

A Comparative Study of The Effects of Sumac Aqueous and Ethanol Extracts on *Candida Albicans* in Laboratory Conditions

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ABSTRACT

Background and purpose: *Candida albicans* is an opportunistic pathogenic fungus that is considered a member of the human intestinal flora that can survive outside the human body. This fungus is usually a harmless organism, but it can become pathogenic in immunocompromised people under various conditions. For oral infections, treatment with topical clotrimazole or nystatin is usually effective. In case of failure, oral or intravenous fluconazole, itraconazole or amphotericin B may be used. Several topical antifungal medications may be used for vaginal infections, including clotrimazole. This study was conducted to investigate the effect of sumac aqueous and ethanol extracts on *Candida albicans*. Methods: In this experimental study, aqueous and alcoholic sumac extracts were prepared and different concentrations were prepared to investigate their effect on *Candida albicans*. The negative control included the culture medium without mushrooms and the positive control included mushrooms without extract. *Candida albicans* fungus was cultured after 24, 48 and 72 hours, and control drug concentrations for fluconazole were 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32 on *Candida albicans*. The effect was given. Methods: In this experimental study, aqueous and alcoholic sumac extracts were prepared and different concentrations were prepared to investigate their effect on *Candida albicans*. The negative control included the culture medium without mushrooms and the positive control included mushrooms without extract. *Candida albicans* fungus was cultured after 24, 48 and 72 hours, and control drug concentrations for fluconazole were 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 millilitres per milligram on *Candida albicans*. The effect was given. Results: After the cultivation of *Candida albicans* mushroom and examination, it was found that this mushroom can grow in all concentrations of aqueous extract of the sumac plant and none of the tested concentrations can prevent its growth, and the alcoholic extract at a concentration of 60 mg/ml can inhibit growth. MIC) of *Candida albicans* fungus, but the fungus was able to grow in other concentrations. The concentration of 0.5 mg/ml of the commercial antibiotic fluconazole could inhibit the growth (MIC) of this fungus. Conclusion: While different concentrations of aqueous and alcoholic extracts of the sumac plant were investigated and it was found that aqueous extract has no effect and alcoholic extract is effective in the same concentration, more and more comprehensive studies are suggested in laboratory scale and living organisms.

1. Introduction

Candidiasis is a range of opportunistic fungal diseases that occur in susceptible people in the form of simple superficial infections to systemic infections. (1 and 2) In general, in the *Candida* genus, there are commensal fungi that coexist with the host, but due to predisposing factors, they can attack the host's tissues and cause disease or make the person a *Candida* carrier. These predisposing factors include a weak immune system, the use of artificial teeth, antibiotic treatment, breastfeeding, AIDS, cancer, bone marrow transplantation or organ transplantation, which cause infections that lead to death. Candidiasis can have different forms, from mucosal candidiasis to disseminated disease, which often depends on the host's immune system, will be associated with the involvement of several organs. *Trichomonas* and bacterial infections are more common. When *Candida albicans* affects the vagina, the symptoms include itching, burning, and sometimes a white discharge from the vagina. Fungal infection of the penis is less common and is usually accompanied by an itchy rash. Rarely, fungal infections may become aggressive and spread to other parts of the body, and depending on the parts involved, may develop along with other symptoms. Vaginal infections occur more often during pregnancy in people who have a weak immune system and after using antibiotics. About three-quarters of women will have at least one yeast infection in their lifetime, but in general, common yeast infections are rare except in people with risk factors. *Candida vaginitis* usually does not have specific clinical symptoms and can manifest itself with symptoms such as itching, burning, cheesy secretions and painful intercourse. Several antifungal drugs such as polyenes, nystatin, azole derivatives (clotrimazole, fluconazole, ketoconazole) and chlorhexidine have been used to treat oral and vaginal candidiasis and other cases, and if it does not work, oral fluconazole or intravenous itraconazole may be used. or amphotericin B should be used. The above drugs, despite their beneficial effects, have side and harmful effects. The bitter taste following the use of nystatin tablets and the possibility of allergic reactions after the use of nystatin creams, (3) burning sensation, swelling, pimple-like swelling after use (clotrimazole cream) have been confirmed in several studies (4 according to The above reasons as well as the drug resistance of this mushroom and the increase in the dosage and the increase in the side effects of the drugs, experts and researchers are looking for effective drugs without side effects. In other studies, it has been determined that they have attracted the attention of researchers.

