

Isolation and identification of some fungal species contaminating dried dates and the impact of some plant extracts on fungal species contaminating dried dates them

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Abstract

The study included the preparation of aqueous extracts (using distilled water) of a group of local plants that included verum (Inamum), mint mint, Anisum pimpinella, Salvia officinalis, for the purpose of studying their effect on a group of pathogenic fungi, using the method of mixing the extracted plant with the nutrient medium at a concentration (5,10,15,20,25) mg/ml. For the effective evaluation of the plant extracts compared to the fungal isolates included in the study and the calculation of the activation ratio and the concentration value.

Thirty (30) samples of dried palm fruits (*Phoenix dactylifera*) were collected from street vendors, shops and markets from different regions of Diyala governorate. Detection of fungal species. The investigation was based on cultural, microscopic and biochemical tests. *C.guilliermondii* and *C.albicans* (7) isolates (23.33%) were diagnosed followed by *C.krusei* and *C.stellatoidea* (5) isolates (16.66%), and *C.* (3) isolates (10%). So there is a need to treat these fruits appropriately before consumption to reduce the possibility of fungal infection.

Anisum-pimpinella extract concentration (5 mg/ml) is the highest, while (15 mg/ml) is the lowest, Salvia officinalis extract (5 mg/ml) is the highest and (25 mg/ml) is the lowest. The substance with the greatest concentration is inamomum ferrum (25 mg/ml). While the learner with a concentrate on (20 mg / ml) is the lowest, while mint with a concentration of (15) is the highest, while the learner with an emphasis on (25 mg / ml) is the lowest.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) A key horticultural crop in the Middle East is the date palm. The majority of the world's date palm exports come from Arab nations. (Dhehibi et al., 2022). Dates are a food of high nutritional value and long lasting quality that contains the most essential nutrients such as protein, fibre, carbohydrates, fats and minerals. (Farsi and Lee Chang-Young, 2008) It can be used in vinegar, sugar, syrups and food flavors. (Cantadori et al., 2022). Many countries pay great attention to increasing farms for the production of dates, especially in Iraq. Dates are mostly consumed by Muslims for both religious and customary reasons since they constitute one of the first religious meals of Ramadan in Islamic nations. As with other fruits, though, it is susceptible to contamination by various fungi, which cause susceptible to rot during the stages of ripening, storage, and processing. (Ahmad., 2003). Hence, even though a number of fungi species have been linked to date fruit harm, it was reported (Jerbi, 1983) the most typical fungus causing spoilage of date fruits is *Aspergillus* sp. and *Alternaria* sp. Certain fungi that grow in agricultural fields and on stored food products can cause damage resulting in a decrease in the fruit's quantity and quality. In addition, a variety of fungi have the capacity to produce mycotoxins (aflatoxins), very hazardous secondary metabolites for both humans and animals (Shamsuddeen and Magashi., 2005; Shenasi et al., 2002) *Aspergillus flavus* and *A. parasiticus*, date goods as well as both of whose products create the aflatoxins discovered to infest dates at all stages of maturity.

The dates are sold by local vendors who store the soft, dry dates in polythene or natural fiber bags, wooden boxes or wrapped trays. Through personal observation, it has been found the

common practice of selling unwashed fruits to consumers, especially dried fruits and some also consume them in unwashed form. Therefore, the aim throughout this study was to cultivate, separate and distinguish fungi found in the dates. The results of this research also aim to reduce or avoid potential health problems associated with these fungi.

Salvia officinalis (garden sage, sage vulgaris) or dalmion sage is known as a panacea with good results from medical treatments. The genus *Salvia* is one of the most important genera in the family Labiatae and includes about 900 species worldwide.

Salvia plants are fragrant and full of essential oils that have been employed in various pharmacological, cosmetic, and food items (Pop et al., 2014). In addition to the therapeutic properties it possesses, *Salvia officinalis* can be utilized as effective substitutes for synthetic fungicidal treatments. Several studies have previously discovered that *Salvia officinalis* has antifungal capabilities when tested against *Aspergillus* sp., *Fusarium* sp., or *Penicillium* sp. (Rus et al., 2015).

An exclusive oil found in *salvia* leaves is efficient against filamentous yeasts like *Candida albicans* and fungus. (Rusul Mohammad and Israa Mohammad ., 2011).

