Evaluation of the activity of alcoholic extract of Gujarat plant (Hibiscussabdariffa L.) against some dermatophytes

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Abstract. The present study highlighted the inhibitory effect of alcoholic extract of the calyx leaves of Hibiscussabdariffa L. against the growth of some dermatophytes fungi isolated from the skin, head hair and nails. During this study four cutaneous fungus was isolated and classified from the skin :- Trichophytonmentagrophytes, Trichophytonrubrum, Microsporumgypseum and Microsporumcanis.

Three concentrations of alcoholic extract of Gujarat plant (5, 10, 15) mg / ml were tested by mixing it with fungus medium. The study showed that the alcohol extract of Gujarat had an inhibitory ability on isolated fungi skin, and the highest rate of extracted effect was at the concentration of 15 mg / ml where the colony appeared as small white fungi isolation of *Trichophytonmentagrophytes* and *Microsporumgypseum*, while the lowest rate of effect extracted at a concentration of 5 mg / ml was on *Microsporumcanis*. The results showed that, using several chemical reagents of the alcoholic extract contains alkaloids, tannins, kalekosides and phenols and did not contain saponins and resins. The present study concludes the high efficiency of alcoholic extract of Gujarat plant in its effect on the growth of dermatophytes.

INTRODUCTION

One of the most important microorganisms are Dermatophytes, which is considered as one of the skin pathogenic fungi that causes many skin infections in humans and animals, *Trichophyton, Microsporum* and *Épidermophyton* [1] are three important geniuses. These geniuses are characterized by being keratin-loving as it is used as food materials because it contains the enzyme Keratinase, which analyzes keratin [2], where fungal infections are on the surface layer, which includes hair, skin and nails, and the skin injury resulting from these fungi is known as dermatophytosis[3] . The main source of dermatophytes infection is the soil and animals, where host interactions vary depending on the source of dermatophytes as well as different metabolic products of the pathogen fungus and virulence of the species and environmental factors [4]. Skin infections have become widespread worldwide, especially in hot and densely populated areas, as well as in areas that lack health care[5] . Fungal diseases are
difficult to control if their treatment needs to use special antifungal drugs such as Ketoconazole and Griseofulvin [6], but the use of these antagonists and for a long period of time leads to the occurrence of genetic mutations in fungal species, making them more resistant to the action of these antibiotics [7]. At the same time, some antibiotics have side effects on humans such as itching, redness and local irritation [8]. In order to avoid complications of chemical drugs and to overcome the absence of antibiotic resistant strains, attention has been drawn to the use of medicinal plants as alternatives in the treatment of fungal infections. Due to its Possession of medicinal value in inhibiting the growth of microorganisms for containing alkaloids, phenols, tannins, Alsaboninat, resins and other active substances [9].

Hibiscus sabdariffa L. is a medicinal plant belonging to the family (Malvaceae), calyxes leaves is one of the important and chemical-rich parts of the active substances containing water, proteins, fats, Carbohydrates, fibers, Thiamine Carotene, Niacin, Riboflavin, and vitamin ascorbic acid (C) and mineral elements such as calcium, phosphorus and iron [11]. and due to the therapeutic importance of fungal skin infections and to identify the impact of some local plants in it, so the present study aimed to test the inhibitory effectiveness of Alcoholic extract of Gujarat plant on fungal species isolated from fungal skin infections.

MATERIALS AND METHODS

1. Sampling
Dermatophytes were isolated from patients with dermatophytes who visited the Al-Hussein Hospital in Karbala governorate, which was 90 samples. The samples were clinically diagnosed by dermatologists from the hospital and samples were collected from skin, head hair and nails.

