliliters of distilled water. For 48 hours, the sample was kept in the Shaker Incubator at a temperature of 37 C., then passed Several layers of cloth to filter it, then filter the solution using a Buchner system and using Whattman No.1 filter paper, then concentrate the solution with a rotary evaporator and use a spray drier to dry it. Once the product is ready to use, gather it and store it in an opaque bottle until you reach the desired dry weight of 4 grams [6]. Alcoholic extract (soaking): Weigh (50) g of ground radish seed powder and put it in a (1) Liter glass beaker and add to it (500) ml alcohol (80%) ethanol and put it in the vibrating incubator at (37) C for (48) hours, then pass On several layers of cloth for filtering, the solution was then filtered by a Buchner system and using Whattman No.1 filter paper. Then the solution was concentrated with a rotary evaporator and dried using a spray dryer. The dry extract was collected and kept in an opaque bottle until use, reaching the dry weight obtained. In order to determine the active chemical components of the plant extracts, a series of qualitative tests were carried out.[8]

IDENTIFYING THE TANNINS TEST

When a white precipitate forms, add one milliliter of 1% aqueous lead acetate to one milliliter of the extract for detection. The outcome is encouraging and shows that tannins are present.

Test for Carbohydrates: Using Mollsch's reagent, (1) milliliters of the extract were combined with five drops of alcoholic α-naphthol in a tube and thoroughly shaken. Next, (2.5 milliliters) of sulfuric acid H2SO4 at a concentration of (96 percent) was added. The presence of carbohydrates is indicated by a blue ring.

Test for Glycoside Detection: Fehling reagent was used to identify glycosides. The presence of glycosides is indicated by the formation of a crimson precipitate.

Phenols Test: This method involves detecting the presence of blue or green color by dissolving (0.1) grams of the extract in (1) milliliters of distilled water and adding 1-2 drops of ferric chloride solution to the mixture. The presence of phenols is indicated by the affirmative result.

Resins Test: This test looks for the presence of resins by adding one milliliter (1 percent) of lead acetate to one milliliter of extract. If a white precipitate forms, the test is positive.

Flavonoid detection: One milliliter of potassium hydroxide (KOH) alcohol reagent (N5) was added to one milliliter of the extract. The presence of flavonoids is indicated by the appearance of a yellow precipitate, which is a positive result.

Saponin detection involves adding one milliliter of aqueous mercury chloride reagent (5 percent) to one milliliter of the extract; if a white precipitate forms, the saponins are present.

Alkaloids can be found by using the Wagner reagent. To do this, add a few drops of the reagent to one milliliter of extract; when turbidity emerges, the alkaloids are present, and the test is positive.

The protein test is used to detect the presence of proteins when the violet hue indicates the presence of proteins. Eighty percent aqueous copper sulfate is dissolved in distilled water and one milliliter of the reagent is used for protein detection.

coumarin detection an amount of the plant's alcoholic extract is put in a test tube, which is then covered with filter paper saturated with diluted sodium hydroxide solution. The tube is then heated to 100 ˚C over a boiling water bath for a few minutes, and the filter paper is finally subjected to radiation. When a leaf is bright greenish yellow in the ultraviolet spectrum, coumarin is present.

Terpene and steroid detection involve dissolving one gram of the extract in a little amount of chloroform, adding a drop of chloroform anhydride, and finally adding a drop of strong sulfuric acid. If the extract turns a dark blue hue after a few minutes, it shows the presence of steroids. If the brown color develops immediately, it indicates the presence of terpenes.

ANTI-BACTERIAL ACTIVITY TEST

Activation of bacterial strains: The bacterial species were activated using the liquid nutrient medium (Nutrient Broth), (100) ml of the mentioned medium was prepared according to the manufacturer's instructions and distributed in sealed glass tubes at a rate of (20) ml per tube according to the number of bacteria available then heated to 121 C in an autoclave for sterilization for (15) minutes, After being allowed to cool at 25°C, the samples were infected with 1 milliliter of the bacterial suspension and incubated for 24 hours at 37°C. [9][10] Examination of biological activity: Make 100 milliliters of Muller-Hinton Agar medium according the manufacturer's directions., then distribute it in sealed glass tubes at a rate of (20) ml per tube according to the number of bacteria available, sterilize with an autoclave at (121) ° C for a period of (15) minutes and allowed to cool to (25)° C. Inoculate each glass tube with (1) ml of the activated bacteria suspension from the previous day. Fill each (9) cm with 20 ml of the medium diameter petri dish. Dishes left to harden, worked. Four holes were done in each dish with eight millimeters of diameter for every hole and using a sterile tool, adding (50) microliters of each extract into each hole using a micropipette), gentamycin 10 mcg tablets were used and nystatin 100 units, following a 24-hour incubation period at 37 ¯C, the diameters of the inhibition areas on the plates were measured. using the ruler. [11][12] Micobes used are Bacteria E. coli bacteria, Staphylococcus aureus, and Streptococcus mutans, which are gram-positive. Gram-positive Staphylococcus epidermidis, the causative agent of skin infections, and Candida albicans yeast. [11][12]

