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Article

Alcoholic Plant Extracts' Impact on Trichophyton rubrum Growth Characteristics

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Abstract: This study investigates the inhibitory effects of alcoholic extracts from Sidr, castor, and sage plants, individually and in combination, on the growth characteristics of Trichophyton rubrum, a dermatophyte fungus. Plant parts were processed into extracts and applied to culture media. Results indicate significant inhibition of fungal colony growth and dry weight, with higher concentrations yielding greater inhibition. The combination of Sidr and castor extracts exhibited the most pronounced inhibitory effect. Microscopic examination revealed varied impacts on fungal morphology, including filament distortion and protoplasm separation. These findings underscore the potential of these plant extracts as antifungal agents, emphasizing the importance of considering both extract type and concentration. This research fills a gap in understanding the efficacy of natural extracts against dermatophyte fungi and suggests avenues for further exploration in antifungal therapy.

Keywords: Trichophyton rubrum, Ziziphus spina-christi, Ricinus communis, Salvia officinalis

1. Introduction

The various traditional medicine systems vary and their philosophies and practices are influenced by social, environmental and geographical conditions. However, all of these systems agree on a holistic approach to health and life, with systems such as traditional Chinese medicine and Ayurveda characterized by the idea of focusing on health rather than disease as a central concept..(Sam, 2019). People use traditional medicine for many reasons, including affordability, consistency with personal beliefs, reducing concerns about side effects of chemical drugs, providing personalized health care, and easy access to medical information. Herbal medicine is mainly used to improve health and treat chronic conditions, while medical therapy is used when conventional medicine is no longer effective, such as in cases of advanced cancer and new infectious diseases. Although traditional treatment is considered safe, using herbs without medical advice can lead to unwanted interactions, especially when taken with prescribed medications.(Cohen & Ernst, 2010)(Loya et al., 2009). Plants contain a variety of compounds, including secondary metabolites such as aromatics and phenols and oxygen derivatives such as tannins, which possess antioxidant and antifungal properties. The use of aromatic plants is fundamental in drug research and development, whether as direct therapeutic agents, as starting materials for the pharmaceutical industry or as models for pharmaceutically active compounds(Li & Vederas, 2009)(Klemow et al., 2011). Fungal infections, also known as mycoses, are disease conditions caused by fungi, whether yeast or mold. These infections are usually common on the skin and nails, however, fungi - plural: fungi - can also cause infections in the mouth, throat, lungs, urinary tract, and

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other areas of the body. Fungal infections may appear on the skin in a variety of forms, such as redness, swelling, or bumps, and may appear as a rash or a palpable lump under the skin. Regarding the nails, they may change color - they can become yellow, brown or white - and become thick or cracked. Consortium (2012).

Ziziphus spina-christi, commonly known as Christ's thistle or sidr, contains many active compounds, including flavonoids, triterpenoids, alkaloids, and saponins. One of the known active compounds found in this plant is betulinic acid, which is a triterpenoid compound found in various plant species, including Ziziphus spina-christi and is known for its pharmacological properties, potential therapeutic applications, and biological anti-inflammatory, antioxidant, antiviral, and anti-cancer properties. It shows promising activity against various types of cancer cells, including melanoma, lung cancer, and leukemia.(Hussein, 2019)(Abdulrahman et al., 2022).Betulinic acid and other bioactive compounds found in Ziziphus spina-christi contribute to its traditional medicinal uses and potential therapeutic benefits. However, more research is underway to explore the full scope of its activities and pharmaceutical applications. (Nagaoka et al., 2002) (Kledecka et al., 2022).

Castor, whose scientific name is Ricinus communis, are the seeds and leaves of the castor plant and are very poisonous. A tree plant belonging to the Euphorbia family, the castor seed contains about 50% of its weight in oil, and this oil is used medicinally. The seeds contain the toxic substance ricin and are soluble in water but not in oil. (Chakrabarty et al., 2021)(McKeon, 2016).Castor plant leaves are used in herbal preparations to treat candidiasis, skin infections and wounds in many countries of the world.(Suurbaar et al., 2017)

Ricinine is an alkaloid found in the seeds and other parts of the castor plant that has been studied for its pharmacological properties and potential uses. The castor plant contains other compounds such as ricinoleic acid, which is the main component of castor oil and has various industrial and pharmaceutical applications. Studies have shown that ricinine has insecticidal properties and may also exhibit anti-inflammatory and antimicrobial effects. These compounds collectively contribute to the pharmacological and toxicological properties of the plant.(Auxtero et al., 2021)

