



Anticandidal efficacy of the Secondary Metabolites Extracted from *Carthamus tinctorius* L. Seeds

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Abstract:

The present study was conducted to investigate the effect of the crude Alkaloids, Flavonoids, and Terpenoids compounds extracted from seeds of *Carthamus tinctorius* L. against *Candida* species isolated from different clinical samples such as mouth and vagina in the province of Babil 2021 in Iraq. Antifungal activity was achieved in vitro by using agar well diffusion method against *Candida* species by preparing three concentrations for each crude compound 25, 50, and 100 mg/ml and compared to positive control represented by Fluconazole 50mg/ml and negative control represented by 10% dimethyl sulfoxide. This study aimed to control *Candida* species

isolated from different clinical samples such as mouth and vagina by using secondary metabolites extracted from seeds of *Carthamus tinctorius* L. The data collected from the study revealed that the crude Alkaloids, Flavonoids, and Terpenoids compounds extracted from seeds of *Carthamus tinctorius* L. showed reduction at $P \leq 0.05$ in the growth of *Candida* species, especially at 100mg/ml compared with negative control. Finally, it can be concluded that *Carthamus tinctorius* L. is most effective in controlling *Candida* species, especially Alkaloids compounds.

Keywords: Anticandidal, *Carthamus tinctorius* L., Alkaloids, Flavonoids, Terpenoids.

Introduction

The use of plant metabolites for medicinal and cosmetic purposes is gaining popularity today (Njila, et al., 2017). Secondary metabolites serve as competitive weapons used against other bacteria, fungi, amoebae, plants, insects, and large animals (Demain, & Fang, 2000). Alkaloids are generally present in higher plants, particularly in dicots, whereas only a few have been noted in lower plants. The alkaloids can occur in the whole plant or the specific plant organ. Alkaloids

are derived from amino acid and mostly contain one or more carbon rings which usually contain nitrogen (Egamberdieva, et al., 2017). Terpenes are the largest class of secondary metabolites having hydrocarbons and the combination of several five-carbon isoprene units resulting from hydrocarbons (Chanda, & Ramachandra, 2019). Terpenes interact with Biomembranes and membrane proteins, and most the terpenoids are lipophilic. In general, terpenes show cytotoxic activities against a wide range of organisms,

ranging from bacteria and fungi to insects and vertebrates have been widely used in herbal medicine against infections (Wink, 2015). Flavonoids are a group of plant secondary metabolites where the molecular framework is categorized by variable phenolic structures (Njila, et al., 2017). Flavonoids are divided into two classes due to the position of the benzenoid substituent, such as (flavone) two position and (isoflavones) three position; Flavonoids be active ingredients of numerous herbal medicines (Wink, 2015). *Carthamus tinctorius* L. (Safflower) of the family Asteraceae is a medicinal plant with great potential. Its extract and oil have many therapeutic uses and have great pharmacological importance. Safflower is mainly cultivated for its seeds, oil, and flowers. It is to cure many day-to-day ailments and has proved importance as purgative, analgesic, anti-inflammatory, antipyretic, menstrual problems, post-partum hemorrhage, osteoporosis, diabetes, hepatoprotection, cancer, fibrosis, and antioxidant. Carthamine, hydroxyl safflower yellow-A, carthamidine, luteolin are the main phytoactive principles of this plant. Flavonoid derivatives and Furanocoumarins are a target for inflammatory, antimicrobial, anticancer, and antidiarrheal drugs (Rashmi Dehariya, & Dixit, 2015). More than 200 compounds have been isolated from *C. tinctorius*, and the commonly known ones are flavonoids, phenylethanoid glycosides, coumarins, fatty acids, steroids, and polysaccharides (Zhou, Zhao, & Tu, 2009).

