A Study Of The Effect Of Sandarac Resin (Tetraclinis Articulate) Extract On Wound Treatment

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ABSTRACT
The main objective of the research is to obtain sandarac resin (Tetraclinis articulate) using traditional techniques (soaking method), and to assess the extract's impact as a bio-inhibitor for some types of bacteria and to know the effective groups such as (Tannins, phenols, resins, flavonoids, saponins, alkaloids and terpenes). The biological activity of the extract against bacterial and fungi species was measured. The results of the antimicrobial activity detection test shows that the effect of the extract at concentrations (50%, 25%) on Staphylococcus aureus ATCC (6538) inhibited activity (15 mm, 14 mm), respectively, and showed the antibiotic (Gentamycin) 17 mm inhibitory activity. The extract showed at concentrations (50%, 25%) activity against Staphylococcus epidermidis (local isolate) inhibitory activity (16 mm, 15 mm), while the inhibitory activity of the antibiotic (Gentamycin) was (17 mm) and the effectiveness of the extract (50%, 25%) has inhibitory activity on Streptococcus mutans (local isolate) (14 mm, 12 mm) and the antibiotic activity (17 mm). The extract (50%, 25%) showed inhibitory activity (15). mm, 14 mm) on E. coli ATCC (105538), respectively while the antibiotic had inhibitory activity (17 mm). The extract (at concentrations of 50%, 25%) showed a higher inhibitory activity on Candida albicans ATCC (10231) where the inhibitory activity was (13 mm, 12 mm), while Nystatin showed inhibitory activity (16 mm).

KEYWORDS
Sandarac, Resin Extract, Wound Treatment, Biological Activity

INTRODUCTION

The interest in medicinal plants has increased with the discovery of modern methods of separation, and scientists have been interested in discovering and researching what is hidden in the therapeutic secrets and discoveries in the world of plants, which have had an impact in eliminating some of the diseases that threatened humanity [1]. There are many uses for medicinal plants, not only as a treatment for many diseases, but also in the human diet, as well as the intervention of medicinal plants in the manufacture of pesticides, perfumes, cosmetics, oils, and textile industries and are also used as animal feed and in decorations [2]. Medicinal plants and herbs for the year 2001 were estimated at about (250,000-500,000) species, only a few of them were studied and researched chemically and biologically [3] and the number is increasing with the progress of scientific research. Sandros is a small tree with slow growth, but it can reach a height of 6 to 15 meters. It belongs to the conifers, with opposite leaves that are curved from the top to the bottom, and flowers on the end of the branches. Sandros is a monogamous plant, bisexual, and the female flowers are smaller than the male [4], Habitat The original plant of North Africa, especially Morocco and Algeria, where it was found that it is used there in the number of

diseases for treatment purposes, the Sandarac tree is known scientifically as (Tetraclinis Articulate) [5]. Sandarac gum is a resinous substance that is very similar to gum, which is secreted by plant branches and concentrated in the peels of the branches [6]. Sandarac gum contains about 95% diterpene acids. The occurrence of the wound is due to the breakdown of the connective tissue from the skin tissue in addition to the opening and destruction of cells. The most important symptoms resulting from it are bleeding, heat, redness around the wound site, a feeling of pain, throbbing, swelling of the tissues in the area and pus [8]. Wound healing is a very complex process that occurs through several factors that result in many cellular and biochemical processes.

The aim of my research is to assess the antimicrobial effectiveness of extracts of sandarac gum extract and detect the responsible components for their action.

MATERIALS AND METHODS

In this research, sandarac gum was purchased from local markets milled by electrical mill and kept at 3-4 °C till use. Two types of bacteria staphylococcus aureus serotype ATCC6538, Ibn Sina center. staphylococcus epidermidis, E. coli ATCC (105538, Candida albicans).

EXTRACT PREPARATION

The solubility of sandarac gum was tested in several solvents, including (water, acetone 36%, ethyl alcohol 96%, methyl alcohol 99%, diethyl ether 99%). The test showed that the substance dissolved in diethyl ether. (80) gm of sandarac gum powder was taken and placed in a (1 liter) glass beaker containing (800) ml of diethyl ether. and filtered through (with Whatman No.1) filter paper on Buchner funnel. The filtrate was taken and focused with a rotating vacuum evaporator. The concentrated material was dried in a steel tray in an electric oven set to a certain temperature of 40°C for (6) hours, then the dry extract was collected (12) grams and placed in an opaque bottle until use.