The scientific name of the sage plant is (Salvia officinalis L.), and it belongs to the Lamiaceae family. It is an ornamental, culinary, medicinal, and aromatic plant (Roby et al., 2013). They have shown various biological activities, including antioxidant, antibacterial, antiviral, and antifungal properties (Martins et al., 2015). The leaves of this important industrial crop are used in the pharmaceutical, perfume, and food industries (Martins et al., 2015). The essential oil of Salvia species is widely used in the treatment of various diseases such as nervous, cardiac, circulatory, respiratory, gastrointestinal, metabolic and endocrine diseases (Vosoughi et al., 2018). The components of the S. officinalis plant, which is usually grown in several regions around the Mediterranean Sea, its leaves contain many important active compounds such as phenolic compounds, volatile oils, and caffeic acid derivatives such as rosmarinic acid, chlorogenic acid, diterpenes, and flavonoids: including: Apigenin and luteolin-7-glucosides, and several methoxylated aglycones, -6-methyl ether and triterpene. Sage exhibits antimicrobial, antioxidant, antiviral, and immunosuppressive effects, making its medicinal and aromatic uses of great interest. Additionally, sage is traditionally used for a variety of purposes, such as preserving natural and processed foods, as a sweetener, or even as a food coloring. (El-Feky & Aboulthana, 2016) (Sharma et al., 2019). (Yilar et al., 2018)(Al-Qudah et al., 2014)(Lakhal et al., 2013)(Paknejadi et al., 2012). Therefore, the aim of this research is to study the effect of some plant extracts in inhibiting the growth of dermatophytes Trichophyton rubrum

2. Materials and Methods

Collection of medicinal plants

Sidr and castor plants were obtained from a home garden in Karbala Governorate, and sage plants were obtained from one of the local markets in Karbala. The used plant parts were washed with distilled water, air-dried, and then ground using an electric grinder for the purpose of obtaining plant powder.Preparation of alcoholic extracts (40) grams of dry powder were taken for each of the plant samples and mixed with (400) ml of ethyl alcohol (70%) in a 1000 ml glass beaker. It was closed tightly and placed in a shaking incubator and left for (24) hours at room temperature, after which the mixture was filtered. Using several layers of medical gauze to get rid of plankton, then centrifuge at a speed of (3000) rpm for (10 minutes), then filter the extract using Whatman No. 0.1 filter papers to obtain a clear solution, dry the extract using an oven at 40°C, then Store in the refrigerator until use (Hernández-Pérez et al., 1994)

Growing media

Sabouraud Dextrose Agar: Prepare by dissolving 65 g of the ready-made medium powder in an amount of distilled water and bringing the volume to 1 liter according to the manufacturer's instructions (HIMEDIA). Then pour it into petri dishes according to the purpose of the experiment.

Sabouraud Dextrose broth: Prepare by dissolving 30 g of the ready-made powder in an amount of distilled water, then increasing the volume to 1000 ml according to the manufacturer's instructions (OXOID), then pouring it into tubes according to the purpose of the experiment.

Sterilization of culture media

The antibiotic Chloramphenicol was added at a rate of 250 mg/L to all the culture media, then it was sterilized in an incubator at a temperature of 121°C and a pressure of 15 atmospheres for 20 minutes, after which it was left to cool.

The effect of extracts on fungi growth on the culture medium

The method of (Kady and El-Maraghy 1993) was followed, where the alcoholic extracts of the studied plants were mixed with the culture Subroid Dexsteroid Agar SDA before hardening, at four concentrations: 0.02, 0.04, 0.06, 0.08 mg/ml, at a rate of three replicates for each concentration, in addition to Control treatment (culture medium only without any addition) and after the medium hardened, the plates were inoculated with the inoculum of the studied fungi grown on SDA medium at two weeks of age for each of them by planting a disc with a diameter of 8 mm in the middle of the plate. All dishes were incubated at a temperature of 25°C for two weeks, and the diameter of the developing colony was measured (an average of two perpendicular diameters), and the results were recorded.