Candida albicans is an oral commensal flora that causes opportunistic local and systemic infections in immunocompromised individuals. Fluconazole is frequently used for treating patients with active infections or preventing recurrent infections. The emergence of resistant strains encouraged scientists to search for compounds that have antifungal properties and can overcome the usual microbial-resistant mechanisms of antimicrobial agents (Abdulaziz, Shaswary, & Muhammad, 2015). From this standpoint, scientists should search for natural sources that are less harmful, environmentally friendly, less expensive, and more efficient in anticipation of the spread of resistant strains in microorganisms to control fungi and reduce as much as possible the use of fungicides. However, this study aimed to control *Candida* species isolated from different clinical samples by using secondary metabolites extracted from *Carthamus tinctorius* L. seeds

Materials and Methods

Plant Material

Safflower seeds (*Carthamus tinctorius* L.) were purchased from local markets and identified based on the taxonomic features of Iraqi Flora (Ghazanfar, Edmondson, & Nicholas Hind, 2019). (Table: 1). Seeds of these plants were cleaned, dried, and kept according to Harborne, Mabray, & Marby, (1975).

Table 1. Scientific, Local, English Name, Family, and Active Parts

Scientific name	Local name	English name	Family	Active part used
<i>Carthamus tinctorius</i> L.	Asfur, Asfoor, Usfur	Safflower	Asteraceae	Seeds

Extraction of the Crude Alkaloid Compounds

Crude Alkaloid compounds were extracted according to Harborne, (1973).

Extraction of the Crude Flavonoid Compounds

Crude Flavonoid compounds were extracted according to Boham, & Kocipai-Abyazan, (1974).

Extraction of the Crude Terpenoid Compounds

Crude terpenoid compounds were extracted according to Al-Jassani, (2017). Stock solution of 100 mg/ml for Alkaloid, Flavonoid, and Terpenoid was prepared in 10% Dimethyl Sulfoxide (DMSO), then sterilized by Millipore filter (0.22µm) and stored at (-20C°) until use (Al-Jassani, 2017).

Anti-Candidiasis Efficacy

The anti-candidiasis activity of the secondary metabolites compounds extracted from the seeds of *Carthamus tinctorius* L. was tested against the isolates of *Candida* species by using the agar-well diffusion method (Perez, Pauli, & Bazerque, 1990). Wells were made by using a cork porer (6mm) in diameter. Dimethyl sulfoxide 10% (DMSO) was used as a negative control, and Fluconazole antibiotic as a positive control.

Candida Isolates

All isolates used in this study were isolated from hospitals located in Hillah City, Iraq (Table: 2).

Table: 2. Types of Candida Isolates and Their Sources

N0	Fungal isolates	Type of specimen
1	Candida Isolates	Mouth Vagina

Statistical Analysis

All data of treatments were dictated by three replicates. Data were subjected to an analysis of variance by using SPSS 16.0 program, a completely randomized design was used, and the least significant difference (L.S.D) was performed at $P \leq 0.05$.

Results

The results of antifungal activity of the crude Alkaloid compounds extracted from the seeds of *Carthamus tinctorius* L. against *Candida* species isolated from different clinical samples such as mouth and vagina are presented in (table 3). The antifungal activity of Alkaloid secondary metabolites with three concentrations (25, 50, and 100mg/ml) was screened by agar well diffusion methods. The results revealed that the crude Alkaloid compounds extracted from the seeds of *arthamus tinctorius* L. showed a significant reduction at $P \leq 0.05$ in the growth of *Candida* species. Growth inhibition represented by zone of inhibition ranging from (22 ± 1.00 mm in 25 mg/ml, 26 ± 1.00 mm in 50 mg/ ml, and 30 ± 1.00 mm in 100 mg/ml) (Figure: 1), compared

with negative control representative by 10% DMSO and positive control representative by Fluconazole 50mg/ml (Figure: 2) where inhibition zone was (0.00 mm for negative control and 35 ± 1.00 mm for positive control). On the other hand, the crude Flavonoid compounds showed 8 ± 1.00 mm of the zone of inhibition at (25 mg/ml) 12 ± 1.00 mm at (50 mg/ml), and 21 ± 1.00 mm at (100 mg/ml) concentration (table 4), Thus, it differed significantly compared to the control treatment (Figure: 3).