Mentha It belongs to the Lamiaceae family and is a well-known genus recognized for its qualities that are both therapeutic and aromatic. In temperate locations all throughout the world, the genus *Mentha* has 25–30 species that are frequently farmed, *Mentha* spp. It is a plant with an important biological activity. Its essential oil, which is obtained from several sections of the plant, including the aerial portions of flowering plants, dried leaves, and fresh flowers, is what gives it its crucial action. (Chavez Gonzalez, 2016)

Pimpinella anisum L., This plant is an Umbelliferae member and is one of the most important and ancient botanical remedies. It is a yearly plant that grows 30 to 50 cm tall. White blossoms and small, green to yellow seeds are present. This plant may be found growing in Mexico, Spain, Egypt, Western Asia, the Middle East, and the Eastern Mediterranean area.(Asie Shojaii and Mehri Abdollahi Fard., 2012)

Anise seeds extracted from the fruits harvested are grown in August and September. Anise seeds utilized as a flavoring, digestive aid, and carminative, and contain 1.5–5% essential oil and antispasmodic substances for stomach cramps. Anise is used extensively by breastfeeding women, as it relieves their children from digestive problems.(Zargari.,1996).

Licium verum: is an 8–15 m tall evergreen tree or shrub with many branches. (Patra et al., 2020). It is a member of the Illiciaceae family (Badianaceae) in the most ancient orderlies. Within the APG IV system (2016), *Illicium* was used to classify *I. verum*. which belongs in the Schisandraceae family (Reveal et al., 2011). The Magnoliopsida family, which includes the well-known traditional herbal remedy *Illicium verum*, has excellent antimicrobial, antioxidant, and insecticidal properties. (Ibrahim et al.,2019; Park et al., 2016)

Cinnamon, also identified as *Cinnamomum verum*, is a little a year-round tree of the Lauraceae family. At the a height about 5-7 meters, it is a medium-sized tree. Sri Lanka is home to the cinnamon plant, whose bark is commonly used as a spice. It has long been understood that cinnamon possesses antifungal, antiviral, and antibacterial properties. The combination of cinnamon essential oil with ortho-methoxycinnamaldehyde may be the

cause of its antibacterial and antifungal properties. The inability of fungal spores to multiply in a medium containing cinnamon was observed. (Bauera and Kirby ., 2012).

Several investigations by various specialists revealed that cinnamon essential oil has the capacity to stop the development of mold on bread.(Bodey.,2011).

Candida: is a fungus-like yeast organism that is naturally present in the mucous membrane of the digestive and reproductive systems and are simple to extract from the oral mucosa, thus it was found that up to about 80 percent of those in good health are most vulnerable to typical fungal illnesses, such candidiasis. Several types of infections caused by *Candida* can range in severity from non-life-threatening to mucosal dermatoses to intrusive situations This might result in a serious illness leading to failure essential organs. (Eckert.,2012).

detected 150 distinct several *Candida* species, just a few of which are know to infect people. Being the most pathogenic of all, *C. albicans*. Several more species that have been identified as being harmful to people are *C. krusei*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. lusitaniae*, *C. dubliniensis*, *C. kefyr*, *C. guilliermondii*, and *C. stellatoidea*. (James.,2006).

Methods:

Collection of Samples: - Samples were taken based on the degree of ripeness (This is connected to the sugar and moisture content of the dates). By using the technique of physical inspection, infested dates were discovered. (Jha.,1995) whereas the fungi in the vicinity were recognized to the extent of (Cheesbrough.,2000).Dried date samples totaling 30 samples were gathered from various Diyala Government districts, namely (Khan

Bani Saad, Kanaan, Katoun Al-Rahma, Sherwin, Al-Tahrir, Shahrban) with 6 samples from each region.

Culture Media: There were 2 media used: Sabouraud Dextrose Agar and Potato Dextrose Agar (PDA), which served as the culture as a whole medium (SDA). They were made in accordance with the directions provided by the manufacturer.

Fungi Isolation: The contaminated date samples were chopped into 3 mm pieces, 2 minutes of surface sterilisation in 1% hypochlorite, then put on Potato Dextrose Agar (PDA), where they were left to incubate for 5 days at room temperature. On the plates after incubation, colonies of various sizes and hues were visible. Each colony type's pure culture was established and kept on each plate. Each of the several colonies was sub-cultured and then incubated once more for five days at room temperature on SDA plates as part of the maintenance process (Jha.,1995).

To distinguish isolated fungus: - A approach was used (James and Natalie, 2001) to identify isolated unidentified fungus using lactophenol to color blue cotton. Using a fixation needle to apply a drop of the stain to a clean slide allowed for identification, on which a compact part of the fungus was removed a drop of lactophenol was added after the fungus cultures. With the needle, evenly distribute the mycelium on the slide. Was a cover slip applied lightly and minimally pressurized to get rid of breath of air. After mounting, During the slide looked at via x10 and x40 objective lenses, respectively. The species that were seen were identified using (Cheesbrough .,2000).

Preparation of aqueous extract.

