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Estimation of the minimum inhibitory concentration (MIC) of the ethanolic extract of *S. monoica* as an antifungal agent for *Candida albicans*

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Abstract

Medicinal plants have shown an important role in treating many different diseases, and the world still depends on many of these plants in primary and advanced health care. These plants have been used as important sources for treating many types of pathogenic fungi. In the current study, the leaves of the *S. monoica* plant were extracted using absolute ethanol (99%). The components of the ethanolic extract of the leaves of the *S. monoica* plant were analyzed using the GC-Mass technique. The effectiveness of this extract was evaluated and the value of the minimum inhibitory concentration (MIC) was estimated as an antifungal agent. *Candida albicans* species. The results showed that the ethanolic content of *S. monoica* leaves contains many active compounds, including: Stigmasterol, 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z, Z), Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl, 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one, Pterin -6-carboxylic acid, Glycine, N,N-dimethyl-methyl ester, Acetyl turicine, 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one, Phenol, 2,2'- methylenebis[6-(1,1- dimethylethyl)-4- methyl. The results also showed that the ethanolic extract has the ability to inhibit the growth of *Candida albicans* fungi. It was observed that the diameters of inhibition of the fungal colonies decreased with the increase in the concentration of the ethanolic extract used, as the value of the minimum inhibitory concentration of the ethanolic extract reached 0.8 mg/ml towards these fungi. Therefore, it is possible to use the *S. monoica* plant as a natural source of treatment for many disease-causing fungi**.**

Keywords: Anti-fungal, candida, monoica, extract, fungi

1. Introduction

Medicinal plants provided a complete medicinal system in ancient times, and these plants are still commonly used to treat many diseases [1, 2]. According to reports from the World Health Organization (WHO), more than 75% of people in the world still depend on common and well-known medicinal plants in primary health care and to treat incurable diseases [3]. Societies orientation towards medicinal plants comes as a result of the belief that they are gentle and harmless and cannot cause other negative aspects. In the field of fungi, many medicinal plants have been used to treat fungal infections, including the oil extracted from the seeds of the celery plant *Apium graveolens* [4]. And the extract of the floral parts of the *Calendula officinalis* plant, which has shown biological activity against fungi, especially skin fungi [5].

S. monoica is an annual plant that adjusts soil salinity and grows in saline areas or dry salty soil. This plant belongs to the *Amaranthaceae* family, which contains 1,300 species, including various trees and herbs [6]. Spread geographically, *S. monoica* can be found along the coasts of tropical Africa, the southern Arabian Peninsula, India and Pakistan's coastlines, and Palestine and Jordan's Dead Sea region. This plant is considered a source of human food and a treatment for some diseases at the same time [7]. Previous studies indicate that the *S. monoica* plant contains many medically important chemical compounds such as tannins, resins, terpenoids, glycosides, and flavonoids [8]. The leaves of *S. monoica* were used as a treatment for liver infections and were also used as an ointment for skin cracks [9, 10]. Its leaves also showed effectiveness against the growth and reproduction of many viruses and healing of wounds [7], and as a pesticide for various insects [11], and antibacterial activities [12]. A laboratory study indicated that the aqueous extract of *S. monoica* has anti-fungal activity [13]. The chemical components found in Iraqi *S. monoica* leaves have been shown in studies to be important as a potential nutritional supplement in nutritional systems to prevent liver cancer and improve the liver's defense against illnesses [14].

In the current study, the ethanolic extract of *S. monoica* leaves was prepared and its effectiveness was evaluated as an anti-growth of *Candida albicans* fungi, and the ethanolic extract's minimum inhibitory concentration (MIC) value for this fungus was estimated.

2. Materials and method

2.1 Collect and prepare plant samples

S. monoica plant samples were collected from one of the gardens of Abu Al-Khasib district in Basra Governorate, southern Iraq, in April 2023. The plant was cleaned of dust using distilled water and then dried in the shade at room temperature. The plant was ground using a mechanical machine so that the plant was form a powder and then store it in opaque plastic containers at a temperature of (-4 °C).