Table 3. Anticandidal Activity of the Crude Alkaloid Compounds Extracted from *Carthamus tinctorius* L. Seeds

Concentrations (mg/ml)	Alkaloids compounds Inhibition Zone/mm
Negative Control	0 ± 0.00
25 mg/ml	22 ± 1.00
50 mg/ml	26 ± 1.00
100 mg/ml	30 ± 1.00
Positive Control	35 ± 1.00
L.S. D	1.62
*Mean $\pm$ standard deviation	

Table 4. Anticandidal Activity of the Crude Flavonoid Compounds Extracted from *Carthamus tinctorius* L. Seeds

Concentrations (mg/ml)	Flavonoid compounds Inhibition zone %
Negative Control	0 ± 0.00
25 mg/ml	8 ± 1.00
50 mg/ml	12 ± 1.00
100 mg/ml	21 ± 1.00
Positive Control	35 ± 1.00
L.S. D	1.60
*Mean $\pm$ standard deviation	

Table 5. Anticandidal Activity of the Crude Terpenoid Compounds Extracted from *Carthamus tinctorius* L. Seeds

Concentrations (mg/ml)	Terpenoid compounds Inhibition zone %
Negative Control	0 ± 0.00
25 mg/ml	21 ± 1.00
50 mg/ml	24 ± 1.00
100 mg/ml	26 ± 1.00
Positive Control	35 ± 1.00
L.S. D	1.61
*Mean $\pm$ standard deviation	

In the same context, the crude Terpenoid compounds showed significant activity at three concentrations (25, 50, and 100 mg/ml) compared with negative control against *Candida* species isolated from different clinical samples (Table 5). The highest zone of inhibition, 26 ± 1.00 mm, was recorded at 100 mg/ml, and 24 ± 1.00 mm was recorded at 50 mg/ml (figure: 4). While the highest zone of inhibition in the crude

Alkaloid compounds reached 30 ± 1.00 mm at 100 mg/ml concentration and the highest zone of inhibition in the crude Flavonoid compounds was reached up to 21 ± 1.00 at 100 mg/ml concentration (Figure: 5). The alkaloid compounds were the most effective compared to the rest of the active compounds such as Terpenoids and Flavonoid came close to the effect of the Fluconazole (35 ± 1.00 mm).

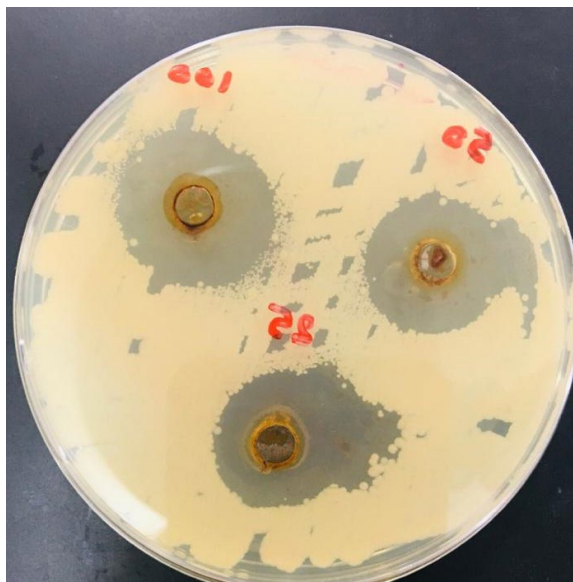


Figure 1. Anticandidal Activity of the Crude Alkaloid Compounds Extracted from (*C. tinctorius L*) seeds at 25, 50, and 100 mg/ml



Figure 2. Fluconazole as a Positive Control at 50mg/ml

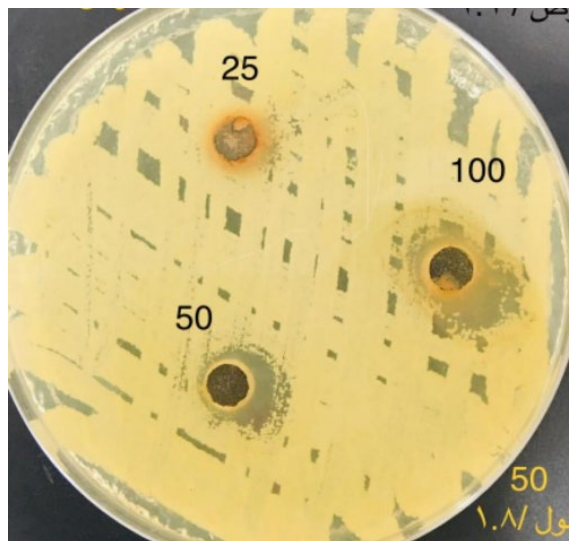


Figure 3. Anticandidal Activity of the Crude Flavonoid Compounds Extracted from *C. tinctorius* Seeds at 25, 50, and 100 mg/ml

