



## Antidermatophyte efficacy of the secondary metabolites extracted from *Citrullus colocynthis* L. fruits

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### ABSTRACT

The present study, was conducted to investigate the effect of the crude alkaloid, terpenoid, and flavonoid extracts from *Citrullus colocynthis* L. fruits against dermatophyte species such as *Chrysosporium* sp., *Trichophyton mentagrophytes*, *Microsporum canis*, *Trichophyton quinckeanum*, and *Trichophyton interdigitale* isolated from clinical samples in the province of Babil, Iraq during 2022. Antifungal activity was achieved *in vitro* using food poisoning method against dermatophyte species by preparing three concentrations for each crude compound including 5, 10, and 15 mg mL<sup>-1</sup> and compared to positive control represented by Fluconazole 150 mg and a negative control represented by 10% dimethyl sulfoxide. The aim of this study was to control of dermatophyte species isolated from clinical samples using secondary metabolites extracted from *Citrullus colocynthis* L. fruits. The data collected from the study revealed that the crude alkaloids, terpenoids, and flavonoids extracts from *Citrullus colocynthis* L. fruits showed significant reduction at  $p < 0.05$  in the growth of dermatophyte species especially at 15 mg mL<sup>-1</sup> compared with negative control and the same effects compared to the Fluconazole. Finally, it can be concluded that *Citrullus colocynthis* L. is most effective in controlling dermatophyte species.

**Keywords:** Antidermatophytal, *Citrullus colocynthis* L, Alkaloids, Flavonoids, Terpenoids.

**Article type:** Research Article.

### INTRODUCTION

Bitter apple, *Citrullus colocynthis* is a useful cucurbit plant that is extensively dispersed throughout the world's arid locations. These plants are mainly found in the deserts of Arabia, the Sahara and the southern part of Asia including India, Pakistan, and southern islands (Coffey *et al.* 2015). *C. colocynthis* is a fruit belonging to the Cucurbitaceae family (Ziyyat *et al.* 1997). It is considered extraordinary compared to other hereditarily various collections of remedial plants in the plant realm (Dhakad 2017). *C. colocynthis* is known as bitter apple in the English language (Patrick *et al.* 1960) and Hindal in Arabic (Al Ghaithi *et al.* 2004). *C. colocynthis* is herbaceous plant packed with abundance of nutrients which plays key role in improvement of well-being. This is an underutilized and less known fruit crop of arid region. Almost all parts of such fruit crop are used for health benefits. Seeds are rich source of oil and protein with superior fatty acid and amino acid profile. Other than nutrients, there are number of bioactive compounds such as cucurbitacin, flavonoids and polyphenols are present in *C. colocynthis* which are further responsible for medicinal properties (Bhasin *et al.* 2020). Millions of people suffer from superficial infections caused by dermatophytes (Grumbt *et al.* 2013), which comprise pathogenic fungi exhibiting a high affinity for the keratinized structures present in nails, skin, and hair, causing superficial infections known as dermatophytosis. A reasonable number of antifungal drugs currently exist on the pharmaceutical market to control mycoses; however, their cellular targets are restricted, and fungi may exhibit tolerance or resistance to these agents. For example, the stress caused by antifungal and cytotoxic drugs in sub-inhibitory concentrations promotes compensatory stress responses, with the over-expression of genes involved in cellular detoxification, drug efflux, and signalling pathways being among the various mechanisms that may contribute to drug tolerance.

In addition, the ATP-binding cassette transporters in dermatophytes that are responsible for cellular efflux can act synergistically, allowing one to compensate for the absence of the other, revealing the complexity of drug tolerance phenomena (Martinez Rossi *et al.* 2018). Plant extracts have shown inhibitory effect on the growth of wide range of fungi. They are represented a good alternative for prevention and treatment of fungal diseases (Al Snafi 2019). From this standpoint, humans should search for natural sources that are less harmful and environmentally friendly in order to control fungi and reduce as much as possible the use of fungicides and antibiotics. However, the aim of this study was to control dermatophytes species isolated from clinical samples using secondary metabolites extracted from *Citrullus colocynthis* L. fruits

## MATERIALS AND METHODS

**Plant material.** Fruits of *Citrullus colocynthis* L., were purchased from local markets, identified based on the taxonomic features in Iraqi Flora (Ghazanfar *et al.* 2019; Table 1). Fruits of these plants were cleaned, dried, and kept according to (Harborne *et al.* 1975; Table 1).