2.2 Preparation of ethanolic extract

The extraction process was carried out by mixing 50 g of *S. monoica* plant powder with 500 ml of pure ethanol (99%) in a conical flask. The flask was placed on a magnetic stirrer with the temperature set to (50 °C) for three hours. Then the filtration process was carried out using a Buechner funnel to get rid of the remains of the extraction process, and the ethanolic extract was stored in plastic containers at a temperature of (-4 °C) until laboratory tests were conducted on it [15].

2.3 Identification of chemical compounds in the ethanolic extract of *S. monoica* by GC-Mass technology

The GC-Mass technique was used to diagnose the compounds present in the ethanolic extract of the *S. monoica* plant. A phenylmethylsiloxane column was used, where the pressure was 6.0799 psi and the temperature gradient in the column began at 40 °C at a rate of 5%. A linear gradient was produced when the temperature was increased from 50 to 280°C at a rate of min\°C10. For four minutes, the injector was maintained at 290 °C after the solvent was turned off. The carrier gas was helium, flowing at a rate of one milliliter per minute. Syringe filters were used to filter the extract before it was injected into a GC column and examined using the NIST 2014 library [16].

2.4 Evaluation of the effectiveness of the ethanolic extract of *S. monoica* against fungal growth

A dilution method was used for the ethanolic extract of *S. monoica* according to the standard protocol recommended by CLSI (Clinical Laboratory Standards Institute) to determine the minimum inhibitory concentration (MIC) for *Candida albicans* fungi (ATCC 11006). A series of dilution concentrations of the ethanolic extract was done. Within the range (0.125-4 mg/ml) by the solvent dimethyl sulfoxide (DMSO) [17]. *Candida albicans* fungi, isolated from fresh yeast, were cultured, and the minimum inhibitory concentration for the growth of these fungi was measured using the ethanolic extract of the *S. monoica* plant.

3. Results and Discussion

The results showed that an ethanolic extract of the *S. monoica* plant was obtained, and its color was green to blue. The weight of the resulting extract amounted to (2 g), where the extraction percentage was calculated through the following equation [18].

Extraction percentage = Weight of the extract / Weight of the plant * 100

According to the above equation, the extraction percentage was 4%. The results of the GC-Mass analysis of the ethanolic extract of the *S. monoica* plant showed that it contains many active chemical compounds and important alcoholic compounds, as Fig (1) shows the histogram spectrum of the ethanolic extract and table (1) shows the most important chemical compounds Which appeared in the abstract, Fig (2), Fig (3), Fig (4), Fig (5), Fig (6), Fig (7), Fig (8), Fig (9), Fig (10) are the most important. Important chemical compounds contained in the extract.