The effect of extracts on the dry weight of fungis

In order to test the effect of the extracts on the dry weight of the fungi, the alcoholic extracts were mixed with the sterile culture medium Sabroid dextoid broth after taking it out of the freezer, at a temperature of 50°C, at four concentrations: 0.02, 0.04, 0.06, 0.08 mg/ml, at a rate of three replicates for each concentration, in addition to the control treatment Cultivation medium only, without any additives. I used 70 ml tubes with 20 ml of culture medium in each. The tubes were inoculated with fungal inoculum by planting a disc with a diameter of 8 mm. The tubes were incubated at a temperature of 25 °C for two weeks after which the dry weight was calculated after filtering the liquid cultures through paper. Filtering the weight information. After that, the filter papers with the fungi were dried in an oven at a temperature of 40°C until dry. After that, the dry weight of the fungi s was calculated using a sensitive electric balance with four decimal places using the following equation Arey (2010) **Weight of mycelium = (Weight of filter paper + Weight of Mycelium) – (Weight of filter paper)**

The fungus Trichophyton rubrum isolate IQT-No.1, identified by molecular method, was obtained from the laboratories of the College of Education for Pure Sciences, Department of Biology, University of Karbala.

Mixing concentrations: Three single alcoholic extracts were used for each of: Sidr, Castor, and Sage, and three synergistic alcoholic extracts: (Sidr + Castor), (Sidr + Sage), and (Castor + Sage). Each treatment had 4 concentrations: 0.02, 0.04, 0.06, 0.08. mg/ml of alcoholic extract added to the control treatment without addition. Statistical analysis A Completely Randomized Block Design (C.R.D) was adopted, and the averages of the coefficients were compared using the Least Significant Difference (L.S.D) at the 0.05 probability level (Steel and Torrie 1986). Genstat program was used for statistical analysis.

3. Results

Table 1 shows the effect of different concentrations of alcoholic extracts of sage, castor, and sidr, and their synergy on the growth diameters of the T.rubrum fungus. The effect of the alcoholic extract of sider + castor was the most inhibitory, with a rate of 24.27 mm, which did not differ significantly from the sidr extract, and the least effect was for the sage extract, with a rate of 36.13. mm. As for the effect of concentration, the concentration of 0.08 was the most inhibitory with an average diameter of 5.50 mm compared to the control treatment. The least effective was the concentration of 0.02 with an average diameter of 47.39 mm. As for the binary interactions between the type of extract and the concentration, the most inhibitory effect was for the Sidr + Castor extract for both concentrations of 0.06. And 0.08 mg/ml, in addition to the sage extract at a concentration of 0.08 mg/ml, which recorded 0.00, and the least inhibition was for the sage extract at a concentration of 0.02 mg/ml, which reached 76.00 mm.

Table 1. The effect of different concentrations of alcoholic extracts of sage, castor, and sidr, and their synergy on the growth diameters, mm, of the T.rubrum fungus.

plant / Con.	Sage + castor	Sage + sider	sidr+ castor	sidr	castor	Sage	Average
0.02	45.33	48.67	34.00	51.33	38.00	76.00	47.39
0.04	13.00	13.67	12.00	8.67	14.33	23.67	14.22
0.06	12.67	12.00	0.00	0.00	8.33	15.33	8.06
0.08	10.33	9.67	0.00	0.00	13.00	0.00	5.50
Cont.	77.33	73.67	75.33	71.67	75.00	74.67	74.61
average	31.73	31.53	24.27	26.33	29.73	36.13	
L.S.D	Plant: 1.29			concentration:1.178		interferes :2.886	

Table 2 shows the effect of the different concentrations of the alcohol extracts of Sage, castor, and sidr and the synergy between them on the dry weight of T. The Sage extract had a weight rate of 0.248, as for the effect of the concentration, the concentration of 0.08 mg / ml was the most inhibition with a weight rate of 0.014 g compared to the control treatment, the lowest effect was a concentration of 0.02 mg / ml with an average weight of 0.337 g. And the concentration, the most inhibition was the extract of sidr and