**Table 1.** Scientific, Local, English name, Family, and active parts.

Scientific name	Local name	English name	Family	Active part used
<i>Citrullus colocynthis</i> L.	Hindal	Bitter apple	Cucurbitaceae	Fruits

**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 terpenoids compounds were extracted according to Harborne (1984). Stock solution of 100 mg mL<sup>-1</sup> for alkaloids, flavonoids, and terpenoids were prepared in 10% dimethyl sulfoxide (DMSO) then sterilized by Millipore filter (0.22 µm) and stored at -20 °C until use (Al Jassani 2017).

**Collection and cultivation of specimens.** A total of 60 scraps of skin scales, hair parts and nail were prepared. Samples were collected from patients with dermatophytosis and kept in sterile containers. For identification of dermatophytes, these specimens were cultured on Sabouraud dextrose agar (SDA) with chloramphenicol and cycloheximide and cultured at 25-30 °C for up to 4 weeks.

**Diagnosing fungal colonies.** After appearance of growth as well as examining colonies of fungi from the colony colour, shape and texture (powdery, granular, cottony), the recorded pigments were examined on foundation at surface of colony and appearance. Fungi isolates were examined microscopically, by taking the fingerprint of the fungus in the colony using adhesive tape touching with the surface of the fungal colonies and then paste the tape on a glass slide containing a drop of lactophenol cotton blue. Slides were examined under magnification 10X, 40X and 100X as described by Astrid (1999).

**Antifungal activity assay of extract.** PDA medium was prepared and autoclaved. Afterward, a known volume (2 mL) of each plant extract concentrations were placed in the centre of the petri dishes and completed the volume to 20 mL with PDA medium to obtain the required final concentrations (5, 10, and 15 mg mL<sup>-1</sup>) of the medicinal plant. After complete solidification of the medium, 5 mm disc of 28 days-old culture of the test fungus was placed aseptically on the centre of the Petri plates and incubated at 25-30 ± 2 °C for 28 days. Simultaneously 0.02 mL of antibiotic solution was added to each assay plate to check the bacterial contamination as suggested by (Gupta & Banerjee 1970). Fluconazole (150 mg) was used as positive control and dimethyl sulfoxide as a negative control. Observations were recorded on 28<sup>th</sup> day. The colony diameter was recorded in terms of millimetres. PDA medium devoid of extract served as control. For each treatment, three replicates were maintained. The fungi toxicity of extracts was calculated in terms of percent inhibition of mycelia growth using the following formula (Singh & Tripathi 1999).

$$\text{Inhibition rate (\%)} = (dc - dt / dc) \times 100$$

where:

dc = Average increase in mycelia growth in control.

dt = Average increase in mycelia growth in treatment.

**Statistical analysis.** All data of treatments were dictated by three replicates. Data were subjected to an analysis of variance using SPSS 16.0 program. A completely randomized design was used and least significant difference (LSD) was performed at  $p \leq 0.05$ .