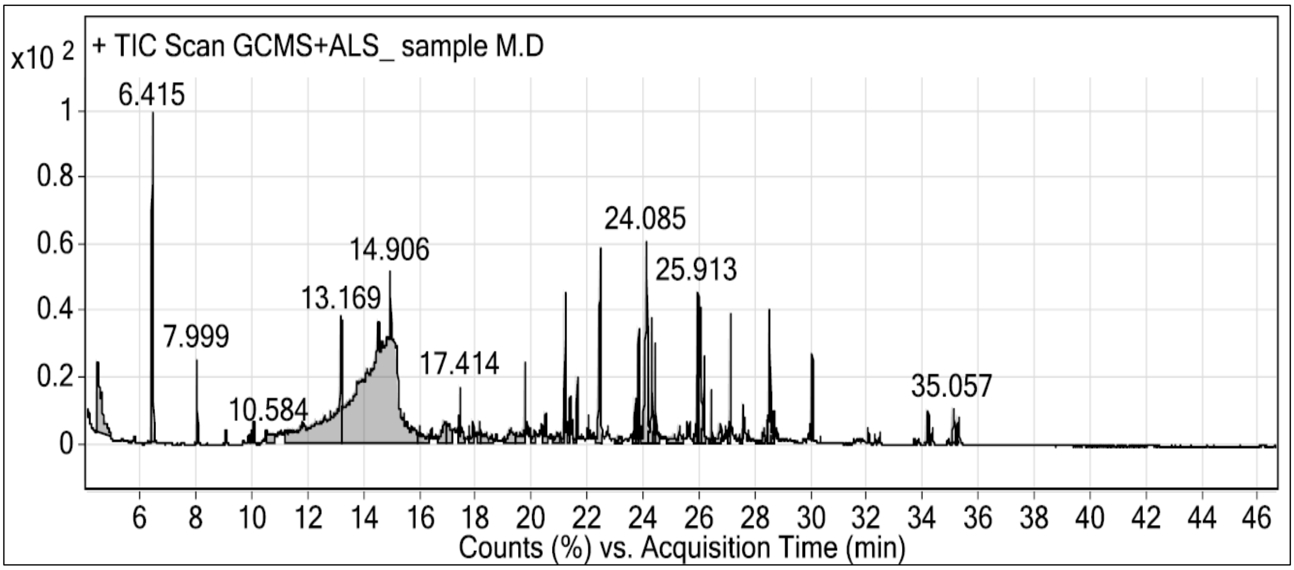

Fig 1: Histogram spectrum of the ethanolic extract of *S. monoica*

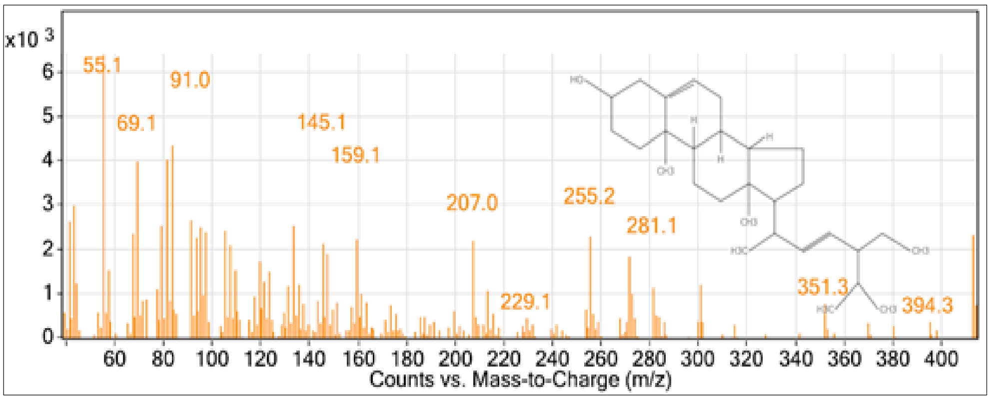

Fig 2: c spectrum of the compound stigmasterol

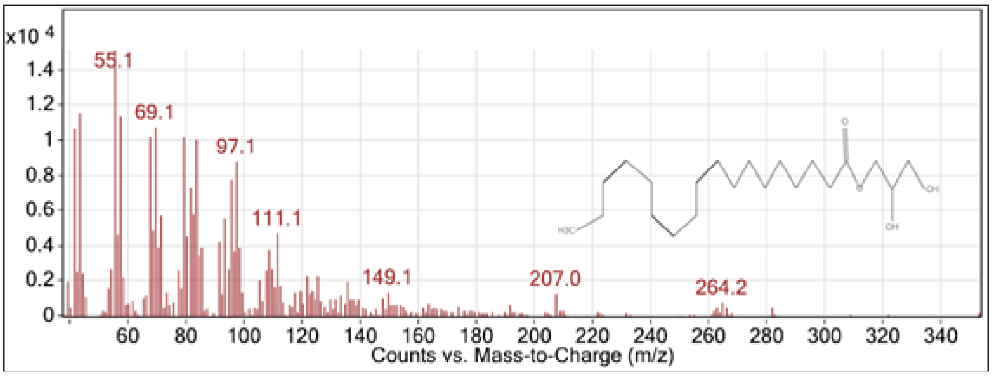

Fig 3: Mass spectrum of the compound 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)

