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EXTRACTION OF THE ANTIMICROBIAL OF 2,4-DECADIENAL FROM CITRULLUS COLOCYNTHIS PLANT

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ABSTRACT

The medicinal properties of Citrullus Colocynthis plant seeds were used, and the compound M-2,4-decenal was extracted and tested in the laboratory against two strains of standard and newly isolated bacteria that are known to cause gastrointestinal and respiratory infections in humans. This investigation included the qualitative detection of the active components in the colocynth fruits, as well as the efficiency of its aqueous and alcoholic extracts in suppressing the bacteria isolated from the gastrointestinal tract. The quantitative detection revealed that, in addition to the compound M-2,4-decenal, which accounts for approximately 10% of the seed weight and is thought to be the most abundant component of colocynth seeds, the aqueous and alcoholic extracts of the colocynth plant's seeds contain phenols, salts, galactose, resins, and terpenes. GC-MS and RP-HPLC were used. The concentrations were 780 mg/kg in water and 950 mg/kg in methyl alcohol.

Three species of pathogenic Gram-negative bacterium, and Staphylococcus aureus, a Gram-positive bacterium were picked from the Food Processing Department's laboratories at the Second Technical College in Qassim, while all normal laboratory preventive and safety procedures were followed. The efficiency of bitter melon against the chosen microorganisms was demonstrated by comparing it to antibiotics such as chloramphenicol, tetracycline and ciprofloxacin. The findings indicated that Proteus mirabilis, a Gram-negative bacterium, and Staphylococcus aureus, a Gram-positive bacterium were the most susceptible to the aqueous and alcoholic extracts of Citrullus Colocynthis plant which were comparable to the effects of the chosen medications.

The plant extracts of Citrullus Colocynthis plant were also found to be highly competitive with antibiotics. The findings demonstrated that the alcoholic and aqueous extracts had the greatest effect on Proteus mirabilis, Gram-negative bacterium, and Staphylococcus aureus, a Gram-positive bacterium with inhibition values of 6.58 and 7.21 mm. Because the inhibition values for the alcoholic and aqueous extracts were 8.01mm and 6.27 mm, respectively, Staphylococcus aureus, bacterium demonstrated the greatest resistance. Noting that Gram-negative bacterium, and Staphylococcus aureus, a Gram-positive bacterium bacteria were more impacted at 5.88 and 7.15 mm and 8.0 and 7.68 against the two types of antibiotics, the antibiotics chloramphenicol and tetracycline showed efficacy in killing the tested bacteria. The study demonstrated that alcoholic extracts are more effective than aqueous extracts and closely resemble the bacterial inhibition rate of the medicines tetracycline and chloramphenicol against Proteus mirabilis, a Gram-negative bacterium, and Staphylococcus aureus, a Gram-positive bacterium. This study demonstrated that trans-2,4-decadienal, an antibiotic, can be easily extracted from colocynth seeds and used safely to eradicate the bacteria under study. This suggests that the colocynth plant is an efficient disease-curing agent.

Key words: Citrullus Plant Seeds Proteus Mirabilis-Staphylococcus Aureus

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1. INTRODUCTION

The bitter apple, sometimes known as colocynth, is an annual creeping desert plant native to Asia and the Mediterranean, primarily in Turkey and Nubia. It resembles a watermelon vine, but it produces heavy, little fruits with bitter pulp. This perennial member of the gourd family grows in deserts across Asia and Africa. Its root structure is level and deep. The leaves alternate and are strong. The black, spherical fruit is very bitter. It has powerful laxative qualities. Colocynth plant stems and roots, particularly those found in herbs and small shrubs. Because the colocynth plant's massive, perennial roots spread out across a few meters in all directions, they have a high survival rate and hunt anything that can be climbed, especially small bushes.