Aqueous extracts of plants are a simple, economical and environmentally friendly

alternative that can be used as a source of antifungal activity. Plant materials were collected from different regions of Diyala Governorate and identified. Leaves from the plant were dried by air. The plant material was ground into a dry powder with a mountain capacity of (100) grams, mixed 500 milliliters of distilled water, and followed by a minute of boiling to produce the aqueous extract. We sterilized the solution by passing 0.22 μ m Millipore filters. This aqueous extract has been stored at 4°C and depending on the plant it can remain effective for a specified period of time. After completion of the above process stages, the aqueous extract is ready for use in bioassays for antifungal activity. (Chang et al., 2001).

Different amounts of distilled water were added to make different concentrations of extracts, and then it was added to foods containing *Candida* spp. The maximum focus has been described as the attentional focus of its visual product. Triplicates of each experiment were run. (Barret et al;1999).

Results:

Identity of *Candida* Using Sabouraud dextrose agar medium:

Every sample that was taken was grown on Sabouraud dextrose agar (SDA). *Candida* colonies are cream-yellow in color, expand quickly, and mature within 24-48 hours. The colony has a supple texture, glossy, according to the species, wet, or dry. These results were consistent with (Bhavan et al., 2010). In particular, On SDA, *C. albicans* colonies were whitish to cream in color with rounded, silky, supple edges attached to the curved cap, smelling of yeast. Yeast growth has the typical shape in a period of three days. 37°C was used to incubate the plates for 24-48 hours during which yeast colonies develop. Next, different yeasts were identified using culture

characteristics, germ tube testing, and other biochemical tests.

Figure 1: Candida spp on Sabouraud dextrose agar medium.



Identification of Candida species using chrom agar

Chromium agar medium is a selective media commonly used for yeast isolation It offers immediate difference while doing both through color contrast and numerous Candida species have been identified. (Seada et al., 2010). This research revealed that the use of Candida chromium agar, It is an agar with differences, showed colonies of Candida spp.(Hospenthal et al., 2006).(figure 2).

Colorimetric media is an effective and rapid test for diagnosing the level of color produced by Candida species after injection and incubation in comparison to other traditional culture techniques. The a shift in color arising from interactions that produce species-specific enzymes while utilizing a special chromogenic substrate and the medium makes detection more easier of samples comprising blends of several yeast strains. Tested grew well, as suggested in the manufacturer's instructions (Iyampillia et al., 2004).

Figure2: Candida spp. on chrom agar medium



Figure 3: Candida spp.

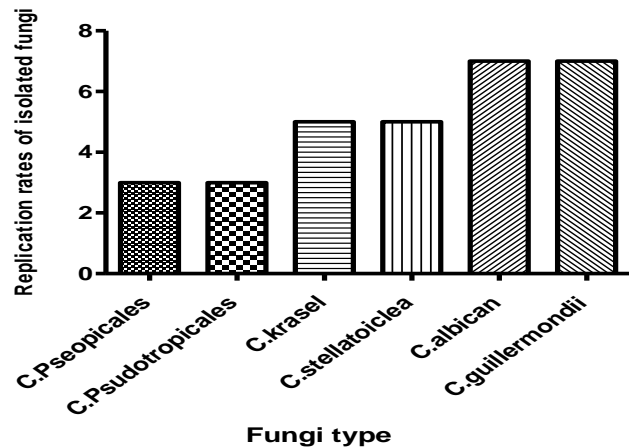


Table 1: The effect of aqueous extract for Salvia officinalis on frequency of fungi isolated from dry dates

Fungi Type	5	10	15	20	25
C. guillermundii	++	+++	++	+	+
C.Krusi	++	+++	+++	++	++
C. Tropical	+++	+++	++	++	++
C. Pseudotropicales	+++	+++	+++	+++	+++
C. Albicans	+++	+++	+++	+++	+++

C.Stellatoidea	+++	+++	++	++	++
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Salvia officinalis aqueous extract at different concentrations prevented the growth of C. Guillermondii. These inhibitors reached a maximum of 100% for the extract at 10 mg/mL.

Minimal prevented at (20-25 mg/ml) of the extract. nonetheless, the greatest inhibition was for C.Krusi at (10-15 mg/ml) and lowest concentration. These inhibitors reached a maximum of 100% extraction at different concentrations against C. Pseudotropicales and C. Albicans, less concentration against C. Tropical and C.Stellatoidea These results were identical with (Rusul Mohammad and Israa Mohammad., 2011).

Figure 4: The effect of aqueous extract for Salvia officinalis on frequency of fungi isolated from dry dates.