## RESULTS

The results of antifungal activity of the crude alkaloid compounds extracted from the *Citrullus colocynthis* fruits against dermatophytes such as *Chrysosporium* sp., *Trichophyton mentagrophytes*, *Microsporum canis*, *Trichophyton quinckeanum*, and *Trichophyton interdigitale* isolated from clinical samples are presented in Table 2. The antifungal activity of alkaloid secondary metabolites with three concentrations (5, 10, and 15 mg mL<sup>-1</sup>) was screened by food poisoning methods. The results revealed that the crude alkaloid compounds extracted from the *C. colocynthis* fruits showed significant reduction ( $p < 0.05$ ) in the growth of dermatophyte species. Antifungal activities were applied at 5, 10, and 15 mg mL<sup>-1</sup>. Inhibitory rate (%) of alkaloids occurred ranging from 54.4% in 5 mg mL<sup>-1</sup>, 73.7% in 10 mg mL<sup>-1</sup>, and 100% in 15 mg mL<sup>-1</sup> once applying *Chrysosporium* sp. It was 52.5% to 100% once applying *Trichophyton mentagrophytes*, while ranging from 73.3% to 100% once applying *Microsporum canis*. In addition, inhibitory rate reached 100% once applying *Trichophyton quinckeanum* and *Trichophyton interdigitale* at 15 mg mL<sup>-1</sup> of alkaloid extract (Fig. 1) compared to negative and positive controls. The inhibitory rate was 0.00% for negative control (Fig. 5), while 100% for positive control (Fig. 4). In the same context, the crude terpenoid compounds showed 43.3% growth inhibition at 5 mg mL<sup>-1</sup>, 60.7% at 10 mg mL<sup>-1</sup>, and 100% at 15 mg mL<sup>-1</sup> once applying *Chrysosporium* sp. It was from 47.7% at 5 mg mL<sup>-1</sup> to 100% at 15 mg mL<sup>-1</sup> once applying *Trichophyton mentagrophytes*. In addition, inhibitory rate reached 100% once applying *Microsporum canis*, *Trichophyton quinckeanum* and *Trichophyton interdigitale* at 15 mg mL<sup>-1</sup> of terpenoid extracts respectively (Table 3). Thus, it differed significantly compared to the control treatment (Fig. 2).

**Table 2.** Anti-dermatophytal efficacy of the crude alkaloid compounds extracted from *Citrullus colocynthis* fruits against dermatophytes isolated from clinical samples.

Concentration (mg mL <sup>-1</sup> )	Pathogenic fungi				
	<i>Chrysosporium</i> sp.	<i>T. mentagrophytes</i>	<i>M. canis</i>	<i>T. quinckeanum</i>	<i>T. interdigitale</i>
	Inhibitory rate (%) of alkaloids				
Control negative (DMSO 10%)	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
5	54.4 ± 1.1	52.5 ± 1.6	73.3 ± 1.1	55.9 ± 1.7	55.5 ± 1.1
10	73.7 ± 2.7	62.6 ± 1.7	77.4 ± 1.6	65.1 ± 1.6	64.4 ± 2.2
15	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Control positive	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
LSD	2.445	1.950	1.648	1.951	2.191

Note: \*Mean ± standard deviation; Mean difference is significant at 0.05 level.

**Table 3.** Anti-dermatophytal efficacy of the crude Terpenoid compounds extracted from *Citrullus colocynthis* fruits against Dermatophytes isolated from clinical samples.

Concentration (mg mL <sup>-1</sup> )	Pathogenic fungi				
	<i>Chrysosporium</i> sp.	<i>T. mentagrophytes</i>	<i>M. canis</i>	<i>T. quinckeanum</i>	<i>T. interdigitale</i>
	Inhibitory rate (%) of terpenoids				
Control negative (DMSO 10%)	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
5	43.3 ± 1.1	47.7 ± 1.1	47.4 ± 0.6	50.3 ± 1.2	53.7 ± 0.7
10	60.7 ± 1.7	61.1 ± 1.1	71.1 ± 1.1	57.4 ± 0.6	58.8 ± 1.1
15	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Control positive	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
LSD	1.652	1.277	1.043	1.170	1.966

Note: \*Mean ± standard deviation; Mean difference is significant at 0.05 level.

In addition, the crude flavonoid compounds showed significant activity at three concentrations (5, 10, and 15 mg mL<sup>-1</sup>) compared to negative control against dermatophyte species isolated from clinical samples (Table 4). The highest rate of inhibition (100%) was recorded at 15 mg mL<sup>-1</sup> once applying *Trichophyton mentagrophytes*, *Trichophyton quinckeanum*, and *Trichophyton interdigitale* (Fig. 3). On the other hand, the effect of secondary

metabolite compounds extracted from *C. colocynthis* was equal to the effect of the antibiotics, which confirms the effectiveness of its metabolites against dermatophytes under study.