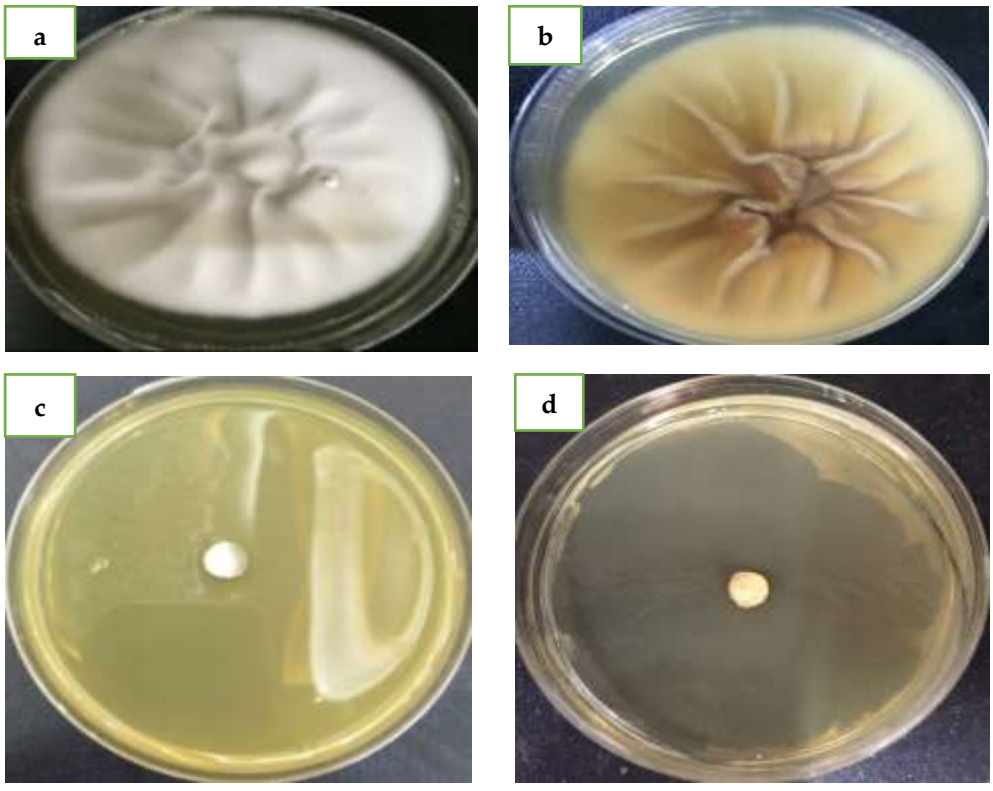
sidr + castor for each of the two concentrations 0.06 and 0.08 mg / ml, in addition to Sage extract at a concentration of 0.08 mg / ml, which scored 0.00, and the least inhibition was for sidr and sidr extract + castor with an average weight of 0.376 g at a concentration of 0.02 mg /Ml.

Table 2. The effect of different concentrations of alcoholic extracts of Sage , castor and sidr, and their synergies on the dry weight of T.rubrum

plant / concentration	Sage + castor	Sage + sidr	sidr +castor	sidr	castor	Sage	average
0.02	0.283	0.356	0.376	0.376	0.286	0.346	0.337
0.04	0.126	0.116	0.136	0.176	0.116	0.160	0.138
0.06	0.086	0.083	0.000	0.000	0.096	0.073	0.056
0.08	0.013	0.036	0.000	0.000	0.036	0.000	0.014
Cont.	0.586	0.543	0.576	0.553	0.620	0.660	0.590
average	0.219	0.227	0.218	0.221	0.231	0.248	
L.S.D	plant :0.0055			concentration : 0.0050			
				interfere: 0.0124			

The effect of alcoholic extract on the growth of fungal colonies

Figure (1) clearly demonstrates the variable impact of alcohol extracts on fungal cultures. The inhibitory effect of alcohols ranges from distorting fungal filaments to causing irregular growth, as opposed to the control, and leads to a decrease in colony diameter. Depending on concentration, certain treated fungal cultures tend to ascend towards the plate cover.



