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Effect of tannin fruit extracts on *Candida albicans* yeast

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Abstract

Background: The results of the chemical detection showed that the active groups in the fruit extracts of the tannin plant contained glycosides, saponins, flavonoids, tannins, terpene and phenols.

Aim: To evaluate Tannins inhibition activity against *Candida albicans.*

Materials and methods: A number of aqueous, alcoholic and acetone fruit extracts of Tannins were prepared, and their effect on inhibition of *Candida albicans* was tested. Four concentrations were used [10, 20, 30, 40 (mg / ml)].

Results: The effect of tested extracts on yeast varied in terms of concentration and type of extract. The inhibition diameter was positively correlated to the increase in extract concentration. The results showed that the acetone extract was with a very high effectiveness and demonstrate higher inhibition effect against *C. albicans*, with an average inhibition diameter of 15.5 and 20 mm at a concentration of 10 and 20 mg / ml, respectively, while it was increased to reach 24.5 and 28 mm at a concentration 30 and 40 mg/ml, respectively. The alcoholic extract came in the second inhibition effect.

Conclusion: The acetone extract shows a higher inhibitory activity than alcoholic and aqueous extracts. The interesting finding of this study was the inhibition against *C. albicans* was exhibited in the first 24 hours, while the nystatin inhibitory effect started in after 48 hours. The inhibition timing was vital as it lead to short course of treatment and less possibility for resistance induction.

Keywords: Tannin fruit , *Candida albicans,* Glycosides.

Introduction

Medicinal plants and herbs are among the most important natural sources approved for obtaining the materials involved in the formulation of treatments and drugs and in the pharmaceutical industry, and since nature is rich in these plants, efforts and attention have been focused on nature and what it contains of herbs and plants that are characterized by their medical and therapeutic effectiveness [1]. The most important of these materials are phenols, alkaloids, tannins, volatile oils, organic acids, and others. These substances are often formed as natural by-products of metabolic processes [2] .Tannin is the main component of tannins, and its percentage ranges from (50-70%) of its various other components [3].Tannins contain

a phenolic acids, such as Gallic acid (3%), Syringic acid (5%), and varying proportions of Quercetin, P-Hydroxybenzoic Gebullic acid , M-Degallic acid , Degallic acid, Glucogallic acid, and others. Starch and resin are common components of tannins, especially in the early stages of Cyrenaica, in addition to flavones [4]. Tannins was used as powder form in the treatment of diarrhea and dysentery. It is also used in the treatment of gonorrhea, bloody sputum, inflammation of the mucous membrane. gastric Catarrh (stomach ulcer), swelling of the spleen, hemorrhoids, polyposis, and in the treatment of eczema [5]. It is also used in the treatment of pharyngitis through gargling, inflammation of the kidneys and ureter, bleeding of the bladder, and in the treatment of putrefactive wounds and the prevention of contamination. It is believed that it has many pharmacological properties, including that it is an astringent, antibacterial, antifungal and anti-inflammatory, and it can also be used as a local anesthetic. Tannins were used in the past in the treatment of typhoid fever and in lowering blood sugar and in the treatment of burns and wounds and studies have shown that the preparations obtained from tannins are useful in treating cases of urinary tract infection and some cases of inflammation of the kidneys, ear and skin. [6]. Thus this study was conducted to evaluate its inhibition activity against *Candida albicans.*

Materials and Methods

Reagents used to detect the plant used in the study:-

1- **Benedict's Reagent** was prepared by dissolving (137) grams of sodium citrate and (100) grams of monohydrate sodium carbonate in (800) ml of distilled water. The solution was filtered and added to the filtrate. Cupric sulphate solution (17.3) gm in (100) ml distilled water, complete the volume to (1000) ml using distilled water. This detector produces a red precipitate at the bottom, indicating the presence of glycosidic compounds [7] .

2- **Marquis Reagent** was prepared according to what was mentioned in [8] and used detecting alkaloids as follows: Mix (1) ml of formaldehyde at a concentration of (40%), and add to (10) ml of concentrated sulfuric acid

3- **Ferric chloride reagent** 1% was prepared according to the method described previously [9]; it was prepared by weighting (1) gm of ferric chloride and placed in a graduated cylinder, then completed the volume to (100) ml. This solution was used to detect phenols, as it gives a bluish-green color .