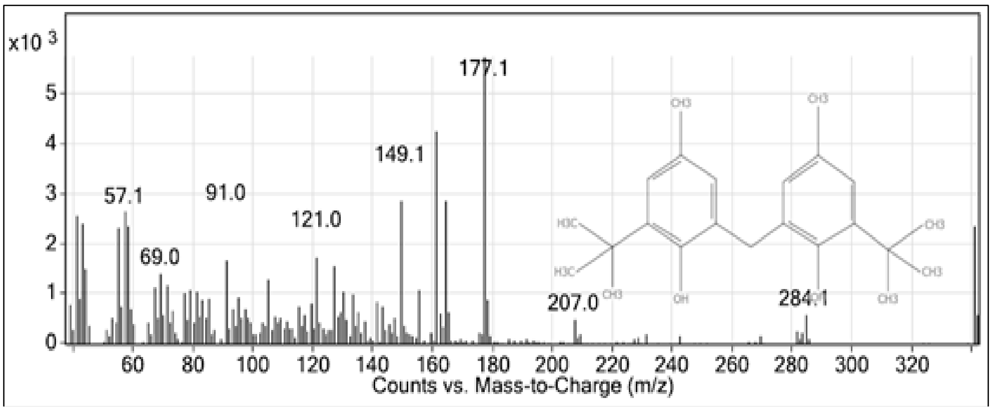

Fig 4: Mass spectrum of the compound Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl

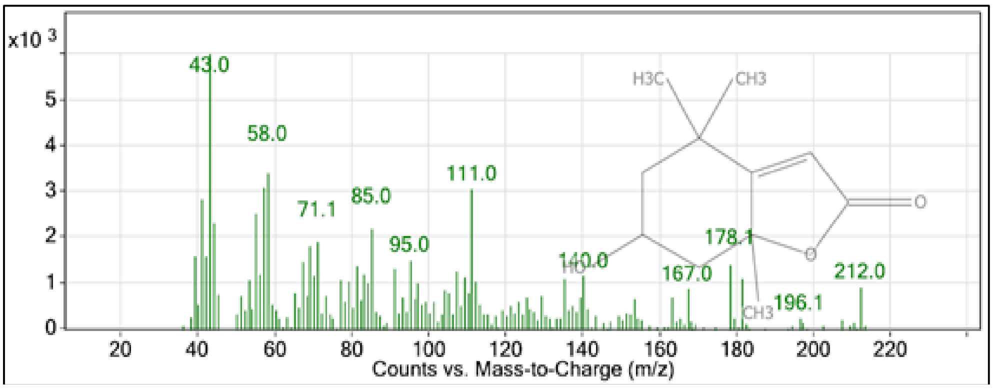

Fig 5: Mass spectrum of the compound 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one