CHEMICAL IDENTIFICATION OF ACTIVE INGREDIENTS

To determine the active chemical components of the sandarac gum, a series of qualitative tests were performed [8]:

Detection of tannins Test: (1ml) of aqueous lead acetate (1%) was added to (1ml) of the alcoholic extract, when a white precipitation as an indication of the presence of tannins

Carbohydrate test: The test was carried out using Molish's reagent, where (1 ml) of alcoholic extraction was mixed about (5) drops of phenethyl alcohol in a test tube, shaken well, in addition to (2.5 ml) of sulfuric acid was added, when the presence of carbohydrates was indicated by the formation of a blue ring.

Glycosides test: The glycosides were detected by means of Fehling reagent, and there are glycosides present when a red precipitate appears.

Phenols test: After dissolving 0.1 g of the extraction in 1 ml of distilled water, add 1-2 drops of ferric chloride solution (FeCl3) when phenols were visible as a blue or green.

Resins test: One ml of the extract was mixed with one ml of lead acetate (1%), and the formation of a white precipitate suggests the presence of resins.

Detection of flavonoids: When one milliliter of the extract is mixed with one milliliter of the alcoholic (5N) potassium hydroxide reagent, a yellow precipitate appears; this indicates the presence of flavonoids.

Saponin test detection involves adding 1 ml of aqueous mercury chloride reagent (5 %) to 1 ml of extract; a white precipitate forming shows the presence of saponins and is regarded as a positive result.

Alkaloid test: Wagner's reagent is used to detect alkaloids. Several drops of the reagent are added to one milliliter of extract, and the extract is considered alkaloid-free when turbidity occurs.

Protein test: Proteins can be found using the Bayuret detection method, which involves dissolving 80% of copper sulfate in distilled water with 1 milliliter of 10% reagent. The presence of proteins is indicated by the violet hue of the solution.

The Coumarins test involves adding a certain amount of the plant's alcoholic extract to a test tube, covering the tube with filter paper moistened with a dilute sodium hydroxide solution, heating the tube in a boiling water bath for a few minutes, and then exposing the filter paper to a source of ultraviolet light. The presence of coumarins is indicated when the paper turns a bright yellow-green color.

Terpene and steroid detection: Combine one gram of the extract with a little amount of chloroform, then add a drop of acetic anhydride and a drop of strong sulfuric acid to the mixture. It contains the terpenes extract when the brown color occurs; however, if the brown hue appears after a dark blue phase, the extract contains steroids [9].

ANTI-MICROBIAL ACTIVITY TEST

The efficacy of the extracts was evaluated using the diffusion with agar method, in which the bacterial species were stimulated in Nutrient Broth medium. As directed by the manufacturer, prepare (250 ml) of the specified medium, sterilize using an autoclave device at (121°) C for fifteen minutes, and then let it cool. After being inoculated with one milliliter of bacterial cell suspension at 25 °C and heated to 37 °C for a full day, (250 milliliters) of agar were made, autoclaved, and allowed to cool. Using a micropipette, 50 microliters of each extract were applied at precise locations. Gentamicin was then added as a control agent, and the mixture was incubated for 24 hours at 37°C [8].

RESULTS AND DISCUSSION

The findings of the chemical identification of the active ingredients in the sandarac gum extract are displayed in Table 1, as this evaluation of the types of detection depends mainly on the components of food metabolism that occur naturally in the cells and tissues of medicinal and aromatic plants, that are called primary metabolic products or natural products. These primary compounds or Natural products by chemical difference with their medically effective groups despite the different types among plants producing these medicinal substances.