2. Direct microscopic examination
The samples were examined using a method [12] by cleaning the area with a cotton swab saturated with alcohol 70% to get rid of external bacteria and fungi Saprophytes, and then take a scrape from the affected skin, head hair or nails infected by a tool Loop pollination and then placed On a clean glass slide with a drop of 0% potassium hydroxide and then put the glass slide cover and warm the sample on the flame of a benzene lamp to melt the host cells and examined by microscope for the presence of spores or dermatophyteshypha, and for large nails parts was taken by using sterile forceps flat end and also took pieces Of infected nails after sterilization and put the infected parts taken in a small amount of potassium hydroxide 10% and left at room temperature for a whole night. Fungi were diagnosed based on the following sources: [14] [13] 15] [16]. The microscopic properties and phenotypic characteristics of spores and fungal colonies were adopted by identifying the forms of hypha and spores, as well as the shape of the farm and the appearance and color of the colony from the bottom of the dish.

3. Preparation of Alcoholic Extract of Gujarat Plant:
The method was followed [17] in the extraction process, where the dry plant parts were grinded using a mill to obtain a powder, soak in 70% ethanol to get the alcoholic extract, 20 g of dry plant powder were used with 100 ml of liquid extract with ratio of 1 g of powder per 5 ml of liquid, and leave the mixture in the shaker incubator at 37 ° C for 24 hours, after which the infusion was filtered using several layers of medical gauze and then using Whatman type 1 filter paper. The filtrate was exposed to the force of 2500 rpm and for 10 minutes with a centrifuge, then the filtrate was placed in clean and sterile glass petri dishes and placed in the incubator at 40
° C and for 2-3 days until the extract was dry, then scraped the dry extract with a clean and sterile knife and keep the dry powder after weighing in clean and tight plastic containers until use and called this dry alcohol extract.

4. Effect of alcoholic extract of Gujarat plant on dermatophytes growth on cultivated medium.
Method [18] were followed, The alcoholic extract of Gujarat was mixed with cultivated media Sabroid Dextrose Agar (SDA) before solidification, and with three concentrations 5, 10, 15mg/ml with an average of three replicates for each concentration. After a hardening of the medium, a hole was made in the center of each dish by a cork borer piercing 5 mm in diameter. A comparison was used as no material was added to the media. The dishes were inoculated with the fungus vaccine studied and grown on the medium of SGA and at the age of 3 weeks each by planting a disk with a diameter of 5 mm each in the hole that worked in the center of the dish. All dishes were incubated at a temperature of 25 ° C and for two weeks, the diameter of the developing colony was measured (the average of two perpendicular diameters). Results were recorded, and the inhibition ratio was calculated by using the following equation:

\[ \text{Inhibition ratio} = \frac{\text{Average diameter of fungus in control dish(1) - Average diameter of fungus in treatment dish}}{\text{Average diameter of fungus in control dish(1)}} \times 100 \]

5. Qualitative Detection of Alcoholic Extract of Gujarat.
The group of qualitative Detection was taken to identify basic chemical ingredients or effective compounds present in the alcoholic extraction of Gujarat, these Detection was :
1. Detection of alkaloids :
Alkaloids were detected using Wagner reagent. by dissolving 1.3 g of iodine with 2 g of potassium iodide in 100 ml of distilled water, then add the plant extract. If the precipitate is brown, this indicates the presence of alkaloids [19].

2. Detection of Tannins
Ferric chloride test was used where several drops of ferric chloride, FeCl3 concentration of 1% were added to a test tube containing 0.5 ml of extract. The appearance of a bluish green color was a sign of tannins[20].

3. Detection of Saponins
Mercuric chloride test was used where 3 ml of the extract was added to 2 ml of mercuric chloride, HgCl3 at a concentration of 1% The appearance of a white precipitate indicates the presence of Saponins [21].

4. Detection of Glycosides
Glycosides were detected using the Molish reagent, taking 2 ml of the extract to be tested and added two drops of α-naphthol solution. shake the solution well, then grab the tube diagonally and add 2 ml of concentrated sulfuric acid In the form of drops on the wall of the tube until the emergence of two layers, acid layer is the bottom and separated between the two layers ring purple color when the presence of Glycosides [22].