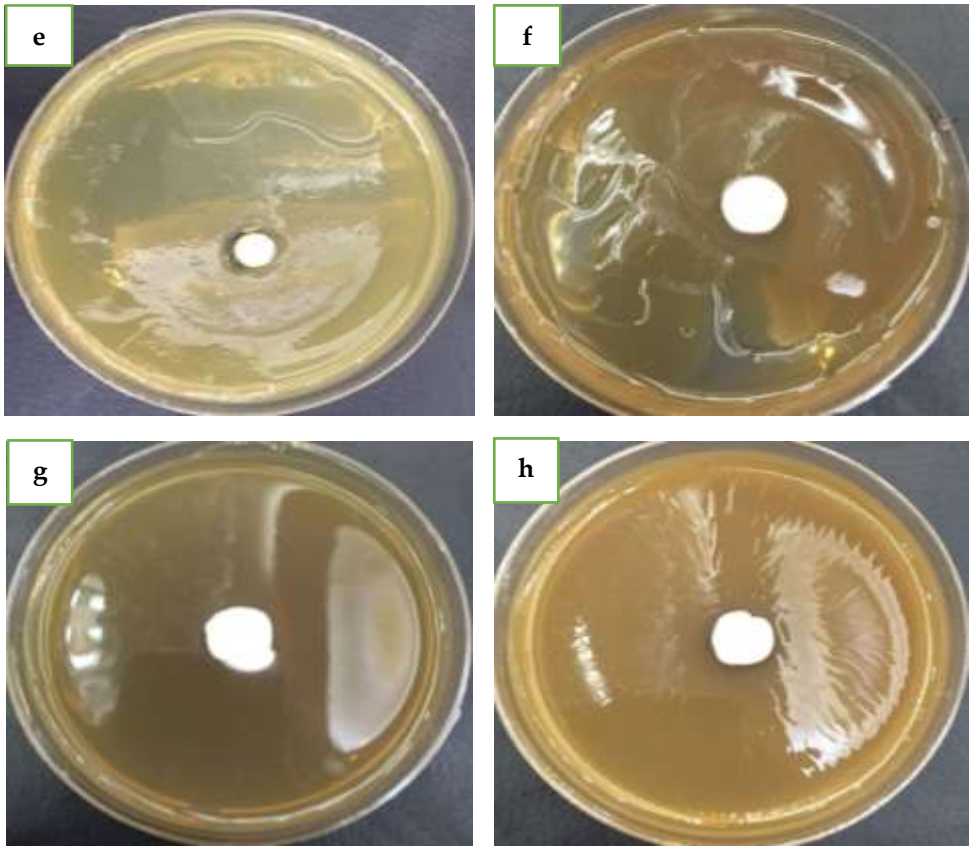
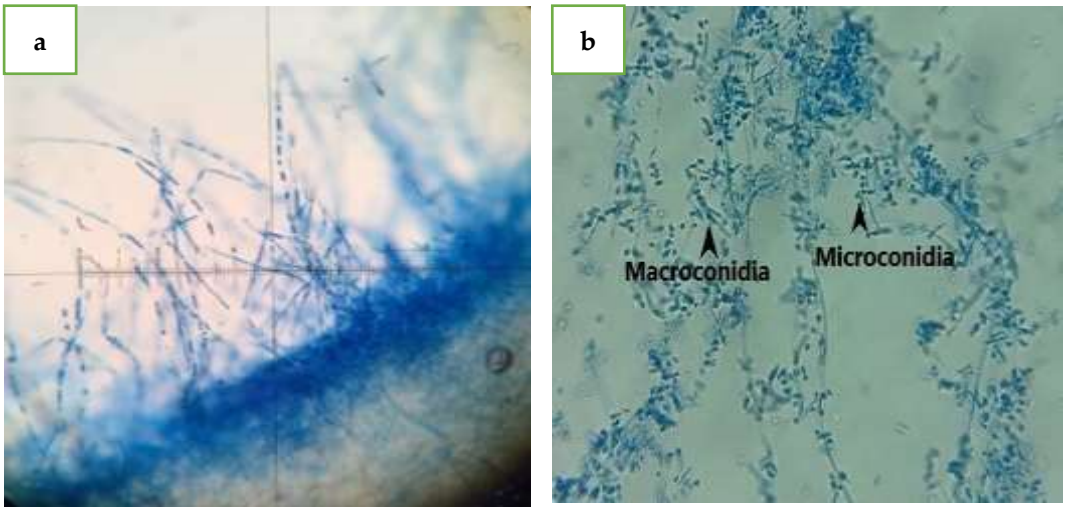
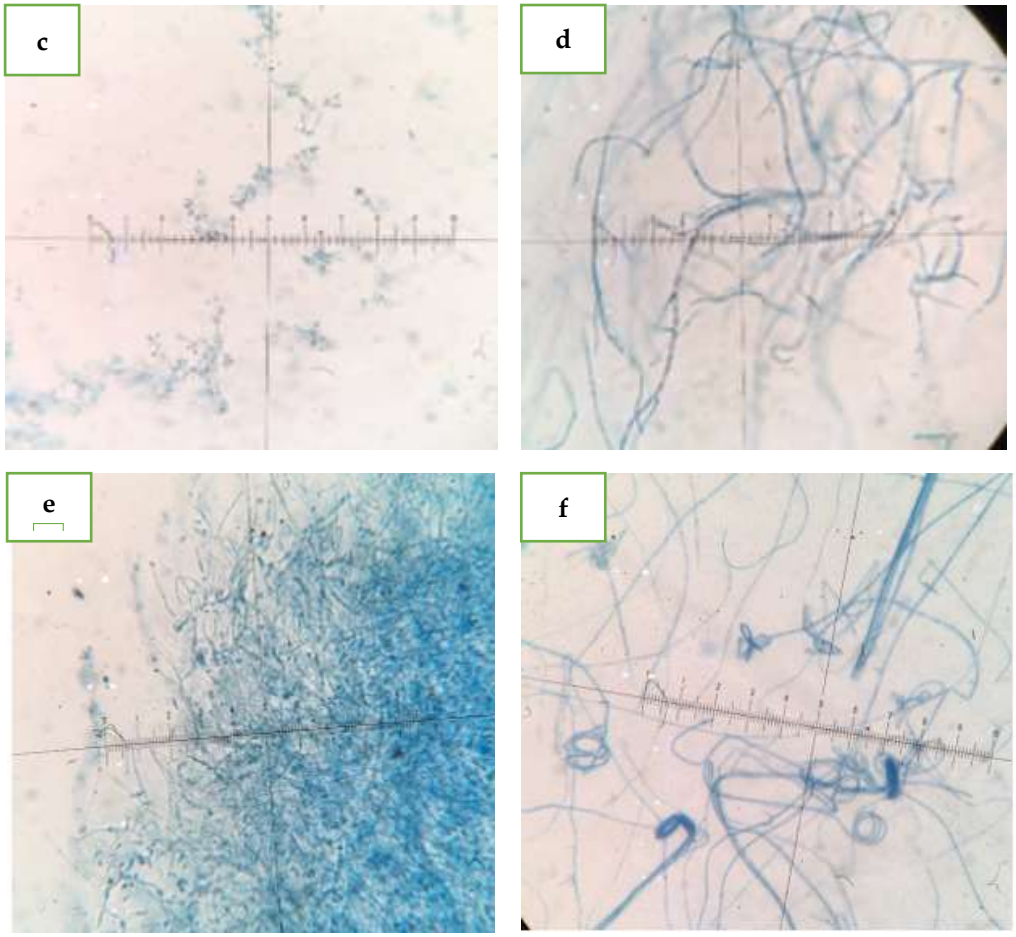