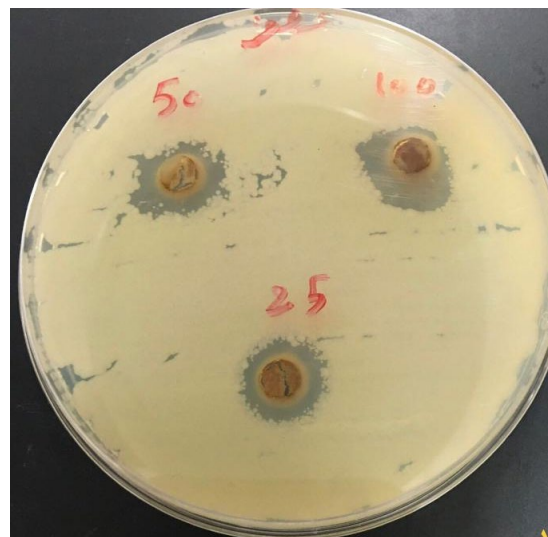


Figure 4. Anticandidal Activity of the Crude Terpenoid Compounds Extracted from *C. tinctorius* Seeds at 25, 50, and 100 mg/ml

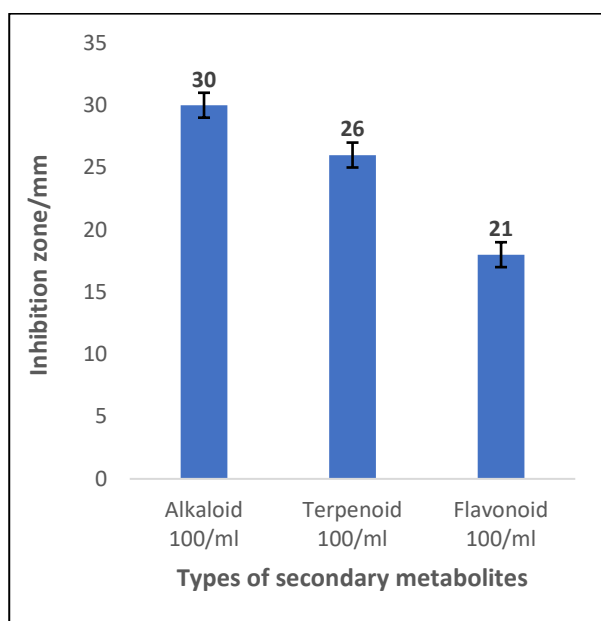


Figure 5. Inhibition Zone of Alkaloid, Terpenoid, and Flavonoid at 100/ml against *Candida* species, LSD= 1.81

Discussion

The present study was proved that, the secondary metabolites include Alkaloids, Flavonoids, and Terpenoids extracted from the seeds of (*Carthamus tinctorius* L.) have powerful antifungal activity against *Candida* species isolated from different clinical samples such as mouth and vagina. The plant kingdom provided and is still providing endless sources of medicinal plants of various uses for example, Secondary metabolites extracted from different active parts of numerous medicinal plants such as (*Lactuca serriola* leaves; *Lepidium sativum* leaves; *Myrtus Communis* leaves; *Cassia senna* leaves; *Ricinus communis* leaves; *Cassia didymobotrya* leaves; *Melia azedarach* leaves; *Dianthus caryophyllus* flowers bud; and *Salvia hispanica* seeds), possess the ability of antibacterials for controlling several pathogenic microorganisms isolated from different clinical samples (Al-Marzoqi, Hussein, & Al-Khafaji, 2015; Al-Marzoqi, Al-Khafaji, & Hussein, 2016; Hussein, et al., 2017; Hussein, Al-Khafaji, & Al-Mamoori, 2018; Hussein, et al., 2018; Hussein, et al., 2020; Hussein, & Al-Marzoqi, 2020; Kamil, Hussein, & Al-Marzoqi,