**Table 4.** Anti-dermatophyte efficacy of the crude Flavonoid compounds extracted from *Citrullus colocynthis* fruits against *Dermatophytes* isolated from clinical samples.

Concentration (mg mL <sup>-1</sup> )	Pathogenic Fungi				
	<i>Chrysosporium</i> sp.	<i>T. mentagrophytes</i>	<i>M. canis</i>	<i>T. quinckeanum</i>	<i>T. interdigitale</i>
	Inhibitory rate (%) of flavonoids				
Control negative (DMSO 10%)	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
5	42.2 ± 1.1	38.4 ± 0.6	56.9 ± 1.6	46.2 ± 1.6	38.8 ± 1.1
10	53.3 ± 2.2	48.1 ± 1.7	60.3 ± 4.2	53.3 ± 1.1	51.1 ± 1.1
15	98.5 ± 0.6	100 ± 0.0	97.7 ± 1.1	100 ± 0.0	100 ± 0.0
Control positive	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
LSD	2.852	2.228	3.832	1.634	1.295

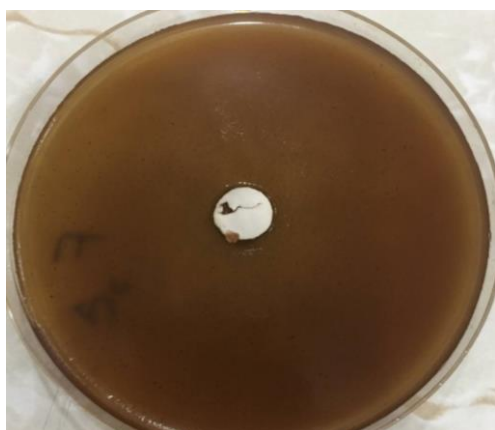
Note: \*Mean ± standard deviation; Mean difference is significant at 0.05 level.



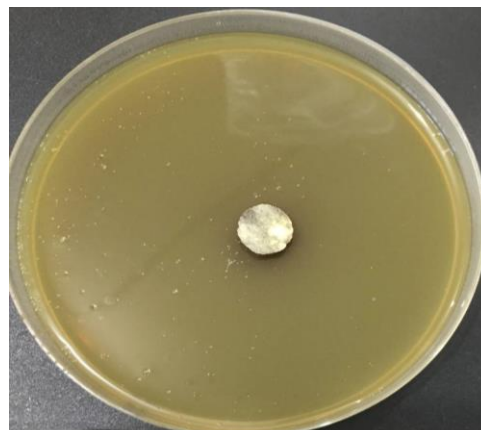
**Fig. 1.** Anti-dermatophyte efficacy of the crude alkaloid compounds at 15 mg mL<sup>-1</sup> against dermatophyte species



**Fig. 2.** Anti-dermatophyte efficacy of the crude Terpenoid compounds at 15 mg mL<sup>-1</sup> against dermatophyte species



**Fig. 3.** Anti-dermatophyte Efficacy of the crude Flavonoid compounds at 15 mg mL<sup>-1</sup> against dermatophyte species