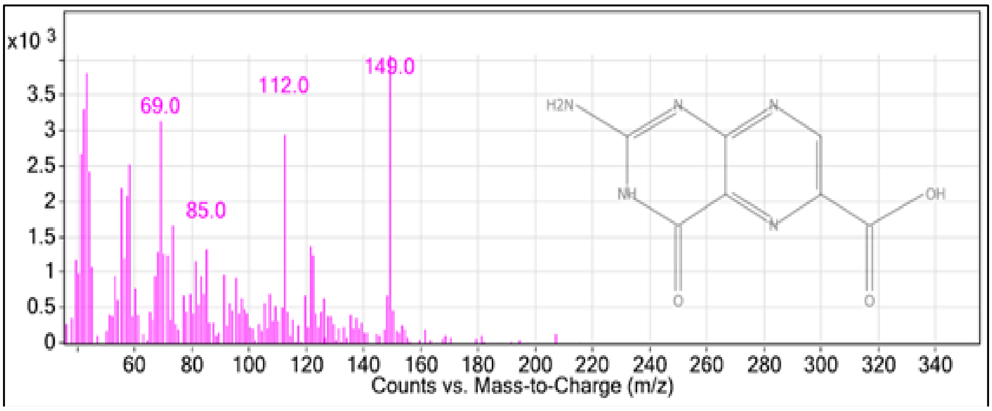

Fig 6: Mass spectrum of the compound Pterin-6-carboxylic acid

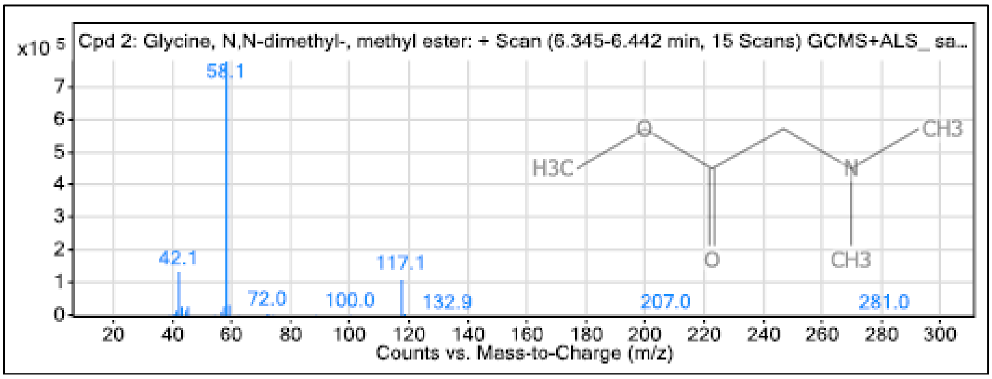

Fig 7: Mass spectrum of the compound Glycine, N,N-dimethyl-methyl ester

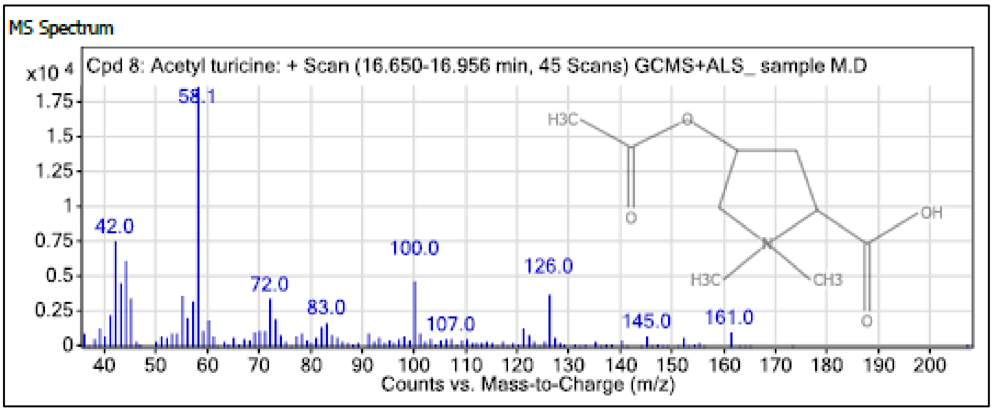

Fig 8: Mass spectrum of the compound acetyl turicine

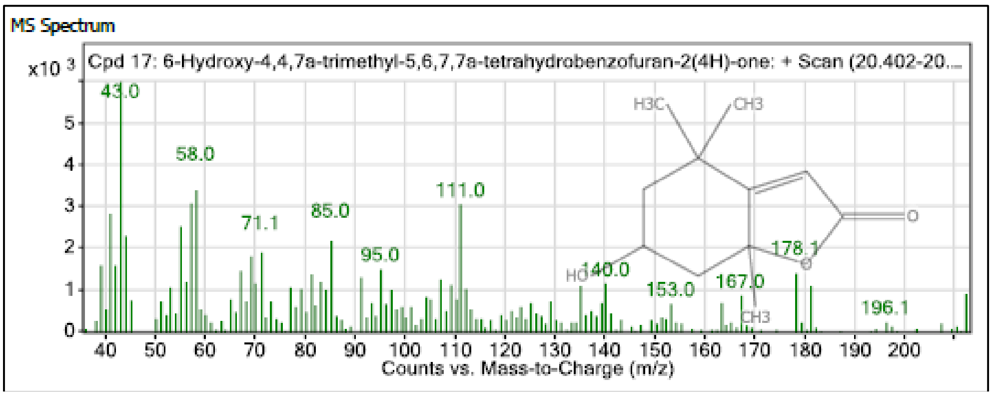

Fig 9: Mass spectrum of the compound 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran- 2(4H)-one

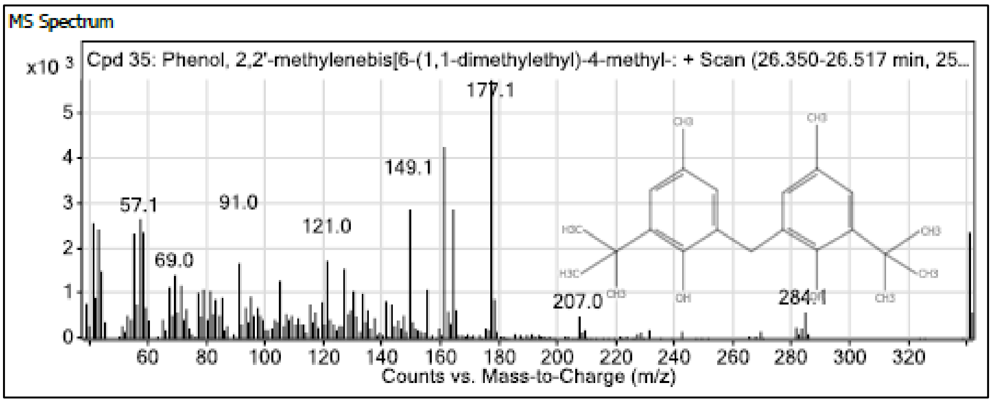

Fig 10: Mass spectrum of the compound Phenol, 2,2'- methylenebis [6-(1,1- dimethylethyl)-4- methyl

The results of testing the effectiveness of the ethanolic extract of the *S. monoica* plant against the growth of *Candida albicans* fungi showed that the extract was able to inhibit the growth of *Candida albicans*. The diameters of inhibition of the fungal colonies were shown to decrease with the increase in the concentration of the ethanolic extract used, as the value of the minimum inhibitory concentration of the ethanolic extract reached mg/ ml 0.8 against these fungi. Fig (10) shows the inhibitory effectiveness of the ethanolic extract against *Candida albicans.*