RESULTS AND DISCUSSION

Table (1) shows the results of the chemical detections of the active substances of radish seed extracts (aqueous and alcoholic). The results showed the presence of flavonoids, which are pigments that dissolve in water, and they are primarily responsible for the medicinal properties of plants, as they act as anti-inflammatory, viruses, bacteria, and antioxidants as well. [13] The results also showed the presence of resins, which are characterized as a tonic and antiseptic for the gums that also inhibits the growth of bacteria on the teeth; also, it contains tannins, which are useful in treating burns and wounds because they function as antiseptics and aid in the production of new tissue and the repair of mucous membranes. Additionally, the presence of glycosides, alkaloids, and saponins, all of which have a significant impact on inhibiting the growth of microorganisms. [14]

TABLE (1) QUALITATIVE CHEMICAL ANALYSES OF EXTRACTS FROM RADISH SEEDS (AQUEOUS AND ALCOHOLIC)

Test	Alcoholic Extract	Aqueous Extract
Tannins Test	+	+
Carbohydrate Test	+	+
Glycosides Test	+	+
Phenols Test	-	-
Resins Test	+	+
Flavonoid 's Test	+	+
Saponin Test	+	+
Alkaloid Test	+	+
Protein Test	-	-
Comarines Test	+	-
Terpenes Test	-	-
Steroids	-	-

Table (2) shows the biological activity of radish seed extracts on intestinal and non-intestinal bacterial species, where the diameters of the inhibition areas were measured using the well diffusion method, and the length of time needed to read the results was established after twenty-four hours. The results revealed a variation in the rates of the inhibition area diameters. This is crucial for the kind and amount of active primary metabolites that are present in the plant, and which is directly related to the sensitivity of each

type of bacteria and the type of solvent utilized. The alcoholic extract showed greater efficacy than the aqueous extract and the antibiotic gentamicin, and the concentration of 100% was the best, as the diameter of the inhibition zone on E. coli bacteria reached (32 mm) compared to gentamycin, which reached the diameter of the inhibition zone (17 mm). The results also showed the effectiveness of the alcoholic extract against other species. of bacteria (as shown in the table), where the diameters of the inhibition zones ranged between (28-32 mm) compared to gentamycin, which reached the diameter of the inhibition zone (17 mm). Nystatin, which was only 16 mm. As for the aqueous extract, the concentration of 100% was also the best, and it showed activity against E. coli bacteria, where the diameter of the inhibition zone was (21 mm) compared to gentamycin, which had a diameter of 17 mm. It also showed activity against S. mutans bacteria, where the diameter of the inhibition zone was (20 mm) compared to gentamycin, while it did not affect the rest of the bacteria and yeasts. These differences in the diameters of the inhibition zones are attributed to the types and concentrations of the active substances in each sample, to the extraction conditions, and to the conditions of the microorganisms themselves.

TABLE (2) ILLUSTRATES THE OUTCOMES OF SEVERAL RADISH SEED EXTRACTS' BIOLOGICAL ACTIVITIES. (AQUEOUS AND ALCOHOLIC) AGAINST BACTERIUM TYPES AND THEIR COMPARISON WITH GENTAMYCIN.

Bacteria	Inhibition zone diameter (mm)								
	Ethanolic extract				Aqueous extract				
	25%	50%	75%	100%	25%	50%	75%	100%	Gentamicin
Escherichia coli ATCC 25922	23	25	28	32	14	16	18	21	17
Staphylococcus aureus NCTC 8325,	22	25	27	32	-	-	-	-	17
Staphylococcus epidermidis ATCC 35984	22	25	26	27	-	-	-	-	17
Streptococcus mutans Clinical isolate	22	24	26	28	-	15	17	20	17
Candida albicans	14	17	18	21	-	-	-	-	Nystatin 16

CONCLUSIONS

Chemical disclosures of the extracts (aqueous and alcoholic) that they are affected by the nature of the solvent in terms of polarity, which affects the extracted groups and the method of extraction, as each group has certain specifications and characteristics. the biological activity's outcomes of the alcoholic extract are better than that of the water to prevent the growth of the intestinal bacteria E. coli that causes diarrhea, colic and urinary tract infections. Therefore, the possibility of benefiting from the extracts of the radish plant seeds should be included in medicinal compositions.

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