2020; Hussein, Kamal, & Sahi, 2020). Hussein, Naji, & Al-Khafaji, (2018) reported that phytochemical compounds extracted from the unicellular primitive plant like *Chlorella vulgaris* possess the ability to antibacterial counter to pathogenic bacteria. Kamal, Hussein, & Tolaifeh, (2019) used phytochemical compounds extracted from *Hibiscus sabdarifa* for controlling *E. coli* and *Proteus* sp. Kamal, Al-Kaim, & Hussein, (2020) used phytochemical compounds extracted from *Ficus carica* L. for controlling *E. coli* and *Pseudomonas aeruginosa*. AL-Masoodi, Hussein, & Al-Rubaye, (2020) used phytochemical compounds extracted from *Boswellia carteri* and *Curcuma longa* for controlling *Fusarium* sp. isolated from seeds of maize. Hussain, A.Y., Hussein, H.J., & Al-Rubaye, A.F. (2021) used terpenoid compounds extracted from *Carthamus tinctorius* seeds and flavonoid compounds extracted from *M. Communis* leaves against *Aspergillus* species isolated from stored medicinal plant seeds. Secondary metabolites represented by Alkaloids and flavonoid compounds extracted from *M. Communis* leaves are respected as a worthy source for controlling pathogenic microorganisms segregated from hemodialysis fluid specimens (Sharara, Al-Marzoqi, & Hussein, 2021). Radhi et.al. (2022) Used *Callistemon viminalis* leaves extracts for controlling isolates of Urinary Tract Infections. *Allium sativum* respected a good source for controlling some bacterial species isolated from infected patients with Corona Virus (Hussein, et al., 2023). Secondary metabolite compounds extracted from the *D. caryophyllus* L. flower buds, such as terpenoid and flavonoid, have powerful antifungal activity against *Candida* species (Karim, Hussein, & Al-Rubaye, 2023). Alkaloids and Terpenoids extracted from the roots of *Saussurea costus* have powerful antifungal activity against *Candida* species (Karim, Hussein, Al-Rubaye, 2022), *S. costus* roots also have powerful antifungal activity against *Aspergillus* species isolated from rice seeds (Al-Nafie, Hussein, & Al-Rubaye, 2024). The antifungal activity of the carthamin natural pigment of safflower was more active against *Candida albicans* than precarthamin (Salem, et al., 2014). Safflower oil

extracted from seeds exhibited significant antifungal activity against *Candida parapsilosis* and *Candida sake* (Khémiri et al., 2020). On the other hand, the mode of the antifungal action of the Alkaloids is usually pleiotropic, where protein synthesis is inhibited, and the fungal DNA is intercalated or by boosting the development of fungi inhibitors (Arif, et al., 2009). Terpenoids reduce the mitochondrial content, thus modifying the level of reactive oxygen species (ROS) and ATP generation. It is also reported that triterpenoid possesses more potent antifungal activity as compared to tetraterpenoid (Haque, et al., 2016). Terpenoids and flavonoids have their effects by disrupting microbial membranes (Okusa, Stévigny, & Duez, 2009). Medicinal plants possess antifungal effects by many mechanisms. They caused membrane disturbance resulting in the loss of membrane integrity, inhibited DNA transcription and reduced the cell populations, inhibited the activity of fungal antioxidant enzymes, and inhibited fungal biofilm formation (Evensen, & Braun, 2009; Wu, et al., 2013). Finally, the anti-candidiasis activity of *Carthamus tinctorius* L. seeds might belong to secondary metabolites like Alkaloids, Flavonoids, and Terpenoids and their effect on proteins and DNA synthesis and disruption in membranes permeability or disturbance in metabolic activity.

Conclusion

Alkaloids, Flavonoids, and Terpenoids extracted from the seeds of *Carthamus tinctorius* L. have powerful antifungal activity against *Candida* species.

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