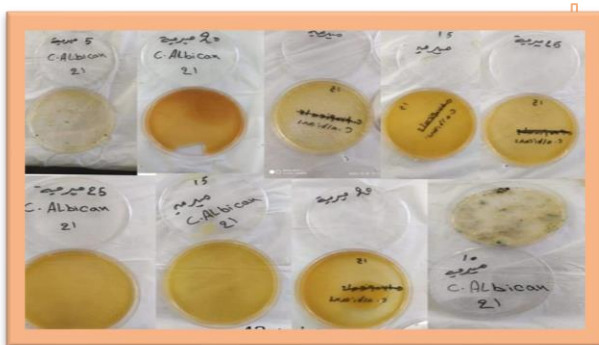


Table 2: The effect of aqueous extract for Illicium verum on frequency of fungi isolated from dry dates

Fungi Type	5	10	15	20	25
C.Stellatoidea	+++	+++	+++	+++	++
C.guillermondii	+++	+++	+++	+++	+++
C.Krusi	+++	+++	+++	++	++
C. Pseudotropicales	+++	+++	++	+++	++

C.Tropical	+++	+++	+++	+++	+++
C. Albicans	+++	+++	+++	+++	+++

At different concentrations, the growth of Candida species was suppressed by the aqueous extract of Illicium verum.

Table 3: The effect of aqueous extract for Mentha on frequency of fungi isolated from dry dates

Fungi Type	5	10	15	20	25
C.guillermondii	+++	+++	+++	++	+
C.Krusi	+++	+++	+++	++	+
C.Tropical	+++	+++	+++	+++	+
C. Albicans	+++	+++	++	+++	+
C. Pseudotropicales	+++	+++	+++	++	+++
C.Stellatoidea	+++	+++	+++	+++	+

The aqueous extract of mint in different concentrations inhibits the growth of Candida fungi.

A maximum of 100% of these inhibitors were present in the extract at (5–10 mg/mL). With the exception of C. pseudotropicales, the lowest inhibitory concentration is 25 mg/ml.

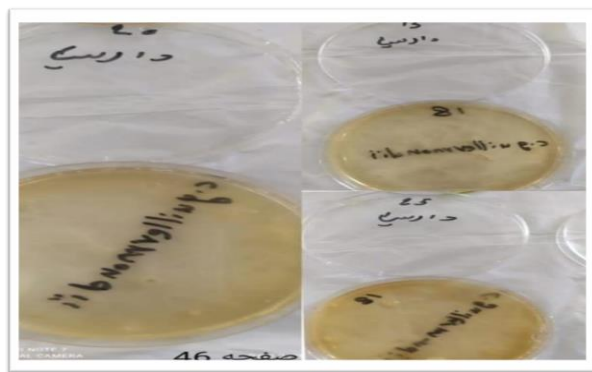
Table 4: Cinnamomum verum's response to an aqueous extract on frequency of fungi isolated from dry dates.

Fungi Type	5	10	15	20	25
C.Stellatoidea	+++	+++	++	+	+
C.guillermondii	+++	+++	++	+	+
C.Krusi	+++	+++	++	++	+
C.Tropical	+++	+++	+	++	+
C. Albicans	+++	+++	++	++	+
C. Pseudotropicales	+++	+++	++	+	+

Aqueous extract of cinnamon in different concentrations inhibited the growth of *Candida* fungi.

These inhibitors obtained a high of 100% for the extract at (5–10 mg/mL). In contrast, 25 mg/mL had the lowest inhibition.

Figure 5: The effect of aqueous extract for *Cinnamomum verum* on frequency of fungi isolated from dry dates.



Discussion:

Content of moisture appears to be one of the main factors supporting dates with fungus growth (Hill and Waller., 1999) being both semi-dry and soft varieties contain all six recognizable ones. It was found that the materials and tools used in storage, such include bags made of polythene, natural fibers, and sacks used by merchants to store all items, may have encouraged the growth of fungi in the dry dates, the subject of the above study. As these storage conditions led to the dates' humidity and temperature have been steadily rising, which consequently leads to the growth of fungi, as mentioned (Ahmed, 2003). But When it came to display, presence in this investigation with a infection rate as a percentage with yeast *C.guilliermondii* and *C.albicans* (23.33%) followed by *C.krusei* and *C.stellatoiclea* (16.66%), and *C. Tropicales*, *C.pseudotropicales* were of the duplicates (10%) respectively. Thus, it is possible that

some of the fungal species identified in the study may have originated from one of these sources mentioned above. Thus, the continual contact that dates have with the outside world during whenever a sale is made can help fungal spores be deposited on them as well. Therefore, spores can germinate when optimum conditions of temperature and humidity are present thus initiating growth processes. Thus, an insect's presence in specific, dangerous circumstances may be very essential for the formation and spread of infection. Accordingly, The suggestions listed below are:- 1. In a cool location, soft dates should be kept, although dried dates are to be kept dry when storing to avoid boosting humidity and to avoid prolonged exposure to air during the sale and then sellers should be encouraged to use hard wrappers. 2. Dates with contamination should be separated and disposed of in order to prevent re-infection or spread. Dates should be washed with pure water before eating them. Competent authorities should be encouraged to monitor closely to stop an illness and the need for a wholesome community.

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