5: *Rhus coriaria*) *Ala_R*, which is called Sumaq in Persian, contains 250 unique species of flowering plants in the Anacardiaceae family, of which Sumac is one of the most common examples. (6) To separate, determine and identify the compounds obtained from the sumac plant, various extracts were taken from the fruit and leaves of this plant. Some of the aqueous extracts and some of the alcoholic extracts and some of the fatty extracts have been isolated. Degradable tannins constitute the highest percentage in sumac fruits, which emphasizes the antioxidant potential of the fruit. About 191 chemical compounds from the sumac fruit plant have been identified by chromatographic methods, among which 78 degradable tannin compounds, 59 flavonoid compounds, 9 anthocyanin compounds, 2 isoflavonoid compounds, 2 terpenoid compounds, 1 diterpene compound and 38 other compounds are still unknown. Plant extracts containing tannins are used as an astringent compound and as anti-inflammatory, antiseptic, antioxidant and hemostatic drugs. In addition, in another study, it was found that the compounds obtained from this plant are more effective than some antifungal compounds such as nystatin. Also, in recent studies on the ethanolic and methanolic properties of sumac, the extraordinary antioxidant (9) and anti-inflammatory (10) properties, antibacterial activity (11), liver protection (12) and DNA protection (13) and soothing (14)) for this plant, this study aims to compare the effect of aqueous and alcoholic extracts of sumac fruit on *Candida albicans* in laboratory conditions based on finding the lowest inhibitory concentration. The effect of aqueous and

alcoholic extracts of sumac fruit on *Candida albicans* in laboratory conditions is based on finding the lowest inhibitory concentration. Materials and methods: The standard strain of *Candida albicans* fungus with numbers 10231 ATCC and PTCC5027 was purchased from Bosina company and checked for purity by microscope and then on subrodextrose agar culture medium which is a medium for growing and counting yeasts and moulds. It was cultured in completely sterile conditions. The cultured mediums were kept in a greenhouse for 24 hours at 25 degrees Celsius for further investigations. After 24 hours, to fully ensure the absence of *Candida albicans* contamination, a sterile loop was removed from the pure culture and added to 500 micrograms of fetal bovine serum. It was well homogenized.

Then the resulting suspension was placed in a greenhouse at a temperature of 37 degrees Celsius, after a period of 2 to 3 hours, a drop of the suspension was placed on the elm, and the elm was placed on it, and the presence of the reproductive tube, which indicates *Candida albicans*, was checked and confirmed. Was obtained.

Preparation of sumac fruit extract:

First, sumac fruit was procured from reputable sellers of medicinal plants in Karaj city and approved by a reputable botanist, and after cleaning and drying, it was ground. Preparation of aqueous extract: This extract was prepared by soaking, first, 50 grams of ground sumac fruit was added to it in the form of powder and 500 ml of distilled water was added to it, then a magnet was placed in it and placed on The thermal shaker was placed for 24 hours. The obtained aqueous extract was centrifuged and filtered with Whatman No. 4 paper and sterilized using a syringe head filter and finally transferred to a freezer at minus 20 degrees Celsius for use.

Preparation of alcoholic extract of sumac fruit:

50 grams of ground sumac fruit was mixed with 500 ml of 96% alcohol (ethanol) in a one-litre beaker, then a magnet was placed inside the beaker and placed on the shaker, after 24 hours the alcoholic extract was Obtained, centrifuged and passed through Whatman No. 4 paper, and then this extract was poured into a glass plate and placed in a greenhouse at 37 degrees Celsius for 24 hours. After 24 hours, the contents inside the plate were scraped and dissolved in distilled water and passed through a needle filter until it was dissolved and finally, it was transferred to a freezer at minus 20 degrees Celsius.

Candida albicans dilution for testing

A colony was removed from a 24-hour culture of bacteria on subrodextrose agar medium and mixed well in 5 ml of sterile distilled water. 1 ml of the resulting suspension was used for analysis using spectrophotometry 3 at a wavelength of 530 nm and a transmittance of 77%. was placed and a dilution of 1-4 ml/10³ cfu* was prepared from *Candida albicans*. To prepare the dilution of 1 ml of the desired suspension, 9 ml of the RPMI solution made with MOPS was diluted and the dilution was 1%. From the resulting dilution, 100 µl was added to 3-10 ml of RPMI solution with MOPS to obtain a dilution of 0.001 and several 10⁻³.

Calculation of the lowest inhibitory concentration (MIC) of aqueous and alcoholic sumac fruit extract on *Candida albicans* mushroom suspension with 1/001 dilution, RPMI medium with double and one-fold concentration, aqueous and alcoholic extract of sumac fruit with a final concentration of 120 mg/ml was prepared.

All of the above in the wells with the final concentration of 15, 30, 60, 7.5, 3.75, 1.875, 0.937, 0.468, 0.234, mg to ml.

The samples were placed in a 37-degree Celsius incubator for 24, 48 and 72 hours and after this time they were analyzed for MIC. The test for each drug combination was repeated 5 times in

duplicate, all test steps were performed in sterile conditions and against control tests including positive and negative and DSMO control.

Calculation of the lowest lethal concentration (MFC) of aqueous and alcoholic sumac extracts on *Candida albicans*.

To check the minimum lethal concentration (MFC) of aqueous and alcoholic sumac extracts on *Candida albicans*, the samples were cultured from the available wells to calculate the MIC on Sabrodextrod culture medium and placed in a greenhouse for 24, 48 and 72 hours at 37 degrees Celsius.

The lowest concentration in which the fungus did not grow. It is considered as the lowest lethal concentration.

- Research findings

In order to evaluate the effect of aqueous and alcoholic extracts of sumac fruit on *Candida albicans* and also to compare their effect with fluconazole in successive concentrations of 60-30-15-75 mg/ml, the compounds were prepared and a total of 10 series of tests were performed for each compound. Was performed.