Like the watermelon plant, the colocynth plant has leaves with three to seven lobes each. Colocynth plants produce yellow flowers on small stems that adhere to the leaf axis. Each plant produces 15 to 30 smooth, spherical fruits with a strong bitter flavor that range in size from 5 to 10 cm across. The gray, 5 mm long by 3 mm wide seeds of the colocynth plant are edible, despite having the same bitter taste as the high-fat, protein-rich fruit. Colocynth, also known as Citrullus colocynthis (L.) Schrad, is a useful plant from the Cucurbitaceae family that flourishes in arid regions around the world. Although it can be found in deserts, its primary habitat is Asia and the Mediterranean region. of North Africa, Southern. It has traditionally been used to cure a variety of diseases, including constipation, cough, diabetes, leprosy, toothaches, and asthma. Citrullus Colocyn The seeds are commonly used as antidiabetic and antihypertensive drugs, while the fruits have been used to treat lung and urinary tract infections [2]. Plants contain protein, carbohydrates, lipids, oils, minerals, vitamins, and water, all of which are required for human and animal growth. Phytochemicals are classified as major or secondary components based on their role in plant metabolism. Protein, general sugars, amino acids, nucleic acids (purines and pyrimidines), and chlorophyll all help with primary metabolism, which is essential for plant growth and development. Secondary metabolites, on the other hand, are vital to plant survival in their environment because they attract pollinators and provide organic defense against diseases and predators [3]. Secondary metabolites include terpenoids, flavonoids, alkaloids, tannins, saponins, steroids, and anthocyanins, which have commercial use in plants, pharmaceuticals, colors, flavors, and scents. Emerging research reveals that numerous phytochemicals protect humans from diseases such as diabetes, cancer, arthritis, aging, and others, despite the fact that plants produce these compounds to defend themselves. [4]. Cardiovascular. Terpenes produced spontaneously via the mevalonate route yield complex combinations of volatile oil components, which are most commonly found in aromatic plants. These volatile chemicals are made up of monoterpenes and sesquiterpenes [5].Oxidation occurs when a substance loses an electron or a hydrogen atom to oxidizing chemicals; these reactions can also result in the formation of free radicals. Many diseases, including cancer, inflammatory ailments, cardiac problems, and premature aging, are linked to oxidative stress. Plant antioxidants protect biological systems from free radical damage, which helps to avoid many diseases [6]. The highest concentration (2500 mg.ml-1) of fruit extracts had the maximum antioxidant and free radical scavenging activity, which may be linked to phenolic components, as validated by [7]. The antioxidant activity of Citrullus colocynthis fruits peaked at 2500 mg/ml, and its ability to scavenge free radicals enhanced with increasing concentrations. Colocynthis, a member of the Cucurbitaceae family, has a high degree of genetic variability. This perennial plant, which thrives in tropical soils, can tolerate harsh environmental conditions [8, 9]. Colocynthis fruits are distinguished by their spherical shape, wide range of colors, soft flesh, and edible seeds. Ancient societies recorded the potential medicinal properties of Colocynthis. It has traditionally been used to cure a wide range of ailments, including toothaches, diabetes, constipation, leprosy, cough, and asthma [11, 12]. In Mediterranean countries, the fruits of the Colocynthis plant have been used to treat lung and urinary tract infections [13]. Early research suggested that colocynthis may have hepatoprotective effects [14]. In Morocco, C. colocynthis seeds are widely used to treat diabetes and hypertension [15,16]. According to phytochemical study, C. colocynthis has a diverse range of chemical families, including sugars, alkaloids, flavonoids, and phenolic acids. Milovanovic and Pekoric-Jovanovic (2005) investigated the nutritional composition of C. colocynthis seeds and discovered that they had a dry weight of 52.3% pericarp and 47.7% kernel, a moisture content of 54.5%, an oil content ranging from 22.1 to 53.5%, a protein content of 21.8%, and the majority of the oilseeds' fatty acid (77.4%), including linoleic acid (18:2) (62.2%) [18].

Because no previous research has evaluated the pharmacological activity of C. colocynthis seeds grown in northern Morocco, the current study was conducted to investigate the phytochemical composition and antibacterial properties. Proteus mirabilis is a Gram-negative bacterium well recognized for its ability to swarm across surfaces in a distinctive bulls'-eye pattern. Clinically, this bacterium is most commonly a pathogen of the urinary system, particularly in patients undergoing long-term catheterization. Proteus mirabilis is a Gram-negative bacterium well recognized for its ability to swarm across surfaces in a distinctive bulls'-eye pattern. Clinically, this bacterium is most commonly a urinary tract infection, especially in patients undergoing long-term catheterization. (19, 20) Staphylococcus aureus is a Gram-positive, coccoid, non-motile, and non-spore-forming facultative. Numerous hazardous doses of enterotoxins, ranging from nanogram to microgram levels, are produced by it (21, 22, 23).

2. MATERIALS AND METHODS

2.1. Preparation of Colocynth Extract

Colocynth fruits were harvested in the Qassim district of Buraidah, Saudi Arabia. 100 grams of degreased and powdered colocynth seeds were steeped for 24 hours at reflux with stirring in 150 milliliters of ethyl acetate and 5% hydrochloric acid. After steeping the mixture, the filtrate was removed by filtration. The filtrate was collected using a separate filter after the acid phase had been treated with NH4OH to reduce the pH to 8.5. Following separation, the plant's seeds were crushed and oven-dried at 40 degrees Celsius. The seeds were ground into a 500 μm fine powder using an electric grinder. Fifty grams of powdered seeds were soaked in a one-liter solution of, water, acetone, ethyl and methyl alcohol for 48 hours. The entire mixture was passed through a Buncher funnel and a Whatman filter. The extract was concentrated using a rotary evaporator set at 40 degrees Celsius and pressure. The seed extract was concentrated to 100 mg/ml and diluted to 50 mg/ml.