Figure 1. Effect of 0.08 concentration of alcoholic extracts on average diameter of colony (mm) on sabrouaud dextrose agar at 28 C for 14 daysT.rubrum morphology : a,b: control , c: Sage, d: sidr , e: sidr + castor , f: castor , g: Sage+ sidr ,h: Sag

Figure (2) illustrates the impact of various concentrations of alcoholic extracts on the microscopic attributes of the fungus. These impacts encompass a spectrum from mycelium distortions to protoplasm separation in select regions, alongside its aggregation in others. Furthermore, it includes the diminishment of small conidia and distortion of large conidia. Noteworthy internal distortions are observable during examination, most prominently the agglomeration of protoplasts within fungal cells without compromising the cell wall integrity. Additionally, there is a notable presence of chlamydospores in abundance, along with the observation of nodular formations on the fungal hyphae.





Figer 2. Effect of 0.08 concentration of alcoholic extracts on microscopic features (40x) of T.rubrum a: control ,b: castor , c: sidr + castor , d: sidr , e: Sage, f: Sage+castor

4. Discussion

The results in Tables 1 and 2 showed that the Sidr + Castor extract achieved the highest inhibitory activity for the diameter and weights of fungal colonies over the rest of the types of extracts. This may be due to the biological effectiveness possessed by the active compounds of these two plants, such as phenols, volatile oils, resins and glycosides, that affect the growth of fungi through mechanisms of action. Various phenolic compounds possess antimicrobial properties, including antifungal effects. They can disrupt fungal cell membranes, interfere with cellular processes such as respiration and nutrient absorption, and inhibit enzyme activity necessary for fungal growth. Phenols achieve these effects by disrupting protein structures and causing oxidative damage to fungal cells.(Isman, 2000)(Mishra & Dubey, 1994). Phenolic substances in plants contain many active compounds that contribute effectively to inhibiting the growth of skin fungi (Yassin & Mohammed, 2021)(Ghani et al., n.d.)