After the cultivation of *Candida albicans* fungus in the wells and after 24, 48, and 72 hours, it was found that this fungus can grow in all concentrations of sumac aqueous extract and none of the tested concentrations can prevent the growth of this fungus. . However, sumac fruit sieve extract at a concentration of 60 mg/ml can inhibit the growth of *Candida albicans*, but it could grow at other concentrations.

While the concentration of 0.5 mg/ml of fluconazole antibiotic could inhibit and kill this fungus.

In addition, after MCI calculations, to investigate the lethal effect of the concentration of 60 mg/ml on Sabrod Doctorose agar culture medium, it was determined that this concentration, in addition to inhibiting the growth of the fungus, also can destroy it. (3-4)

A statistical comparison of the results of commercial antibiotics with aqueous and alcoholic sumac fruit extracts on *Candida albicans* fungus at the 95% level indicated that there is a significant difference in the effect of commercial antibiotics with aqueous and alcoholic extracts. (P-value<005)

In the present study, a comparative study of the effect of aqueous and alcoholic extracts of sumac fruit on the standard strain of *Candida albicans* with numbers ATcc70237 and PTcc5087 was conducted, which can be noted that *Candida albicans* is the main fungal pathogen in humans, especially in patients with immunodeficiency. Candidiasis can have different forms, from mucosal candidiasis to widespread disease, which often involves several organs depending on the immune system.

Since medicinal plants are widely used all over the world since these plants have the least side effects, many people have accepted them, so the main purpose of this study was to investigate the antifungal effects of sumac fruit extract and *Candida albicans* kidney. Considering that the effectiveness of both alcoholic and (ethanolic) extracts of sumac fruit in the production of *Candida albicans* growth is higher in concentration compared to the drug fluconazole and therefore it should be determined that there is a significant difference in the effect of the commercial antibiotic with aqueous and alcoholic extracts. There is. (P-value <0.05)

Conclusion

Sumac fruit, having compounds such as alkaloids, terpenes, flavonoids, lingans, plant steroids, curcumins, saponises, tesfoni compounds, and leukocosides, is a potential plant stain for the

treatment of these infections, and the aqueous extract of sumac contains anti-inflammatory compounds. It is not fungal, but the high-concentration sieved extract has an inhibitory effect on the growth of candida albicans due to the extraction and antifungal compounds and sumac, but this inhibitory effect is weaker compared to those containing fluconazole, therefore, considering the side effects and resistance of chemical antifungal drugs It can be more widely used against candida albicans yeast in traditional soap.

The discussion of increasing drug resistance of this mushroom, therefore, the increase in the dosage of common drugs, followed by the increase in the side effects of drugs, has led to the fact that today the most attention is paid to factors with a natural basis, such as medicinal plants with very few side effects.

A study conducted by Salehi et al. in 2021 aimed at the antifungal effect of methanolic sumac extract on *Candida albicans*. The antifungal properties of sumac were proven and the results showed that the methanolic extract of sumac had MCI, 512 to 2048 mg/ml. If the drug nystatin at a concentration of 0.063 mg/ml inhibited the growth of 50% of *Candida albicans* isolates (15).

In another study conducted by Derodrigyze et al. in 2015, the antifungal properties of sumac were proven and the results showed that *Rhus muelleri* can have an inhibitory effect on *Fusarium oxysporum* (19).

In Naseri Farnaz Khalkhali's study in 2015, the effect of an aqueous extract of sumac on increasing the shelf life of raw meat during storage at refrigerator temperature to replace chemical preservatives and ensure the health of consumers showed that with the increase in the concentration of antimicrobial extract, the reduction of the population with kettle Ferrophiles, lactic acid bacteria and total bacteria, the overall form of mesophilic bacteria and lactic acid bacteria are significantly reduced in the highest concentrations of the extract, 0.67, 2.5, and 3.53 logarithmic units, respectively, according to the results As a result, it can be acknowledged that sumac blue essential oil can be used as a natural preservative compound for meat and meat products. (18)

A study by Behzadi and his colleagues in 2015 to investigate the inhibitory effect of aqueous and alcoholic extracts of the sumac plant on *Candida albicans* showed that the alcoholic extract of the sumac plant, unlike its aqueous extract, at concentrations of 250 and 500 mg/ml has an inhibitory effect on *Candida albicans*. *Candida albicans* growth. In the studies, the MCI of ethanol extract of the sumac plant was 60 mg/litre. (17)

In a study conducted by Rashid et al. in 2018, the results showed that sumac plant extract at a concentration of 100 µg/ml reduces the growth of *Coltotricum acutum* hyphae, so it has a fungal effect. (16)

Also, the results of the study by Onkar and his colleagues in 2011 showed that the methanolic extract of sumac seeds can have an inhibitory effect against *Aspergillus*, *Candida albicans* and *Penicillium cistrutum* in concentrations of 20, 10, 5, 1 mg/ml, which The feature is due to the presence of many phytochemicals in the sumac plant.

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