Chemical detection of some active substances in plants

1- Detection of tannins

The detection was carried out according to the method described previously [10], where 1 ml of aqueous lead acetate solution (1%) was added to 1 ml of the extract and when a white precipitate is formed, the result is positive, which indicates the presence of tannins

2- Detection of Saponins

The saponins were detected by following the following two methods:

a- Shake the aqueous solution vigorously in the test tube, and the presence of saponins is indicated by the appearance of thick foam that remains for a long time [11].

b- Add 1 ml of mercuric chloride solution (1%) to 1 ml of the extract, the appearance of a white precipitate indicates a positive detection [8].

3- Detection of resins

The presence of resins was detected by the weight of 10 g of vegetable powder and 50 ml of ethyl alcohol with a concentration of 95% and leave the mixture in a water bath for two minutes. Then it was filtered and 100 ml of distilled water acidified with hydrochloric acid at a concentration of 4% was added to it. The presence of resins is indicated by the appearance of turbidity in it [12].

4- Detection of flavonoids

Flavonoids were detected according to the method described previously [13] by adding 1 ml of potassium hydroxide (Ethanolic KOH) solution to 1 ml of plant extract. When a yellow precipitate appears, the result is positive, indicating the presence of Flavonoids

5- Detection of alkaloids

Previously described methods [8] followed, the method by adding 3 ml of plant extract in a test tube and 2 ml of Marquise's reagent was added to it. When the tube was shaken, a pale gray color was formed indicating the presence of alkaloids.

6- Detection of terpenes and steroids

One gram of the dry extract is dissolved in a little chloroform and a drop of anhydrous acetic acid is added to it, then a drop of concentrated sulfuric acid is added to it, as the appearance of a brown color is evidence of The presence of terpenes, but if it turns blue after (3-5) minutes, it indicates the presence of steroids [14].

7- Detection of Glycosides

The method of Ahmed and Sulaiman [7] was followed by adding 1 ml of aqueous plant extract to 5 ml of Benedict's reagent, where the appearance of a red precipitate confirms the presence of sugars, while the appearance of a blue color indicates the absence of sugars

8. Detection of phenolic compounds

A (3) g of the extract was added to (2) ml of ferric chloride prepared by dissolving (1) g of ferric chloride in (100) ml of distilled water, as the green color appeared The bluish color indicates the presence of these substances.[8]

Preparation of Plant Extracts

Three types of solvents were used, distilled water, 95% alcohol, and 80% acetone. Forty gram of plant form was mixed with 160 ml of distilled water, and the mixture was stirred by a shaker. Leave the mixture in the refrigerator to soak for 24 hours. It was then filtered through several layers of gauze and filtered again using filter papers (Whatman No 1) to get rid of the unpulverized plant parts and remaining fibers. Then the extract was placed in the oven at a temperature of 40 °C until all the water evaporated and the extract remained in the base of Al-Baker [15] . Then the extracts, after drying, were placed in glass vials with tightly closed lids and kept in the freezer until use.

Preparation of Alcoholic Extract

The alcoholic extract was prepared in the same way as in the preparation of the previous aqueous extract, except for replacing the distilled water with ethyl alcohol at a concentration of 95% [15].

Preparation of Acetone Extract

The acetone extract was prepared in the same way as in the previous preparation of the aqueous and alcoholic extract, except for the replacement of distilled water and ethyl alcohol with acetone at a concentration of 80% [15] .

Concentrations used in the study:

Four concentrations were used (10, 20, 30 and 40) in mg/ml.

Sterilization of extracts and preparation of dilutions:

Prepare a stocking solution by taking 1 gm of dry plant extract powder and dissolving it in 10 ml of sterile distilled water. The concentration of the storage solution became 100 mg / ml. Sterilize the solution by filtration using sterile filter papers (Whatman No 1) to get rid of contaminants and obtain a sterile storage solution. And used as a source for the preparation of fears.[16]. The method of diffusion in the pits was performed as described before [17], as follows:

1- Pour (25) ml of the SDA agar into each plate.
2- Inoculated the feeding acres by spreading (0.1) ml by means of a sterile spreader from the yeast culture containing (1.5 x 108) cells/ml, by comparing it with the standard stable turbidity solution, then the plates were left to dry at room temperature.
3- A hole with a diameter of (5) mm was made in the culture medium with a sterile cork (borer)
4- An amount of (0.2) ml of graduated concentrations prepared for plant extracts was added using a micropipette. A positive control hole was made by adding anti-nystatin 30 mg / ml.
5- Three replications were made for each plate, then the plates were incubated at a temperature of (37) C for a period of (48) hours, in a manner that determined the effectiveness of each concentration of the extract by measuring the inhibition zone.