TABLE (1) CHEMICAL TESTS ON SANDARAC GUM

Test	Extract
Tannins Test	+
Carbohydrate Test	-
Glycosides Test	-
Phenols Test	+
Resins Test	+
Flavonoid 'S Test	+
Saponin Test	+
Alkaloid Test	+
Protein Test	-
Coumarins Test	-
Terpenes Test	+
Steroids	-

The results showed the presence of the active groups (tannins, phenols, resins, flavonoids, saponins, alkaloids, terpenes). Flavonoids have therapeutic effects due to presence of pigments, so They have antiviral, anti-inflammatory, antihistamine, and antioxidant properties [17]. The tannins function as antiseptics and are useful in the treatment of wounds because they stop the growth of germs [18]. The findings demonstrated that the rates of the inhibition zone widths varied depending on the sensitivity of the various bacterial species. The following bacteria were used: streptococcus mutans, E. coli ATCC (105538), Candida albicans ATCC (1023), and Staphylococcus epidermidis (ATCC 6538). Table 2 revealed that the biological activity results of the various extract types under investigation varied, as did the examined bacteria's inhibitory role in relation to Gentamycin control. Good efficacy, as it showed an inhibitory activity of (15 ml, 14 ml) and the antibiotic activity was 17 ml. While the extract at concentration (50%, 25%) gave activity against Staphylococcus epidermidis (local isolate) inhibitory activity (16 mm, 15 mm), while the activity of the antibiotic was 17 mm. The alcoholic extract also showed an inhibitory activity at a concentration of (50%, 25%) on

Streptococcus mutants (local isolate), an inhibitory activity (14 mm, 12 mm) and the inhibitory activity of the antibiotic (Gentamycin) was 16 mm. The inhibitory activity of the alcoholic extract at a concentration (50%, 25%) on E-coli bacteria (15 ml, 14 ml) and the inhibitory activity of the antibiotic was 17 ml, and the alcoholic extract gave activity against Candida albicans ATCC (1023) at a concentration (50%, 25%) had a diameter of inhibition (13 mm, 12 mm) and Nystatin provided an inhibition diameter (16 mm). The presence of active groups such as alkaloids effectively prevents the development of certain inflammatory organisms. The presence of phenols and tannins has an effective effect in inhibiting inflammatory bacteria and thus helps in the healing process, and this confirms the findings of the effectiveness of the extract on bacteria and Candida albicans [19].

TABLE (2) SHOWS THE RESULTS OF THE BIOLOGICAL EFFICACY OF SANDARAC GUM EXTRACT AGAINST TYPES OF BACTERIA

Bacteria	Inhibition Zone Diameter (mm)		
	Extract (50%)	Extract (25%)	Positive Gentamycin Ct
E. coli ATCC (105538)	15	14	17
Staphylococcus aureus ATCC (6538)	15	14	17
Staphylococcus epidermidis local isolate	16	15	17
Streptococcus mutans local isolate	14	12	16

100% concentration means 1 gram dissolved in 1 ml, 50% means (0.5) grams dissolved in 1 ml, and 25% means (0.25) grams dissolved in 1 ml.

The antimicrobial efficacy of sandarac gum extract in my study was as high as that of Derakhshan S et al., who assessed the activity of dill seed essential oil against certain pathogenic bacteria, such as Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus, based on the inhibition zone diameter and minimum inhibitory concentration. Additionally, the impact of sub-inhibitory doses of the alcoholic extract of cumin seeds was evaluated on K. pneumoniae's capacity to build biofilms. When evaluated against the identified strains, dill essential oil exhibited good to moderate activity. The antibacterial activity against S. aureus was found to be the highest (inhibition zone of 15 mm) and V. cholerae (inhibition zone of 14 mm) [16]. The antimicrobial properties of Rumex abyssinicus extracts and their combinations with ciprofloxacin and fluconazole were isolated, characterized, and assessed using the broth microdilution method by calculating the minimum inhibitory concentration (MIC) and minimum microbicidal concentration. Kengne CI et al (MMC). Using a spectrophotometric approach, the extracts' effects on the bacterial cell membrane and the activity of the enzyme microbial respiratory chain dehydrogenase were assessed [17].

CONCLUSIONS

Depending on the results of the antibacterial activity assay that sandarac gum extract is highly effective against Staphylococcus epidermidis that causes skin infections (wounds), as it gave a diameter of 16 mm inhibition. While its efficacy was limited compared to Candida albicans. So, benefiting from the high biological effectiveness of the extract of sandarac gum against the growth of bacteria for skin infections and making an ointment or cream to treat wounds.

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