5. Detect of Resins
Mix 1 g of dry plant powder with 10 ml of 95% ethyl alcohol and leave the solution for one minute in a 100 °C water bath, then filter the solution and add 10 ml of a 4% hydrochloric acid aqueous solution. Infer the presence of resin materials by the appearance of turbidity. [23]

6 - Detecting of flavonoids
1 ml of extract was dissolve in 1 ml of concentrated sulfuric acid, if dark yellow color appeared infer the presence of flavonoids. [21].

RESULT AND DISCUSSION

Qualitative detection of alcoholic extract of Gujarat plants
The qualitative detection revealed that alcoholic extract of Gujarat plants(table 1) contained alkaloids, tannins, glycosides and phenols and did not contain saponins and resins. These results are matched with the results of [24] from the presence of alkaloids and flavonoids but it is not matched Where there are no saponins. Plants containing alkaloids are one of the most important groups of medicinal plants because of their role in therapeutic efficacy[25] . Tannins have a role in protecting the plant from microorganisms, and it also protects vital plant compounds. [26] While Glycosides are important compounds of the plant play a protective role against some pests and insects that affect the plant[27] . Flavonoids have high inhibitory activity against microorganisms because of their ability to dissolve cellular proteins and break down the cell membrane[28] .

| Flavonoids | Resins | Glycosides | Saponins | Tannins | Alkaloids |
|------------|--------|------------|----------|---------|-----------|
| +          | -      | +          | -        | +       | +         |

Effect of alcoholic extract of Gujarat plant on dermatophytes growth
The results showed that the alcohol extract of the Gujarat plant was highly efficient in inhibiting the studied fungi at concentrations 15, 10, 5mg / ml.
The colony had a diameter of 0 mm where the colony appeared as a small, white isolation of all concentrations for Trichophytonmentagrophytes and Microsporumgypseum while Trichophytonrubrum The colony diameter was 1.5, 1.5, 1.75 cm at concentrations 15, 10, 5 mg / ml respectively. Microsporumcanis was colony diameter 1.25 cm at concentration 5 mg / ml and 1.57 at concentration 10 mg / ml and 2 mm at concentration 15 mg / ml Table (2) Fi ger (1).
Consider the inhibitory activity of the plant Al-Gujarat contains many active substances as the flowers of the plant contains compounds: Anthocyanins Phenolic acid and Flavonoids and also contain many organic acids such as citric acid, Malik, tartaric and hibiscisuc acid also contains a high percentage of ascorbic acid [ 30, 29, 31]
Some studies suggest that the plant contains high acidic substances, which in turn affect the speed of growth of fungi [32]. The study found that the plant extraction has inhibition activity against different enzymes [33]. These results are consistent with what many researchers have found [34]. The aqueous and alcoholic extract of the Gujarat plant has an anti-growth effect on all pathogenic microorganisms such as *Candida albicans*, *Enterococcus fecalis, Escherichia coli, Serratiamarcesers, Salmonella typhi*. It was also found by [35] that the aqueous extract of the Gujarat plant has inhibitory activity against *Candida albicans*, as well as the increased antifungal activity of ketoconazole and fluconazole when mixed with the Gujarat plant.

**Table (2): Effect of alcoholic extract of Gujarat plant against dermatophytes**

| Concentration of alcoholic extract | Dermatophytes          | Control | 5 mg/ml | 10 mg/ml | 15 mg/ml |
|-----------------------------------|------------------------|---------|---------|----------|----------|
|                                   | *Trichophyton mentagrophytes* | 9.00    | 0.00    | 0.00     | 0.00     |
|                                   | *Trichophyton rubrum*     | 9.00    | 1.75    | 1.5      | 1.5      |
|                                   | *Microsporum gypseum*     | 9.00    | 0.00    | 0.00     | 0.00     |
|                                   | *Microsporum canis*       | 9.00    | 2.00    | 1.57     | 1.25     |
Figer (1): Effect of Alcoholic Extract of Gujarat Plant on Growth of dermatophytes on Cultivars Sabroid Dextrose Agar
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