**Fig. 4.** Growth of dermatophyte species in antibiotic control positive



**Fig. 5.** the growth of dermatophyte species in control negative treatment

## DISCUSSION

Although the resistance of dermatophytes to antifungals and cytotoxic drugs in different ways such as modifications of target enzymes, over-expression of genes encoding ATP-binding cassette transporters and stress-response-related proteins, the present study confirmed that, the secondary metabolites including alkaloids, terpenoids, and flavonoids extracted from the *Citrullus colocynthis* L. fruits have powerful antifungal activity against dermatophyte species isolated from clinical samples. The plant kingdom provided and is still providing endless sources of medicinal plants of various application. For example, secondary metabolites extracted from different active parts of numerous medicinal plants such as leaves of *Lactuca serriola*, *Lepidium sativum*, *Myrtus Communis*, *Cassia senna*, *Ricinus communis*, *Cassia didymobotrya*, *Melia azedarach*, as well as flower buds of *Dianthus caryophyllus*; and seeds of *Salvia hispanica* possess ability of antibacterials for controlling several pathogenic microorganisms isolated from different clinical samples (Al Marzoqi *et al.* 2015; Al Marzoqi *et al.* 2016; Hussein *et al.* 2017; Hussein *et al.* 2018; Hussein *et al.* 2018; Hussein *et al.* 2019; Hussein & Al Marzoqi 2020; Kamil *et al.* 2020; Hussein *et al.* 2020; Hamad *et al.* 2023; Kadhim & Younis 2023; Iriani *et al.* 2023; Lattef Ismaeel 2024). Hussein *et al.* (2018) reported that phytochemical compounds extracted from the unicellular primitive plant such as *Chlorella vulgaris* possess ability of antibacterial counter to pathogenic bacteria. Kamal *et al.* (2019) used phytochemical compounds extracted from *Hibiscus sabdarifa* for controlling Enterobacteriaceae. Kamal *et al.* (2020) employed phytochemical compounds extracted from of *Ficus carica* L. for controlling *E. coli* and *Pseudomonas aeruginosa*. AL Masoodi *et al.* (2020) used phytochemical compounds extracted from *Boswellia carteri* and *Curcuma longa* for controlling *Fusarium* spp. isolated from seeds of maize. Hussain *et al.* (2021) employed terpenoids 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 the alkaloid and flavonoid compounds extracted from *M. Communis* leaves are a worthy source for controlling pathogenic microorganisms segregated from hemodialysis fluid specimens (Sharara *et al.* 2021). Safa *et al.* (2022) used *Callistemon viminalis* leaf extracts for controlling isolates of urinary tract infections. Alkaloids and terpenoids extracted from the roots of *Saussurea costus* exhibited powerful antifungal activity against *Candida* species (Karim *et al.* 2022). The terpenoid and flavonoid compounds are most effective in controlling *Candida* species (Mohammed Karim *et al.* 2023). The extracts of *C. colocynthis* seeds used at low concentration may have significant potential for biological control of fungi and their toxins (Gacem *et al.* 2013). *C. colocynthis* seeds display inhibitory effect against *Rhizopus* spp. (Prasad 2014). Its fruits have inhibitory effects against different *Candida* and *Aspergillus* strains (Eidi *et al.* 2015). and also have antifungal properties against resistant *Candida* spp. (Alsubhi *et al.* 2019). It has an inhibitory effect on bacteria and *Candida albicans* (Tahmasebi *et al.* 2022). *C. colocynthis* seed methanol extract from Sinai Desert, Egypt combined with 0.5  $\mu\text{g mL}^{-1}$  of the antifungal drug fluconazole was more effective against dermatophytes than the extract or fluconazole each on its own (Ouf *et al.* 2022). 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 modify the level of reactive oxygen species (ROS) and ATP generation. It is also reported that triterpenoid possesses more potent antifungal activity as compared to the tetraterpenoid (Haque *et al.* 2016). Terpenoids and flavonoids make their effects by

disruption of microbial membranes (Okusa *et al.* 2009). Medicinal plant possessed antifungal effects by many mechanisms, causing membrane disturbance resulting in the loss of membrane integrity, inhibiting DNA transcription and reducing cell populations, as well as inhibiting the activity of fungal antioxidant enzymes and also fungal biofilm formation (Braun 2009; Wu *et al.* 2013). Alternatively, the presence of alkaloid compounds such as colocyntidin and colocyntin may disrupt cytoplasmic membrane of the microorganisms through their action on lipids and protein (Anthony 1976). Finally, antifungal activity of *C. colocynthis* L. might be belonging to secondary metabolites like alkaloids, flavonoids, and terpenoids and their effects on proteins and DNA synthesis and disruption in membrane permeability or disturbance in metabolic activity.

## CONCLUSION

Alkaloids, flavonoids, and terpenoids extracted from the fruits of *Citrullus colocynthis* L. have powerful antifungal activity against dermatophytes species.

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