This is similar to (Ads et al., 2022) found that the Sidr plant contains biological activity that acts as an antifungal, especially against Aspergillus and Candida fungi. The study showed that the Sidr plant (Ziziphus spina-christi) and its plant components, especially triterpenoids, could be promising antimicrobial candidates in the fields of pharmaceutical and clinical application.and It is similar to (Carolina et al., 2019) found in that the methanolic extract of the castor plant at a concentration of 500 µg/ml was able to inhibit the growth of the Aspergillus niger fungus by (71.46%) This may be due to the substance ricinine.

Volatile oils contain volatile compounds such as terpenes and phenylpropanoids, which exhibit antifungal properties. These oils can penetrate fungal membranes, disrupt

membrane integrity, and interfere with vital cellular functions such as enzyme activity and ion transport. In addition, some volatile oils can inhibit the germination of fungal spores and fungal growth. Resins: Resins contain a variety of compounds, including terpenoids and phenols, that contribute to their antifungal activity.(Bakkali et al., 2008)(Cox et al., 2000).

Similar to volatile oils, resins can disrupt fungal membranes, inhibit enzyme activity, and interfere with fungal cell wall synthesis. They may also induce oxidative stress and disrupt cellular metabolism in fungi, leading to growth inhibition or cell death.(Thombre et al., 2016)(Lou ZaiXiang et al., 2011) .(Easa et al., 2018) concluded the effectiveness of some plant extracts in inhibiting the growth of some skin fungi Microsporum gypseum, Microsporum boullardii, Trichophyton mentagrophytes, Trichophyton terrsetre, and Trichophyton verrucosum

Some plant glycosides possess antifungal properties through mechanisms such as inhibiting fungal cell wall synthesis, disrupting membrane integrity, or interfering with fungal enzyme systems. For example, saponins, a type of glycoside, can disrupt fungal membranes and inhibit fungal growth. In general, the mechanisms of action of these active compounds involve targeting key structures and processes in fungal cells, resulting in growth inhibition, cell damage, or cell death. However, it is important to note that the effectiveness of these compounds can vary depending on factors such as the specific fungal species, concentration of the active compound, and environmental conditions. (Siddiqui 2012) (Zornberg,1985). The results of a study conducted by (Jirovetz et al., 2007) showed the antifungal activities of the essential oils of several types of sage, namely Salvia lavandulifolia, Salvia officinalis, and Salvia sclarea, against different types of pathogenic Candida. It has been shown that the antifungal activity of sage essential oils depends on their chemical composition and that the strongest antifungal activity is possessed by the essential oil of S. lavandulifolia, followed by the essential oils of S. sclarea and S. officinalis.Also, the fungal strains varied in their resistance, as it was found that the Candida albicans strain responsible for pharynx and mouth infections was the most resistant, while the Candida albicans strains ATCC 10231 and C.albicans, responsible for skin infections, were evaluated as the most susceptible to resistance. The results of a study conducted by (Badiee et al., 2012)showed that Salvia officinalis oil extract has good antifungal activity, and can serve as a natural alternative to synthetic fungicides to combat some important fungal diseases. The minimum inhibitory concentrations of essential oil extracts were 15.6, 3.9, 31.3, 31.3 and 1.9 μg/ml, respectively. Against C. albicans, C. parapsilosis, C. krusei (standard species), C. albicans and C. glabrata (isolated from patients)

5. Conclusion

Different concentrations of alcoholic extracts of sage, castor and sidr showed inhibitory effects on the growth diameters of T. rubrum fungi. They have varying degrees of inhibitory effects on fungal growth and dry weight, with sidr showing a more significant inhibition compared to the sage extract. The most inhibitory effect was observed when using a combination of Sidr and Castor extract, which leads to increased inhibition of fungal growth

These conclusions provide insights into the potential use of these extracts as antifungal agents and highlight the importance of considering both extract type and concentration in studying their inhibitory effects on fungal growth

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