Statistical analysis

The results of the study were analyzed statistically according to the Duncan polynomial test [18], at a probability level of 5%, and this was implemented using the Excel program in the electronic calculator.

Results and Discussion

Chemical detection of some active substances in the extracts of the fruits of the tannins plant The results of the chemical detection showed some of the active substances in the extracts of the fruits of the tannins plant. The plant contained glycosides, saponins, tannins, phenols, terpene and flavonoids, but it did not contain alkaloids and resins, and this is consistent with others [19], who found that the fruits of tannins do not contain alkaloids and resins.

Glycosides are among the important compounds in the plant, and they are considered as one of the storage sources of sugar materials, which in turn are involved in the process of regulating osmotic pressure, and the transfer of some substances necessary for the plant's metabolism. It also plays a "protective" role against some pests and insects that infect plants [20] , It is decomposed by acids or yeasts into two substances: a sugary substance called Glycone, which is dextrose and has no pharmacological efficacy, except that it carries the non-sugar part of the glycoside to its area of influence in the human body, and one or several

non-sugar substances called Aglycone or Genine, which is the pharmacologically active part, from the glucoside [21].

Table 1:- Chemical detection of some active substances of fruit extracts of tannins

Compound	Used detector	Detection guide	Acetone	Alcoholic	Aqueous
Glycosides	**Benedict**	**Red precipitate**	+	+	+
Alkaloids	**Marquis**	**Grainy lead color**	-	-	-
Phenols	**Ferric chloride %**	**Bluish green**	+	+	+
Tannins	**Lead acetate**	**White precipitate**	+	+	+
Saponins	**Mercuric chloride**	**White precipitate**	+	+	+
Flavonoids	**Ethyl alcohol (KOH)**	**Yellow precipitate**	+	+	+
Resins	**Ethyl alcohol**	**Turbidity**	-	-	-
Turbines & Steroids	**Chloroform Anhydrous acetic acid &H_2so_4**	**Terpene brown and steroid blue**	+	+	+

Tannins are polyphenols, and the term Tannin is derived from the ancient use of these phenols in the manufacture of animal skins, where tannins were used for tanning leather (tanning agent). , It is believed that tannins have a role in "water absorption, as is the case in colloids. Thus, they protect the plant from dehydration. They have a role in inhibiting the growth of microorganisms, and tannins have a diuretic effect." They are antioxidants and have the ability to inhibit mutagenic susceptibility to some mutations. Thus, they are considered anti-cancer [22]. As for the saponins, they produce soapy foam when shaken with water. They are chemical compounds of triterpenes or steroids found in many plants. Detergents were used before the discovery of soap. Saponins protect plants from insects and microorganisms as they affect the permeability of the cell membrane and thus facilitate the entry of toxic substances or cause a deficiency in the vital components of microorganism cells [23].

Phenols are organic compounds due to the presence of a hydroxyl group (OH) linked to a benzene ring or aromatic ring structures, and they form a hydrogen bond with the active part of the enzyme. Thus, the volumes of these enzymes change as well as their properties change, and therefore they are not effective in the cell, which leads to the stopping of certain pathways In the cell, which leads to its death, and phenols may bind with each other, stopping cancerous tumors, as they play an “important” role in stopping cancer and inhibiting the representation of mutations. It also has an anti-inflammatory role [24].

Flavonoids are organic compounds with a chemical composition consisting of three hexagonal rings, three hydroxyl groups, and two oxygen atoms. Flavonoids are derived from Flavanone, and there are more than (4000) flavonoids isolated from plants .It also has many effects, as it was found that it has the ability to inhibit the aggregation of blood platelets (Antithrombotic effect), and encourages the expansion of blood vessels and anti-

inflammatory and anti-mutagenic [25]. Terpene are non-nitrogenous chemical compounds, characterized by their sharp taste, anti-microbial, food preservatives, appetite stimulants, facilitating digestion, analgesics and tonics. Terpene are the largest group of natural products in plants, with more than 20,000 compounds, including essential oils, flavorings, fragrances, and fat-soluble plant pigments [26].