2.2. Preservation of microorganisms

Solid Mueller-Hinton Bacteria were cultured at 37°C using agar and liquid peptone medium. Bacterial growth was supported by Sigma dextrose agar medium, which contained 3 g/liter of solid beef extract and 5 g of liquid peptone at pH 7. This study used two types of bacteria: Proteus mirabilis, a Gram-negative bacterium, and Staphylococcus aureus, a Gram-positive bacterium. After growth, all samples are stored on dextrose nutritional agar media at 4 °C, with the germs replenished every six months.

2.3. Chemicals

Sigma Chemicals supplied acetone, formaldehyde, toluene, formic acid, as well as 99% pure ethyl and methyl alcohol. This study used colocynth seeds as a positive control at a concentration of 0.01 μg/ml, equivalent to common medicines such as ciprofloxacin, tetracycline, and chloramphenicol. Linalool improves the essential oil's antibacterial qualities through additive and synergistic effects. The study aimed to assess the antibacterial properties of the essential oil alone and in combination with linalool. The essential oil's antibacterial properties against the microorganisms being studied were explored.

2.4. Preparation of the microbial suspension

A standardized concentration of 5.0 is used to make a microbiological suspension. Three to five pure bacterial colonies are collected using a sterile cotton swab and placed in a glass tube with five milliliters of sterile distilled water. After shaking the tube with the sample suspension until it got close to the McFarland suspension concentration of 5.0, the concentration was changed by adding more water or microbes until the microbial suspension reached the standard McFarland concentration of 5.0, which is equivalent to a bacterial concentration of 10 CFU/ml.

2.5. Effect of antibiotics against microbes

We utilized 15 cc of sterile solid nutrition agar material and sterile Petri dishes with a diameter of 90 mm. To ensure that the microorganisms were evenly spread, a cotton swab was taken from the inoculum suspension and ran over the entire medium surface after the microbial culture was refreshed. Three 7-mm-diameter holes were punched in each dish with a sterile cork punch to ascertain the medium. Using a micropipette, add 50 µl of antibiotic (10 mg/ml) to the perforations. The antibiotic was then allowed to spread throughout the medium by leaving the plates at room temperature for 45 minutes. Following that, the fungal plates were incubated for 48 hours at 28°C.

2.6. Antimicrobial effect of colocynth seeds

This study used sterile Petri dishes with a 90 mm diameter and 15 cc of sterile solid nutrition agar medium to investigate the antibacterial activity of colocynth seeds. Once the microbial cultures had been regenerated, a cotton swab was taken from the inoculum suspension and ran over the entire surface to ensure that the bacteria distributed evenly across the medium. To determine the medium, three 7-mm-diameter holes were drilled in each plate with a sterile cork drill. Using a micropipette, apply 50 µl of the antibacterial colocynth seed extract (10 mg/ml) to each hole. The plates were then left at room temperature for 45 minutes to let the colocynth seed extract penetrate into the medium. The bacterial plates were then incubated at 28°C for 48 hours, while the bacterial and yeast plates were incubated at 37°C for 24 hours each. The average diameter of the inhibitory zone was then determined. In mm, all experiments were repeated three times.

2.7. Chemical analysis

Results of GC-MS analysis of colocynth seed extract. At the Second Technical College in Buraidah, the 2,4-decadienal material was examined using a GC TRACE 1300 TSQ 8000 evo, which integrated mass spectrometry and gas chromatography. A 30 m × 0.25 mm × 0.25 µm Thermo TR-5 MS capillary column was used.

3. RESULTS

3.1. Effect of Antibiotics on microbes

Ciprofloxacin, tetracycline, and chloramphenicol antibiotics have an effect on germs of gram-positive bacteria, Staphylococcus aureus, and Gram-negative bacteria, Proteus mirabilis. It demonstrated an effective inhibitory impact on both 7.0-8.0 mm in diameters.