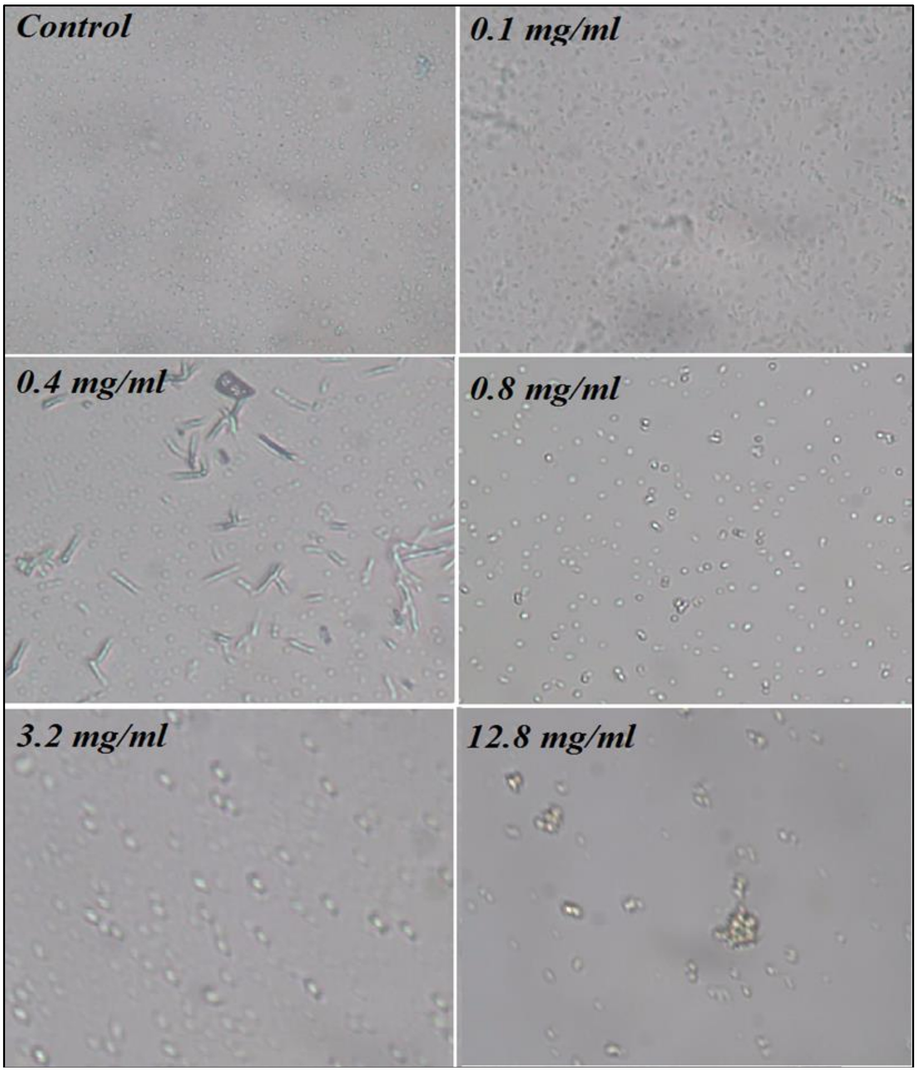

Fig 10: Inhibitory activity of the ethanolic extract against *Candida albicans*

The reason for the ethanolic efficiency of the *S. monoica* plant can be attributed to it containing active metabolic compounds in it, especially alcoholic and alkaloid, and this confirms the validity of previous studies that showed that phenolic and alkaloid compounds have inhibitory activity against laboratory-isolated fungi. Which is important in inhibiting the growth of fungi.

The mechanisms that are the reason behind the efficiency of medicinal plant extracts in inhibiting microorganisms differ and depend on the presence of these compounds [19]. Some effective compounds work to inhibit the action of enzymes through oxidizing compounds or by acting on as a source of stable free radicals, which ultimately lead to the production of proteins in an inactive state, losing their basic function [20].

4. Conclusion
The ethanolic extract of the *S. monoica* plant showed many effective medicinal compounds. It was noted through GC-MS analysis of the ethanolic extract of the *S. monoica* plant that it contains important chemical compounds, especially phenolic ones. These compounds, through a synergistic effect, had good effectiveness in inhibiting the growth of colonies. *Candida albicans* fungi found in the respiratory system, intestines, and vagina. The minimum inhibitory concentration (MIC) of the ethanolic extract reached (0.8 mg/ml). Therefore, this extract can be considered the best natural drug for respiratory and vaginal fungi because it does not have any potential side effects, as further studies have suggested. The future is in identifying the compounds responsible for inhibiting the fungi *Candida albicans* and studying the mechanism of inhibition through knowledge of the inhibitory proteins.

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