The alkaloids are nitrogenous compounds that are colorless and odorless, and have a bitter and toxic taste (the toxicity of most plants is due to the presence of alkaloids in them)[27]. Resins are plant materials with a complex chemical composition that result from the oxidation of some essential oils. It dissolves in alcohol, ether, and volatile oils, and it has become possible to manufacture many of them in the form of solid or semi-solid materials that are used in the manufacture of paints and plastics [28].

Effect of extracts of the fruits of the tannins plant in the inhibition of *C. albicans* yeast

Recently a strong tendencies have emerged towards plant extracts and biologically active compounds isolated from local plant species, because the use of medicinal plants plays a vital role in protecting the health needed by developing countries, because these plants may provide a new source as agents against pathogenic bacteria, fungi, and viruses [29]. The results showed that the effect depended on the type and concentration of the extract. The acetone extract showed a high inhibitory activity and demonstrate the strongest inhibition, followed by the alcoholic and then the aqueous extract. The diameters of growth inhibition of *C. albicans* increased with the increase in the concentration of the extract, Table (2). The average diameter of acetone inhibition was (20,15.5) mm, at concentrations of 10 and 20 mg/ml, respectively. While it increased to reach (24, 28) mm, at a concentration of 30, 40 mg / ml respectively.

The alcoholic extract showed a good inhibitory activity against *C. albicans*, the average diameter of inhibition was (5.66, 4.5) mm, at a concentration of 20 mg / ml and 10 mg/ml. The inhibitory effect was increased to (8.5, 6.16) mm for concentration of 40 and 30 mg/ml respectively. The aqueous extract shows a higher inhibition of 6.66 mm at concentration of 40 mg/ml, while the other concentration induced lower inhibition activity. The interesting finding of this study was the inhibition against *C. albicans* was exhibited in the first 24 hours, while the nystatin inhibitory effect started in after 48 hours. The inhibition timing was vital as it lead to short course of treatment and less possibility for resistance induction.

The results of the statistical analysis of Dunkin's multinomial test showed that there are significant differences at the level of probability 0.05 between the aqueous, alcoholic and acetone extracts, as the results indicated that the acetone extract was more efficient than the alcoholic and aqueous extracts, which did not show significant differences between them. It is known that tannin is a phenolic compound soluble in water, alcohol and acetone and gives a precipitate "with protein". It appears to be dependent on the presence of tannin in plant extracts [30]. And the high amounts of tannin content in the lobules of the tannin plant indicate that tannin may be responsible for the antibacterial activity in this study. As a result, the extracts of the lobules of the tannin plant have a high potential as an antibacterial agent, and this gives a reason for the use of the tannin plant in traditional medicine for diseases

associated with bacterial infection [31]. The current study agreed with the other study [32], where it was found that plant galls represent the best antibacterial when used at an inhibitory concentration between (5-40) mg.

Table :- 2 Average of inhibition diameters of fruit extracts of tannins plant against *C. albicans* yeast

Extract type	Positive control	Negative control	Concentrations used are mg\ ml			
			40	30	20	10
Aqueous	0.00 L	29 A	6.66 G	4.50 I	3.50 J	2.0 * K
Alcoholic	0.00 L	29 A	8.50 F	6.16 GH	5.66 H	4.50 I
Acetone	0.00 L	29 A	28.0 B	24.5 C	20.0 D	15.5 E

***Numbers that share the same letters of the alphabet are not significantly different at the level of probability 0.05.**
***As the results in the above table represent the average of three replications.**

Conclusion

- The acetone extract of the fruits of the tannins plant comes first in inhibiting *C. albicans* yeast, and the alcoholic extract ranks second, then the aqueous extract .
- Nystatin showed inhibitory activity against *C. albicans* yeast after 48 hours, while tannins extracts showed inhibitory activity within 24 hours.
- The fruits of the tannins plant contained many effective compounds such as phenols, glycosides, tannins, saponins and flavonoids.

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