3.2. Effect of Citrullus Colocynthis plant seeds on harmful microbes

The effects of the antibacterial standard ciprofloxacin, tetracycline, and chloramphenicol on bacteria were first tested and then compared with the tested Citrullus Colocynthis plant seeds to determine the strength of the effect of this plant's seeds on bacteria in the three extracts—water, alcoholic, and acetone. When employed separately at doses ranging from 10% to 100%, it inhibited both Gram-positive bacteria, Staphylococcus aureus, and Gram-negative bacteria, Proteus mirabilis, with an average inhibition diameter of 6 to 8 mm (Tables 1 and 2). Citrullus Colocynthis plant seeds frequently had a larger effect on Gram-positive bacteria, particularly Staphylococcus aureus, than on Gram-negative bacteria, namely Proteus mirabilis. Although the Albicans extract has an average area diameter of 7.50 mm and the effect of the extract water is 7.00 mm, the alcoholic extract of the Citrullus Colocynthis plants was frequently the most prevalent in its effect on the microorganisms used, followed by the alcitonic extract and water alcohol. Because the average volunteer area diameter was 7.0 mm and the aqueous extract of this plant had an inhibitory influence of 6.0 mm on the two antibiotics, tetracycline and chloramphenicol, the alcoholic form of colocynth oil outperformed the two antibiotics on bacteria. The effect of the colocynth plant's aqueous extract on bacterial inhibition. Ciprofloxacin is being researched. The 6.5 ml acetone extract of the colocynth plant's seeds and the 7.54 ml acetone extract of the colocynth plant were both around Cipro, which was positively impacted by the average suppression area diameter (6.17 mm) by 0.5 ml. Antibacterial colocynth seeds have an average disinfection area diameter of 6.0 mm.

3.3. Effect of concentration of alcoholic extract of Citrullus Colocynthis seeds

Antibiotics like ciprofloxacin, tetracycline, chloramphenicol, Proteus mirabilis, Staphylococcus aureus, and Gram-positive and Gram-negative bacteria Microorganisms' response to Citrullus Colocynthis plant seed extract. The alcoholic extract of Citrullus Colocynthis plant seeds exhibited the greatest inhibitory effect on both Gram-positive Staphylococcus aureus and Gram-negative Proteus mirabilis. The acetone extract from Citrullus Colocynthis plant seeds showed the greatest effect on both of these bacteria. The acetonic extract (7.16 mm) was followed by A (7.23) and 6.54 mm, and then the aqueous extract (13 mm). Citrullus' acetonic extract was the most effective against Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Proteus mirabilis), measured 6.54 and 617 mm in diameter, respectively. On the other hand, the alcoholic extract was the most effective at inhibiting Gram-positive bacteria, Staphylococcus aureus, and Gram-negative bacteria, Proteus mirabilis, with diameters of 6.54 and 617 mm, respectively.

Table 1: Effect of aqueous, alcoholic, and acetone extracts of colocynth seeds in inhibiting the two species of bacteria under investigation (mm)

	trans-2,4-decadienal in water	trans-2,4-decadienal in alc.	trans-2,4-decadienal in acetone	ciprofloxacin	tetracycline	chloramphenicol
Staphylococcus Aureus	7.25	8.42	7.93	9.32	5.23	7.08
Proteus mirabilis,	6.25	6.53	6.42	8.41	4.85	6.10

Table (2): Effect of quantities of aqueous extract of colocynth seeds on suppression of the two species of bacteria under investigation (mm)

	trans-2,4-decadienal in water	trans-2,4-decadienal 0.8g/L	trans-2,4-decadienal 0.6g/L	trans-2,4-decadienal 0.4g/L	trans-2,4-decadienal 0.2g/L	trans-2,4-decadienal 0.1g/L
Staphylococcus Aureus	7.25	7.24	6.82	4.21	2.24	1.89
Proteus mirabilis,	6.25	6.23	5.41	4.32	3.12	3.08

Table (3) Effect of concentrations of alcoholic extract of colocynth seeds on inhibition of the two types of bacteria under examination(mm)

	trans-2,4 decadienal 100 الكحول	trans-2,4-decadienal 80%	trans-2,4-decadienal 60%	trans-2,4-decadienal 40%	trans-2,4-decadienal 20%	trans-2,4-decadienal 10%
Staphylococcus Aureus	8.42	8.41	7.32	5.35	2.24	1.02
Proteus mirabilis,	6.53	6.55	4.62	2.14	1.17	0.95

Table (4) Effect of concentrations of acetone extract of colocynth seeds on suppression of the two species of bacteria under study (mm)

	trans-2,4 decadienal Acetone100	trans-2,4-decadienal 80% Acetone	trans-2,4-decadienal 60% Acetone	trans-2,4-decadienal 40% Acetone	trans-2,4-decadienal 20% Acetone	trans-2,4-decadienal 10% Acetone
Staphylococcus Aureus	7.93	7.92	5.56	2.82	1.54	0.85
Proteus mirabilis,	6.42	6.43	4.25	1.93	1.17	0.43

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